Eur. J. Med. Chem. **24** (1989) 479–484

Original paper

Synthesis and β-adrenergic blocking activity of 1,4-benzothiazine oxime ethers**

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(Received 19 July 1988, accepted 13 February 1989)

Summary — Synthesis of 6-, 7-, 8-acetimidoyloxypropanolamines of 3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine and 8-iminoxypropanolamines of 3,4,5,6,7,8-hexahydro-3-oxo-2*H*-1,4-benzothiazine are reported. All of the synthesized compounds were tested *in vitro* for their ability to displace [^{3}H]dihydroalprenolol from turkey erythrocyte membranes and *in vivo* for their β-adrenoceptor blocking activity by the inhibition of isoprenaline-induced tachycardia and compared with the corresponding oxypropanolamines which we had previously described as β-blockers.

Résumé — Synthèse et activité β-bloquante adrénergique de certains éthers d'oximes dérivés de la benzothiazine-1,4. *Les auteurs décrivent la synthèse d'acétimidoyloxy-6, -7 et -8 propanolamines dérivées de la dihydro-3,4-oxo-3-2H-benzothiazine-1,4 et iminoxy-8-propanolamines dérivées de l'hexahydro-3,4,5,6,7,8-oxo-3-2H-benzothiazine-1,4. Tous les composés synthétisés, ont fait l'objet d'essais* in vitro, *pour étudier leur capacité à déplacer le [^{3}H]dihydroalprenolol des membranes d'érythrocytes de dindon et,* in vivo, *pour étudier l'activité β-bloquante, par l'inhibition de la tachycardie induite par l'isoprénaline.*

oxime ethers / β-blockers / 1,4-benzothiazine

Introduction

An ethanolamine side-chain or, more frequently, an oxypropanolamine one linked to an aromatic ring are the chemical features required for β-blocking activity; however the alteration of these features as the intercalation of an iminic group in the side-chain to give aromatic [1–5], alicyclic or aliphatic [6–8] iminoxypropanolamines does not abolish the interaction on β-adrenoceptors but can lead, in some cases, to potent β-antagonists [7, 9].

With the aim of evaluating the effect caused by a different type of insertion of the pharmacophore oxypropanolamine chain in the same 1,4-benzothiazine moiety, we prepared oxime ether derivatives as 6-, 7-, 8-acetimidoyloxypropanolamines of 3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine **(A)** in order to compare them with the corresponding oxypropanolamines **(B)** which we had previously prepared and which showed a strong β-blocking activity [10] (Fig. 1).

In order to extend our investigation on oxime ether derivatives, 8-iminoxypropanolamines of 3,4,5,6,7,8-hexahydro-3-oxo-2*H*-1,4-benzothiazine **(C)**, in which the iminoxypropanolamine chain was directly linked to an alicyclic nucleus, were prepared. Such compounds may allow us to verify if, in spite of the missing aromatic portion, the π area of the iminic group can by itself guarantee the electronic distribution necessary for interaction with β-adrenoceptors, as already reported by other authors for aliphatic oxime ether derivatives [6–8].

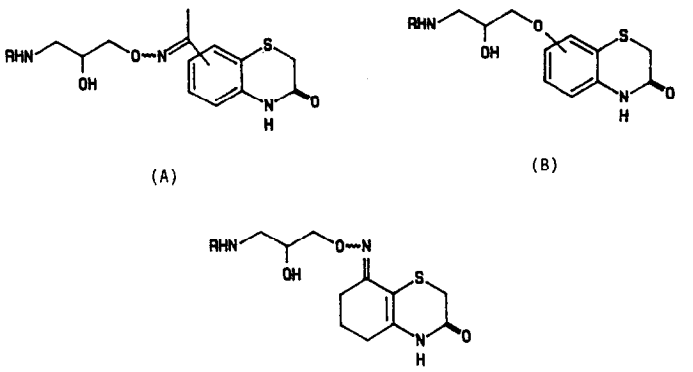

Fig. 1.

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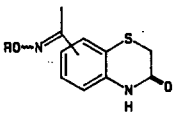
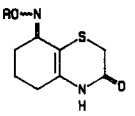
**A preliminary account of this work was presented at the French-Italian Joint Meeting on Medicinal Chemistry, Pisa, Italy, September 1987.

Table I. Physical properties of oxime and epoxide intermediates.



compd	position	R	mp, °C	crystn solvent	yield %	formula
7	6	H	218-222	MeOH	78	$C_{10}H_{10}N_2O_2S$
8	7	H	273-275	DMF	87	$C_{10}H_{10}N_2O_2S$
9	8	H	228-236	EtOH/pyridine	93	$C_{10}H_{10}N_2O_2S$
11	6		165-166	cyclohexane/EtOAc	40	$C_{13}H_{14}N_2O_3S$
12	7		166-168	EtOH	70	$C_{13}H_{14}N_2O_3S$
13	8		164-166	EtOH	69	$C_{13}H_{14}N_2O_3S$



10		H	263-265 dec	AcOH	68	$C_8H_{10}N_2O_2S$
14			171-173	EtOH	47	$C_{11}H_{14}N_2O_3S$

[a]Substituted position on the aromatic nucleus.

All of the synthesized oxime ether derivatives **15–34** (Tables II, III) and, for comparison, those of the oxypropanolamine series **35–49** (Table IV) were first tested at the receptor level to determine their ability to displace the binding of [^{3}H]dihydroalprenolol from turkey erythrocyte membranes (β_1-adrenoceptors) and then *in vivo* to evaluate their β-adrenoceptor blocking activity by the inhibition of isoprenaline-induced tachycardia.

Chemistry

The oxime ether derivatives **15–34** were prepared according to a classic synthetic procedure, as indicated in Scheme 1.

The oxime derivatives **7–10**, obtained by reaction of hydroxylamine hydrochloride with 6-, 7-, 8-acetyl-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazines **(1, 2, 5)** and 3,4,5,6,7,8-hexahydro-3,8-dioxo-2*H*-1,4-benzothiazine **(6)** respectively, were allowed to react as sodium salts with epibromohydrin in dry dimethylformamide and the obtained intermediate epoxides **11–14** were treated with an excess of the required amine in ethanol to give the oxime ether derivatives **15–34** as a mixture of E and Z isomers[a].

[a]The oxime ether derivatives **15–34** move as double spots in an approximate 6:4 ratio on TLC using chloroform: methanol 1:1 as eluent. The ^{1}H NMR are inexpressive for assignment of E and Z isomers.

Among the ketone precursors, 6-acetyl **1** and 7-acetylbenzothiazine **2** were prepared according to the literature [11, 12]. With a procedure similar to that used for the preparation of the 6-acetylbenzothiazine **1**, the unknown 8-acetylbenzothiazine **5** was synthesized by reaction of 2-chloro- 3-nitroacetophenone **(3)** with thioglycolic acid followed by reductive cyclization of the resulting *S*-(2-nitro-6-acetyl)-phenylthioglycolic acid **(4)** with ferrous sulfate and ammonia (Scheme 2). On the other hand, the 3,4,5,6,7,8-hexahydro-3,8-dioxo-2*H*-1,4-benzothiazine **(6)** was obtained in one step by condensation of 2-bromo-1,3-cyclohexanedione with thioglycolamide (Scheme 3).

Results and Discussion

Binding assays showed that the different type of insertion of the oxypropanolamine chain markedly affects affinity to β_1-adrenoceptors (Table V). The highest affinity is observed with oxypropanolamine derivatives in which the side chain is bound to position 8 of the 3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine moiety (compounds **45–49**): derivatives **47** and **48** have indeed an IC_{50} of $7.06 \cdot 10^{-8}$ M and $8.2 \cdot 10^{-10}$ M, respectively. Among 7-derivatives **(40–44)** only compounds **40** and **43** show an IC_{50} in the 10^{-7} M range, while for 6-derivatives **(35–39)** affinity dramatically decreases. This is in agreement with our previous findings obtained for this series by pA_2 measurement which showed that the 8-oxypropanolamine derivatives were more active than the corresponding 7-isomers while the 6-isomers showed the lowest potency as adrenergic β-blockers [10].

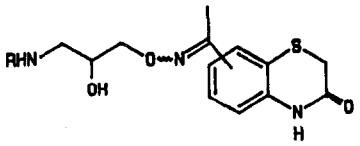
On the contrary, when the oxypropanolamine chain is bound to the same 1,4-benzothiazine moiety by means of an acetimidoyl linkage (compounds **15a–28a, 19, 24, 29**) these correlation are not valid as position 8 is no longer the prefered one; indeed, among 8-derivatives **(25a–28a, 29)**, only **27a** shows an IC_{50} of $2.33 \cdot 10^{-6}$ M. Affinity, however, remains modest for 6- and 7-derivatives **(15a–23a, 19, 24)** also with IC_{50} values in the 10^{-6} M range and an IC_{50} of $3.51 \cdot 10^{-7}$ M only for the 7-derivative **22a**.

For the iminoxypropanolamine derivatives **30a–34a**, in which the oxypropanolamine chain is linked by an iminic group to position 8 of 3,4,5,6,7,8-hexahydro-3,8-dioxo-2*H*-1,4-benzothiazine moiety lacking the aromatic portion, the IC_{50} values are again in some instances significant (**32a**, **34a** $IC_{50} \approx 10^{-7}$ M).

Furthermore, the *in vivo* anti-adrenergic effects (Table VI) showed that the 8-oxypropanolamine derivatives **(45–49)** are more active in inhibiting isoprenaline-induced tachycardia with total inhibition for compounds **46**, **48** and **49** at the dose of 4 mg/kg, i.v. For compounds **45** and **47**, with an IC_{50} of 10^{-7} and 10^{-8} M respectively, inhibition is not complete (79.3 and 66.3% respectively). It becomes total for compound **47** at the dose of 0.4 and 0.04 mg/kg, possibly suggesting a partial agonistic activity at the dose of 4 mg/kg; on the contrary, on decreasing the dose, compound **45** shows no significant inhibition.

Among the acetimidoyloxypropanolamine derivatives **(15a–28a, 19, 24, 29)** and iminoxypropanolamine deriva-

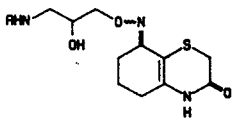
Table II. Physical properties of acetimidoyloxypropanolamine derivatives.



compd	position [a]	R	mp, °C	crystn solvent	yield %	formula [b]
15	6	*i*-Pr	153-155	benzene	98	$C_{16}H_{23}N_3O_3S$
15a	6	*i*-Pr	213-214 dec	MeOH	48	$C_{16}H_{23}N_3O_3S \cdot \frac{1}{2} C_4H_4O_4 \cdot H_2O$
16	6	*i*-Bu	147-148	cyclohexane/EtOAc	89	$C_{17}H_{25}N_3O_3S$
16a	6	*i*-Bu	141-142 dec	EtOH	51	$C_{17}H_{25}N_3O_3S \cdot \frac{1}{2} C_4H_4O_4 \cdot H_2O$
17	6	*tert*-Bu	181-183	EtOAc	85	$C_{17}H_{25}N_3O_3S$
17a	6	*tert*-Bu	254 dec	DMF	68	$C_{17}H_{25}N_3O_3S \cdot \frac{1}{2} C_4H_4O_4$
18	6	*sec*-Bu	139-141	benzene	57	$C_{17}H_{25}N_3O_3S$
18a	6	*sec*-Bu	197 dec	EtOH	49	$C_{17}H_{25}N_3O_3S \cdot \frac{1}{2} C_4H_4O_4 \cdot H_2O$
19	6	3,4-dimethoxyphenylethyl	164-167	benzene	53	$C_{23}H_{29}N_3O_5S$
20	7	*i*-Pr	133-136	benzene	82	$C_{16}H_{23}N_3O_3S$
20a	7	*i*-Pr	209-211 dec	EtOH/DMF	76	$C_{16}H_{23}N_3O_3S \cdot \frac{1}{2} C_4H_4O_4$
21	7	*i*-Bu	109-112	EtOAc	95	$C_{17}H_{25}N_3O_3S$
21a	7	*i*-Bu	188-190 dec	EtOH	54	$C_{17}H_{25}N_3O_3S \cdot \frac{1}{2} C_4H_4O_4$
22	7	*tert*-Bu	188-190	EtOH	98	$C_{17}H_{25}N_3O_3S$
22a	7	*tert*-Bu	237-239 dec	EtOH/DMF	72	$C_{17}H_{25}N_3O_3S \cdot \frac{1}{2} C_4H_4O_4$
23	7	*sec*-Bu	117-118	benzene	92	$C_{17}H_{25}N_3O_3S$
23a	7	*sec*-Bu	190-193 dec	EtOH	74	$C_{17}H_{25}N_3O_3S \cdot \frac{1}{2} C_4H_4O_4$
24	7	3,4-dimethoxyphenylethyl	138-142	EtOH	53	$C_{23}H_{29}N_3O_5S$
25	8	*i*-Pr	133-136	benzene	61	$C_{16}H_{23}N_3O_3S$
25a	8	*i*-Pr	210-211 dec	EtOH/DMF	77	$C_{16}H_{23}N_3O_3S \cdot \frac{1}{2} C_4H_4O_4$
26	8	*i*-Bu	93-95	benzene	55	$C_{17}H_{25}N_3O_3S$
26a	8	*i*-Bu	197-199 dec	EtOH	47	$C_{17}H_{25}N_3O_3S \cdot \frac{1}{2} C_4H_4O_4$
27	8	*tert*-Bu	132-135	EtOH	59	$C_{17}H_{25}N_3O_3S$
27a	8	*tert*-Bu	244-245 dec	EtOH/DMF	81	$C_{17}H_{25}N_3O_3S \cdot \frac{1}{2} C_4H_4O_4$
28	8	*sec*-Bu	78-81	benzene	49	$C_{17}H_{25}N_3O_3S$
28a	8	*sec*-Bu	199 dec	EtOH	64	$C_{17}H_{25}N_3O_3S \cdot \frac{1}{2} C_4H_4O_4$
29	8	3,4-dimethoxyphenylethyl	130-133	EtOH	42	$C_{23}H_{29}N_3O_5S$

[a]Substituted position on the aromatic nucleus. [b]$C_4H_4O_4$ = fumaric acid.

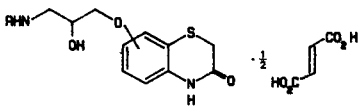
Table III. Physical properties of 8-iminoxypropanolamine derivatives.



compd	R	mp, °C	crystn solvent	yield %	formula [a]
30	*i*-Pr	173-176	EtOH	78	$C_{14}H_{23}N_3O_3S$
30a	*i*-Pr	232-234 dec	EtOH/DMF	50	$C_{14}H_{23}N_3O_3S \cdot \frac{1}{2}C_4H_4O_4 \cdot H_2O$
31	*i*-Bu	153-154	EtOAc	68	$C_{15}H_{25}N_3O_3S$
31a	*i*-Bu	192-195 dec	EtOH	48	$C_{15}H_{25}N_3O_3S \cdot \frac{1}{2}C_4H_4O_4 \cdot H_2O$
32	*tert*-Bu	163-165	EtOH	68	$C_{15}H_{25}N_3O_3S$
32a	*tert*-Bu	249-251 dec	EtOH/DMF	49	$C_{15}H_{25}N_3O_3S \cdot \frac{1}{2}C_4H_4O_4 \cdot H_2O$
33	*sec*-Bu	125-128	EtOAc	68	$C_{15}H_{25}N_3O_3S$
33a	*sec*-Bu	207-208 dec	EtOH/DMF	52	$C_{15}H_{25}N_3O_3S \cdot \frac{1}{2}C_4H_4O_4$
34	3,4-dimethoxy-phenylethyl	119-122	benzene	39	$C_{21}H_{29}N_3O_5S$
34a	3,4-dimethoxy-phenylethyl	165-168 dec	EtOH	40	$C_{21}H_{29}N_3O_5S \cdot \frac{1}{2}C_4H_4O_4 \cdot H_2O$

[a] $C_4H_4O_4$ = fumaric acid.

Table IV. Oxypropanolamine derivatives[a].

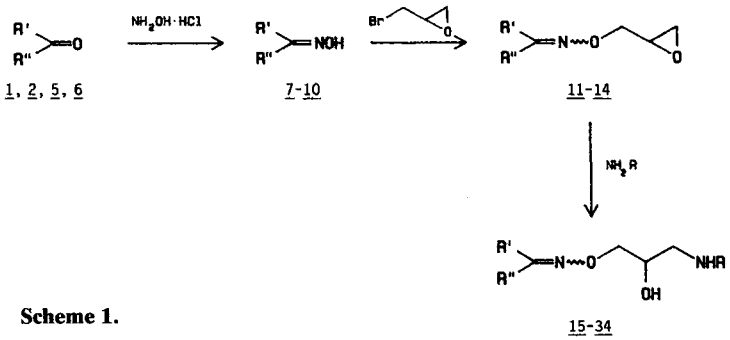


compd	position [b]	R	compd	position [b]	R
35	6	*i*-Pr	43	7	*sec*-Bu
36	6	*i*-Bu	44	7	3,4-dimethoxy-phenylethyl
37	6	*tert*-Bu	45	8	*i*-Pr
38	6	*sec*-Bu	46	8	*i*-Bu
39	6	3,4-dimethoxy-phenylethyl	47	8	*tert*-Bu
40	7	*i*-Pr	48	8	*sec*-Bu
41	7	*i*-Bu	49	8	3,4-dimethoxy-phenylethyl
42	7	*tert*-Bu			

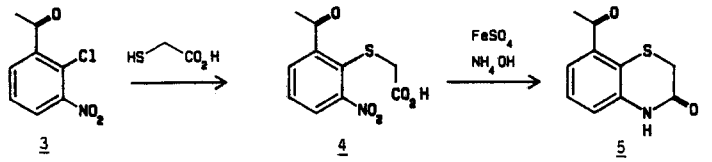
[a] See literature [10]. [b] Substituted position on the aromatic nucleus.

tives **(30a–34a)** only compounds **22a** and **32a** with IC_{50} values in the 10^{-7} M range show inhibition equal to 82.6 and 95.2% respectively that decrease, however, on decreasing the dose, thus ruling out a possible partial agonistic activity. For all the other compounds, block is only partial or non-existing.

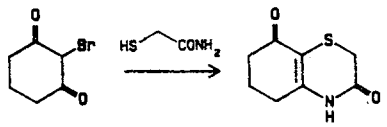
The above data showed that the modification of the oxypropanolamine chain linked to the 1,4-benzothiazine moiety by intercalation of an iminic group is of no advantage. Indeed, type **A** oxime ethers always are less active



Scheme 1.



Scheme 2.



Scheme 3.

than the corresponding type **B** oxypropanolamines. It was also shown that position 8 of 1,4-benzothiazine moiety, which was best for type **B** derivatives, is not the preferred one for type **A** derivatives, as in this case it gives pratically inactive compounds. On the other hand, the comparison of type **A** and **C** oxime ether derivatives showed that the aromatic structure is not indispensable for β-adrenoceptor blocking activity. Indeed, type **C** compounds display an activity similar and, in some case, superior to that of type **A** compounds.

Experimental protocols

Chemistry

Melting points were determined in capillary tubes (Buchi melting point apparatus) and are uncorrected. ^1H NMR spectra were recorded on a 90 MHz Varian EM 390 spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane and the spectral data were consistent with the assigned structures. Column chromatographic separations were carried out on Merck silica gel 40 (mesh 70–230). Analytical thin-layer chromatography (TLC) was carried out on Merck aluminium sheets silica gel 60 F–254 and visualized by UV or iodine vapours. Yields are of purified products and are not optimized. All compounds were analyzed for C, H, N and the analytical values are within ± 0.4% of the theoretical values. The characteristics of the synthesized compounds are summarized in Tables I–III, while the chemical structures of the oxypropanolamine derivatives used for comparison are reported in Table IV.

Table V. Inhibition of [^{3}H]DHA binding in turkey erythrocytes.

compd	IC_{50},(M) [a]	compd	IC_{50},(M) [a]
15a	$3.03 \cdot 10^{-6}$	35	NA
16a	$5.28 \cdot 10^{-5}$	36	$8.40 \cdot 10^{-5}$
17a	$1.80 \cdot 10^{-6}$	37	NA
18a	$1.85 \cdot 10^{-6}$	38	NA
19	NA [b]	39	NA
20a	$2.40 \cdot 10^{-6}$	40	$3.40 \cdot 10^{-7}$
21a	$2.49 \cdot 10^{-6}$	41	NA
22a	$3.51 \cdot 10^{-7}$	42	NA
23a	$2.19 \cdot 10^{-6}$	43	$1.10 \cdot 10^{-7}$
24	$1.80 \cdot 10^{-6}$	44	NA
25a	NA	45	$1.07 \cdot 10^{-7}$
26a	NA	46	$4.23 \cdot 10^{-7}$
27a	$2.33 \cdot 10^{-6}$	47	$7.06 \cdot 10^{-8}$
28a	NA	48	$8.20 \cdot 10^{-10}$
29	$1.15 \cdot 10^{-5}$	49	$4.29 \cdot 10^{-7}$
30a	$9.87 \cdot 10^{-5}$	PROP [c]	$4.15 \cdot 10^{-8}$
31a	$1.34 \cdot 10^{-5}$	CART [d]	$2.33 \cdot 10^{-8}$
32a	$5.21 \cdot 10^{-7}$		
33a	$2.85 \cdot 10^{-6}$		
34a	$3.21 \cdot 10^{-7}$		

[a]Drugs were run at 7 concentrations in each experiment, and IC_{50} values were determined by linear regression of log probit analysis of radioligand displacement. [b]NA indicates no active compound ($IC_{50} \geqq 10^{-4}$ M). [c] Propranolol. [d]Carteolol.

Table VI. Anti-isoprenaline activity in normal rats.

compd	dosage (mg/kg,i.v.)	% inhibition [a,b] of tachycardia	compd	dosage (mg/kg,i.v.)	% inhibition [a,b] of tachycardia
15a	4	NA [c]	35	4	NA
16a	"	37.2 ± 12.11	36	"	NA
17a	"	NA	37	"	NA
18a	"	NA	38	"	NA
19	"	NA	39	"	NA
20a	"	4.7 ± 0.94	40	"	13.6 ± 3.18
21a	"	NA	41	"	NA
22a	"	82.6 ± 8.74	42	"	NA
	0.4	30.3 ± 4.55	43	"	14.0 ± 1.49
23a	4	29.7 ± 3.95	44	"	NA
24	"	12.2 ± 4.43	45	"	79.3 ± 4.19
25a	"	NA		0.4	32.0 ± 2.15
26a	"	NA	46	4	100 ± 0
27a	"	NA	47	"	66.3 ± 8.87
				0.4	100 ± 0
28a	"	NA		0.04	100 ± 0
29	"	NA	48	4	100 ± 0
30a	"	49.4 ± 8.16	49	"	100 ± 0
31a	"	NA	PROP [d]	2	100 ± 0
32a	"	95.2 ± 4.77	CART [e]	"	100 ± 0
	0.4	24.2 ± 5.50			
33a	4	NA			
34a	"	NA			

[a]Mean (±) SEM for 3 separate observations are given. [b]Two minutes after INA (0.12 μg/kg) administration. [c]NA indicates no active β-blocking compound. [d]Propranolol. [e]Carteolol.

S-*(2-Nitro-6-acetyl)-phenylthioglycolic acid* ***4***

Sodium hydrogen carbonate (22 g, 0.262 mol) was added to a solution of thioglycolic acid (11.45 g, 0.124 mol) in ethanol (10 ml) and water (15 ml). The resulting solution was added portion wise to the ethanolic solution of **3** [13] (22 g, 0.110 mol). This was refluxed for 6 h, concentrated to half its volume, diluted with water and then extracted with chloroform. The organic layer was removed and the aqueous solution acidified and extracted 3 times with chloroform. The combined organic layers were washed with water, dried and evaporated to give 24 g (85%) of virtually pure **4** as an oil which was used without further purification. ^{1}H NMR ($CDCl_3$) δ: 2.65 (3H, s, CH_3); 3.65 (2H, s, SCH_2); 7.48–7.80 (3H, m, H–aromatic); 10.12 (1H, s, OH). Anal. $C_{10}H_9NO_5S$ (C, H, N).

*8-Acetyl-3,4-dihydro-3-oxo-2*H*-1,4-benzothiazine* ***5***

An aqueous solution of ferrous sulfate heptahydrate (178 g dissolved in 390 ml of water) was added to a solution of the above acid **4** (16.50 g, 0.065 ml) in ammonia (130 ml) while stirring for 15 min. The reaction mixture was heated in a water bath for 1 h and then filtered with charcoal. The filtrate was acidified with dilute chloride acid and the resulting solid collected, washed with water, dried and recrystallized from ethanol to give 7.40 g (55%) of **5**, mp 213–215°C; ^{1}H NMR (DMSO–d_6) δ: 2.64 (3H, s, CH_3); 3.25 (2H, s, SCH_2); 7.05–7.30 (2H, m, H–6 and H–7); 7.65 (1H, dd, J=7.2 and 2.0 Hz, H–8); 10.60 (1H, s, NH). Anal. $C_{10}H_9NO_2S$ (C, H, N).

*3,4,5,6,7,8-Hexahydro-3,8-dioxo-2*H*-1,4-benzothiazine* ***6***

A mixture containing 2-bromo-1,3-cyclohexanedione [14] (15 g, 0.078 mol), thioglycolamide [15] (7.14 g, 0.078 mol) and a small amount of dry dimethylformamide (20 ml) was stirred mechanically and heated at 100–110°C for 30 min. After cooling, the reaction mixture was treated with acetone. The resulting solid was filtered off and recrystallized from ethanol to give 7.10 g (49%) of **6**, mp 219–221°C; ^{1}H NMR (DMSO–d_6) δ: 1.70–2.10 (2H, m, $CH_2CH_2CH_2$); 2.20–2.60 (4H, m, $CH_2CH_2CH_2$); 3.30 (2H, s, SCH_2); 10.40 (1H, s, NH). Anal. $C_8H_9NO_2S$ (C, H, N).

Preparation of the oximes ***7–10***

The following method used to synthesize 3,4-dihydro-3-oxo-2*H*-1,4-benzothiazin-6-acetoxime **7** is standard.

A solution of 6-acetylbenzothiazine **1** (10.35 g, 0.050 mol) and hydroxylamine hydrochloride (6.95 g, 0.100 mol) in ethanol (130 ml) and pyridine (40 ml) was refluxed with stirring for 2 h. The mixture was concentrated and then poured into ice-water. The precipitated solid was collected, dried and recrystallized from methanol to give 8.61 g (78%) of **7**, mp 218–222°C; ^{1}H NMR (TFA) δ: 2.88 (3H, s, CH_3); 3.73 (2H, s, SCH_2); 7.40–7.73 (3H, m, H–aromatic). Anal. $C_{10}H_{10}N_2O_2S$ (C, H, N).

Preparation of the epoxides ***11–14***

This preparation is illustrated by the synthesis of 6-{1-[(2,3-epoxy)-propoxy]-iminoethyl}-3,4-dhydro-3-oxo-2*H*-1,4-benzothiazine **(11)**.

Under an atmosphere of nitrogen, a solution of oxime **7** (3.80 g, 0.017 mol) in dry dimethylformamide (30 ml) was slowly added to a suspension of sodium hydride (0.72 g, 0.030 mol) in dry dimethylformamide (10 ml) and the reaction mixture was stirred for 4 h at room temperature. A solution of epibromohydrin (2.80 g, 0.020 mol) in dry dimethylformamide (10 ml) was added. The reaction mixture was stirred for a further 5 h and then poured into ice-water. The separated solid was collected, washed with water, dried and finally purified by silica gel column chromatography eluting with cyclohexane–ethyl acetate (7:3) to obtain 1.90 g (40%) of **11**, mp 165–166°C; ^{1}H NMR (DMSO–d_6) δ: 2.20 (3H, s, CH_3); 2.70–2.90 (2H, m, CH_2-oxiranic); 3.10–3.40 (1H, m, CH-oxiranic); 3.50 (2H, s, SCH_2); 3.83–4.48 (2H, m, OCH_2); 7.17–7.40 (3H, m, H-aromatic); 10.55 (1H, broad s, NH). Anal. $C_{13}H_{14}N_2O_3S$ (C, H, N).

Preparation of the oxime ether derivatives ***15–34***

The procedure is illustrated by the synthesis of 6-{1-[(3-*tert*-buthylamino-2-hydroxy)-propoxy]-iminoethyl}-3,4-dhydro-3-oxo-2*H*-1,4-benzothiazine fumarate **(17a)**.

A large excess of *tert*-buthylamine (5 ml) was added to the ethanolic solution of the epoxide **11** (0.80 g) and stirred at 50–60°C for 5 h. The solvent and the excess of amine were removed under reduced pressure and the solid residue crystallized from ethyl acetate to give 0.86 g (85%) of **17**, mp 181–183°C: ^{1}H NMR (DMSO–d_6) δ: 1.03 [9H, s, $C(CH_3)_3$]; 2.15 (3H, s, CH_3); 2.45–2.60 [2H, m, $OCH_2CH(OH)CH_2$]; 3.48 (2H, s, SCH_2); 3.68–3.90 [1H, m, $OCH_2CH(OH)CH_2$]; 4.02–4.15 [2H, m, $OCH_2CH(OH)CH_2$]; 7.15–7.38 (3H, m, H-aromatic). Anal. $C_{17}H_{25}N_3O_3S$ (C, H, N).

A saturated solution of fumaric acid in dry acetone was added to a solution of crude base **17** (0.58 g) in dry acetone until no more precipitation was formed. The precipitated salt was collected and recrystallized from dimethylformamide to give 0.46 g (68%) of **17a**, mp 254°C dec. Anal. $C_{17}H_{25}N_3O_3S$. 1/2 $C_4H_4O_4$ (C, H, N).

Pharmacology

Binding determination

Pellets containing β_1 type adrenoceptors were obtained from turkey erythrocyte membranes as described in the literature [16]. [^{3}H] dihydroalprenolol ([^{3}H]DHA) obtained from New England Nuclear, having a specific activity of 99.9 Ci/mmol and a radiochemical purity > 98.5% was used as ligand.

β-Adrenoceptor binding activity was determined as follows: 100 μl of $6 \cdot 10^{-8}$ M [^{3}H]DHA were incubated for 15 min at 37°C with 100 μl of membranes (431 μg/ml of protein diluted 1:8 v/v) and 100 μl of a solution of the substance dissolved in water or dimethylsulfoxide, at a range of concentrations, to a final volume of 1 ml with saline buffer (sodium chloride 90 mM, Tris 12 mM; pH=7.5). The reaction was stopped by adding 3 ml of cold buffer. The material was filtered under reduced pressure through glass fiber filter disks (Whatman GF/B). Afterwards, the samples were 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 a scintillation spectrometer (Packard TRI-CARB 300C). Control (blank) experiments were carried out to determine the effect of the solvent (5%) on the binding. The concentration of the test compounds that inhibited [^{3}H]DHA binding by 50% (IC_{50}) were determined by log-probit analysis with 7 concentrations of the displacers, each performed in duplicate. The IC_{50} values of propranolol and carteolol were also included as reference compounds (Table V).

Anti-isoprenaline activity

The β-adrenoceptor blocking activity was studied *in vivo* by the inhibition of isoprenaline-induced tachycardia in rats [17]. For this purpose 0.12 μg/kg of isoprenaline was injected intravenously (jugular vein) into male Wistar rats (250–300 g) anesthetized with sodium nembutal (55 mg/kg, i.p.) and the increase in heart rate (HR) was evaluated by electrocardiograph. After several min, when the normal heart rate was restored, the compound under examination, dissolved in dimethylsulfoxide at a prestabilite dose, was injected intravenously; two min later, the isoprenaline injection was repeated. Control experiments were carried out to determine the effect of the solvent on the test. The comparison was made with two known β-blockers: propranolol and carteolol (2 mg/kg, i.v.). Three rats were used per group and a single % variation was calculated from the mean values (Table VI).

Acknowledgments

We thank Mr. Roberto Bianconi for skillful synthetic assistance. This research was assisted by a grant from Ministero della Pubblica Istruzione, Rome, Italy.

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