Eur. J. Med. Chem. 35 (2000) 815–826

S0223523400001732/FLA

Original article

Synthesis and β-blocking activity of (R,S)-(E)-oximeethers of 2,3-dihydro-1,8-naphthyridine and 2,3-dihydrothiopyrano[2,3-b]pyridine: potential antihypertensive agents – Part IX

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Received 8 June 1999; revised and accepted 22 March 2000

Abstract – The synthesis of oximeethers of 2,3-dihydro-1,8-naphthyridine and 2,3-dihydrothiopyrano[2,3-b]pyridine is described. These compounds exhibit a selective β-blocking activity, with a selectivity towards β_2-receptors. Groups in the N_1 position giving rise to a considerable steric hindrance led to a higher β_2-blocking selectivity, whereas groups creating a moderate hindrance caused a weak but significant decrease in β_2-antagonist potency. Substitution of the N_1-R group with a sulfur atom led to compounds possessing β_1-, β_2- and β_3-blocking properties. Compounds **9c$_1$** and **10a$_1$** showed a β_3-antagonist activity slightly lower than that of propranolol. © 2000 Éditions scientifiques et médicales Elsevier SAS

β_1-, β_2- and β_3-adrenergic blockers / oximeethers / 1,8-naphthyridine derivatives / thiopyrano[2,3-b]pyridine derivatives

1. Introduction

In a previous paper [1], we described the synthesis of a series of (R,S)-(E)- and (R,S)-(Z)-oximeethers of 1,8-naphthyridine **1** and **2** (*figure 1*) and their β_2-stimulating and β_1-blocking properties as evaluated in vitro on isolated preparations. Recently, we have synthesized the (R)- and (S)- enantiomers of oximeethers **1** and **2** in order to examine their behaviour towards β-adrenergic receptors. The enantiomeric compounds obtained showed an interesting β-blocking activity: compounds with bromine in positions 6 and/or 7 exhibited pK_b values for the β_1-adrenoceptor similar to that of practolol [2].

On the basis of these results, it appeared to be of interest to continue our chemical and pharmacological investigation in this field. We therefore decided to prepare a new series of oximeethers of 1,8-naphthyridine with an N-alkyl group or a sulfur atom in the 1-position of the 1,8-naphthyridine nucleus and with a methyl group in the 7-position, to study the influence of these substituents on the activity versus β-adrenoceptor subtypes. We chose the latter substituent, because, in previous papers, the oximeethers **1a** and **2a** (R = CH_3, R_1 = H) were considered as lead compounds [1, 2].

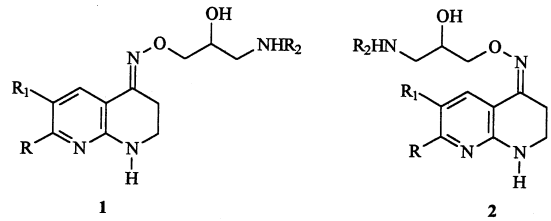

Figure 1. Structures of (R, S)-(E)- and (R, S)-(Z)-4(1H)oximeether-2,3-dihydro-1,8-naphthyridines.

Moreover, we decided to prepare only the (R,S)-(E)-oximeethers, because the (R)- and (S)-enantiomers of **1** and **2** exhibited no chiral preference, and the (E)-isomer **1** generally presented a slightly higher affinity for both

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β-adrenoceptor subtypes with a slight selectivity for β_2-receptors [2].

As compounds binding to β_3-adrenoceptors, whose propanolamine side chain is attached to the oxygen of an oxime function, have not been prepared so far [3], we decided to examine this series of compounds for their β_3-activity.

2. Chemistry

The ketones **3** were prepared following a new, less complicated route than that reported by us in a previous paper [4].

Starting from 2-amino-6-methylpyridine **4**, the alkylamines **5a** [5], **5b** [6], **5c** [7] and **5d** (*figure 2*) were obtained by reductive amination with sodium borohydride and the appropriate aldehyde. The subsequent reaction with ethyl acrylate, in the presence of glacial acetic acid, led to derivatives **6**. The corresponding crude acids, obtained by alkaline hydrolysis of **6**, were then allowed to react in polyphosphoric acid (PPA) to give the target compounds **3a–c** [4] and **3d** (*figure 2*, tables I and II). The ketones **3** were converted, by refluxing with hydroxylamine hydrochloride into a mixture of anhydrous ethanol and pyridine, to yield the pure (E)-oximes **7** (*figure 3*, *tables I* and *II*), as demonstrated by ^{1}H-NMR analysis and in agreement with a previous report [1]. The sodium salt of the (E)-oximes **7** was allowed to react with epichlorohydrin in anhydrous toluene to give the (E)-epoxides **8** (*figure 3*, *tables I* and *III*). Under these conditions, the corresponding (Z)-epoxides are not obtained, as reported in a previous paper [1]. The ring-opening addition reaction of epoxides **8** with the suitable amine carried out at room temperature in acetonitrile and catalysed by lithium perchlorate, as reported in a previous paper [2], gave the target compounds **9** in a good yield (*figure 3*, *table IV* and *V*). It was also of interest to prepare oximeethers of thiopyrano[2,3-b]pyridine **10**, in which the N-R group, present in the 1-position of the 1,8-naphthyridine nucleus, was substituted by a sulfur atom to establish the influence of this part of the structure as regards the β-activities. As illustrated in *figure 4*, the 2-mercapto-6-methylpyridine **11** [8], was allowed to react with ethyl acrylate, in the presence of glacial acetic acid to give the derivative **12**. The corresponding acid **13** was then obtained by alkaline hydrolysis. Compound **13** was also obtained by reaction of the potassium salt of **11** with sodium chloropropionate. The cyclization of **13** in PPA at 170 °C gave the expected ketone **14** in a moderate yield (*figure 4*, *tables I* and *II*).

The oxime **15** and epoxide **16** (*figure 5*, *tables I–III*) were prepared as reported above for compounds **7** and **8**,

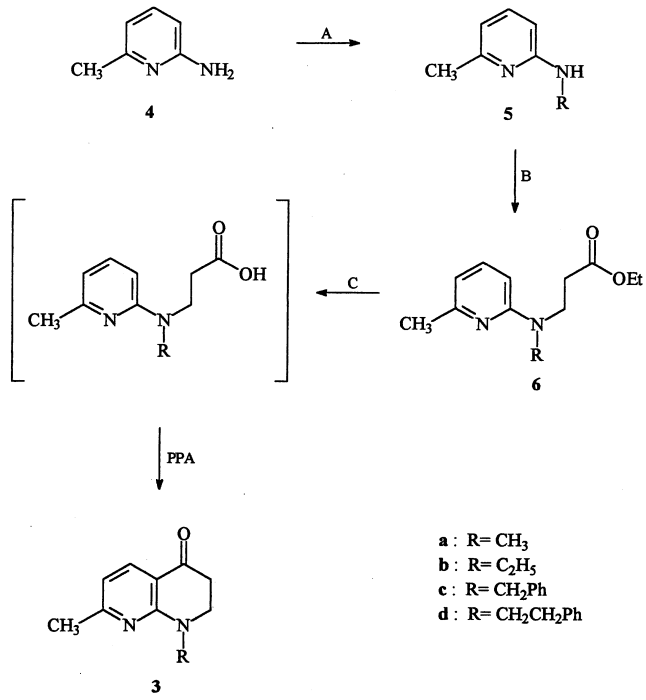


Figure 2. Reagents: A) $NaBH_4$, MeOH, (HCHO, CH_3CHO, $PhCHO$, $PhCH_2CHO$); B) Ethyl acrylate, $AcOH_{(glac.)}$; C) NaOH (6N) / EtOH.

respectively. The ring-opening addition reaction of epoxide **16** with the appropriate amine, carried out at 80 °C in toluene, led to the target compounds **10** (*figure 5*, *tables IV* and *V*).

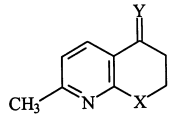
The structures assigned were fully confirmed by elemental analysis, IR and ^{1}H-NMR spectra.

The ^{1}H-NMR spectrum of ester **6b** is quoted as an example: δ 1.22 (CH_3, 6H, m), 2.35 (6-CH_3, 3H, s), 2.63 (C–CH_2, 2H, t), 3.77 (CH_2–CH_2, 2H, t), 3.48 (N–CH_2, 2H, q); 4.11 (O–CH_2, 2H, q), 6.29 (H_3 + H_5, 2H, m); 7.26 (H_4, 1H, m).

The esters **6** all exhibit analogous ^{1}H-NMR spectra.

As reported in previous papers for similar compounds [9–11], the ^{1}H-NMR spectra of compounds **3d**, **7**, **14** and **15** (table II) show two doublets ranging from δ 6.37–6.95 and δ 7.80–8.15, due to H_6 and H_5, respectively, and two multiplets ranging from δ 2.82–2.99 and δ 3.20–3.40, due to H_3 and H_2, respectively. The (E)- conformation was assigned to oximes **7** and **15**, epoxides **8** and **16** (*table III*) and the final compounds **9** and **10** (table V), on the basis of their ^{1}H-NMR spectra, which show the chemical shift of H_5, ranging from δ 7.81–8.07, close to that of ketones **3** and **14**. This behaviour was analogous to that of similar compounds previously reported [1].

Table I. Physical data of ketones, (E)-oximes and (R,S)-(E)-epoxides.



Compound	X	Y	Flash chromatography eluent	Yield (%)	M.p. (°C)
3a	NCH_3	O	AcOEt	68	[4]
3b	NC_2H_5	O	AcOEt	51	[4]
3c	NCH_2Ph	O	AcOEt	61	[4]
3d	$N(CH_2)_2Ph$	O	AcOEt/pet. ether (1:4)	40	oil
7a	NCH_3	NOH	–	85	128–130[a]
7b	NC_2H_5	NOH	–	87	132–135[a]
7c	NCH_2Ph	NOH	–	88	142–145[a]
7d	$N(CH_2)_2Ph$	NOH	AcOEt/pet. ether (1:4)	52	oil
8a	NCH_3	$NOCH_2CH(O)CH_2$	AcOEt/pet. ether (1:6)	67	oil
8b	NC_2H_5	$NOCH_2CH(O)CH_2$	AcOEt/pet. ether (1:6)	72	oil
8c	NCH_2Ph	$NOCH_2CH(O)CH_2$	AcOEt/pet. ether (1:6)	60	oil
8d	$N(CH_2)_2Ph$	$NOCH_2CH(O)CH_2$	AcOEt/pet. ether (1:9)	36	oil
14	S	O	AcOEt/pet. ether (2:3)	42	90–92[b]
15	S	NOH	–	86	234–236[c]
16	S	$NOCH_2CH(O)CH_2$	AcOEt\pet. ether (3:2)	48	oil

[a] Petroleum ether 100–140 °C; [b] cyclohexane; [c] MeOH.

3. Theoretical calculations

The molecular electrostatic potential (MEP) of the N-unsubstituted **1a$_3$** and **10a$_3$** analogues of **1a$_1$** [1] and **10a$_1$** (*figure 6*) were calculated, in accordance with the method used for other adrenergic drugs [12]. Compounds **1a$_3$** and **10a$_3$** were considered in their free base form, and their conformation was optimized at the semi-empirical PM3 level [13]. In the case of compound **1a$_3$** it was also taken into consideration that it could exist in the tautomeric form (A) and (B) (*figure 7*), but a PM3 calculation including full geometry optimisation indicated that form (B) was not favoured by more than 10 kcal/mol.

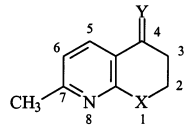
The MEP of **1a$_3$** and **10a$_3$** were calculated from ab initio SCF STO-3G* wave functions [13] in a three-dimensional grid around the molecules. *Figure 8* shows the isopotential MEP surfaces corresponding to a value of –10 kcal/mol [12], indicating the molecular regions possessing a nucleophilic reactivity. The MEP of **1a$_3$** and **10a$_3$** is very similar in the region of the ethanolaminic and the oximeether moiety as well as in that of the pyridinic nitrogens, which should indicate that the two molecules possess a similar reactivity in these regions. The only difference between the MEPs of **1a$_3$** and **10a$_3$** is found in the region where the molecules are structurally different: in compound **1a$_3$**, a negative MEP is localized above and below the ring plane in the region of the N_1, whereas in compound **10a$_3$**, an analogous negative MEP region is localized in the plane of the ring in the region of the sulphur atom.

4. Pharmacological results

The pharmacological results are shown in table VI. Compound **1a$_1$** [1], showing a β_1-, β_2- and β_3-blocking activity, was taken as the lead compound.

Many of the compounds tested, **9a$_1$**–**9c$_2$** and **9d$_1$**–**10a$_2$**, exhibited a β-blocking activity, but showed a different selectivity profile toward the β_1-, β_2- or β_3-subtypes. The molecules with the 1,8-naphthyridine moiety, **9a$_1$**–**9c$_2$** and **9d$_1$**, **9d$_2$**, were more selective as β_2-antagonists than as β_1-antagonists. Furthermore, it was observed that the presence of steric hindrances in the N_1-position abolished the β_1-activity. Nevertheless, the N_1-substitutions giving rise to a considerable steric hindrance (benzyl or phenyl-

Table II. ^{1}H-NMR chemical shifts (δ) of ketones and (E)-oximes.



Compound	X	Y	H_2 (m)	H_3 (m)	H_5 (d)	H_6 (d)	7-CH_3 (s)	Other
3d	$N(CH_2)_2Ph$	O	3.40	2.93	7.90	6.45	2.42	1-CH_2: 3.89 (t); CH_2: 2.52 (t) Ph: 7.22 (m)
7a	NCH_3	NOH	3.20	2.99	7.81	6.41	2.40	1-CH_3: 3.10 (s)
7b	NC_2H_5	NOH	3.27	2.83	7.86	6.37	2.37	1-CH_2: 3.69 (q); CH_3: 1.15 (t)
7c	NCH_2Ph	NOH	3.22	2.82	7.82	6.47	2.37	1-CH_2: 4.88 (s); Ph: 7.25 (m)
7d	$N(CH_2)_2Ph$	NOH	3.24	2.86	7.80	6.41	2.41	1-CH_2: 3.78 (t), CH_2: 2.79 (t) Ph: 7.23 (m)
14	S	O	3.28	2.91	8.15	6.93	2.53	–
15	S	NOH	3.26	2.99	8.00	6.95	2.38	N–OH: 11.40 (s)

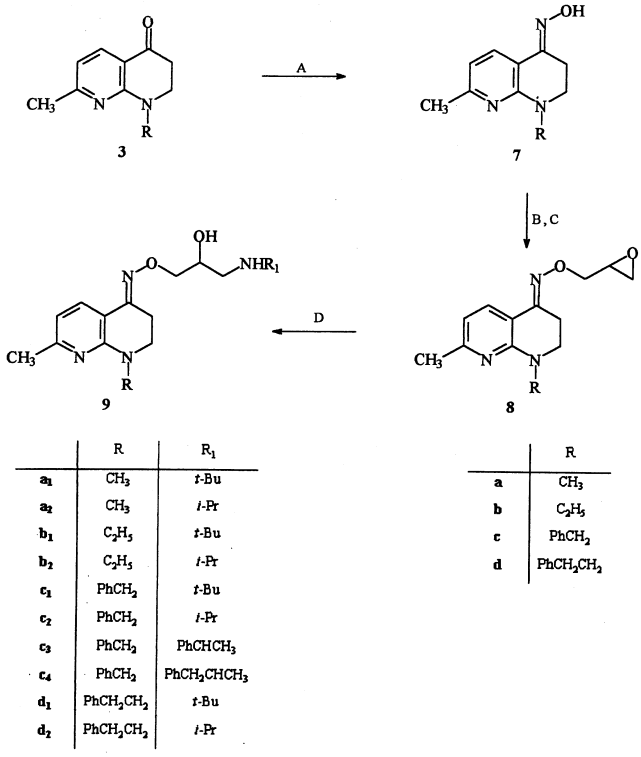


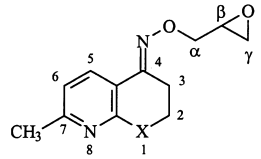
Figure 3. Reagents: A) H_2NOH HCl, EtOH, Py; B) EtONa/EtOH; C) Epichlorohydrin, toluene; D) (*t*-butylamine, *i*-propylamine, α-methylbenzylamine or α-methylphenethylamine), $LiClO_4$ CH_3CN.

ethyl group **9c$_1$**, **9c$_2$**, **9d$_1$** and **9d$_2$**) led to higher β_2-blocking properties (*figure 9*). On the other hand, the presence of a moderate steric hindrance in position N_1 of the 1,8-naphthyridine heterocyclic system (methyl or ethyl substituents, **9a$_1$**–**9b$_2$**), caused a weak, but significant, decrease in the β_2-antagonist potency. Interestingly, the thiopyranopyridine derivatives showed both β_1- and β_2-blocking properties. The *i*-Pr substituted compound **10a$_2$** blocked the two receptor subtypes with almost similar potencies. The *t*-Bu substituted compound **10a$_1$** showed a β_1-blocking activity (*figure 10*), together with a weak, albeit significant, β_1-selectivity. Furthermore its potency was similar to that exhibited by the known antagonist practolol.

Compounds **1a$_1$**, **9a$_1$**, **9b$_1$**, **9c$_1$**, **9c$_3$**, **9c$_4$** and **10a$_1$** were assayed for agonistic and antagonistic activity on β_3-adrenoceptors of rat white adipose tissue; isoprenaline and propranolol were taken as reference drugs.

All the compounds were tested in a concentration range of 10 nM–0.1 mM, except **9c$_1$**, for which an interference with the spectrophotometric determination was observed at the concentration of 0.1 mM. Compounds **1a$_1$**, **9a$_1$**, **9b$_1$**, **9c$_1$**, **9c$_3$**, **9c$_4$** and **10a$_1$**, showed β_3-blocking properties but with significantly different potencies. Compounds **9c$_1$** and **10a$_1$** showed the highest potency, since they blocked isoprenaline-stimulated lipolysis at lower concentrations in comparison with the remaining compounds tested. Furthermore, their potency values were slightly lower than that of propranolol, the non-selective antagonist taken as the reference drug [14]. All the remaining compounds exhibited lower potencies

Table III. ^{1}H-NMR chemical shifts (δ) of (R,S)-(E)-epoxides.



Compound	X	H_2 (m)	H_3 (m)	H_5 (d)	H_6 (d)	7-CH_3 (s)	a-CH_2 (m)	β-CH (m)	γ-CH_2 (m)	Other
8a	NCH_3	3.21	2.75	7.82	6.42	2.38	4.18	3.17	2.95, 2.55	1-CH_3: 3.07 (s)
8b	NC_2H_5	3.22	2.79	7.86	6.37	2.37	4.19	3.26	2.96, 2.61	1-CH_2: 3.67 (q) CH_3: 1.14 (t)
8c	NCH_2Ph	3.18	2.80	7.90	6.42	2.38	4.18	3.21	2.90, 2.55	1-CH_2: 4.87 (s) Ph: 7.25 (m)
8d	$N(CH_2)_2Ph$	3.20	2.77	7.85	6.40	2.40	4.19	3.24	2.96, 2.58	1-CH_2: 3.82 (t) CH_2: 2.30 (t) Ph: 7.22 (m)
16	S	3.12	2.75	8.07	6.83	2.49	4.25	3.16	3.40, 3.40	–

in blocking isoprenaline lipolysis, in comparison with the previous drugs. In particular, the pIC_{50} value of the reference compound **1a$_1$** was found to be 5.14 ± 0.17. The remaining compounds **9a$_1$** and **9b$_1$** showed the lowest degree of activity ($pIC_{50} \leq 4.5$), and a great variability in their action, as indicated by the high value of SEM. Compounds **9c$_3$** and **9c$_4$** did not show any blocking activity on β_3-adrenoceptors.

None of the compounds tested showed any lipolytic activity in our suspension test system.

5. Conclusions

The pharmacological functional study of (R,S)-4(1H)-oximeethers **9** and **10** showed a β_1-, β_2- and β_3-blocking activity for many compounds tested. An examination of the pK_b values reported in table VI indicates that the substitution of H in the N_1 position by an alkyl group determined the total absence of any β_1-activity (compounds **9**), with a generally lower affinity for β_2- and β_3-receptors compared with compound **1a$_1$**, and a moderate selectivity towards β_2-receptors. The presence of a moderate steric hindrance in the N_1-position (compounds **9a$_1$–9b$_2$**) caused a significant decrease in the β_2-antagonist potency (maximum 14-fold) and the β_3-antagonist potency (maximum 10-fold). The presence of a greater steric hindrance (compounds **9c$_1$–9c$_2$**) led to higher β_2- and β_3-blocking properties similar to those of **1a$_1$**. The substitution of the N_1H group by a sulfur atom (compounds **10a$_1$** and **10a$_2$**) leads to a good affinity versus β_1-, β_2- and β_3-receptors, analogous to that of compound **1a$_1$**, but with a slight selectivity toward the β_1-receptor for compound **10a$_1$**. A further increase in the hindrance of the side chain leads to a complete abolition of β_3-activity.

The loss of β_1-activity for compounds **9** is explainable by the steric hindrance due to the substituents present in the N_1-position, whereas the same steric hindrance does not seem to have a great influence on the β_2- or β_3-activity.

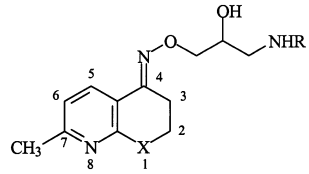
Moreover, the different biopharmacological profile versus β_1-adrenergic receptors of **1a$_1$** and **10a$_1$** could be correlated to the different MEP trend in the region of the aromatic system corresponding to the 1-position. The presence of the proton donor N_1H group in **1a$_1$** makes an interaction possible with an electrophilic region of the receptor above and/or below the ring plane, whereas the presence of the sulphur in **10a$_1$** moves this kind of interaction to the ring plane.

6. Experimental protocols

6.1. Chemistry

All compounds were routinely checked for their structure by IR and ^{1}H-NMR spectroscopy. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. The IR spectra were measured with a Genesis Series FTIR ATI Mattson spectrometer. The ^{1}H-NMR spectra were determined in DMSO-d_6 or $CDCl_3$ with TMS as the internal standard, on a Varian CFT-20 NMR spectrometer. Analytical TLC was carried out on Merck 0.2 mm pre-coated silica-gel glass plates (60 F-254) and location of spots was detected by illumination with a UV lamp. Elemental analysis of all synthesized compounds for C, H and N were within ± 0.4% of theoretical values and were performed by our analytical laboratory.

Table IV. Physical data of (R,S)-(E)-oximeether derivatives.



Compound	X	R	Flash chromatography eluent	Yield (%)[a]
9a$_1$	NCH_3	*t*-Bu	AcOEt:DEA (20:1)	61
9a$_2$	NCH_3	*i*-Pr	AcOEt:DEA (20:1)	64
9b$_1$	NC_2H_5	*t*-Bu	AcOEt:DEA (20:1)	65
9b$_2$	NC_2H_5	*i*-Pr	AcOEt:DEA (20:1)	62
9c$_1$	NCH_2Ph	*i*-Pr	AcOEt:pet. eth:DEA (3:1:0.1)	54
9c$_2$	NCH_2Ph	*t*-Bu	AcOEt:pet. eth:DEA (3:1:0.1)	52
9c$_3$	NCH_2Ph	$Ph(CH_3)CH$	AcOEt:MeOH:DEA (20:1:0.5)	29
9c$_4$	NCH_2Ph	$PhCH_2(CH_3)CH$	AcOEt:MeOH:DEA (20:1:0.5)	30
9d$_1$	$N(CH_2)_2Ph$	*t*-Bu	AcOEt:DEA (3:0.1)	30
9d$_2$	$N(CH_2)_2Ph$	*i*-Pr	AcOEt:DEA (3:0.1)	27
10a$_1$	S	*t*-Bu	AcOEt:pet. eth:DEA (3:3:0.5)	62
10a$_2$	S	*i*-Pr	AcOEt:pet. eth:DEA (3:3:0.5)	67

[a] Oil.

6.1.1. General procedure
for the preparation of 2-alkylamino-6-methylpyridine **5**

Sodium borohydride (100 mmol) was added in small amounts to a solution of 50 mmol of 2-amino-6-methylpyridine **4** and 100 mmol of appropriate aldehyde in 10 mL of anhydrous methanol. After stirring for 48 h at room temperature, water was added, methanol was eliminated by evaporation in vacuo and the mixture was extracted with chloroform. The combined extracts were dried (magnesium sulfate) and evaporated to dryness in vacuo, and the crude oil was purified by flash chromatography to obtain compounds **5**.

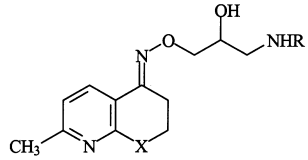
6.1.2. General procedure
for the preparation of 3-alkylamino-3-(6-methylpyrid-2-yl)aminopropionic ethyl esters **6**

A mixture of 5.0 mmol of the appropriate 6-methyl-2-alkylaminopyridine **5**, 9.5 mL of ethyl acrylate (8.6 mmol) and 2.0 mL of glacial acetic acid was heated at 100 °C for 72 h. After cooling, the solution was treated with 10% sodium hydroxide and extracted with chloroform. The combined extracts were dried (magnesium sulfate) and evaporated to dryness in vacuo, and the crude oil was purified by flash chromatography to obtain the target compounds **6**.

6.1.3. General procedure
for the preparation of substituted 7-methyl-2,3-dihydro-1,8-naphthyridine-4(1H)-one **3**

A mixture of **6** (10 mmol) and 20 mL of 6 N aqueous sodium hydroxide solution was refluxed for 60 min. After cooling, the solution was acidified with 10% hydrochloric acid (pH 4–5) and the mixture was extracted with chloroform. The combined extracts were dried (magnesium sulfate) and the solvent was evaporated to dryness in vacuo. The crude propionic acid derivative obtained and 15 g of polyphosphoric acid were heated at 120 °C for 40 min. After cooling, the mixture was poured into

Table V. ^{1}H-NMR chemical shifts (δ) of (R,S)-(E)-oximeether derivatives.



Compound	X	R	H_2 (m)	H_3 (m)	H_5 (d)	H_6 (d)	7-CH_3 (s)	OCH_2 (d)	HOCH (m)	$RNCH_2$ (m)	Other
9a$_1$	NCH_3	*t*-Bu	3.20	2.84	7.83	6.39	2.38	4.12	3.90	2.65	$(CH_3)_3C$: 1.11 (s) 1-CH_3: 3.07 (s)
9a$_2$	NCH_3	*i*-Pr	3.20	2.84	7.83	6.39	2.38	4.12	3.90	2.65	CH: 2.62 (m); $(CH_3)_2C$: 1.08 (d) 1-CH_3: 3.07 (s)
9b$_1$	NC_2H_5	*t*-Bu	3.27	2.82	7.81	6.36	2.36	4.12	3.92	2.72	$(CH_3)_3C$: 1.11 (d) 1-CH_2: 3.67 (q); CH_3: 1.22 (t)
9b$_2$	NC_2H_5	*i*-Pr	3.27	2.82	7.81	6.36	2.36	4.12	3.92	2.72	CH: 2.57 (m); $(CH_3)_2C$: 1.08 (d) 1-CH_2: 3.67 (q); CH_3: 1.22 (t)
9c$_1$	NCH_2Ph	*t*-Bu	3.20	2.78	7.86	6.42	2.38	4.11	3.93	2.68	$(CH_3)_3C$: 1.11 (s) 1-CH_2: 4.87 (s); Ph: 7.25 (m)
9c$_2$	NCH_2Ph	*i*-Pr	3.20	2.78	7.86	6.42	2.38	4.11	3.93	2.68	CH: 2.78 (m); $(CH_3)_2C$: 1.08 (d) 1-CH_2: 4.87 (s); Ph: 7.25 (m)
9c$_3$	NCH_2Ph	A	3.18	2.80	7.85	6.45	2.38	4.06	3.80	2.65	CH_3: 1.32 (d); CH: 4.06 (m) 1-CH_2: 4.87 (s); Ph: 7.25 (m)
9c$_4$	NCH_2Ph	B	3.20	2.70	7.85	6.45	2.38	4.06	3.80	2.69	CH:2.70 (m); CH_3: 1.34 (d); CH_2: 2.43 (d) 1-CH_2: 4.86 (s); Ph: 7.25 (m)
9d$_1$	$N(CH_2)_2Ph$	*t*-Bu	3.22	2.73	7.83	6.40	2.40	4.11	3.97	2.80	$(CH_3)_3C$: 1.10 (s) 1-CH_2: 3.81 (t); CH_2: 2.73 (m) Ph: 7.22 (m)
9d$_2$	$N(CH_2)_2Ph$	*i*-Pr	3.22	2.73	7.83	6.40	2.40	4.11	3.97	2.77	CH: 2.73 (m); $(CH_3)_2C$: 1.07 (d) 1-CH_2: 3.81 (t); CH_2: 2.73 (m) Ph: 7.23 (m)
10a$_1$	S	*t*-Bu	3.04	3.04	8.03	6.83	2.48	4.15	3.95	2.70	$(CH_3)_3C$: 1.11 (s)
10a$_2$	S	*i*-Pr	3.04	3.04	8.03	6.83	2.48	4.15	3.95	2.70	CH: 2.75 (m); $(CH_3)_2C$: 1.06 (d)

A: $CH(CH_3)Ph$; B: $CH(CH_3)CH_2Ph$.

crushed ice and made basic (pH = 8) with cold concentrated ammonium hydroxide. The solution was extracted with chloroform and the combined extracts were dried (magnesium sulfate) and evaporated in vacuo to obtain compounds **3**, as crude oils, which were purified by flash chromatography (*tables I* and *II*).

6.1.4. 3-(6-Methylpyrid-2-yl)-mercaptopropionic ethyl ester **12**

A mixture of 1.25 g (10 mmol) of 6-methyl-2-mercaptopyridine **11** [8], 10 mL of ethyl acrylate (9.0 mmol) and 0.5 mL of glacial acetic acid was heated at 170 °C for 5 h. After cooling, the solution was treated with 10% sodium hydroxide (pH = 7–8) and extracted with chloroform. The combined extracts were dried (magnesium sulfate) and evaporated to dryness to obtain an oily residue which was purified by distillation at 120 °C and 0.3 mm Hg to give **12** in an 86% yield.

6.1.5. 3-(6-Methylpyrid-2-yl)mercaptopropionic acid **13**

A) From **12**:

A mixture of **12** (10 mmol) and 3 mL of 10% potassium hydroxide was stirred at room temperature for 5 days. The solution was then acidified with hydrochloric acid (pH = 4–5) and extracted with chloroform. The combined extracts were dried (magnesium sulfate) and evaporated in vacuo. The crude residue was purified by crystallization from petroleum ether 100–140 °C to give **13** in a 14% yield, m.p. 58–59 °C.

B) From **11**:

A solution of 2-mercapto-6-methylpyridine **11** (4.0 mmol) in 3 mL of 10% potassium hydroxide was added to a solution of β-chloropropionic acid (5.0 mmol) and $NaHCO_3$ (0.5 mmol) in 3 mL of water and the mixture was stirred at room temperature for 4 days.

The solution was treated with hydrochloric acid until the pH was 7 and extracted with chloroform. The combined extracts were dried (magnesium sulfate) and evaporated in vacuo: the starting 2-mercapto-6-methylpyridine (9% yield) was recovered. The aqueous solution was then acidified with hydrochloric acid (pH = 4) and extracted with chloroform. The combined extracts were dried

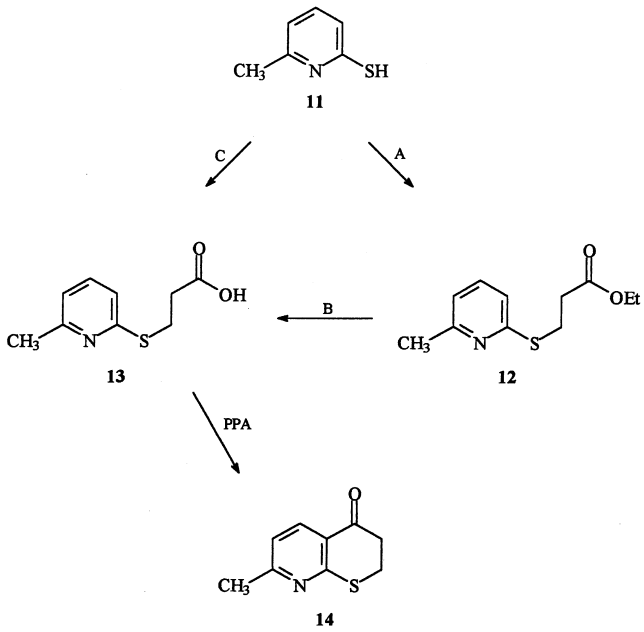


Figure 4. Reagents: A) Ethyl acrylate, $AcOH_{(glac.)}$; B) 10% KOH_{aq}, EtOH; C) $ClCH_2CH_2COONa$, H_2O.

(magnesium sulfate) and evaporated to dryness in vacuo, and the residue was crystallized from petroleum ether 100–140 °C to give **13** in a 63% yield.

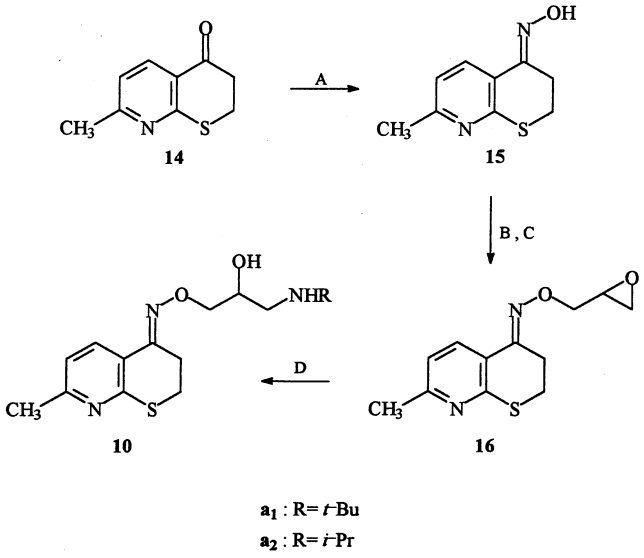


Figure 5. Reagents: A) H_2NOH HCl, EtOH, Py; B) EtONa/EtOH; C) Epichlorohydrin, toluene; D) (*t*-Butylamine or *i*-propylamine), toluene.

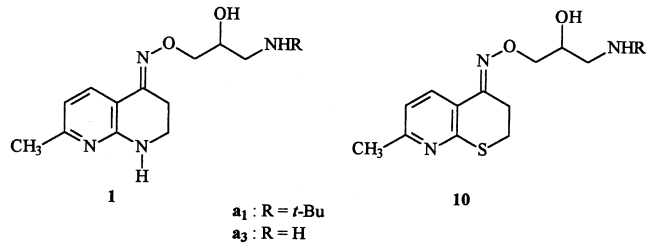


Figure 6. Compounds used for the theoretical calculations.

6.1.6. 7-Methyl-2,3-dihydrothiopyrano[2,3-b]pyridine-4(4H)-one **14**

A mixture of **13** (5.0 mmol) and 10 g of polyphosphoric acid was heated at 170 °C for 3 h. After cooling, the mixture was poured into crushed ice and made basic (pH = 8) with cold concentrated ammonium hydroxide. The solution was extracted with chloroform. The combined extracts were dried (magnesium sulfate) and evaporated in vacuo, and the residue was purified by flash chromatography to give **14** (*tables I* and *II*).

6.1.7. General procedure for the preparation of substituted (E)-4(1H)-hydroxyimino-7-methyl-2,3-dihydro-1,8-naphthyridine **7** *and (E)-4(4H)-hydroxyimino-7-methyl-2,3-dihydrothiopyrano[2,3-b]pyridine* **15**

A suspension of 1.0 mmol of the appropriate ketone **3** or **14** and 2.5 mmol of hydroxylamine hydrochloride in 15 mL of anhydrous ethanol and 1 mL of anhydrous pyridine was refluxed for 90 min. The mixture was evaporated to dryness in vacuo and the crude residue was treated with a saturated $NaHCO_3$ solution. The (E)-oximes **7** or **15** were collected, washed with water and purified by crystallization or flash chromatography (*tables I* and *II*).

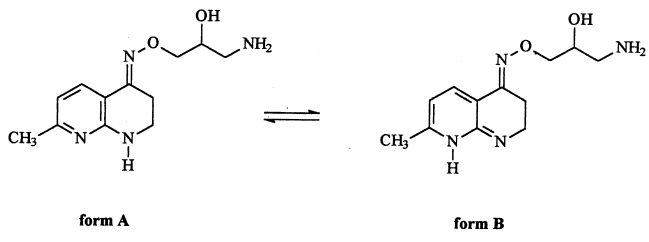


Figure 7. Tautomeric forms of **1a$_3$**.

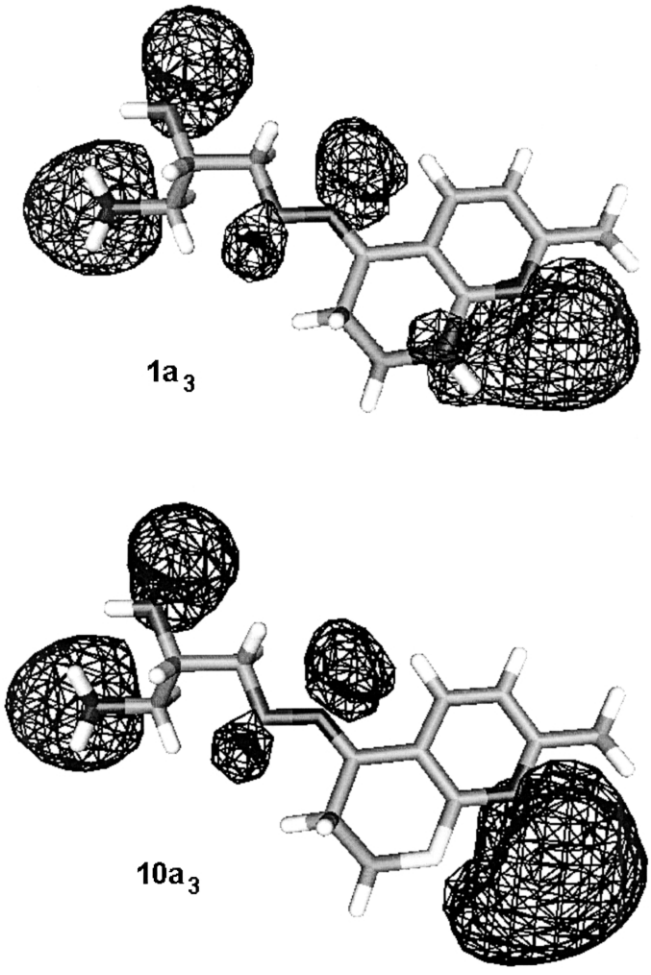


Figure 8. Compounds **1a$_3$** and **10a$_3$** in their preferred conformations. MEP contours corresponding to a value of – 10 kcal/mol are shown.

6.1.8. General procedure for the preparation of (R,S)-(E)-4(1H)-[(2,3-epoxypropyl)oxyimino]-7-methyl-2,3-dihydro-1,8-naphthyridine derivatives **8** *and (R,S)-(E)-4(4H)-[(2,3-epoxypropyl)oxyimino]-7-methyl-2,3-dihydro thiopyrano[2,3-b]pyridine* **16**

Compounds **7** or **15** (1.0 mmol) were added to a solution of 1.0 mmol of freshly prepared sodium methoxide in 15 mL of anhydrous methanol and the mixture was refluxed for 1 h. The evaporation of the solvent in vacuo produced the sodium salt, to which 30 mL of anhydrous toluene and 10 mmol of epichlorohydrin were added; the resulting suspension was allowed to react at 100 °C for 24 h. The solvent was evaporated in vacuo and the crude products were purified by flash chromatography to obtain compounds **8** and **16** (*tables I* and *III*).

6.1.9. General procedure for the preparation of (R,S)-(E)-4(1H)-[1-(3-alkylamino-2-hydroxypropyl)oxyimino]-7-methyl-2,3-dihydro-1,8–naphthyridine derivatives **9**

Two millimoles of the appropriate alkylamine was added to a mixture of 1.0 mmol of epoxypropyloximino derivative **8** and 2.0 mmol of lithium perchlorate in 5 mL of acetonitrile and the mixture was allowed to react at room temperature for 24 h. The organic solution was evaporated to dryness in vacuo and the crude residue was treated with water and extracted with chloroform. The combined extracts were dried (magnesium sulfate) and evaporated in vacuo, and the residue was purified by flash chromatography, to obtain compounds **9** (*tables IV* and *V*).

6.1.10. (R,S)-(E)-4(4H)-[1-(3-alkylamino-2-hydroxypropyl)oxyimino]-7-methyl-2,3-dihydrothiopyrano[2,3-b]pyridine derivatives **10**

A solution of 1.0 mmol of **16** and 3.0 mmol of suitable amine in 10 mL of anhydrous toluene was heated at 80 °C for 24 h in a sealed tube. After cooling, the solution was evaporated to dryness in vacuo to give an oily residue, which was purified by flash chromatography to obtain compounds **10** (*tables IV* and *V*).

6.2. Pharmacological methods

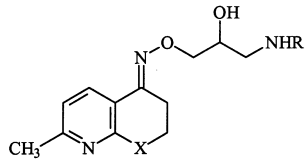
All the procedures performed in the pharmacological screening follow the guidelines of European Community Council directive 86-609. For the pharmacological study, male Dunkin-Hurtley guinea-pigs (300–350 g) and male Wistar rats (200–230 g) were killed by cervical dislocation under light ether anaesthesia. The β-agonist L-isoprenaline hydrochloride (IPNA) was dissolved (1 mM) in distilled water, while the compounds tested were dissolved (1 mM) in dimethylsulfoxide. All the further dilutions were performed in distilled water. The solutions were prepared immediately before the experiments.

6.2.1. Isolated guinea-pig atria

A possible β_1-blocking activity was evaluated on spontaneously beating isolated atria of guinea-pigs, as previously described [2].

The hearts were rapidly explanted and the atria were separated from the ventricular tissue and from the major blood vessels. The left atrium was sutured to a wire-mounting rod, fixed to a 10 mL chamber of the isolated organ bath. The right atrium was connected by inextensive thread to an isometric force transducer (Basile mod. 7005) under a pre-load of 1 g. The atrial inotropic tension developed was recorded on a microdynamometer (Basile mod. 7050). The bathing fluid (Tyrode saline solution, composition in mM: NaCl 136.8, KCl 2.95, $CaCl_2$ 1.80,

Table VI. Antagonistic activity on β-adrenoceptor.



Compound	R	X	pK_b vs β_1	pK_b vs β_2	pIC_{50} vs β_3
1a$_1$	*t*-Bu	NH	6.48 ± 0.15	6.69 ± 0.20	5.14 ± 0.17
9a$_1$	*t*-Bu	NCH_3	≤ 5	5.50 ± 0.17	4.51 ± 0.34
9a$_2$	*i*-Pr	NCH_3	≤ 5	5.75 ± 0.38	n.t.
9b$_1$	*t*-Bu	NC_2H_5	≤ 5	5.32 ± 0.33	4.14 ± 0.49
9b$_2$	*i*-Pr	NC_2H_5	≤ 5	5.73 ± 0.27	n.t.
9c$_1$	*t*-Bu	NCH_2Ph	≤ 5	6.46 ± 0.23	5.80 ± 0.22
9c$_2$	*i*-Pr	NCH_2Ph	≤ 5	6.11 ± 0.08	n.t.
9c$_3$	$Ph(CH_3)CH$	NCH_2Ph	≤ 5	≤ 5	≤ 4
9c$_4$	$PhCH_2(CH_3)CH$	NCH_2Ph	≤ 5	≤ 5	≤ 4
9d$_1$	*t*-Bu	$N(CH_2)_2Ph$	≤ 5	6.38 ± 0.24	n.t.
9d$_2$	*i*-Pr	$N(CH_2)_2Ph$	≤ 5	6.38 ± 0.12	n.t.
10a$_1$	*t*-Bu	S	7.65 ± 0.41	6.70 ± 0.11	5.68 ± 0.21
10a$_2$	*i*-Pr	S	5.76 ± 0.46	6.13 ± 0.11	n.t.
Propranolol			8.71 ± 0.15	8.25 ± 0.12	6.15 ± 0.09
Practolol			7.51 ± 0.19	n.t.	n.t.

n.t.: not tested.

$MgSO_4 \cdot 7H_2O$ 1.05, NaH_2PO_4 0.41, $NaHCO_3$ 11.9, glucose 5.5) was thermostated at 32 °C and continuously gassed with O_2. The preparation was left to equilibrate for 1 h before starting the experimental protocol. A first

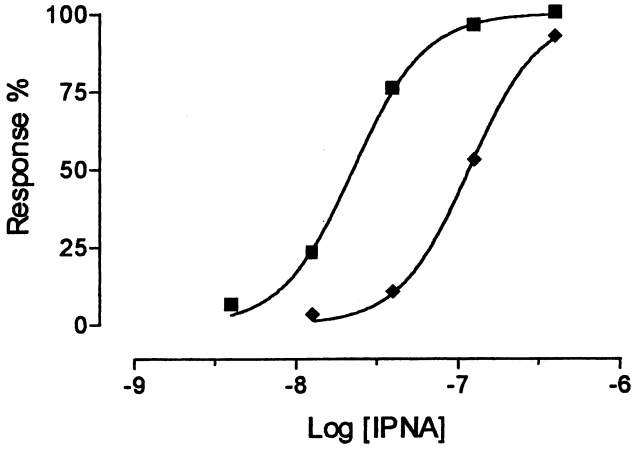


Figure 9. Concentration–response curves for IPNA, in guinea-pig tracheal preparations (β_2-adrenoceptor stimulation), in control conditions (■) and in the presence of **9c$_2$** 1 μM (♦). The points represent the mean of four experiments; values of SEM, not drawn for clarity, are < 10%. The responses are expressed as % of the maximal effect evoked by IPNA.

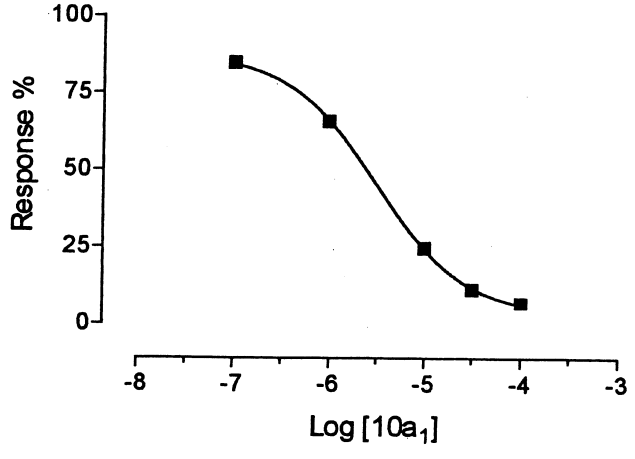


Figure 10. Inhibition curve for compound **10a$_1$** versus the β_1-mediated positive inotropic effect evoked by the reference concentration of IPNA, in guinea-pig spontaneously beating atria. The points represent the mean of four experiments; values of SEM, not drawn for clarity, are < 10%. The responses are expressed as % of the maximal effect evoked by the reference concentration of IPNA.

concentration–response curve for IPNA was obtained by the method of single-concentration administration (starting from 1 nM, with 3-fold increasing steps). β_1-antagonism was evaluated as the inhibition curve obtained by the progressive reduction of the positive inotropic response to a reference concentration (Ar) of IPNA, induced by increasing concentrations (starting from 30 nM, with 3-fold increasing steps) of the compound tested. The chosen Ar of IPNA was 100 nM.

6.2.2. Isolated guinea-pig trachea

The compounds were tested on isolated guinea-pig tracheal smooth muscle in order to evaluate a possible β_2-antagonist activity, as previously described [2]. The trachea, explanted and freed from extraneous tissues, was cut length-wise through the anteromedial cartilage. Finally, a zig-zag-shaped preparation was obtained by alternated partial cuts, perpendicular to the length of the organ. One extremity of the preparation was sutured to a wire-mounting rod, fixed to a 10 mL chamber of the isolated organ bath. The other was connected by inextensive thread to an isotonic force transducer (Basile mod. 7006) under a pre-load of 0.5 g. The isotonic changes of tension were recorded by means of a microdynamometer (Basile mod. 7050). The bathing fluid was Krebs saline solution (composition in mM: NaCl 118, KCl 4.75, $CaCl_2$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.19, $NaHCO_3$ 25, glucose 11.5), thermostated at 37 °C and continuously gassed with a mixture of O_2 (95%) and CO_2 (5%).

The preparations were left to equilibrate for 1 h before starting the experimental protocol. In each preparation, two concentration–response curves for IPNA were obtained. The equilibration time between the two curves was 1 h. The second curve was obtained in the presence of a reference concentration of the test antagonist. Previous experiments showed that the administration of the vehicle did not cause any shift of the second concentration–response curve.

The concentration–relaxing-response curves for IPNA were obtained as follows: the tracheal smooth muscle was pre-contracted by the administration of a single concentration (1 mM) of the muscarinic agonist carbamylcholine. When the contraction reached a steady plateau, the trachea was relaxed by the cumulative administration of increasing concentrations of IPNA (starting from 1 nM, with 3-fold increasing steps).

6.2.3. Rat adipocytes

The compounds were tested on adipose cells isolated from male Wistar rats fed ad libitum, in order to evaluate a possible β_3-antagonist activity. Epididymal fat pads were immediately removed and isolated adipose cells were prepared by the method originally described by Rodbell [15] and subsequently modified by Cushman [16]. After removal of the major blood vessels, the finely cut tissue pieces were incubated for 30 min at 37 °C with collagenase at a concentration of 1 mg/mL in a shaking water bath gassed with 5% CO_2 and 95% O_2. Digestion was terminated by filtration followed by three washes, each with 10 mL of buffer. Tyrode buffer supplemented with 30 mM Hepes containing 10 mg/mL bovine serum albumin at pH 7.4 (THA) was used during collagenase digestion and incubation. Buffer without bovine serum albumin was used for the wash. Washed cells were counted in a Burker chamber and diluted to obtain 10^6 cells/mL.

Adipocyte lipolysis was measured by incubating 2×10^5 cells in a shaking waterbath at 37 °C for 90 min in a final volume of 400 μL containing THA buffer and the agents to be tested. In the tests in which the antagonistic action was assayed, the cells were pre-incubated for 15 min with the antagonist or with the vehicle, before the addition of the agonist. When IPNA was used, the same concentration of L-ascorbic acid was added to the incubation tube as an anti-oxidant.

The agents to be tested were dissolved in Tyrode buffer or in dimethylsulphoxide in a final concentration ranging from 0.00025–0.025%, and in this case the experiments were performed with the appropriate control containing dimethylsulphoxide. At the end of the incubation period, the reaction was stopped in ice and 200 μL of incubation medium was taken for enzymatic determination of glycerol [17] released into the incubation medium, using a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer at 366 nm.

Collagenase (Type II), (–)isoprenaline hydrocloride, bovine serum albumin (fraction V), ATP (magnesium salt), β-NAD, hydrazine hydrate, glycine, and glycerol were purchased from Sigma (St. Louis, USA). Glycerophosphate dehydrogenase (GDH) and glycerokinase (GK) were obtained from Boehringer (Mannheim, FRG).

6.2.4. Data analysis

6.2.4.1. β_1- and β_2-antagonism

The antagonist potency was expressed as pK_b, representing the value of –log of the dissociation constant, and it was expressed as the mean ± standard error, for at least four experiments. The value of the dissociation constant for the β_1-adrenoceptor/antagonist complex was calculated by means of the inhibition curve, as previously described [18]. The value of the dissociation constant for the β_2-adrenoceptor/antagonist complex was calculated by means of Gaddum's method [19]. Data were statisti-

cally analysed by ANOVA or by Student's two-tail *t*-test; a value of $P < 0.05$ was considered as representative of significant differences. Raw data interpolations and statistical analyses were performed by a computerized method (program Graph-Pad Prism TM 2.0).

6.2.4.2. β_3-antagonism

Different concentrations of IPNA and of the compounds tested were added to adipocyte preparations to obtain concentration–response curves. The concentration–response curve to IPNA was taken as the reference for agonistic activity and the concentration that induced about 80% (100 nM) of the maximal response was employed to assay the activity of antagonists. Antagonistic potency was expressed as $pIC_{50} \pm SEM$ of *n* experiments, that is the negative logarithm of the molar concentration of the antagonist that inhibited the stimulant action of IPNA by 50%.

The concentration–response curves were calculated by non-linear regression analysis of data by means of a computer-aided program (GraphPad Prism 2.0). Statistical analysis was carried out using Student's *t*-test, with a probability value (*P*) of less than 0.05 regarded as significant.

Acknowledgements

This work was supported by a grant from the Ministero dell'Università e della Ricerca Scientifica (40%).

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