Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 4921–4931

BIOORGANIC & MEDICINAL CHEMISTRY

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: Identification of β_3-Antagonists

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Received 13 June 2003; accepted 12 September 2003

Abstract—Drugs acting on β_1- and β_2-adrenergic receptors are widely used for the clinical management of a large number of cardiovascular and respiratory pathologies. In the last decade, the discovery of the third subtype of β receptors, the β_3-adrenoceptor, gave a further pharmacological target for the development of new selective drugs. Initially, a potential therapeutic use of β_3-selective agents seemed to be restricted to agonists, for the treatment of metabolic diseases, such as obesity, non-insulin-dependent diabetes, urinary frequency and incontinence. More recently, some interesting theories about a negative role played by the cardiodepressant activity of myocardial β_3-adrenoceptors in heart failure, seemed to justify a clinical use of β_3-antagonists in the last phases of this cardiac disease. Following the indications deriving from previous experimental work, the β-antagonist properties of newly-synthesised (*R*,*S*)-(*E*)-oximeethers of 2,3-dihydro-1,8-naphthyridine and of 2,3-dihydrothiopyrano[2,3-*b*]pyridine were evaluated, in order to identify some useful structure–activity relationships, which might account for selectivity towards the three β-subtypes and, in particular, the β_3-adrenoceptor. Among the various observations regarding possible structure–activity relationships, able to explain the pharmacodynamic patterns of the synthesised compounds on the three subtypes of β-adrenoceptors, the most significant data derived from the evaluation of the β_3-blocking properties of some oximeethers of 1,8-naphthyridine derivatives. In these molecules, although the presence of the large substituents in position 7, such as 4-chloro-phenoxy- or 4-*t*-butyl-phenoxy groups determined a dramatic decline in both the β_1- and β_2-activities, this structural characteristic had a modest influence on the β_3-affinity, which was only slightly lower. Hence, this last structural requirement of oximeethers of 1,8-naphthyridine derivatives seems to represent a useful expedient to induce an appreciable selectivity towards the β_3-receptor, through a markedly negative effect on the β_1- and β_2-activities rather than an increase in the β_3-affinity.

Introduction

β-Adrenoceptor antagonists are used clinically[1,2] in view of their efficacy in the treatment of various cardiovascular diseases, as hypertension, ischemic heart disease, and certain arrhythmias. β-Adrenergic blocking agents are very homogeneous in their chemical structures, which generally belong to the arylethanolamine and aryloxypropanolamine classes.

In contrast, only few β-blockers have been described in which the characteristic propanolamine side chain is attached to the oxygen of an oxime function.[3–17] The role of an imino group in the side chain of β-blocking agents has already been examined in previous studies, which showed that this group did not abolish the β-adrenoceptor activity, and, in some cases, led to β_2-selective antagonists.[2,3,9,18,19] This was exemplified by cyclopropylketoxime propanolamines, which displayed potent activities,[2,20] and in particular the dicyclopropyl analogue falintol, which was found to be useful in the clinical treatment of glaucoma.[2,21]

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doi:10.1016/j.bmc.2003.09.017

The first evidence for the existence of the β_3-adrenoceptor derived from the discovery that some β_1- and β_2-adrenoceptor antagonists showed a very low potency in various gut preparations and were poorly effective as antagonists of β-adrenoceptor agonist-induced lipolysis. Furthermore, novel β-adrenoceptor agonists were discovered to be potent stimulants of rat white or brown adipocyte lipolysis, and to exert a modest influence on atrial contraction, or tracheal or uterine relaxation.[28] These compounds were found to stimulate the rate of metabolism and to possess anti-obesity, insulin-sensitising (anti-diabetic) and hypolipidemic effects in rodent experimental models.[22–25,30,31] The definitive confirmation of the β_3-adrenoceptor was obtained in 1989, when this adrenoceptor subtype was cloned.[26] Nowadays, it is widely accepted that β_3-agonists may be effective in the treatment of obesity, type 2 diabetes and urinary incontinence,[27,28,32] though no β_3-adrenoceptor agonist has advanced beyond phase II clinical studies.

An intriguing hypothesis of a potential therapeutic use of β_3-antagonists has recently been proposed for heart failure.[29] The presence of the β_3-adrenoceptor has been demostrated in cardiac tissue, where the activation of cAMP-dependent positive (principally β_1-adrenoceptors, but also β_2-adrenoceptors) and nitric oxide-dependent negative (β_3-adrenoceptor) inotropic pathways within the same cardiomyocyte are thought to provide a fine-tuning of the adrenoceptor-mediated stimulation of cardiac activity. Hence, the β_3-adrenoceptor-mediated pathway seems to represent a modulatory 'rescue' mechanism preventing myocardial damage due to a hyper-stimulation of β_1- and β_2-adrenoceptors. In the failing heart, β_1- and β_2-adrenoceptors are either downregulated or desensitized, while the β_3-adrenoceptors seem to increase. This upregulation of the β_3-adrenoceptor could represent a compensatory mechanism to prevent further myocyte damage. However, as heart failure progresses to a later stage, this compensatory mechanism can become maladaptive, with a persistent negative inotropic effect and further myocardial depression.

Hence, β-blockers specifically targeting β_1- and β_2-adrenoceptors can be more appropriate to preserve the counterveiling β_3-adrenoceptor-mediated pathway at earlier stages of the disease. Conversely, when the β_3-adrenoceptor-mediated pathway becomes maladaptive, specific β_3-adrenoceptor antagonists can be more desirable.[29]

If this hypothetical therapeutic potential of β_3-antagonists needs further experimental confirmation, the availability of selective β_3-blocking agents undoubtedly seems to be an essential goal for experimental purposes. These compounds could allow a better comprehension of the physiopathological role of this receptor and to obtain selective tools for accurate pharmacological screening procedures, aiming at the classification and characterisation of novel β_3-stimulating drugs.

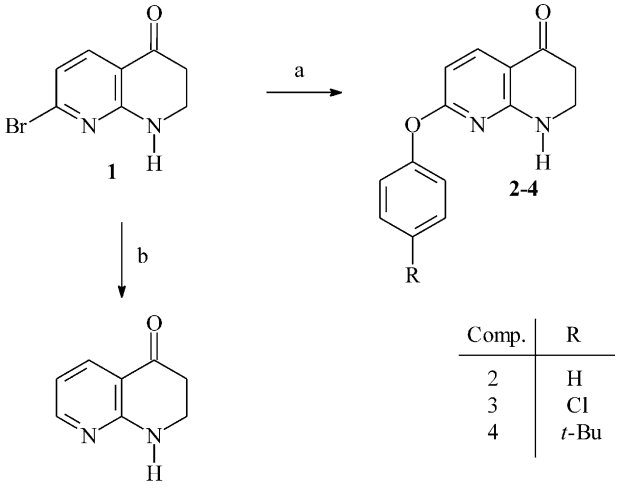
In the field of oximeethers, we recently reported the β_3-adrenoceptor antagonist activity of a series of (*R*,*S*)-(*E*)-oximeethers of 2,3-dihydro-1,8-naphthyridine and of 2,3-dihydrothiopyrano[2,3-*b*]pyridine.[12]

In the present article, we describe the synthesis and the biological results in vitro towards β-adrenergic receptors of a new series of (*R*,*S*)-(*E*)-oxyiminoethers of 2,3-dihydro-1,8-naphthyridine and of 2,3-dihydrothiopyrano[2,3-*b*]pyridine. Furthermore, some previously synthesized compounds, which were tested only as β_1-antagonists[10] without evaluating their possible β_2-blocking properties, have been submitted to a new pharmacological screening as β_2-antagonists, with the aim of formulating an overview of their β_1/β_2-selectivity. Finally, we discuss the structure–activity relationships (SARs) of all the compounds described by us in this paper and in previous papers.[10–12]

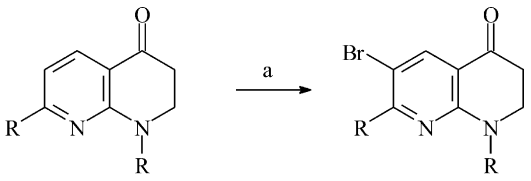
Chemistry

7-Bromo-2,3-dihydro-1,8-naphthyridin-4(1H)-one **1**[33] was allowed to react at 80 °C with ArONa, prepared by treatment of the appropriate phenol with sodium hydride in THF, to give ketones **2–4**. Dehalogenation of **1** with Raney–Ni in refluxing dioxane led to 2,3-dihydro-1,8-naphthyridin-4(1H)-ones **5** (Scheme 1). Bromination of **5** and **6**[12] with *N*-bromosuccinimide in refluxed chloroform gave **7** and **8,** respectively (Scheme 2).

The ketones **2–5** and **7–9**[34] were converted, by refluxing with hydroxylamine hydrochloride, into a mixture of anhydrous ethanol and pyridine, to yield the pure (*E*)-oximes **10–16** (Scheme 3), as demonstrated by ^{1}H NMR analysis and in agreement with a previous report.[9–11] The sodium salt of the (*E*)-oximes **10–16** was allowed to react with epichlorohydrin in anhydrous toluene to give the (*E*)-epoxides **17–23** (Scheme 3). Under these conditions, the corresponding (*Z*)-epoxides are not obtained, as reported in a previous paper.[10] The ring-opening addition reaction of epoxides **13–23** with the suitable amine carried out at room temperature in acetonitrile and catalyzed by lithium perchlorate, as reported in a previous paper,[11] gave the



Scheme 1. (a) NaH, THF, ArOH; (b) Raney–Ni, dioxane, $NaHCO_3$.



Comp.	R
5,7	H
6,8	CH_3

Scheme 2. (a) NBS, $CHCl_3$.

target compounds **24**–**30** in a good yield (Scheme 3). The structures assigned were fully confirmed by elemental analysis, IR and ^1H NMR spectra.

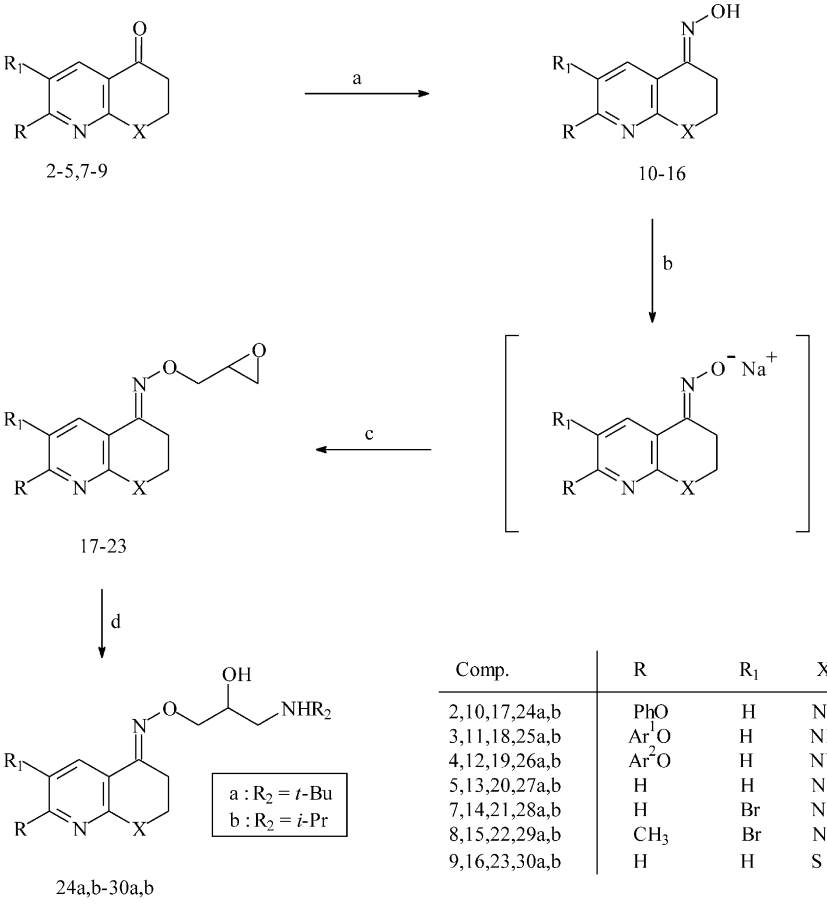
The ^1H NMR spectra of all the compounds prepared, **2**–**5, 7**–**23** and **24a**,**b**–**30a**,**b**, were analogous to those of similar compounds previously reported.[10–12] In particular, the (*E*)- conformation of oximes **10**–**16**, epoxides **17**–**23** and final compounds **24a**,**b**–**30a**,**b** was assigned on the basis of their ^1H NMR spectra, which show a chemical shift of H_5, ranging at δ 7.81–8.07, close to those of ketones **2**–**5** and **7**–**9** and of analogous compounds previously reported.[10–12]

Pharmacology

The antagonism against the positive inotropic effects of the non-selective β-agonist isoprenaline (IPNA) in guinea-pig spontaneously beating isolated atria was tested, in order to evaluate a possible β_1-blocking activity of the test compounds and of the reference drugs. The antagonism against the broncho-relaxing effects of IPNA in guinea-pig tracheal strips, pre-contracted with the muscarinic agonist carbamylcholine, was tested, to evaluate a possible β_2-blocking activity of the test compounds and of the reference drugs. Finally, a possible β_3-antagonism was investigated in isolated rat adipocytes, by the measurement of the decrease in IPNA-induced lipolysis caused by the test compounds and the reference drugs.

Results and Discussion

The pharmacological screening made it possible to evaluate the β_1-blocking activity of the newly-synthesized compounds **27a,b**, which was significantly lower than that observed for the reference drugs propranolol and practolol. Also the 6-Br analogues **28a**,**b** showed the same profile (Table 1). The replacement of the H atom in position 7 with a phenoxy-group (compounds

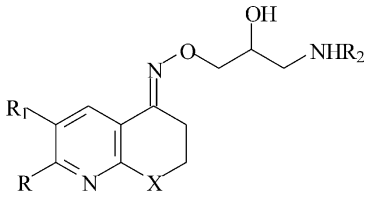


Comp.	R	R_1	X
2,10,17,24a,b	PhO	H	NH
3,11,18,25a,b	Ar^1O	H	NH
4,12,19,26a,b	Ar^2O	H	NH
5,13,20,27a,b	H	H	NH
7,14,21,28a,b	H	Br	NH
8,15,22,29a,b	CH_3	Br	NCH_3
9,16,23,30a,b	H	H	S

Ar^1O = 4-chlorophenoxy ; Ar^2O = 4-*t*-butylphenoxy

Scheme 3. (a) $H_2NOH \cdot HCl$, dry EtOH, pyridine; (b) NaH, THF; (c) epichlorohydrin; (d) R_2NH_2, CH_3CN, $LiClO_4$.

Table 1. Antagonistic activity on β-adrenoceptor



Compd	X	R	R_1	R_2	pKb[a] verses β_1	pKb verses β_2	pIC_{50} verses β_3
24a	N–H	PhO	H	*t*-Bu	<5	6.85±0.13	5.03±0.10
24b	N–H	PhO	H	*i*-Pr	<5	6.64±0.11	5.03±0.12
25a	N–H	4-Cl-PhO	H	*t*-Bu	<5	<5	5.18±0.11
25b	N–H	4-Cl-PhO	H	*i*-Pr	<5	<5	5.13±0.11
26a	N–H	4-*t*-Bu-PhO	H	*t*-Bu	<5	<5	5.33±0.05
26b	N–H	4-*t*-Bu-PhO	H	*i*-Pr	<5	<5	nt
27a	N–H	H	H	*t*-Bu	5.99±0.41	7.22±0.09	5.91±0.14
27b	N–H	H	H	*i*-Pr	5.69±0.07	6.53±0.20	nt
28a	N–H	H	Br	*t*-Bu	6.46±0.12	6.60±0.18	5.75±0.17
28b	N–H	H	Br	*i*-Pr	6.41±0.19	6.88±0.22	nt
29a	N–CH_3	CH_3	Br	*t*-Bu	<5	6.44±0.53	<4
29b	N–CH_3	CH_3	Br	*i*-Pr	<5	5.32±0.28	nt
30a	S	H	H	*t*-Bu	<5	6.56±0.49	5.04±0.15
30b	S	H	H	*i*-Pr	<5	6.40±0.50	nt
31a	NH	CH_3	H	*t*-Bu	6.48±0.15	6.69±0.20	5.14±0.17 [b]
31b	NH	CH_3	H	*i*-Pr	6.27±0.16	6.23±0.12	nt
32a	NH	Br	H	*t*-Bu	7.41±0.19	7.37±0.16	5.21±0.11
32b	NH	Br	H	*i*-Pr	6.23±0.05	6.05±0.15	nt
33a	NH	Cl	H	*t*-Bu	6.97±0.09	5.90±0.52	nt
33b	NH	Cl	H	*i*-Pr	6.10±0.18	7.18±0.02	nt
34a	NH	CH_3O	H	*t*-Bu	7.44±0.16	6.67±0.52	nt
35a	NH	C_2H_5O	H	*t*-Bu	6.39±0.21	6.70±0.10	5.21±0.16
35b	NH	C_2H_5O	H	*i*-Pr	6.51±0.18	5.24±1.06	nt
36a	NH	CH_3	Br	*t*-Bu	8.06±0.10	7.14±0.35	5.47±0.11
37a	NH	Br	Br	*t*-Bu	6.94±0.21	6.68±0.04	nt
38a	NH	CH_3	H	*t*-Bu	6.24±0.13	5.99±0.01	5.70±0.08
38b	NH	CH_3	H	*i*-Pr	5.79±0.09	6.01±0.52	nt
39a	NH	Br	H	*t*-Bu	7.12±0.18	5.42±0.73	nt
40a	NH	Cl	H	*t*-Bu	6.30±0.20	7.18±0.02	nt
41a	NH	C_2H_5O	H	*t*-Bu	6.21±0.08	7.00±0.69	5.03±0.17
Propranolol					8.71±0.15	8.25±0.12	6.22±0.10
Practolol					7.51±0.19	nt	nt

[a]pKb verses β_1 values of compounds **31a**,**b**–**41a** are reported in a previous paper.[10]
[b]pKb verses β_3 values of compounds **31a** are reported in a previous paper.[12]

24a,**b**), as well as with a 4-chloro-phenoxy- (compounds **25a**,**b**) or a 4-*t*-butyl-phenoxy- group (compounds **26a**,**b**) led to the almost complete abolition of the β_1-antagonist properties, indicating that a large steric hindrance in this position is not tolerated in this pharmacodynamic pattern.

Also the substitution of the N_1 atom of the 1,8-naphthyridine nucleus with an S heteroatom led to the abolition of the β_1-blocking activity but spared the β_2-activity, as observed for the thiopyranopyridines **30a**,**b**. Since previously synthesised thiopyranopyridines, closely related to compound **30a**,**b** but bearing a methyl group in position 7, showed an appreciable β_1-blocking activity,[12] the decline in the affinity for the β_1-receptor could be probably ascribed to the presence of the H atom in position 7.

As regards the β_2-antagonism and the β_2/β_1 selectivity of the 1,8-naphthyridine derivatives, 7- and N_1-unsubstituted compounds generally exhibited a poor β_2-selectivity, irrespective of the substitution in position 6 (comparison of compounds **27a**,**b** vs **28a**,**b**). The presence of the PhO– group in position 7, which abolished the β_1-activity as discussed above, did not influence the β_2-antagonist properties (compounds **24a**,**b**). Only a further increase in the steric hindrance in this position, that is the presence of 4-chloro-PhO– (compounds **25a**,**b**) or 4-*t*-butyl-PhO– (compounds **26a**,**b**) groups, determined the abolition of the β_2-activity.

The Br atom in position 6 cannot account for the β_2-selectivity of compounds **29a**,**b**, because the same structural characteristic shown in compounds **28a**,**b** did not lead to any selectivity. On the contrary, the presence of a methyl substituent in position N_1 of compounds **29a**,**b** could represent the key to interpret the β_2-selectivity, which might result from an almost complete absence of any β_1-activity. This evidence is strengthened by previous experimental observations, indicating that the presence of methyl, ethyl, benzyl or phenylethyl substituents in position N_1 determined a dramatic decline in the affinity for the β_1-receptor, without altering the β_2-activity.[12] However, these compounds also showed a

methyl substituent in position 7, but this structural characteristic is unlikely to be the cause of the β_2-selectivity, because no drop in the β_1-activity was observed for previously synthesised 1,8-naphthyridine derivatives, bearing methyl, Br, Cl, methoxyl or ethoxyl groups in this position (compounds **31–41**).[10] Unfortunately, these compounds were tested only as β_1-antagonists, but their possible β_2-blocking properties were not evaluated and hence were unpredictable. Therefore, in order to complete the experimental overview of their β_1/β_2-antagonism, they were submitted to a new pharmacological screening. Not surprisingly, these compounds (all exhibiting β_1-antagonist properties) were also β_2-antagonists, with comparable potencies, confirming that the presence of relatively small steric hindrances in position 7 does not play any key role in conferring any β_1- or β_2-selectivity. On the contrary, the substitution of the nitrogen atom in position 1 of the 1,8-naphthyridine nucleus with a sulfur atom led to the thiopyranopyridine compound **30a**,**b**, which exhibited significant β_2-selective antagonist properties, although quite modest in potency.

As regards the β_3-blocking activity, the *t*-butyl and the *i*-propyl substituents in the N atom of the 2-hydroxypropyloxyimino chain seem to be almost equivalent (as clearly shown by the couples of analogues **24a**,**b** and **25a**,**b**), and consequently the screening was carried out only on the remaining *t*-butyl derivatives **26a**, **27a**, **28a**, **29a**, **30a**, **31a**, **32a**, **35a**, **36a**, **38a** and **41a**.

Previous experiments showed that compound **31a**[12] was a β_3-antagonist. The structural changes, which determined a decline in the β_1-activity but spared the β_2-activity, induced a decrease (but not the abolition) of the affinity for the β_3-receptors.[12] Coherently, the newly-synthesized β_1- and β_2-blocking agents **27a** and **28a** also showed β_3-blocking properties, with potency parameters almost the same as those exhibited by the reference compound propranolol (Table 1).

Compounds **24a**,**b**, which include a PhO– group in position 7, sparing the β_2-activity and abolishing the β_1-activity, exhibited appreciable, albeit slightly lower, β_3-blocking properties. The same profile was exhibited by the thiopyranopyridine **30a**.

As observed in the previous experiments,[12] the presence of a methyl substituent in position N_1 of compound **29a** led to a significant decrease in the β_3-activity.

In our opinion, the most significant data are those of the pharmacological tests on the β_3-blocking profile of compounds **25a**,**b** and **26a**. In these molecules, the presence of the large substituents in position 7 (4-chloro-phenoxy or 4-*t*-butyl-phenoxy group) was accompanied by a dramatic decline in both the β_1- and β_2-activities; however, this structural characteristic had a modest influence on the β_3-affinity, which was only slightly lower. The compounds reported in this paper reached a β_3-selectivity, which resulted quite weak, in comparison with the β_3-selectivity ratios shown by other antagonists, such as L-748,328 and L-748,337, published by Candelore et al.[35] Nevertheless, this last structural characteristic seems to be a promising expedient to induce an appreciable selectivity towards the β_3-receptor, through a profoundly negative effect on the β_1- and β_2-activities rather than an increase in the β_3-affinity, for oximeethers of 1,8-naphthyridine derivatives.

Experimental

Chemistry

General information. 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 Bruker AC-200 NMR spectrometer. Analytical TLC was carried out on Merck 0.2 mm precoated 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 $\pm 0.4\%$ of theoretical values and were performed by our analytical laboratory.

General procedure I: synthesis of 7-aryloxy-2,3-dihydro-1,8-naphthyridin-4(1H)-one 2–4. Sodium hydride (4 mmol, 50% in mineral oil) was added to a solution of the appropriate phenol (4 mmol) in THF (20 mL) . After stirring for 1 h at room temperature, a solution of 7-bromo-2,3-dihydro-1,8-naphthyridin-4(1H)-one **1** (1.4 mmol) in THF (30 mL) was added and the mixture was refluxed for 24 h. The THF was then removed under reduced pressure to obtain a crude solid.

The analytically pure product was isolated by an appropriate method of purification as indicated below.

7-Phenoxy-2,3-dihydro-1,8-naphthyridin-4(1H)-one 2. General procedure I was used to convert ketone **1** into the title compound. The crude solid was dissolved in a small volume of toluene and, purified by flash chromatography (ethyl acetate/petroleum ether 60–80 °C 2:3), afforded **2** (0.77 g, 88.8%); mp 158–159 °C (petroleum ether 100–140 °C); ^{1}H NMR ($CDCl_3$) δ 2.62 (m, 2H), 3.53 (m, 2H), 5.40 (br, 1H), 6.15 (d, 1H), 7.28 (m, 5H), 8.02 (d, 1H). Anal. calcd for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66. Found: C, 69.78; H, 5.14; N, 11.41.

7-(4-Chloro-phenoxy)-2,3-dihydro-1,8-naphthyridin-4(1H)-one 3. General procedure I was used to convert ketone **1** into the title compound. Purification of the crude solid by flash chromatography (ethyl acetate/toluene/diethylamine 1:2:0.04) afforded **3** (0.10 g, 28.7%); mp 148–150 °C (petroleum ether 100–140 °C); ^{1}H NMR ($CDCl_3$) δ 2.94 (m, 2H), 3.35 (m, 2H), 5.20 (br, 1H), 6.24 (d, 1H), 7.20 (d, 2H),7.47 (d, 2H), 7.93 (d, 1H). Anal. calcd for $C_{14}H_{11}N_2O_2Cl$: C, 61.31; H, 4.01; N, 10.21. Found: C, 60.81; H, 4.32; N, 10.19.

7-(4-*t*-Butyl-phenoxy)-2,3-dihydro-1,8-naphthyridin-4(1H)-one 4. General procedure I was used to convert ketone **1** into the title compound. The crude solid was treated with NaOH 6 N, collected and washed with water. Purification of the crude product by crystallization from petroleum ether 100–140 °C afforded **4** (0.27 g, 69.2%); mp 198–200 °C; ^{1}H NMR ($CDCl_3$) δ 1.61 (s, 9H), 2.67 (m, 2H), 3.59 (m, 2H), 5.24 (br, 1H), 6.19 (d, 1H), 7.06 (d, 2H), 7.40 (d, 2H), 8.07 (d, 1H). Anal. calcd for $C_{18}H_{20}N_2O_2$: C, 72.91; H, 6.75; N, 9.46. Found: C, 72.64; H, 6.52; N, 9.22.

2,3-Dihydro-1,8-naphthyridin-4(1H)-one 5. Raney–Ni was added to a stirred mixture of 7-bromo-2,3-dihydro-1,8-naphthyridin-4(1H)-one **1** (0.9 mmol) and $NaHCO_3$ (0.2 g) in dioxane (10 mL), heated at 100 °C, until compound **1** finished up (controlled by TLC). The mixture was then filtered and the solution was evaporated to dryness at reduced pressure to give a crude product. Purification by flash chromatography (ethyl acetate) afforded **5** (0.09 g, 74.5%); mp 136–140 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 2.70 (m, 2H), 3.62 (m, 2H), 5.50 (br, 1H), 6.65 (m, 1H), 8.10 (m, 2H). Anal. calcd for $C_8H_8N_2O$: C, 64.86; H, 5.40; N, 10.81. Found: C, 64.74; H, 5.61; N, 10.89.

General procedure II: synthesis of 6-bromo-2,3-dihydro-1,8-naphthyridin-4(1H)-ones 7 and 8. A solution of 1.0 mmol of **5** or **6**[12] and 1.1 mmol of NBS in 15 mL of chloroform was refluxed for 2 h. After cooling, the solution was washed with water, dried over $MgSO_4$ and evaporated to dryness in vacuo. The product was collected, washed with water and purified.

6-Bromo-2,3-dihydro-1,8-naphthyridin-4(1H)-one 7. General procedure II was used to convert ketone **5** into the title compound. Purification of the crude product by crystallization from ethanol afforded **7** (0.22 g, 97.0%); mp 176–178 °C; ^{1}H NMR ($CDCl_3$) δ 2.68 (m, 2H), 3.64 (m, 2H), 5.40 (br, 1H), 8.17 (m, 2H). Anal. calcd for $C_8H_7N_2OBr$: C, 42.29; H, 3.08; N, 12.33. Found: C, 41.91; H, 2.96; N, 12.09.

6-Bromo-1,7-dimethyl-2,3-dihydro-1,8-naphthyridin-4(1H)-one 8. General procedure II was used to convert ketone **6** into the title compound. Purification by flash chromatography (ethyl acetate/hexane 1:2) afforded **8** (0.18 g, 69.5%); 88–90 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ 2.53 (s, 3H), 2.67 (m, 2H), 3.14 (s, 3H), 3.48 (m, 2H), 8.04 (s, 1H). Anal. calcd for $C_{10}H_{11}N_2OBr$: C, 46.87; H, 4.29; N, 10.93. Found: C, 46.65; H, 4.35; N, 10.72.

General procedure III: preparation of substituted (*E*)-4(1H)-hydroxyimino-2,3-dihydro-1,8-naphthyridines 10–15 and (*E*)-4(4H)-hydroxyimino-7-methyl-2,3-dihydrothiopyrano[2,3-*b*]pyridine 16. A suspension of 1.00 mmol of the appropriate ketone **2**–**5**, **7**, **8** or **9**[34] and 3.4 mmol of hydroxylamine hydrochloride in 30 mL of anhydrous ethanol and 1.5 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 **10**–**15** or **16** were collected, washed with water and purified.

(*E*)-4(1H)-Hydroxyimino-7-phenoxy-2,3-dihydro-1,8-naphthyridine 10. General procedure III was used to convert ketone **2** into the title compound. Purification of the crude product by crystallization from ethanol afforded **10** (0.22 g, 86.0%); mp 220–222 °C; ^{1}H NMR (DMSO) δ 2.67 (m, 2H), 3.19 (m, 2H), 6.10 (d, 1H), 6.60 (br, 1H), 7.25 (m, 5H), 7.89 (d, 1H), 10.73 (br, 1H). Anal. calcd for $C_{14}H_{13}N_3O_2$: C, 65.87; H, 5.13; N, 16.46. Found: C, 65.58; H,5.24; N, 16.30.

(*E*)-4(1H)-Hydroxyimino-7-(4-chloro-phenoxy)-2,3-dihydro-1,8-naphthyridine 11. General procedure III was used to convert ketone **3** into the title compound. Purification of the crude product by crystallization from petroleum ether 100–140 °C afforded **11** (0.27 g, 93.2%); mp 216–218 °C; ^{1}H NMR (DMSO) δ 2.62 (m, 2H), 3.13 (m, 2H), 6.17 (d, 1H), 6.76 (br, 1H), 7.14 (d, 2H), 7.44 (d, 2H), 7.92 (d, 1H), 10.87 (br, 1H). Anal. calcd for $C_{14}H_{12}N_3O_2Cl$: C, 58.13; H, 4.15; N, 14.53. Found: C, 58.31; H, 4.32; N, 14.31.

(*E*)-4(1H)-Hydroxyimino-7-(4-*t*-butyl-phenoxy)-2,3-dihydro-1,8-naphthyridine 12. General procedure III was used to convert ketone **4** into the title compound. Purification of the crude product by crystallization from petroleum ether 100–140 °C afforded **12** (0.29 g, 97.6%); mp 237–239 °C; ^{1}H NMR (DMSO) δ 1.28 (s, 9H), 2.63 (m, 2H), 3.18 (m, 2H), 6.10 (d, 1H), 7.01 (d, 2H), 7.78 (d, br, 3H), 10.81 (d, 1H). Anal. calcd for $C_{18}H_{21}N_3O_2$: C, 69.45; H, 6.75; N, 13.50. Found: C, 67.87; H, 6.90; N, 13.34.

(*E*)-4(1H)-Hydroxyimino-2,3-dihydro-1,8-naphthyridine 13. General procedure III was used to convert ketone **5** into the title compound. Purification of the crude product by crystallization from ethanol afforded **13** (0.14 g, 86.0%); mp 240–242 °C; ^{1}H NMR (DMSO) δ 2.69 (m, 2H), 3.21 (m, 2H), 5.50 (br, 1H), 6.57 (m, 1H), 7.88 (m, 2H), 10.97 (br, 1H). Anal. calcd for $C_8H_9N_3O$: C, 58.80; H, 5.52; N, 25.76. Found: C, 58.53; H, 5.66; N, 25.69.

(*E*)-4(1H)-Hydroxyimino-6-bromo-2,3-dihydro-1,8-naphthyridine 14. General procedure III was used to convert ketone **7** into the title compound. Purification of the crude product by crystallization from ethanol afforded **14** (0.22 g, 81.0%); mp 251–253 °C; ^{1}H NMR (DMSO) δ 2.67 (m, 2H), 3.23 (m, 2H), 6.91 (br, 1H), 7.96 (m, 2H), 11.20 (br, 1H). Anal. calcd for $C_8H_8N_3OBr$: C, 39.70; H, 3.30; N, 17.35. Found: C, 39.58; H, 3.45; N, 17.10.

(*E*)-4(1H)-Hydroxyimino-6-bromo-1,7-dimethyl-2,3-dihydro-1,8-naphthyridine 15. General procedure III was used to convert ketone **8** into the title compound. Purification of the crude product by crystallization from petroleum ether 100–140 °C afforded **15** (0.25 g, 93.4%); mp 151–153 °C; ^{1}H NMR (DMSO) δ 2.41 (s, 3H), 2.73 (m, 2H), 2.97 (s, 3H), 3.24 (m, 2H), 7.87 (s, 1H). Anal. calcd for $C_{10}H_{12}N_3OBr$: C, 44.60; H, 4.46; N, 15.61. Found: C, 44.43; H, 4.63; N, 15.58.

(*E*)-4(1H)-Hydroxyimino-2,3-dihydrothiopyrano[2,3-b]pyridine 16. General procedure III was used to con-

vert ketone **9**[34] into the title compound. Purification of the crude product by crystallization from toluene afforded **16** (0.17 g, 93.6%); mp 218–220 °C; ^{1}H NMR (DMSO) δ 2.96 (m, 2H), 3.10 (m, 2H), 7.13 (m, 1H), 8.34 (m, 1H), 8.48 (m, 1H), 11.66 (br, 1H). Anal. calcd for $C_8H_8N_2OS$: C, 53.33; H, 4.44; N, 15.55. Found: C, 53.08; H, 4.48; N, 15.69.

General procedure IV: preparation of substituted (*R*,*S*)-(*E*)-4(1H)-[(2,3-epoxypropyl)oxyimino]-2,3-dihydro-1,8-naphthyridine derivatives 17–22 and (*R*,*S*)-(*E*)-4(4H)-[(2,3-epoxypropyl)oxyimino]-2,3-dihydrothiopyrano[2,3-b]pyridine 23. Sodium hydride (1.1 mmol, 50% in mineral oil) was added to a solution of the appropriate hydroxyimino derivative **10–15** or **16** (1.0 mmol) in anhydrous THF (20 mL), the mixture was refluxed for 1 h and then epichlorohydrin (10 mmol) was added. The resulting suspension was refluxed for 72 h. The solvent was evaporated to dryness in vacuo; the crude products were dissolved in a small volume of the appropriate solvent and purified by flash chromatography to obtain compounds **17–22** or **23**.

(*R*,*S*)-(*E*)-4(1H)-[(2,3-epoxypropyl)oxyimino]-7-phenoxy-2,3-dihydro-1,8-naphthyridine 17. General procedure IV was used to convert the hydroxyimino derivative **10** into the title compound. Purification by flash chromatography (petroleum ether 60–80 °C/ethyl acetate/diethylamine 6:2:0.5) afforded **17** (0.23 g, 72.0%); mp 145–147 °C (petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 2.62–3.03 (m, 4H), 3.20–3.57 (m, 3H), 4.27 (m, 2H), 6.10 (d, 1H), 7.70 (m, 5H), 8.03 (d, 1H). Anal. calcd for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50. Found: C, 65.54; H, 5.66; N, 13.42.

(*R*,*S*)-(*E*)-4(1H)-[(2,3-epoxypropyl)oxyimino]-7-(4-chloro-phenoxy)-2,3-dihydro-1,8-naphthyridine 18. General procedure IV was used to convert the hydroxyimino derivative **11** into the title compound. Purification by flash chromatography (petroleum ether 60–80 °C/ethyl acetate/diethylamine 2:1:0.07) afforded **18** (0.10 g, 41.4%); 131–133 °C (petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 2.44–2.78 (m, 4H), 3.34 (m, 2H), 3.98 (m, 1H), 4.35 (m, 2H), 6.19 (d, 1H), 6.88 (br, 1H), 7.15 (d, 2H), 7.44 (d, 2H), 7.93 (d, 1H). Anal. calcd for $C_{17}H_{16}N_3O_3Cl$: C, 59.13; H, 4.63; N, 12.17. Found: C, 58.83; H, 4.80; N, 11.92.

(*R*,*S*)-(*E*)-4(1H)-[(2,3-epoxypropyl)oxyimino]-7-(4-*t*-butyl-phenoxy)-2,3-dihydro-1,8-naphthyridine 19. General procedure IV was used to convert the hydroxyimino derivative **12** into the title compound. Purification by flash chromatography (petroleum ether 60–80 °C/ethyl acetate 3:2) afforded **19** (0.10 g, 39.0%); 136–138 °C (petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 1.34 (s, 9H), 2.67–2.90 (m, 4H), 3.38 (m, 2H), 4.06 (m, 1H), 4.36 (m, 2H), 4.82 (br, 1H), 6.13 (d, 1H), 7.06 (d, 2H), 7.39 (d, 2H), 8.02 (d, 1H). Anal. calcd for $C_{21}H_{25}N_3O_3$: C, 68.66; H,6.81; N,11.44. Found: C, 68.41; H, 7.01; N, 11.12.

(*R*,*S*)-(*E*)-4(1H)-[(2,3-epoxypropyl)oxyimino]-2,3-dihydro-1,8-naphthyridine 20. General procedure IV was used to convert the hydroxyimino derivative **13** into the title compound. Purification by flash chromatography (petroleum ether 60–80 °C/ethyl acetate/diethylamine 3:1:0.5) afforded **20** (0.06 g, 48.6%); mp 120–122 °C (petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 2.60–2.94 (m, 4H), 3.23–3.46 (m, 3H), 4.02 (m, 2H), 6.59 (d, 1H), 8.02 (m, 2H). Anal. calcd for $C_{11}H_{13}N_3O_2$: C, 60.27; H, 5.94; N, 19.18. Found: C, 60.04; H, 5.66; N, 19.42.

(*R*,*S*)-(*E*)-4(1H)-[(2,3-epoxypropyl)oxyimino]-6-bromo-2,3-dihydro-1,8-naphthyridine 21. General procedure IV was used to convert the hydroxyimino derivative **14** into the title compound. Purification by flash chromatography (petroleum ether 60–80 °C/ethyl acetate/diethylamine 3:1:0.5) afforded **21** (0.06 g, 48.6%); mp 160–161 °C (petroleum ether 100–140 °C); ^{1}H NMR ($CDCl_3$) δ 2.63–2.93 (m, 4H), 3.19–3.43 (m, 3H), 4.22 (m, 2H), 8.06 (m, 2H). Anal. calcd for $C_{11}H_{12}N_3O_2Br$: C, 44.29; H, 4.02; N, 14.09. Found: C, 44.15; H, 4.29; N, 14.25.

(*R*,*S*)-(*E*)-4(1H)-[(2,3-epoxypropyl)oxyimino]-6-bromo-1,7-dimethyl-2,3-dihydro-1,8-naphthyridine 22. General procedure IV was used to convert the hydroxyimino derivative **15** into the title compound. Purification by flash chromatography (petroleum ether 60-80 °C/ethyl acetate/diethylamine 30:10:0.2) afforded **22** (0.18 g, 67.4%); 143–145 °C (petroleum ether 100–140 °C); ^{1}H NMR ($CDCl_3$) δ 2.35–2.85 (m, 7H), 2.99 (s, 3H), 3.30 (m, 2H), 4.38 (m, 2H), 7.94 (s, 1H). Anal. calcd for $C_{13}H_{16}N_3O_2Br$: C, 48.00; H, 4.92; N, 12.92. Found: C, 47.60; H, 5.17; N, 12.53.

(*R*,*S*)-(*E*)-4(4H)-[(2,3-epoxypropyl)oxyimino]-2,3-dihydrothiopyrano[2,3-*b*]pyridine 23. General procedure IV was used to convert the hydroxyimino derivative **16** into the title compound. Purification by flash chromatography (petroleum ether 60–80 °C/ethyl acetate / diethylamine 2:3:0.1) afforded **23** (0.15 g, 64.4%); oil; ^{1}H NMR (DMSO) δ 2.65–2.85 (m, 2H), 3.02 (m, 2H), 3.11 (m, 2H), 4.03 (m, 1H), 4.43 (m, 2H), 7.19 (m, 1H), 8.20 (m, 1H), 8.37 (m, 1H). Anal. calcd for $C_{11}H_{12}N_2O_2S$: C, 55.93; H, 5.08; N, 11.86. Found: C, 55.71; H, 5.21; N, 11.62.

General procedure V: preparation of substituted (*R*,*S*)-(*E*)-4(1H)-[1-(3-alkylamino-2-hydroxypropyl)oxyimino]-2,3-dihydro-1,8 naphthyridine derivatives 24a,b–29a,b. Two millimoles of the appropriate alkylamine (isopropylamine or tertbutylamine) was added to a mixture of 1.0 mmol of epoxypropyloxyimino derivatives **17–22** 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 crude products were dissolved in a small volume of appropriate solvent and purified by flash chromatography, to obtain compounds **24a**,**b–29a**,**b**.

(*R*,*S*)-(*E*)-4(1H)-[1-(3-*t*-butylamino-2-hydroxypropyl)oxyimino]-7-phenoxy-2,3-dihydro-1,8 naphthyridine 24a. General procedure V was used to convert the 2,3-epoxypropyloxyimino derivative **17** into the title compound. Purification by flash chromatography (petroleum ether 60–80 °C/ethyl acetate/diethylamine 2:3:0.2) afforded **24a** (0.27 g, 68.7%); mp 134–136 °C (petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 1.23 (s, 9H), 2.77–3.02 (m, 5H), 3.36 (m, 2H), 4.16 (m, 2H), 6.20 (d, 1H), 7.85 (m, 5H), 8.06 (d, 1H). Anal. calcd for $C_{21}H_{28}N_4O_3$: C, 65.60; H, 7.34; N, 14.57. Found: C, 65.72; H, 7.13; N, 14.69.

(*R*,*S*)-(*E*)-4(1H)-[1-(3-*i*-propylamino-2-hydroxypropyl)-oxyimino]-7-phenoxy-2,3-dihydro-1,8 naphthyridine 24b. General procedure V was used to convert the 2,3-epoxypropyloxyimino derivative **17** into the title compound. Purification by flash chromatography (ethyl acetate/diethylamine 20:1) afforded **24b** (0.19 g, 68.5%); oil; ^{1}H NMR (DMSO) δ 1.21 (d, 6H), 2.79–3.04 (m, 6H), 3.36 (m, 2H), 4.16 (m, 2H), 6.21 (d, 1H), 7.85 (m, 5H), 8.06 (d, 1H). Anal. calcd for $C_{20}H_{26}N_4O_3$: C, 64.85; H, 7.07; N, 15.13. Found: C, 64.64; H, 7.19; N, 15.28.

(*R*,*S*)-(*E*)-4(1H)-[1-(3-*t*-butylamino-2-hydroxypropyl) oxyimino]-7-(4-chlorophenoxy)-2,3-dihydro-1,8 naphthyridine 25a. General procedure V was used to convert the 2,3-epoxypropyloxyimino derivative **18** into the title compound. Purification by flash chromatography (ethyl acetate/diethylamine 20:1) afforded **25a** (0.29 g, 77.7%); mp 152–154 °C (triturated with petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 1.22 (s, 9H), 2.80–3.00 (m, 6H), 3.34 (m, 2H), 4.17 (m, 2H), 6.15 (d, 1H), 7.16 (d, 2H), 7.43 (d, 2H), 7.93 (d, 1H). Anal. calcd for $C_{21}H_{27}N_4O_3Cl$: C, 60.28; H, 6.45; N, 13.39. Found: C, 60.01; H, 6.13; N, 13.05.

(*R*,*S*)-(*E*)-4(1H)-[1-(3-*i*-propylamino-2-hydroxypropyl)-oxyimino]-7-(4-chlorophenoxy)-2,3-dihydro-1,8 naphthyridine 25b. General procedure V was used to convert the 2,3-epoxypropyloxyimino derivative **18** into the title compound. Purification by flash chromatography (ethyl acetate/diethylamine 20:1) afforded **25b** (0.25 g, 61.0%); mp 148–149 °C (triturated with petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 1.20 (d, 6H), 2.79–3.02 (m, 6H), 3.34 (m, 2H), 4.17 (m, 2H), 6.15 (d, 1H), 7.16 (d, 2H), 7.43 (d, 2H), 7.93 (d, 1H). Anal. calcd for $C_{20}H_{25}N_4O_3Cl$: C, 59.40; H, 6.18; N, 13.86. Found: C, 59.22; H,6.25; N, 13.53.

(*R*,*S*)-(*E*)-4(1H)-[1-(3-*t*-butylamino-2-hydroxypropyl)oxyimino]-7-(4-*t*-butylphenoxy)-2,3-dihydro-1,8 naphthyridine 26a. General procedure V was used to convert the 2,3-epoxypropyloxyimino derivative **19** into the title compound. Purification by flash chromatography (ethyl acetate/diethylamine 20:1) afforded **26a** (0.33 g, 77.3%); mp 162–164 °C (triturated with petroleum ether 100–140 °C); ^{1}H NMR ($CDCl_3$) δ 1.25 (s, 9H), 1.34 (s, 9H), 2.80–3.01 (m, 5H), 3.35 (m, 2H), 4.18 (m, 2H), 6.11 (d, 1H), 7.04 (d, 2H), 7.39 (d, 2H), 7.96 (d, 1H). Anal. calcd for $C_{25}H_{36}N_4O_3$: C, 68.18; H, 8.18; N, 12.70. Found: C, 67.93; H, 8.41; N, 12.41.

(*R*,*S*)-(*E*)-4(1H)-[1-(3-*i*-propylamino-2-hydroxypropyl)-oxyimino]-7-(4-*t*-butylphenoxy)-2,3-dihydro-1,8 naphthyridine 26b. General procedure V was used to convert the 2,3-epoxypropyloxyimino derivative **19** into the title compound. Purification by flash chromatography (ethyl acetate/diethylamine 20:1) afforded **26b** (0.33 g, 77.0%); mp 155–157 °C (triturated with petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 1.19 (d, 6H), 1.34 (s, 9H), 2.81–3.03 (m, 6H), 3.35 (m, 2H), 4.18 (m, 2H), 6.11 (d, 1H), 7.04 (d, 2H), 7.39 (d, 2H), 7.96 (d, 1H). Anal. calcd for $C_{24}H_{34}N_4O_3$: C, 67.60; H, 7.98; N, 13.14. Found: C, 67.55; H, 7.71; N, 12.98.

(*R*,*S*)-(*E*)-4(1H)-[1-(3-*t*-butylamino-2-hydroxypropyl)oxyimino]-2,3-dihydro-1,8 naphthyridine 27a. General procedure V was used to convert the 2,3-epoxypropyloxyimino derivative **20** into the title compound. Purification by flash chromatography (ethyl acetate/diethylamine 20:1) afforded **27a** (0.21 g, 70.1%); a waxlike solid (triturated with petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 1.11 (s, 9H), 2.78–3.04 (m, 5H), 3.34 (m, 2H), 4.16 (m, 2H), 6.58 (m, 1H), 7.97 (m, 2H). Anal. calcd for $C_{15}H_{24}N_4O_2$: C, 61.62; H, 8.27; N, 19.16. Found: C, 61.90; H, 8.03; N, 18.99.

(*R*,*S*)-(*E*)-4(1H)-[1-(3-*i*-propylamino-2-hydroxypropyl)-oxyimino]-2,3-dihydro-1,8 naphthyridine 27b. General procedure V was used to convert the 2,3-epoxypropyloxyimino derivative **20** into the title compound. Purification by flash chromatography (ethyl acetate/diethylamine 20:1) afforded **27b** (0.17 g, 58.5%); a waxlike solid (triturated with petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 1.07 (d, 6H), 2.81–3.03 (m, 6H), 3.34 (m, 2H), 4.15 (m, 2H), 6.58 (m, 1H), 7.09 (m, 1H), 7.96 (m, 1H). Anal. calcd for $C_{14}H_{22}N_4O_2$: C, 60.41; H, 7.97; N, 20.13. Found: C, 60.73; H, 7.69; N, 20.37.

(*R*,*S*)-(*E*)-4(1H)-[1-(3-*t*-butylamino-2-ydroxypropyl)oxyimino]-6-bromo-2,3-dihydro-1,8 naphthyridine 28a. General procedure V was used to convert the 2,3-epoxypropyloxyimino derivative **21** into the title compound. Purification by flash chromatography (ethyl acetate/petroleum ether 60–80 °C/diethylamine 2:2:0.1) afforded **28a** (0.25 g, 68.2%); mp 143–145 °C (petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 1.10 (s, 9H), 2.76–3.01 (m, 5H), 3.32 (m, 2H), 4.12 (m, 2H), 8.04 (m, 2H). Anal. calcd for $C_{15}H_{23}N_4O_2Br$: C, 48.53; H, 6.24; N, 15.09. Found: C, 48.74; H, 6.31; N, 14.88.

(*R*,*S*)-(*E*)-4(1H)-[1-(3-*i*-propylamino-2-hydroxypropyl)-oxyimino]-6-bromo-2,3-dihydro-1,8 naphthyridine 28b. General procedure V was used to convert the 2,3-epoxypropyloxyimino derivative **21** into the title compound. Purification by flash chromatography (ethyl acetate/diethylamine 20:1) afforded **28b** (0.23 g, 66.5%); mp 145–147 °C (petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 1.08 (d, 6H), 2.79–3.01 (m, 6H), 3.33 (m, 2H), 4.14 (m, 2H), 8.04 (m, 2H). Anal. calcd for $C_{14}H_{21}N_4O_2Br$: C, 47.07; H, 5.92; N, 15.68. Found: C, 46.85; H, 5.76; N, 15.40.

(*R*,*S*)-(*E*)-4(1H)-[1-(3-*t*-butylamino-2-hydroxypropyl)oxyimino]-6-bromo-1,7-dimethyl-2,3-dihydro-1,8 naphthyri-

dine 29a. General procedure V was used to convert the 2,3-epoxypropyloxyimino derivative **22** into the title compound. Purification by flash chromatography (ethyl acetate/diethylamine 20:1) afforded **29a** (0.26 g, 65.2%); 157–159 °C (petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 1.01 (s, 9H), 2.42 (s, 3H), 2.50 (m, 2H), 2.80 (m, 2H), 2.98 (s, 3H), 3.32 (m, 3H), 4.08 (d, 2H), 7.94 (s, 1H). Anal. calcd for $C_{17}H_{27}N_4O_2Br$: C, 51.12; H, 6.76; N, 14.03. Found: C, 51.40; H, 7.17; N, 13.75.

(*R*,*S*)-(*E*)-4(1H)-[1-(3-*i*-propylamino-2-hydroxypropyl)-oxyimino]-6-bromo-1,7-dimethyl-2,3-dihydro-1,8 naphthyridine 29b. General procedure V was used to convert the 2,3-epoxypropyloxyimino derivative **22** into the title compound. Purification by flash chromatography (ethyl acetate/diethylamine 20:1) afforded **29b** (0.26 g, 67.5%); mp 160–162 °C (petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 1.21 (d, 6H), 2.43 (s, 3H), 2.50 (m, 3H), 2.80 (m, 2H), 2.99 (s, 3H), 3.31 (m, 3H), 4.08 (d, 2H), 7.94 (s, 1H). Anal. calcd for $C_{16}H_{25}N_4O_2Br$: C, 49.88; H, 6.54; N, 14.54. Found: C, 49.52; H, 6.72; N, 14.26.

General procedure VI: preparation of substituted (*R*,*S*)-(*E*)-4(4H)-[1-(3-alkylamino-2-hydroxypropyl)oxyimino]-2,3-dihydrothiopyrano[2,3-*b*]pyridine derivatives 30a,b. A solution of 1.0 mmol of **23** and 3.0 mmol of the suitable alkylamine (isopropylamine or tertbutylamine) 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 **30a** and **30b**.

(*R*,*S*)-(*E*)-4(4H)-[1-(3-*t*-butylamino-2-hydroxypropyl)oxyimino]-2,3-dihydrothiopyrano[2,3-*b*]pyridine 30a. General procedure VI was used to convert the 2,3-epoxypropyloxyimino derivative **23** into the title compound. Purification by flash chromatography (ethyl acetate/petroleum ether 60–80 °C /diethylamine 3:2:0.5) afforded **30a** (0.21 g, 66.7%); a waxlike solid (triturated with petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 1.00 (s, 9H), 2.43–2.54 (m, 2H), 3.09 (m, 2H), 3.15 (m, 1H), 3.75 (m, 2H), 4.12 (m, 2H), 7.16 (m, 1H), 8.14 (m, 1H), 8.36 (m, 1H). Anal. calcd for $C_{15}H_{23}N_3O_2S$: C, 58.25; H, 7.44; N, 13.59. Found: C, 58.65; H, 7.32; N, 13.49.

(*R*,*S*)-(*E*)-4(4H)-[1-(3-*i*-propylamino-2-hydroxypropyl)-oxyimino] - 2,3 - dihydrothiopyrano[2,3 - *b*]pyridine 30b. General procedure V was used to convert the 2,3-epoxypropyloxyimino derivative 23 into the title compound. Purification by flash chromatography (ethyl acetate/petroleum ether 60–80 °C/diethylamine 3:2:0.5) afforded 30b (0.20 g, 67.3%); a waxlike solid (triturated with petroleum ether 100–140 °C); ^{1}H NMR (DMSO) δ 0.95 (d, 6H), 2.07 (m, 1H), 2.41–2.70 (m, 2H), 3.15 (m, 3H), 3.32 (m, 2H), 4.01 (m, 2H), 7.16 (m, 1H), 8.15 (m, 1H), 8.36 (m, 1H). Anal. calcd for $C_{14}H_{21}N_3O_2S$: C, 56.94; H, 7.11; N, 14.23. Found: C, 56.78; H, 6.80; N, 13.93.

Pharmacology

General information. 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 test compounds were dissolved (1 mM) in dimethylsulfoxide. All further dilutions were performed in distilled water. The solutions were prepared immediately before the experiments.

Isolated guinea-pig atria. A possible β_1-blocking activity was evaluated on spontaneously beating isolated atria of guinea-pigs, as previously described.[11] 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, $MgSO_4$ $7H_2$ O 1.05, NaH_2 PO_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 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 by an 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.

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.[11] 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 tested 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 μM) 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).

Rat adipocytes. To evaluate a possible β_3-blocking activity, adipose cells were isolated from male Wistar rats (200–230 g) fed ad libitum. The rats were killed by cervical dislocation. Epididymal fat pads were immediately removed and isolated adipose cells were prepared by the method originally described by Rodbell[36] and subsequently modified by Cushman.[37] 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 waterbath gassed with 5% CO_2 in 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 the collagenase digestion and the 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 2×105 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 preincubated for 15 min with the antagonist or with the vehicle, before the addition of the agonist. When isoprenaline 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 dimethyl sulphoxide at a final concentration ranging from 0.00025 to 0.025%, and in this case the experiments were performed with the appropriate control containing dimethyl sulphoxide. 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[38] released into the incubation medium, using a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer at 366 nm.

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

Analysis of data

β_1- and β_2-Antagonism. The antagonist potency was expressed as pKb, representing the value of −Log of the dissociation constant (calculated as molar concentration), 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.[39] The value of the dissociation constant for the β_2-adrenoceptor/antagonist complex was calculated by means of Gaddum's method.[40] Data were statistically 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 GraphPad Prism TM 2.0).

β_3-Antagonism. Different concentrations of isoprenaline and of the compounds under test were added to adipocyte preparations to obtain concentration–response curves. The concentration–response curve to isoprenaline 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 evaluated by an inhibition curve and was expressed as $pIC_{50}\pm$SEM of three experiments in duplicate, that is the negative logarithm of the molar concentration of the antagonist that inhibited the stimulant action of isoprenaline by 50%. ± SEM.

The concentration–inhibitory response curves were calculated from non-linear regression analysis of data by means of a computer-aided program (GraphPad Prism 2.0), forcing the analysis between the top and bottom limits of 100 and 0, respectively, to avoid a possible over-estimation of the potency parameters. To allow the possibility of a correct comparison between the results obtained with these newly-synthesised compounds and other analogous molecules previously reported,[10] the above calculation procedures were used for the re-evaluation of the old data. This justifies some slight differences between the data reported in this paper and those already published (which were calculated without forcing the concentration–inhibitory response curves within any top–bottom limit).

Statistical analysis was carried out using ANOVA and Student's *t*-test, with a probability value (*p*) less than 0.05 regarded as significant.

References and Notes

1. Hoffman, B.B.; Lefkowitz, R. J. In *The Pharmacological Basis of Therapeutics*, 9th ed.; Hardman, J. G., Goodman Gilman, A., Limbird, L. E., Eds.; McGraw-Hill: New York, 1995; p 232.
2. Blanc, M.; Tamir, A.; Aubriot, S.; Michel, M. C.; Bouzou-

baa, M.; Leclerc, G.; Demenge, P. *J. Med. Chem.* **1998**, *41*, 1613.
3. Leclerc, G.; Mann, A.; Wermuth, C. G. *J. Med. Chem.* **1977**, *20*, 1657.
4. Fravolini, A.; Schiaffella, F.; Orzalesi, G.; Selleri, R.; Volpato, I. *Eur. J. Med. Chem.* **1978**, *13*, 347.
5. Martani, A.; Magli, M.; Orzalesi, G.; Selleri, R. *Farmaco Ed. Sci.* **1975**, *30*, 370.
6. Baldwin, J. J.; McClure, D. E.; Gross, D. M.; Williams, M. *J. Med. Chem.* **1982**, *25*, 931.
7. Amlaiky, N.; Leclerc, G.; Decker, N.; Schwartz, J. *Eur. J. Med. Chem.* **1984**, *19*, 341.
8. Rakhit, S.; Bouzoubaa, M.; Leclerc, G.; Leger, J. M.; Carpy, A. *Eur. J. Med. Chem.* **1986**, *21*, 411.
9. Jamart-Gregoire, B.; Caubere, P.; Blanc, M.; Gnassounou, J. P.; Advenier, C. *J. Med. Chem.* **1989**, *32*, 315.
10. Ferrarini, P. L.; Mori, C.; Primofiore, G.; Da Settimo, A.; Breschi, M. C.; Martinotti, E.; Nieri, P.; Ciucci, M. A. *Eur. J. Med. Chem.* **1990**, *25*, 489.
11. Ferrarini, P. L.; Mori, C.; Badawneh, M.; Manera, C.; Saccomanni, G.; Calderone, V.; Scatizzi, R.; Barili, P. L. *Eur. J. Med. Chem.* **1997**, *32*, 955.
12. Ferrarini, P. L.; Mori, C.; Badawneh, M.; Calderone, V.; Greco, R.; Manera, C.; Martinelli, A.; Nieri, P.; Saccomanni, G. *Eur. J. Med. Chem.* **2000**, *35*, 815.
13. Balsamo, A.; Breschi, M. C.; Chini, M.; Domiano, P.; Giannaccini, G.; Lucacchini, A.; Macchia, B.; Macchia, M.; Manera, C.; Martinelli, A.; Martini, C.; Martinotti, E.; Nieri, P.; Rossello, A. *Eur. J. Med. Chem.* **1992**, *27*, 751.
14. Macchia, B.; Balsamo, A.; Breschi, M. C.; Chiellini, G.; Macchia, M.; Martinelli, A.; Martini, C.; Nardini, C.; Nencetti, S.; Rossello, A.; Scatizzi, R. *J. Med. Chem.* **1994**, *37*, 1518.
15. Balsamo, A.; Gentili, D.; Lapucci, A.; Macchia, M.; Martinelli, A.; Orlandini, E. *Farmaco* **1994**, *49*, 759.
16. Balsamo, A.; Lapucci, A.; Macchia, B.; Macchia, M.; Orlandini, E.; Rossello, A. *Farmaco* **1995**, *50*, 239.
17. Gentili, D.; Lapucci, A.; Macchia, B.; Macchia, M.; Martinelli, A.; Nencetti, S.; Orlandini, E.; Ferni, G.; Pinza, M. *Farmaco* **1995**, *50*, 519.
18. Bouzoubaa, M.; Leclerc, G.; Decker, N.; Schwartz, J.; Andermann, G. *J. Med. Chem.* **1984**, *27*, 1291.
19. Macchia, B.; Balsamo, A.; Lapucci, A.; Macchia, F.; Breschi, M. C.; Fantoni, B.; Martinotti, E. *J. Med. Chem.* **1985**, *28*, 153.
20. Bouzoubaa, M.; Leclerc, G.; Rakhit, S.; Andermann, G. *J. Med. Chem.* **1985**, *28*, 896.
21. Himber, J.; Sallee, V.; Andermann, G.; Bouzoubaa, M.; Leclerc, G.; De Santis, L. *J. Ocul. Pharmacol.* **1987**, *3*, 111.
22. Arch, J. R. S.; Ainsworth, A. T.; Ellis, R. D. M.; Piercy, V.; Thody, V. E.; Thurlby, P. L.; Wilson, C.; Wilson, S.; Young, P. *J. Obes.* **1984**, *8* (Suppl. 1), 1.
23. Meier, M. K.; Alig, L.; Burgi-Saville, M. E.; Muller, M. *J. Obes.* **1984**, *8* (Suppl. 1), 215.
24. Yen, T. T.; McKee, M. M.; Stamm, N. B. *J. Obes.* **1984**, *8* (Suppl. 1), 65.
25. Cawthorne, M. A.; Carroll, M. J.; Levy, A. L.; Lister, C. L.; Sennitt, M. V.; Smith, S. A.; Young, P. *J. Obes.* **1984**, *8* (Suppl. 1), 93.
26. Emorine, L. J.; Marullo, S.; Briend-Sutren, M.-M.; Patey, G.; Tate, T.; Delavier-Klutchko, C.; Strosberg, A. D. *Science* **1989**, *245*, 1118.
27. Arch, J. R. S.; Ainsworth, A. T. *J. Obes.* **1983**, *7*, 85.
28. Arch, J. R. S.; Ainsworth, A. T.; Cawthorne, M. A.; Piercy, V.; Sennitt, M. V.; Thody, V. E.; Wilson, C.; Wilson, S. *Nature* **1984**, *309*, 163.
29. Gauthier, C.; Langin, D.; Balligand, J. L. *Trends Pharmacol. Sci.* **2000**, *21*, 426.
30. Zulet, M. A.; Berraondo, B.; Milagro, F. I.; Martinez, J. A. *Farmaco* **1999**, *54*, 710.
31. He, J.; Nikulin, I.; Vansal, S. S.; Feller, D. R.; Miller, D. D. *J. Med. Chem.* **2000**, *43*, 591.
32. Tanaka, N.; Tamai, T.; Mukaiyama, H.; Hirabayshi, A.; Muranaka, H.; Akahane, S.; Miyata, H.; Akahane, M. *J. Med. Chem.* **2001**, *44*, 1436.
33. Da Settimo, A.; Biagi, G.; Primofiore, G.; Ferrarini, P. L.; Livi, O. *Farmaco Ed. Sci.* **1978**, *33*, 770.
34. Da Settimo, A.; Marini, A. M.; Primofiore, G.; Da Settimo, F.; La Motta, C.; Pardi, G.; Ferrarini, P. L.; Mori, C. *J. Heterocyclic Chem.* **1999**, *37*, 379.
35. Candelore, M. R.; Deng, L.; Tota, L.; Guan, X.-M.; Amend, A.; Liu, Y.; Newbold, R.; Cascieri, M. A.; Weber, A. E. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 649.
36. Rodbell, M. *J. Biol. Chem.* **1964**, *239*, 375.
37. Cushman, S. *J. Cell Biol.* **1970**, *46*, 326.
38. Wieland, O. *Biochem. Z* **1957**, *239*, 313.
39. Calderone, V. J. *Pharmacol. Toxicol. Methods* **1998**, *39*, 129.
40. Gaddum, J. H. *Pharmacol. Rev.* **1957**, *9*, 211.