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Original article

Synthesis of 1,8-naphthyridine derivatives: potential antihypertensive agents – Part VIII

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Abstract – A series of (ethoxycarbonylpiperazinyl)- and piperazinyl-1,8-naphthyridine derivatives, variously substituted, has been synthesized and pharmacologically investigated for anthihypertensive activity. Some of them exhibited a significant and prolonged decrease of the mean arterial pressure (MAP) on spontaneously hypertensive rats. On the basis of the pharmacological results, no structure-activity relationship can be deduced at this time. Moreover, the most active compound **4e**, was investigated by means of in vitro pharmacological functional studies and in vivo, as a diuretic agent, to determine a possible mechanism of the antihypertensive activity, which results in a probably non-competitive antagonism against α_1 vascular adrenoceptors. This mechanism was also shown by the compounds **8** and **13**.

antihypertensive derivatives / piperazinyl / 1,8-naphthyridine derivatives

1. Introduction

Previously, we described the synthesis and the antihypertensive activity of 2-piperazinyl-1,8-naphthyridine derivatives, variously substituted [1, 2].

Some of them exhibited a significant and prolonged decrease of the mean arterial pressure (MAP) in spontaneously hypertensive rats (SHR).

In consideration of the pharmacologically interesting activity shown by this class of compounds, we decided to continue our studies directed toward the synthesis of the products with potential antihypertensive activity.

In this paper we report the preparation and pharmacological results of a new series of 1,8-naphthyridine derivatives, carrying the piperazino group in the 2- and 4-position, with the aim of obtaining further information on the antihypertensive activity of 1,8-naphthyridine derivatives and verifying the influence of the position of the piperazino group on this pharmacological activity.

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2. Chemistry

The hydrolysis of the known 2-acetamido-5-chloro-1,8-naphthyridine **1** [3] with 10% hydrochloric acid gave the amino derivative **2a**, which by diazotization in concentrated sulfuric acid was converted, in good yield, to the corresponding 2-hydroxynaphthyridine **2b** (*figure 1, table I*).

The chloro derivatives **2a, 2b, 2c** [4], **2d** [1], **2e** [1] and **2f** [5] were allowed to react with ethoxycarbonylpiperazine (CEP) in a sealed tube to give the ethoxycarbonylpiperazine derivatives **3** and then the alkaline hydrolysis of **3** gave the piperazine derivatives **4** (*figure 2, table I*).

Chloro derivative **2a** [4] was also treated with methylpiperazine in analogous conditions, as reported above for compounds **2**, to give **5**.

The reaction of **3e** with phosphoryl chloride gave compound **6**, which was allowed to react with sodium methoxide in order to obtain the corresponding 7-methoxyderivative, but under these conditions compound **6** was converted by transesterification to **7**, which

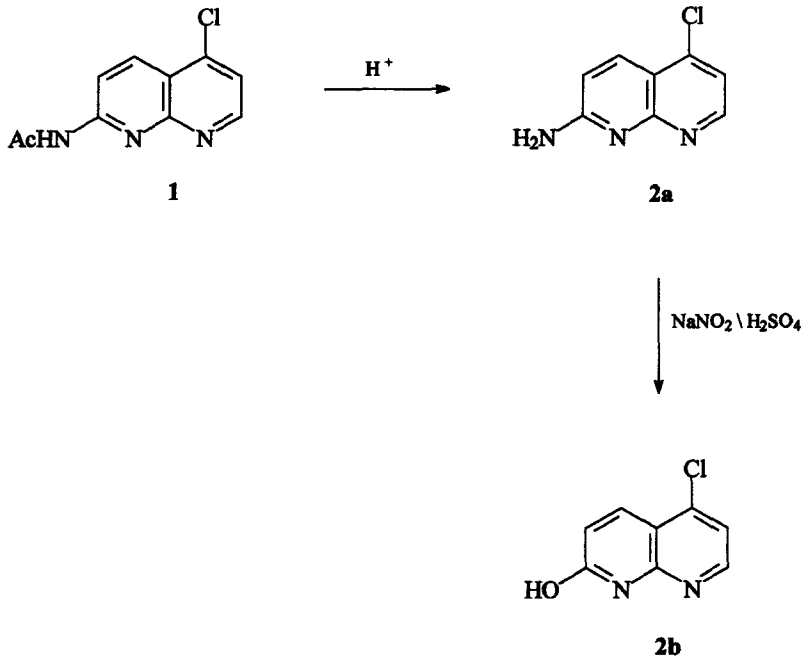
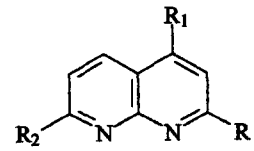


Figure 1. Synthesis of Chloronaphthyridine **2b**.

Table I. Physical data of substituted 1,8-naphthyridines.



Compound	R	R_1	R_2	Yield (%)	M.p. (°C)	Recryst. solvent
2a	H	Cl	NH_2	98	173–175	H_2O
2b	H	Cl	OH	70	234–235	EtOH
3a	H	CEP	NH_2	69	245–247	H_2O
3b	H	CEP	OH	70	239–241	*i*-PrOH
3c	H	CEP	CH_3	31	oil	
3d	CF_3	CEP	NH_2	93	252–253	AcOEt
3e	CF_3	CEP	OH	73	215–216	Benzene
3f	CF_3	CEP	CH_3	97	190–192	Benzene
4a	H	PIP	NH_2	84	213–215	*i*-PrOH
4b	H	PIP	OH	66	220–222	*i*-PrOH
4c	H	PIP	CH_3	76	182–183	Petr. ether[a]
4d	CF_3	PIP	NH_2	89	249–251	AcOEt
4e	CF_3	PIP	OH	88	264–266	EtOH
4f	CF_3	PIP	CH_3	94	168–170	AcOEt
5	H	MPIP	CH_3	73	oil	
6	CF_3	CEP	Cl	99	190–191	Petr. ether[a]
7	CF_3	CMP	OCH_3	76	180–182	Benzene
8	CF_3	PIP	OCH_3	77	248–250	EtOH\H_2O (2:1)

[a]Petroleum ether 100–140°. CEP, N-ethoxycarbonylpiperazine; PIP, piperazine; CMP, N-methoxycarbonylpiperazine; MPIP, N-methylpiperazine.

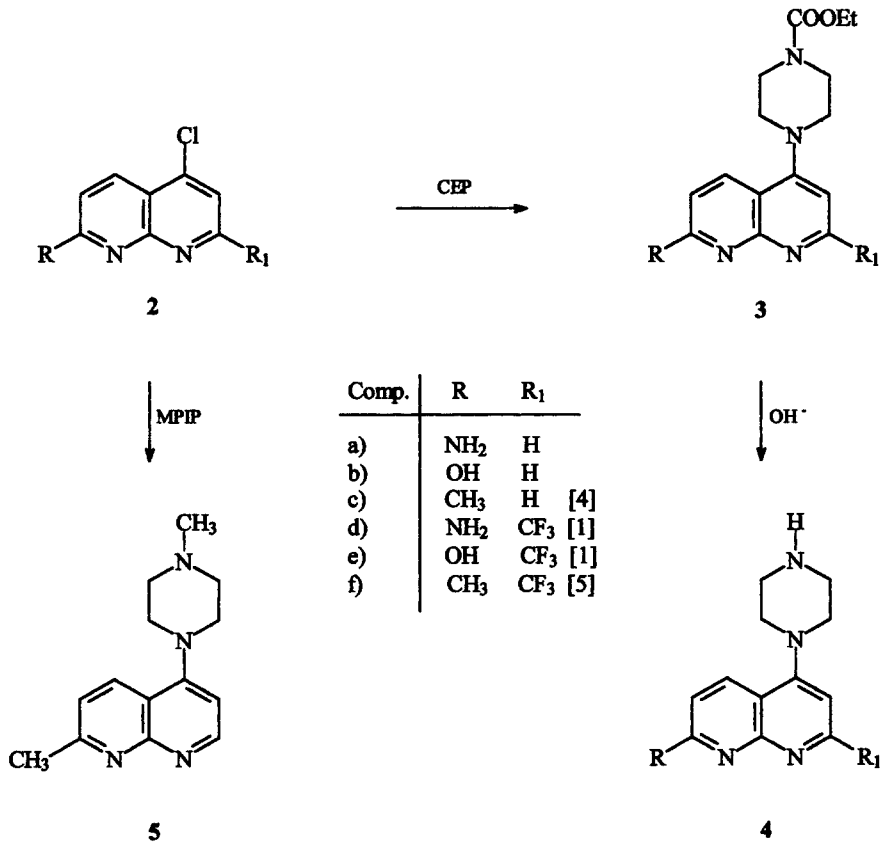


Figure 2. Synthesis route to piperazinyl derivatives **3**, **4** and **5**. Reagents: CEP, N-ethoxycarbonylpiperazine; MPIP, N-methylpiperazine.

gave the piperazine derivative **8** by alkaline hydrolysis (*figure 3, table I*).

The N-methyl derivative **9** was prepared by reaction of the hydroxyderivative **3b** with methyl iodide and then converted to the corresponding piperazino derivative **10** (*figure 4, table II*).

The analogous treatment of hydroxy compound **11** [1] gave, via **12**, the piperazino derivative **13** (*figure 5, table II*).

The assigned structures were fully confirmed by elemental analyses, IR and ^{1}H-NMR spectra. The ^{1}H-NMR spectra of **3d–f**, **4d–f**, **5**, **8**, **12** and **13** show a singlet ranging from δ 6.66–7.33 due to H_3 and two doublets ranging from δ 7.83–8.46 and δ 6.40–7.60 due to H_5 and H_6, respectively (*tables III* and *IV*).

The ^{1}H-NMR spectra of **2a–b**, **3a–c**, **4a–c**, **9** and **10** show four doublets ranging from δ 8.23–8.90, from δ 6.76–7.90, from δ 7.93–8.83 and from δ 6.50–7.65 due to H_2, H_3, H_5 and H_6, respectively. The coupling constants J_{34} of the compounds **9**, **10**, **12** and **13** are about 9.6 cps, according to that reported by us for analogous 1-alkylnaphthyridin-2-ones [6, 7] (*tables III* and *IV*).

3. Pharmacological results

Many compounds, among these newly synthesized 1,8-naphthyridines, showed an interesting antihypertensive effect: compounds **3a, 3d** and **4d** showed weak and transient antihypertensive effects, while compounds **4a, 8** and **13** determined a significant and prolonged decrease of the basal MAP (*figure 6, table V*).

Surprisingly, compounds **3b, 4b** and **5** caused an unexpected hypertensive response (*table V*).

For the determination of the possible mechanism of action of such an antihypertensive activity, the effective compound **4e** underwent a further pharmacological investigation.

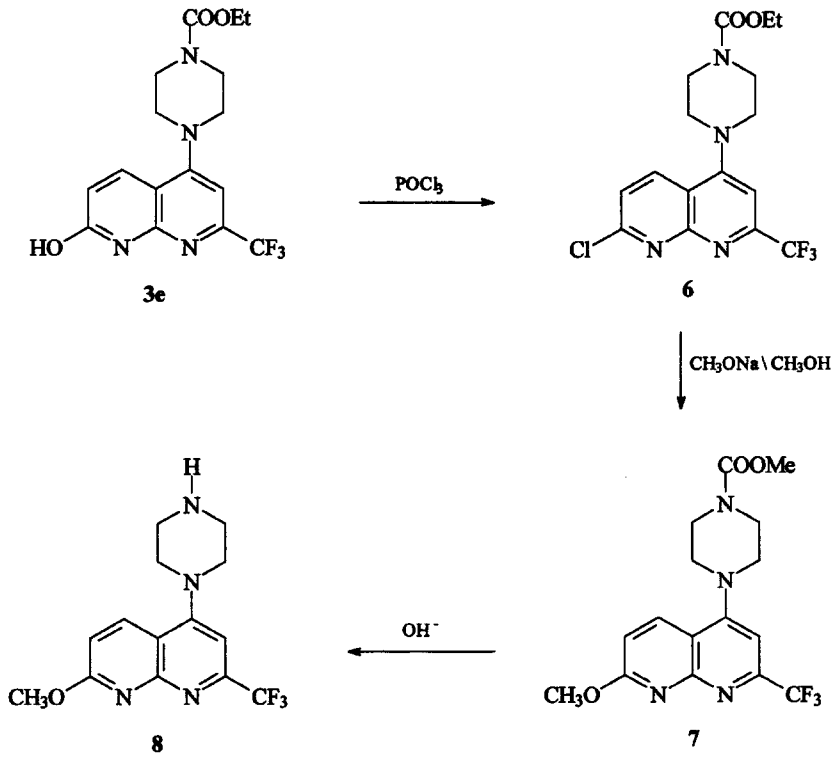


Figure 3. Synthesis of methoxy derivatives **7** and **8**.

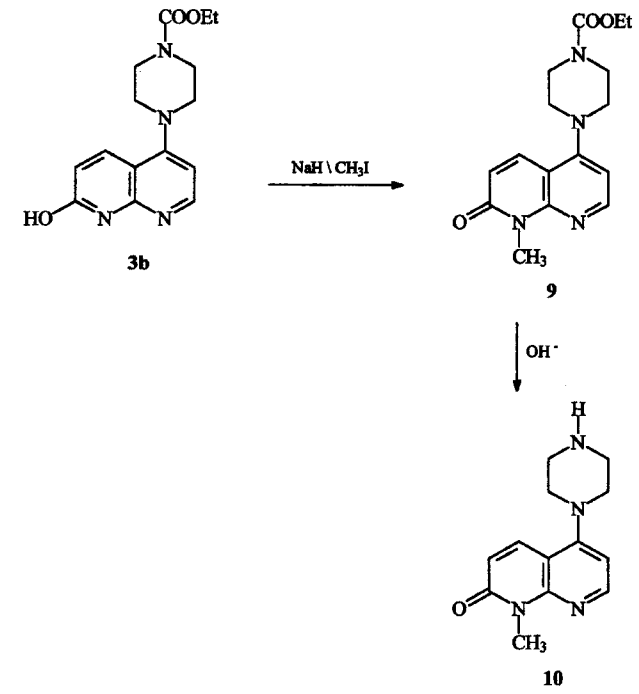


Figure 4. Synthesis of 1-methyl derivatives **9** and **10**.

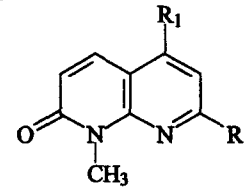
A diuretic activity was discarded, as well as a direct vasorelaxing effect in vessels precontracted by norepinephrine (NE) or KCl, excluding a mechanism of action linked to an agonism for the receptor systems of endogenous vasodilators or to different causes determining vascular smooth muscle relaxation. Compound **4e** did not show an α_2-agonist profile. A possible involvement of an antagonism for β_1 or nicotinic receptors was also excluded, as well as an ACE-inhibiting activity.

Compound **4e** determined a rightward shift of the concentration-response curve for NE, with a significant depression of the maximal effect (n = 6), showing a pharmacodynamic profile which probably can be linked to a non-competitive antagonism against the α_1 vascular adrenoceptors. Such an activity could also be observed for the compounds **13** (n = 6) and **8** (n = 5) (*figure 7, table VI*).

4. Conclusion

As shown in *table V* compounds **3b**, **4b**, **4c**, **4f**, **5**, **9**, **10** and **12** were devoid of the antihypertensive activity and

Table II. Physical data of substituted piperazinyl-1,8-naphthyridines.



Compound	R	R_1	Yield (%)	M.p. (°C)	Recryst. solvent
9	H	CEP	57	140–141	H_2O
10	H	PIP	71	120–121	Petr. ether 100–140°
12	CEP	Cl	84	178–180	*i*-PrOH
13	PIP	Cl	58	186–188	H_2O

CEP, N-ethoxycarbonylpiperazine; PIP, piperazine.

in particular, compounds **3b**, **4b**, and **5** showed hypertensive effects. Compound **3c** induced convulsive effects. Compounds **3a**, **3d** and **4a** showed a weak antihypertensive activity, whereas compounds **4a**, **4e**, **8** and **13** showed the most antihypertensive properties, since their effect was strong and prolonged in time. For this series of compounds, on the basis of the biological results obtained, no structure-activity relationship could be deduced.

Regarding the possible mechanism of action, functional studies were performed for the compound **4e**. This compound was not a direct vasodilator, because it did not relax the isolated precontracted vessels, excluding an action on vasorelaxing receptor systems or ion channels. Furthermore, it did not exhibit an ACE-inhibitor effect, β_1-blocking, α_2-stimulating activity, or ganglioplegic effects. The compounds were not only devoid of diuretic activity, they elicited a significant reduction of the

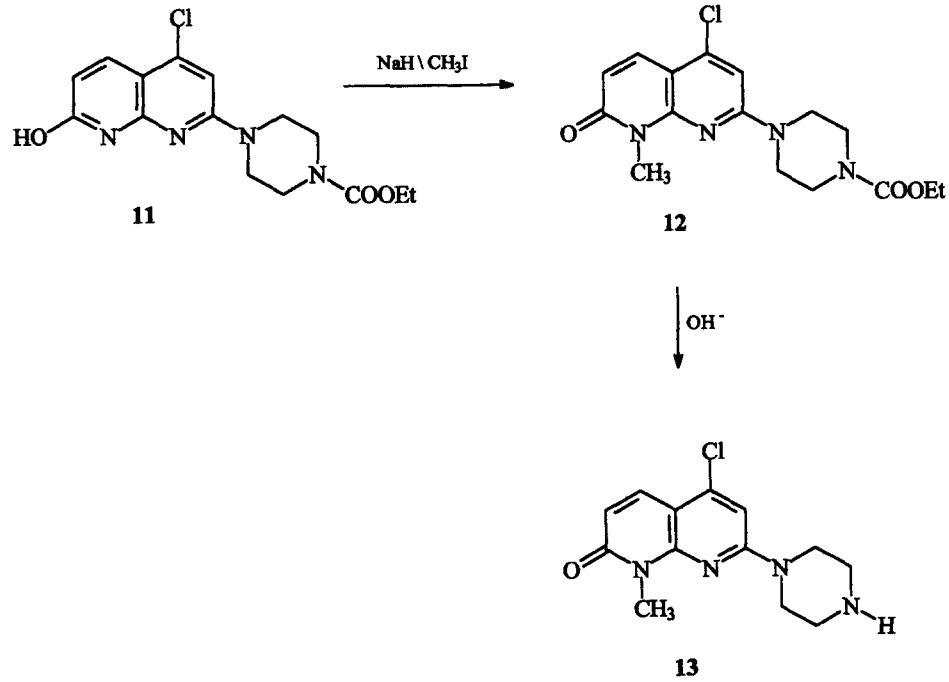
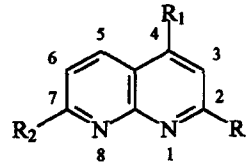


Figure 5. Synthesis of 1-methyl derivatives **12** and **13**.

Table III. ^{1}H-NMR chemical shifts (δ).



Compound	H_2(d)	H_3	H_5(d)	H_6(d)	Pip(m)[a]	Others
2a	8.90	7.90(d)	8.06	7.65		NH_2 4.97
2b	8.70	7.60(d)	8.26	6.83		OH 12.53
3a	8.86	6.80(d)	8.26	6.93	3.46–3.23	C_2H_5 1.33(t), 4.30(q); NH_2 5.76
3b	8.76	6.83(d)	8.03	6.76	3.76–3.23	C_2H_5 1.26(t), 4.26(q); OH 12.93
3c	8.23	7.30(d)	8.83	7.16	3.70–3.16	C_2H_5 1.30(t), 4.20(q); CH_3 2.76(s)
3d		7.03(s)	8.13	6.90	3.73–3.26	C_2H_5 1.33(t), 4.23(q); NH_2 5.80
3e		7.03(s)	7.86	6.76	3.73–3.23	C_2H_5 1.33(t), 4.20(q); OH 10.46
3f		7.26(s)	8.36	7.53	3.83–3.33	C_2H_5 1.36(t), 4.26(q); CH_3 2.83(s)
4a	8.56	6.81(d)	8.06	6.73	3.30	NH_2 6.86
4b	8.33	6.76(d)	7.93	6.50	3.06	OH 11.71
4c	8.86	6.86(d)	8.26	7.30	3.20	CH_3 2.76(s)
4d		7.36(s)	8.13	6.96	3.13	NH_2 7.01
4e		7.10(s)	7.93	6.60	3.23–2.93	OH 10.63
4f		7.33(s)	8.46	7.60	3.36–3.00	CH_3 2.73(s)
5	8.71	6.93(s)	8.24	7.36	3.42–3.13	CH_3 2.65(s) ; NCH_3 2.28(s)
6		7.23(s)	8.40	7.53	3.83–3.40	C_2H_5 1.33(t), 4.23(q)
7		7.13(s)	8.23	7.33	3.80–3.26	OCH_3 3.80(s) ; CH_3 4.16(s)
8		7.20(s)	8.06	6.73	3.30–3.20	OCH_3 3.83(s)

[a]Pip, piperazine.

secreted volume of urine, probably due to the lowering of the systemic blood pressure.

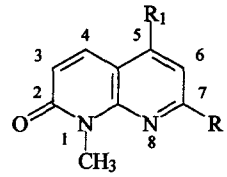
Thus, the possible mechanism of action for the compounds **4e**, **8** and **13** is probably linked to a non-competitive antagonism against α_1 vascular adrenoceptors, because of the shift of the concentration-response curve for NE, with a significant depression of the maximal effect.

5. Experimental protocols

5.1. Chemistry

All compounds were routinely checked for their structure by IR and ^{1}H-NMR spectroscopy. Melting points were determined in a Köfler hot-stage apparatus and are uncorrected. The IR spectra were measured with Perkin-

Table IV. ^{1}H-NMR chemical shifts (δ).



Compound	H_3(d)	H_4(d)	H_6	H_7(d)	Pip(m)[a]	Others
9	6.78	8.00	6.81(d)	8.60	3.76–3.16	CH_3 3.86 (s); C_2H_5 1.33 (t), 4.26 (q)
10	6.73	7.93	6.80(d)	8.50	3.16	CH_3 3.83 (s); NH 2.60
12	6.40	7.83	6.96(s)		3.73–3.43	CH_3 3.50 (s); C_2H_5 1.20 (t), 4.10 (q)
13	6.60	7.96	6.66(s)		3.76–3.03	CH_3 3.76 (s)

[a]Pip, piperazine.

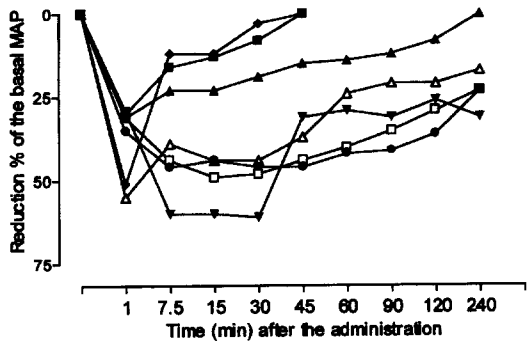


Figure 6. Decrease of MAP (expressed as % of the basal MAP), following the administration of the compounds **3a** (■), **3d** (▲), **4a** (▼), **4d** (◆), **4e** (●), **8** (□) and **13** (△). All the points represent the mean value of four experiments; all the values of SEM (not drawn for clarity) are < 10%.

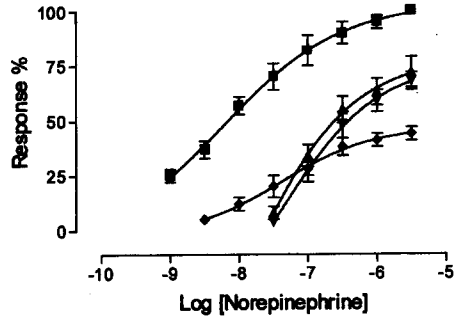


Figure 7. Concentration-response curves for norepinephrine in control conditions (■) and in the presence of compounds **4e** (3 μM, ▲), **8** (3 μM, ▼) and **13** (3 μM, ◆). The points represent the mean value of four to six experiments. The vertical bars indicate the SEM.

Table V. Responses (expressed as variation % of the basal MAP) versus time (min), following the administration of the compounds (30 mg/kg i.p.). The values represent the mean (n = 4/group); all the SEM values (not shown for clarity) are < 10 %.

Compound	Basal MAP	Variation % of MAP vs. time									
		1	7.5	15	30	45	60	90	120	240	notes
3a	155	–30	–16	–13	–8	0	0	0	0	0	
3b	147	+15	+22	0	0	+12	+9	+5	0	0	
3c	167										[a]
3d	130	–31	–23	–23	–19	–15	–14	–12	–8	0	
4a	140	–29	–60	–60	–61	–31	–29	–31	–26	–31	[b]
4b	164	+16	–9	+10	+10	0	0	0	0	0	
4c	171										[c]
4d	162	–51	–12	–12	–3	0	0	0	0	0	[b]
4e	145	–35	–46	–44	–46	–46	–42	–41	–36	–23	
4f	169										[c]
5	140	+21	+14	+22	+28	+27	+16	+16	+16	+10	
8	184	–31	–44	–49	–48	–44	–40	–35	–29	–23	
9	142										[c]
10	158										[c]
12	175										[c]
13	160	–55	–39	–44	–44	–37	–24	–21	–21	–17	

Notes: [a] the compound induced convulsive effects and the animals were immediately killed by an overdose of sodium penthobarbital; [b] immediately after the administration, the compound determined a marked and transient hypertensive peak; [c] no effect has been observed.

Table VI. Maximal responses to NE, obtained in the presence of the three reference concentrations of the compounds under test and expressed as % (mean ± SEM) of the maximal response to NE obtained in control conditions.

Tested compound	Maximal responses to NE vs. conc. of the tested compounds		
	0.3 μM	1 μM	3 μM
4e	100 ± 0	84 ± 3	72 ± 7
8	97 ± 2	78 ± 5	68 ± 3
13	100 ± 0	81 ± 7	44 ± 3

Elmer Infracord Model 1310. The ^{1}H-NMR spectra were determined in DMSO-d_6 or deuteriochloroform with TMS as the internal standard, on a Varian EM 360A 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 analyses of all synthesized compounds for C, H and N were within ± 0.4% of the theoretical values and were performed by our analytical laboratory.

5.1.1. 7-Amino-5-chloro-1,8-naphthyridine **2a**

A solution of 5.0 mmol acetamido-1,8-naphthyridine derivatives [1], in 20 mL of 10% sulfuric acid was refluxed for 2 h and then, after cooling, the pH was adjusted to 9 with concentrated ammonium hydroxide. The solid was separated by filtration and washed with water to give **2a**.

5.1.2. 2-Hydroxy-5-chloro-1,8-naphthyridine **2b**

To a cooled solution (0 °C) of 2.0 mmol of amino-1,8-naphthyridine derivative **2a** in 10 mL of concentrated sulfuric acid, was added 5.0 mmol of sodium nitrite portionwise. After standing for 4 h at room temperature the mixture was poured into crushed ice and made basic (pH 8) with concentrated ammonium hydroxide. The compound **2b** was collected by filtration and washed with water.

5.1.3. General procedure for the preparation of (4-ethoxycarbonylpiperazin-1-yl)-1,8-naphthyridine derivatives **3a–f**

A mixture of 1.0 mmol of **2** and 1.2 mmol of N-ethoxycarbonylpiperazine was heated for 24 h at 140 °C in a sealed tube. After cooling, the mixture obtained was treated with water, the solid compounds **3a, 3b** and **3d–f** were collected by filtration and washed with water. For the compound **3c** the mixture, after treatment with water, was extracted with chloroform. The combined extracts were washed with water, dried (magnesium sulfate) and evaporated to dryness in vacuo, and the crude residue was purified by flash chromatography (eluent: petroleum ether/ethyl acetate/diethylamine (3:8:1)).

5.1.4. General procedure for the preparation of piperazin-1-yl-1,8-naphthyridine derivatives **4a–f, 8, 10** *and* **13**

A suspension of 1.0 mmol of suitable 4-ethoxycarbonylpiperazinyl derivative **3**, 15 mL of ethanol and 15 mL of 10% aqueous sodium hydroxide was refluxed for 4 h and the organic solvent was evaporated in vacuo. The products were then obtained by the following methods. In the case of **4a, 4c, 4d, 8** and **10** the pH of the aqueous solution was adjusted to 8 and the mixture extracted with chloroform. For the other compounds **4b, 4e, 4f**, and **13** the solution was extracted with chloroform. The combined extracts were washed with water, dried (magnesium sulfate) and evaporated to dryness in vacuo to obtain the target compounds.

5.1.5. 4-(4-methylpiperazin-1-yl)-2-methyl-1,8-naphthyridine **5**

A mixture of 1.0 mmol of **2c** and 3.0 mmol of N-methylpiperazine was heated for 12 h at 140 °C in a sealed tube. After cooling, the mixture obtained after treatment with water, was extracted with chloroform. The combined extracts were washed with water, dried (magnesium sulfate) and evaporated to dryness in vacuo, and the crude residue was purified by flash chromatography (eluent: petroleum ether/ethyl acetate/diethylamine (1:2:1)) to give **5**.

5.1.6. 7-Chloro-4-(4-ethoxycarbonylpiperazin-1-yl)-2-trifluoromethyl-1,8-naphthyridine **6**

A mixture of 10 mL of phosphoryl chloride and 5.0 mmol of hydroxy-1,8-naphthyridine **3e** was heated for 120 min at 80 °C. After cooling, the solution was poured into crushed ice and treated with concentrated ammonium hydroxide until pH 7. The solid compound **6** was then collected and washed with water.

5.1.7. 7-Methoxy-4-(4-methoxycarbonylpiperazin-1-yl)-2-trifluoromethyl-1,8-naphthyridine **7**

To a solution of 50 mL of absolute methanol, in which 10 mmol of sodium metal were dissolved, 1.0 mmol of **6** was added and the mixture was refluxed for 48 h. The methanol was evaporated to dryness in vacuo and water was added. Compound **7** was then collected and washed with water.

5.1.8. General procedure for the preparation of substituted 1-methyl-1,8-naphthyridine-2-ones **9** *and* **12**

To a solution of 1.0 mmol of **3b** or **11** and 10 mL of DMF, in a stream of nitrogen, was added 1.2 mmol of sodium hydride and the mixture was heated for 40 min at 60 °C. After the addition of a solution of 4 mL of methyl iodide in 4 mL of DMF, the mixture was heated for 12 h at 60 °C. The DMF was evaporated to dryness in vacuo and water was added. Compounds **9** or **12** were then collected and washed with water.

5.2. Pharmacological methods

All the procedures on experimental animals were performed following the guidelines of the European Community Council directive 86-609.

5.2.1. Evaluation of the antihypertensive activity

Adult male spontaneously hypertensive Wistar Kyoto rats (SHR) (250–300 g) were anaesthetized with diethyl ether and implanted both with a carotid arterial catheter for blood pressure recording and with a jugular venous catheter for the administration of drugs, by a cut in the antero-medial region of the neck. Subcutaneously, the catheters were exteriorized at the back of the neck and protected by spring wires. The arterial catheter was fixed to a pressure transducer (Bentley-Trantec Basile mod. 800), which was connected to a 2-channel pressure recorder (Basile mod. Gemini 7070).

After awakening, the animals were housed individually with water and food ad libitum. Small volumes of heparin solution (20 U.I./mL, in physiological saline) were injected in the arterial catheter at 30 min intervals, to avoid possible blood coagulation. 3–4 h after the surgical protocol, the tested compounds were dissolved in the vehicle (physiological saline, tween 80 10% and dimethylsulfoxide 2%) and were administered in bolus (30 mg/kg i.p.), in a volume of 0.5 mL to the conscious animals. The recording was performed for at least 4 h. Preliminary experiments showed that the administration of 0.5 mL of the vehicle did not determine any response. An overdose i.v. of sodium penthobarbital was used to kill the animals at the end of the experiments, or to kill the animals showing convulsive effects after the administration of the tested compounds.

5.2.2. Determination of the mechanism of action

The representative antihypertensive compound **4e** was investigated to identify a possible diuretic effect, vasodilator activity, ACE-inhibitor action, α_2-agonism, β_1-antagonism or a nicotinic antagonism, by means of the pharmacological procedures previously described [1].

A possible α_1-antagonist activity was also evaluated for the compounds **4e**, **8** and **13**, by the following protocol.

The compounds under test were dissolved (1 mM) in ethanol (96%) and further diluted in bi-distilled water.

Male normotensive Wistar rats (250–350 g) were killed by cervical dislocation, under light diethyl ether anaesthesia, and bled. The thoracic aorta was immediately excised, freed of extraneous tissues and of the endothelial layer and prepared as multiple-ring preparations [8].

Then the vessel was suspended, under a preload of 2 g, in a 10 mL organ bath containing Tyrode saline (composition in mM: NaCl 136.8, KCl 2.95, $CaCl_2$ 1.80, $MgSO_4.7H_2O$ 1.05, NaH_2PO_4 0.41, $NaHCO_3$ 11.9, glucose 5.50), thermostated at 37 °C and continuously bubbled with a mixture of O_2 (95%) and CO_2 (5%).

Changes in tension were recorded by an isometric transducer (Basile mod. 7005), connected with a unirecord microdynamometer (Basile mod. 7050).

After an equilibration time (1 h, wash-out at 15 min intervals), a control concentration-response curve (CRC) for NE was obtained cumulatively (0.1 nM–1 μM). Then, three further CRCs for NE were obtained in the presence of increasing reference concentrations of the compounds under test (0.3, 1 and 3 μM). Before each CRC, the vessels underwent an equilibration time (1 h, wash-out at 15 min intervals). In preliminary experiments, no significant difference could be observed for the four CRCs, obtained in the absence of any compound.

5.2.3. Data analysis

The blood pressure parameters were recorded as systolic (SP) and diastolic (DP) pressure, and were expressed as mean arterial pressure (MAP), calculated as MAP = DP + 1/3 (SP – DP). The antihypertensive activity was shown as decrease % relative to the basal MAP.

The in vitro responses to NE, both in the absence and in the presence of the tested compounds, was evaluated as % of the maximal response obtained in control conditions.

The significance of differences was evaluated by Anova and two-tailed Student's t test. P values < 0.05 were considered statistically significant.

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