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Short communication

Synthesis and evaluation of antihypertensive activity of 1,8-naphthyridine derivatives. Part X

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Abstract – A series of 4-(*N*-methylencycloalkylamino)-1,8-naphthyridine derivatives variously substituted in positions 2 and 7 were synthesized and pharmacologically investigated for possible antihypertensive activity. These compounds were tested to determine a possible vasodilator mechanism of action. Compounds **22**, **23**, **27–29**, **47** and **48** showed satisfactory levels of potency ($pIC_{50}>5$), which in one case (compound **23**) reached a really interesting value (pIC_{50} 6.92). Furthermore, for some selected compounds (**19**, **22**, **23**, **26**, **28**, **29**, **47**), the vasorelaxing activity was also evaluated in the presence of the guanylate cyclase blocker ODQ or of the adenylate cyclase blocker SQ 22536, and some of these can be considered as possible guanylate–cyclase inhibitors. Finally, compounds **19**, **22** and **23** were also tested in the presence of the ATP-sensitive potassium channel blocker glybenclamide and seem to possess activating properties on these potassium channels. © 2001 Éditions scientifiques et médicales Elsevier SAS

antihypertensive / vasodilator / 1,8-naphthyridine / piperidinyl / piperazinyl / *N*-ethoxycarbonylpiperazinyl

1. Introduction

Research in the field of hypertension has continued to grow in recent years because hypertension is a major cardiovascular disease. A considerable number of bi- and tricyclic molecules incorporating piperazine units and exhibiting an interesting antihypertensive activity have been synthesized during the course of the last few years [1–5].

Previously, we have reported the synthesis and the antihypertensive activity of several 2- and 4-piperazinyl-1,8-naphthyridine derivatives, variously substituted. Some of them exhibited a significant, prolonged decrease in the mean arterial pressure (MAP) in spontaneously hypertensive rats (SHR) [6–8].

In the light of the interesting properties shown by this class of compounds, we decided to continue our research directed toward the study of a new series of 1,8-naphthyridine derivatives. With the aim of obtaining further information about the antihypertensive activity of this type of compounds, we focused our research on a series of 1,8-naphthyridine derivatives with the following characteristics: (i) presence of a *N*-methylencycloalkylamino group as a substituent in position 4; (ii) different groups in positions 2 and 7.

2. Chemistry

The *N*-methylencycloalkylamino derivatives **4–13** were prepared starting from compound **1** [9] as described in *figure 1*. Compound **1** [9] was converted with a good yield to the acetamido derivative **2**, which, by treatment with phosphoryl chloride, gave the 2-chloro derivative **3**. Compound **3** was allowed to react with appropriate cycloalkylamines in toluene in a sealed tube to give derivatives **4** and **5**, which

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were converted to the corresponding acetamido derivatives **6** and **7**. In the same manner, compounds **8–10** and **11–13** were prepared from **1** (*figure 1, tables I and II*). Compounds **14** and **15** were obtained by chlorination of compounds **11** and **12** respectively, and then converted to their cycloalkylamino derivatives **16** and **17** (*figure 2, tables I and II*).

The treatment of **9** with phosphorous pentasulfide in anhydrous pyridine afforded the mercapto derivative **18** which, by reaction with Raney Nickel gave compound **19**. Compound **9** was converted by diazotization in concentrated sulfuric acid to the dihydroxynaphthyridine **20**, which by treatment with phosphoryl chloride gave compound **21**. The dimethoxy derivative **22** was prepared by reaction of **21** with sodium methoxide in methanol (*figure 3, tables I, II and III*). Dehalogenation of the 2-chloro derivative **15** was performed in glacial acetic acid, in the presence of Pd–C as a catalyst, to give compound **23** (*figure 4, tables I, II and III*). The treatment of compound **15** with diluted sulfuric acid or sodium methoxide in methanol gave the amino derivative **24** or the methoxy derivative **25** respectively (*figure 4, tables I and II*). Diazotization of **4** and **17** gave a mixture of the hydroxynaphthyridines **26** and **27** and hydroxynitronaphthyridines **28** and **29**, as reported in a previous paper [10]. By treatment of **26** and **27** with phosphoryl chloride the chloro derivatives **30** and **31** were obtained, which, by reaction with sodium methoxide, were converted to compounds **32** and **33** (*figure 5, tables I, II and III*). Diazotization of **5** and **16** gave the hydroxynaphthyridines **34** and **35** respectively. By treatment of compounds **34** and **35** with phosphoryl chloride, the chloro derivatives **36** and **37** were obtained, which, by reaction with sodium methoxide, were converted to compounds **38** and **39** (*figure 6, tables I and II*). The piperazinylnaphthyridines **40–43** and **45–52** were obtained by alkaline hydrolysis of the corresponding carbethoxypiperazinyl derivatives **5**, **9**, **16**, **17**, **22**, **25**, **27**, **33–35**, **38** and **39**. The hydrolysis of compounds **19** and **23** gave the compound **44** (*figure 7, tables I and II*).

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

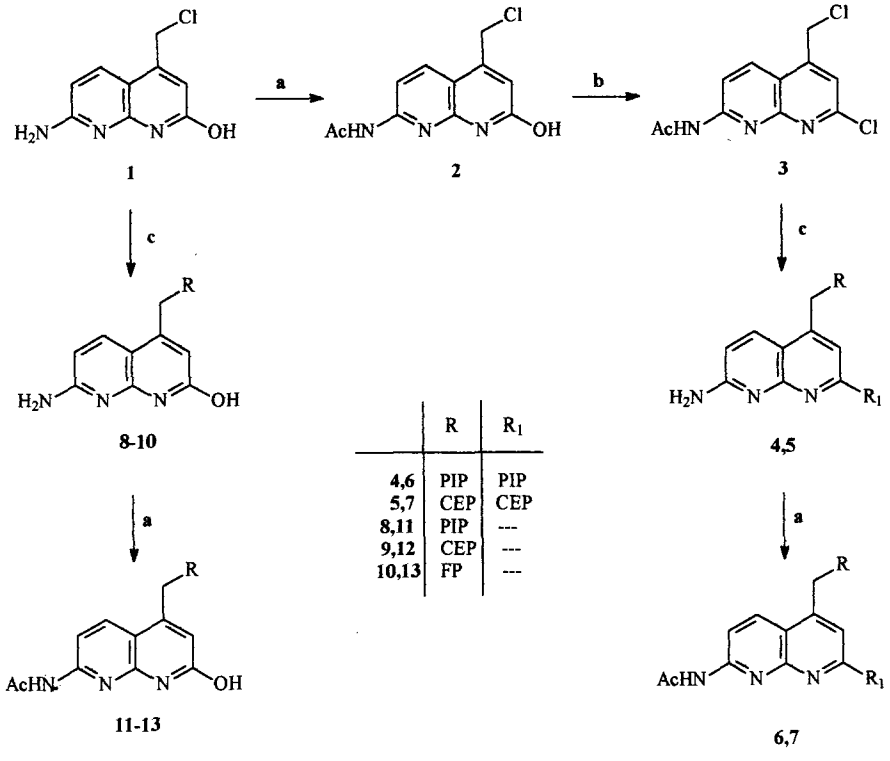
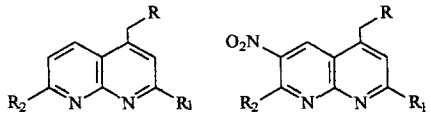


Figure 1. Reagents: (a) Ac_2O; (b) $POCl_3$; (c) *N*-cycloalkylamine. Cep = *N*-ethoxycarbonylpiperazin-1-yl. Pip, piperidin-1-yl. FP, *N*-formylpiperazin-1-yl.

Table I. Physical data of 1,8-naphthyridine derivatives.



2-27, 30-52 28, 29

Compound	R	R_1	R_2	Yield (%)	m.p. (°C)	Recrystallization solvent
2	Cl	OH	NHAc	75	>320	DMSO
3	Cl	Cl	NHAc	55	210–212	DMSO
4	PIP	PIP	NH_2	88	215–217	toluene
5	CEP	CEP	NH_2	43	191–193	toluene
6	PIP	PIP	NHAc	77	315–317	toluene
7	CEP	CEP	NHAc	25	195–196	toluene
8	PIP	OH	NH_2	90	310 dec.	DMSO
9	CEP	OH	NH_2	57	>320	DMSO
10	FP	OH	NH_2	65	>320	DMSO–H_2O
11	PIP	OH	NHAc	82	304–306	DMSO
12	CEP	OH	NHAc	85	>320	DMSO
13	FP	OH	NHAc	86	>320	DMSO
14	PIP	Cl	NHAc	65	231–233	EtOH
15	CEP	Cl	NHAc	83	210–212	EtOH–H_2O
16	PIP	CEP	NH_2	59	174–176	toluene
17	CEP	PIP	NH_2	93	201–203	toluene
18	CEP	SH	NH_2	90	264–265	EtOH
19	CEP	H	NH_2	65	>320	toluene
20	CEP	OH	OH	95	220–222	DMSO–H_2O
21	CEP	Cl	Cl	84	180–181	DMSO–H_2O
22	CEP	OCH_3	OCH_3	78	109–110	petroleum ether 100–140 °C
23	CEP	H	NHAc	72	224–227	2-Propanol
24	CEP	Cl	NH_2	41	230–231	AcOEt
25	CEP	OCH_3	NH_2	72	235–237	toluene
26	PIP	PIP	OH	23	178–180	toluene
27	CEP	PIP	OH	43	205–208	Toluene
28	PIP	PIP	OH	13	233 dec.	toluene
29	CEP	PIP	OH	19	217–219	toluene
30	PIP	PIP	Cl	53	158–160	EtOH–H_2O
31	CEP	PIP	Cl	94	185–187	petroleum ether 100–140 °C
32	PIP	PIP	OCH_3	79	140–141	petroleum ether 100–140 °C
33	CEP	PIP	OCH_3	85	137–139	petroleum ether 100–140 °C
34	CEP	CEP	OH	51	118–120	toluene
35	PIP	CEP	OH	52	93–95	toluene
36	CEP	CEP	Cl	96	102–104	EtOH
37	PIP	CEP	Cl	83	99–101	petroleum ether 100–140 °C
38	CEP	CEP	OCH_3	89	82–84	petroleum ether 100–140 °C
39	PIP	CEP	OCH_3	88	111–113	petroleum ether 100–140 °C
40	PIPZ	PIPZ	NH_2	41	152–154	EtOH
41	PIPZ	OH	NH_2	90	225 dec.	toluene
42	PIP	PIPZ	NH_2	89	212–214	toluene
43	PIPZ	PIP	NH_2	88	218–220	Toluene
44	PIPZ	H	NH_2	93	65–66	petroleum ether 100–140 °C
45	PIPZ	OCH_3	OCH_3	93	65–66	petroleum ether 100–140 °C
46	PIPZ	OCH_3	NH_2	78	78–80	petroleum ether 100–140 °C
47	PIPZ	PIP	OH	61	125–128	toluene
48	PIPZ	PIP	OCH_3	85	137–139	petroleum ether 100–140 °C
49	PIPZ	PIPZ	OH	44	212–214	toluene
50	PIP	PIPZ	OH	84	193–196	toluene
51	PIPZ	PIPZ	OCH_3	96	70–72	petroleum ether 100–140 °C
52	PIP	PIPZ	OCH_3	83	139–141	petroleum ether 100–140 °C

FP, *N*-formylpiperazinyl; CEP, *N*-ethoxycarbonylpiperazinyl; PIP, piperidinyl; PIPZ, piperazinyl.

The ^{1}H-NMR spectra of following compounds were determined in DMSO-d_6.

Table II. Parameters of vasorelaxing potency, expressed as pIC_{50}, and efficacy, expressed as E_{max} (–, uneffective).

Compound	pIC_{50}	E_{max}
4	–	–
5	4.44 ± 0.02	96 ± 2
6	–	–
7	3.02 ± 0.06	94 ± 3
8	–	–
9	3.13 ± 0.11	99 ± 3
10	3.59 ± 0.08	89 ± 6
11	–	–
12	4.03 ± 0.05	100
13	not tested	not tested
14	–	–
15	3.73 ± 0.03	100
16	4.60 ± 0.03	100
17	not tested	not tested
18	3.78 ± 0.07	92 ± 3
19	4.68 ± 0.09	100
20	3.53 ± 0.02	100
21	3.10 ± 0.03	96 ± 1
22	5.05 ± 0.02	100
23	6.92 ± 0.02	99 ± 2
24	–	–
25	not tested	not tested
26	4.52 ± 0.04	100
27	5.29 ± 0.02	100
28	5.45 ± 0.10	93 ± 6
29	5.51 ± 0.05	100
30	–	–
31	–	–
32	–	–
33	4.34 ± 0.03	100
34	–	–
35	not tested	not tested
36	4.67 ± 0.09	94 ± 9
37	not tested	not tested
38	–	–
39	not tested	not tested
40	not tested	not tested
41	–	–
42	–	–
43	4.95 ± 0.07	100
44	4.68 ± 0.09	100
45	3.75 ± 0.08	99 ± 1
46	4.15 ± 0.01	99 ± 1
47	5.84 ± 0.04	100
48	5.19 ± 0.02	100
49	–	–
50	not tested	not tested
51	–	–
52	not tested	not tested

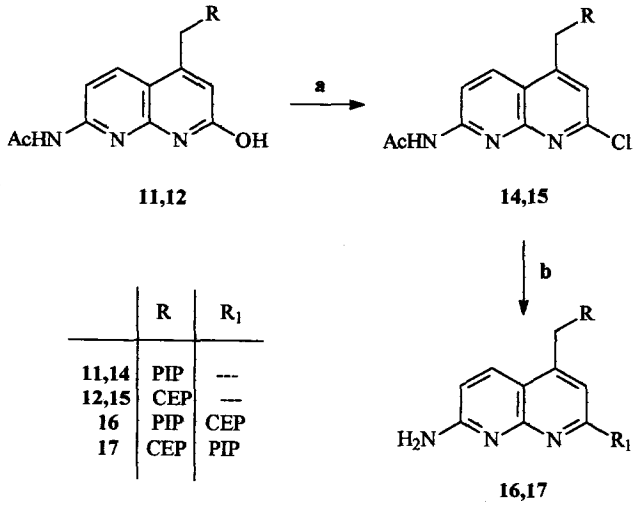


Figure 2. Reagents: (a) $POCl_3$; (b) *N*-cycloalkylamine. Cep, *N*-ethoxycarbonylpiperazin-1-yl. Pip, piperidin-1-yl.

The ^{1}H-NMR spectrum of **42** is reported, as an example of ^{1}H-NMR of the final compounds. This spectrum shows the following signals: δ 7.99 (d, H_5, 1H), 6.70 (s, H_3, 1H), 6.44 (d, H_6, 1H), 6.26 (brs, NH_2, 2H), 3.59 (s, Ar–CH_2–, 2H), 3.57 and 2.87 (m, piperazin-1-yl, 8H), 2.33 (m, $N(CH_2)_2$, 4H), 1.55 (m, $-(CH_2)_3-$, 6H).

In particular the ^{1}H-NMR spectra of acetamido derivatives **2**, **3**, **6**, **7**, **11**–**15** and **23** exhibit the signals of H_5 and H_6 at a lower field due to the deshielding effect of the acetamido group; see e.g. for **15** 8.86 (d, H_5, 1H), 8.46 (d, H_6, 1H). Compounds **28** and **29**, with a nitro group in position 6, instead of the two doublets due to H_5 and H_6 show a singlet at a lower field, δ 9.10 (s, H_5, 1H) and 9.04 (s, H_5, 1H) respectively.

Compound **44** shows two doublets at about δ 8.71 (d, H_7, 1H) and 7.23 (d, H_6, 1H); compounds **19** and **23** exhibit analogous ^{1}H-NMR spectra.

3. Results and discussion

Among the tested compounds, **4**, **6**, **8**, **11**, **14**, **24**, **30**–**32**, **34**, **38**, **41**, **42**, **49** and **51** were devoid of any significant activity, and proved to be almost ineffective to induce a vasorelaxing response. Weak values of potency (pIC_{50} 3–4) were shown by compounds **7**, **9**, **10**, **15**, **18**, **20**, **21** and **45**; while compounds **5**, **12**, **16**, **19**, **26**, **33**, **36**, **43**, **44** and **46** exhibited more significant parameters of potency (pIC_{50} 4–5). Only

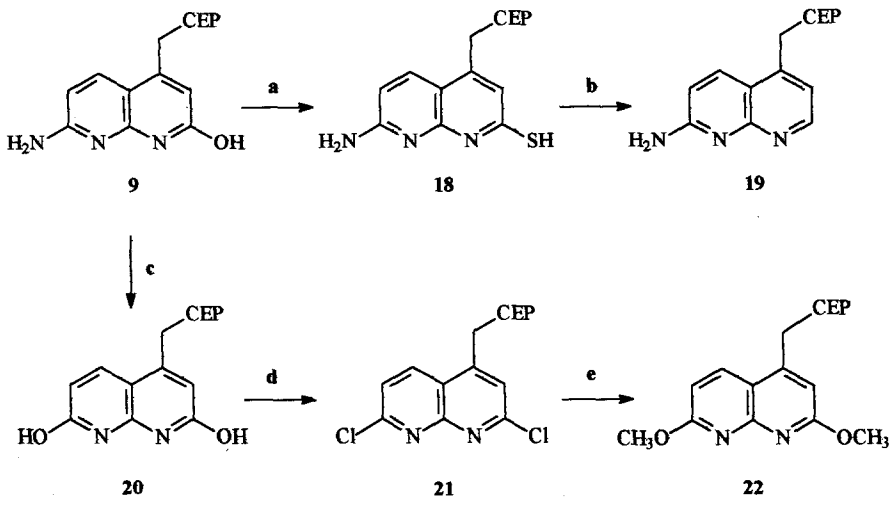


Figure 3. Reagents: (a) P_2S_5; (b) Ni-Raney, EtOH; (c) $NaNO_2$, H_2SO_4; (d) $POCl_3$; (e) MeONa, MeOH. Cep, *N*-ethoxycarbonylpiperazin-1-yl.

compounds **22**, **23**, **27–29**, **47** and **48** showed satisfactory levels of potency ($pIC_{50}>5$), which in one case (compound **23**) reached a really interesting value (results are summarised in *table II*). Besides the wide dispersion of the parameters of potency recorded for the effective vasorelaxing molecules (ranging from the highest value of pIC_{50} of 6.92 to the lowest ones, which were around three), the parameters of efficacy were always very high. Indeed, all the effective compounds showed a profile of full vasodilators, determining an almost complete abolition of the contractile tone induced by KCl.

The wide number of structural variables, linked to several molecular regions, does not allow an exhaustive and coherent explanation of the structure–activity relationships, although some general and preliminary considerations can be indicated. As regards the substituent binding to position 4, through a methylene bridge, the presence of a piperidinyl group seems to determine a negative influence, while a carboxyethylpiperazinyl group or a piperazinyl group appear to be neutral factors, because these groups have a substantially equivalent distribution in all the classes of effectiveness.

The presence of a methoxyl group or of an hydrogen atom in position 2 seems to be well tolerated, while an increase in steric hindrance, with the introduction of a carboxyethylpiperazine or a piperidine, leads to more contradictory results. The piperazinyl group clearly determines a negative influence, as does the presence of the polar groups OH and SH and of the halogen Cl.

A very different consideration may be made as regards the behaviour of the substituent in position 7, where an OH group presents the best profile. The presence of a methoxyl or of an aminic group is substantially indifferent, while the acetamido substituent, and more clearly the Cl atom, can be viewed as negative factors; surprisingly, it has to be pointed out that the most active compound **23** possesses an acetamido group in position 7.

Because of the analogy between the tested compounds and previously synthesised 1,8-naphthyridine derivatives which showed inhibitory effects against

Table III. Values of efficacy and potency, recorded in the presence of ODQ or glybenclamide.

Compound	pIC_{50}	E_{max}
+ODQ 1 μM		
26	3.79 ± 0.03	84 ± 8
28	4.91 ± 0.03	81 ± 5
29	3.87 ± 0.05	91 ± 3
47	5.84 ± 0.04	86 ± 3
+Glybenclamide 1 μM		
19	3.94 ± 0.04	91 ± 5
23	6.37 ± 0.06	100

The relative control values are reported in *table II*. ODQ was unable to antagonize compounds **19**, **22** and **23**, while glybenclamide was unable to antagonize compound **22**.

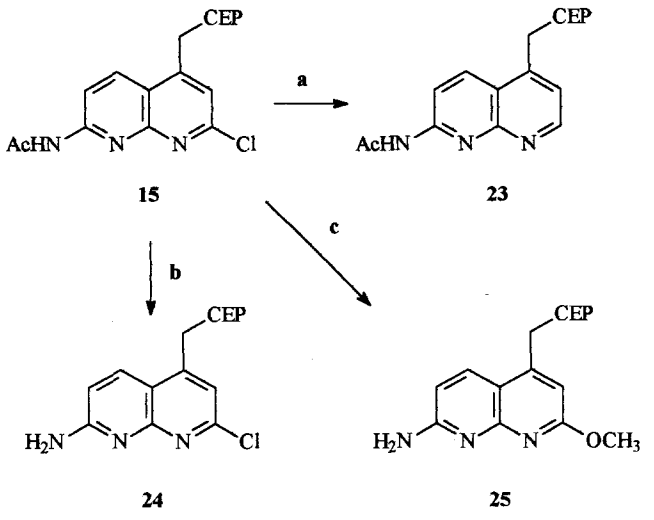


Figure 4. Reagents: (a) H_2/Pd–C 10%; (b) H_2SO_4 10% (c) MeONa, MeOH Cep, *N*-ethoxycarbonylpiperazin-1-yl.

platelet aggregation, due to a possible increase in cyclic nucleotides [11], it seemed of interest to evaluate a possible link between the vasorelaxing properties and the activation of the guanylate cyclase and/or of the adenylate–cyclase systems. Therefore, some of the most potent and effective compounds (**19**, **22**, **23**, **26**, **28**, **29** and **47**) were also tested as vasodilators, after the administration of the guanylate–cyclase inhibitor ODQ or of the adenylate–cyclase inhibitor SQ 22536.

SQ 22536 did not produce any significant inhibition of the responses evoked by the 1,8-naphthyridines in any of the tests, excluding a mechanism of action due to an increase in the levels of cyclic AMP, through adenylate–cyclase activation.

On the contrary, ODQ determined a significant decrease in the responses induced by compounds **26**, **28**, **29** and **47** (*table III*), which therefore can be considered as possible guanylate–cyclase inhibitors.

ODQ did not produce any effect on the responses of compounds **19**, **22** and **23**.

The above observations allowed us to advance a preliminary hypothesis about a possible structure-activity relationship, as all the 'ODQ-sensitive' compounds, but not the 'ODQ-insensitive' ones, have a hydroxylic substituent in position 7 of the 1,8-naphthyridine nucleus. This structural characteristic is probably a fundamental requirement for the guanylate–cyclase activity of this class of molecules.

As regards the mechanism of action of compounds **19**, **22**, and **23**, casual experimental observation on the pharmacodynamic profile of **23**, widely investigated because of its high pIC_{50}, showed that its potency and its efficacy were dramatically reduced, when the compound was tested on aortic rings contracted by KCl 40 mM, i.e. by a level of membrane depolarization higher than that used in the standard protocol (KCl 20 mM) (data not shown). This behaviour was defined as a clear indication of vasorelaxing drugs acting through membrane hyperpolarization, such as potassium channel activators [12, 13]. In agreement with the above hypothesis, compounds **19**, **22**, and **23** were tested in the presence of the sulphanylurea gly-

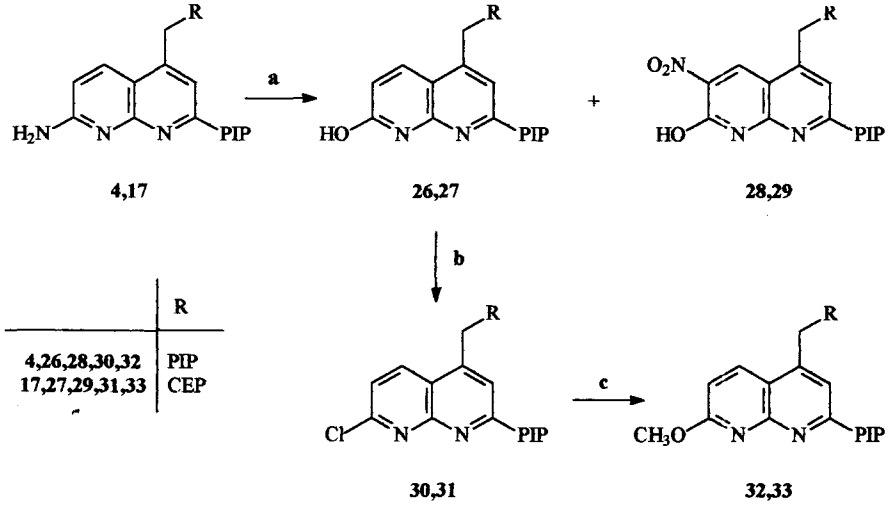


Figure 5. Reagents: (a) $NaNO_2$, H_2SO_4; (b) $POCl_3$; (c) MeONa, MeOH. Cep, *N*-ethoxycarbonylpiperazin-1-yl. Pip, piperidin-1-yl.

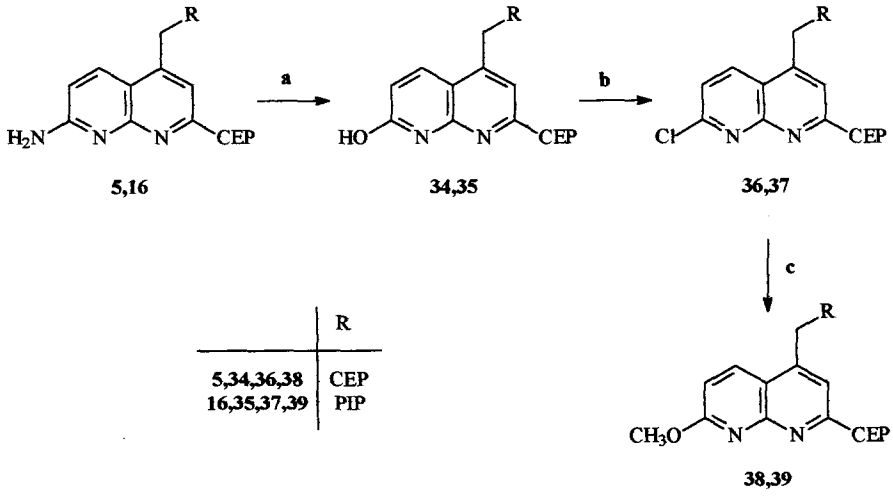


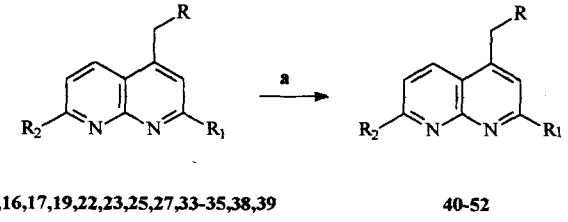
Figure 6. Reagents: (a) $NaNO_2$, H_2SO_4; (b) $POCl_3$; (c) MeONa, MeOH. Cep, *N*-ethoxycarbonylpiperazin-1-yl. Pip, piperidin-1-yl.

benclamide (Gly), a well-known antagonist of the ATP-sensitive potassium channel [14]. In these experimental conditions, the concentration–response curves for compounds **19** and **23**, but not for **22** (whose mechanism of action remain unknown), underwent a significant parallel rightward shift (*table III*). Therefore, compounds **19** and **23**, both binding a nitrogen atom to position 7 of the 1,8-naphthyridine heterocycle (NH_2 and NHAc, respectively) can be viewed as original moieties possessing activating properties on the ATP-sensitive potassium channel. On the one hand, this difference in the mechanism of action may justify the surprising observations about the structure–activity relationships exhibited by compound **23** (i.e. a high potency, which seems to be inconsistent with the presence of the acetamido group, indicated as a negative factor by a preliminary classification). On the other hand, this unexpected pharmacodynamic behaviour may represent a new field of research, in order to recognise original structure–activity relationships, linking the 1,8-naphthyridine moiety to a potassium channel–opener activity.

4. Experimental protocols

4.1. Chemistry

All compounds were routinely checked for their structure by IR and ^{1}H-NMR spectroscopy. Melting points were determined on a Köfler hot-stage apparatus and are uncorrected. The IR spectra were measured with a Genesis Series FTIR ATI Mattson. 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 precoated silica-gel glass plates (60 F-254) and



	R	R_1	R_2
5	CEP	CEP	NH_2
9	CEP	OH	NH_2
16	PIP	CEP	NH_2
17	CEP	PIP	NH_2
19	CEP	H	NH_2
22	CEP	OCH_3	OCH_3
23	CEP	H	NHAc
25	CEP	OCH_3	NH_2
27	CEP	PIP	OH
33	CEP	PIP	OCH_3
34	CEP	CEP	OH
35	PIP	CEP	OH
38	CEP	CEP	OCH_3
39	PIP	CEP	OCH_3

	R	R_1	R_2
40	PIPZ	PIPZ	NH_2
41	PIPZ	OH	NH_2
42	PIP	PIPZ	NH_2
43	PIPZ	PIP	NH_2
44	PIPZ	H	NH_2
45	PIPZ	OCH_3	OCH_3
46	PIPZ	OCH_3	NH_2
47	PIPZ	PIP	OH
48	PIPZ	PIP	OCH_3
49	PIPZ	PIPZ	OH
50	PIP	PIPZ	OH
51	PIPZ	PIPZ	OCH_3
52	PIP	PIPZ	OCH_3

Figure 7. Reagents: (a) NaOH 10%, EtOH. Cep, *N*-ethoxycarbonylpiperazin-1-yl. Pip, piperidin-1-yl. Pipz, piperazin-1-yl.

location of spots was detected by illumination with a UV lamp. Elemental analyses of all compounds synthesized for C, H and N were within ±0.4% of theoretical values and were performed by our analytical laboratory.

*4.1.1. General procedure for the preparation of acetamido 1,8-naphthyridine derivatives (**2**, **6**, **7**, **11–13**)*

A mixture of 10 mmoles of the appropriate 7-amino-1,8-naphthyridine derivative (**1**, **4**, **5**, **8–10**) and 20 mL of acetic anhydride was refluxed for 4 h.. After cooling, the solid obtained (**2**, **6**, **7**, **11–13**) was collected, washed with water and purified by crystallization (*table I*).

*4.1.2. General procedure for the preparation of chloro 1,8-naphthyridine derivatives (**3**, **14**, **15**, **21**, **30**, **31**, **36**, **37**)*

A mixture of 5 mmoles of the appropriate hydroxy-1,8-naphthyridine (**2**, **11**, **12**, **20**, **26**, **27**, **34**, **35**) and 10 mL of $POCl_3$ was kept at 90 °C for 4 h. After cooling, the solution obtained was treated with ice and water and then akalinized with concentrated NH_4OH. The solid obtained (**3**, **14**, **15**, **21**, **30**, **31**, **36**, **37**) was washed with water and purified by crystallization (*table I*).

*4.1.3. General procedure for the preparation of carbethoxypiperazinyl (**5**, **9**, **16**), piperidinyl (**4**, **8**, **17**) and formylpiperazinyl (**10**) 1,8-naphthyridine derivatives*

A mixture of 5 mmoles of the appropriate chloro-1,8-naphthyridine (**1**, **3**, **14**, **15**) and 20 mmoles of 4-carbethoxypiperazine or piperidine, or 10 mmoles of 4-formylpiperazine was kept for 16 h in a sealed tube, at 140 °C when 4-carbethoxypiperazine or piperidine was used and at 80 °C when 4-formylpiperazine was used. After cooling, the solution obtained was treated with water; when piperidine or 4-formylpiperazine was used a solid was obtained that was collected, washed with water and purified by crystallization (*table I*), whereas when 4-carbethoxypiperazine was employed, a suspension was obtained that was extracted several times with chloroform. The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give the target compounds (**4**, **5**, **8–10**, **16**, **17**), which were purified by crystallization (*table I*).

*4.1.4. 7-Amino-4-[methyl(4-carbethoxypiperazin-1-yl)]-2-mercapto-1,8-naphthyridine (**18**)*

A mixture of 3 mmoles of 2-hydroxy-1,8-naphthyridine derivative (**9**), 5 mmoles of P_2S_5 and 100 mL of pyridine was refluxed for 12 h. The reaction mixture was evaporated under reduced pressure obtaining a crude solid (**18**), which was treated with water, filtered and purified by crystallization (*table I*).

*4.1.5. 2-Amino-5-[methyl(4-carbethoxypiperazin-1-yl)]-1,8-naphthyridine (**19**)*

A mixture of 2 mmoles of 2-mercapto-1,8-naphthyridine derivative (**18**), 5 g of Raney Nickel and 50 mL of ethanol was refluxed for 12 h. From the reaction mixture obtained, the inorganic residue was filtered off, and the solution was evaporated to dryness under reduced pressure, obtaining **19**, which was purified by crystallization (*table I*).

*4.1.6. 2-Acetamido-5-[methyl(4-carbethoxypiperazin-1-yl)]-1,8-naphthyridine (**23**)*

A mixture of 1 mmole of 2-chloro-1,8-naphthyridine derivative (**15**), 0.05 g of 10% Pd–C and 60 mL of glacial acetic acid was kept with magnetic stirring at r.t. for 8 h under hydrogen at atmospheric pressure. The catalyst was filtered off and the solution was evaporated to dryness under reduced pressure, obtaining **23**, which was purified by crystallization (*table I*).

*4.1.7. 7-Amino-2-chloro-4-[methyl(4-carbethoxy-piperazin-1-yl)]-1,8-naphthyridine (**24**)*

A mixture of 3 mmoles of 2-chloro-1,8-naphthyridine derivative (**15**) and 10 mL of 10% H_2SO_4 was refluxed for 30 min. The solution obtained was treated with concentrated NH_4OH until the pH was 9. The solid precipitate (**24**) was collected by filtration, washed with water and purified by crystallization (*table I*).

*4.1.8. General procedure for the preparation of methoxy-1,8-naphthyridine derivatives (**22**, **25**, **32**, **33**, **38**, **39**)*

A solution of 10 mmoles of freshly prepared sodium methoxide and 10 mmoles of the appropriate chloro-1,8-naphthyridine (**15**, **21**, **30**, **31**, **36**, **37**) in 20 mL of anhydrous methanol was refluxed for 4 h. The reaction mixture was evaporated to dryness under reduced pressure, obtaining a crude residue, which was treated with water and extracted three times with chloroform. The combined extracts were dried ($MgSO_4$) and evaporated to dryness under reduced pressure, obtaining the target compounds (**22**, **25**, **32**, **33**, **38**, **39**), which were purified by crystallization (*table I*).

*4.1.9. General procedure of diazotization of 7-amino-1,8-naphthyridine derivatives (**4**, **5**, **9**, **16**, **17**), to obtain the corresponding 7-hydroxy-1,8-naphthyridine derivatives (**20**, **26** and **28**, **27** and **29**, **34**, **35**)*

$NaNO_2$ (10 mmoles) was added portionwise to a solution of 2 mmoles of the appropriate 7-amino-1,8-naphthyridine derivative (**4**, **5**, **9**, **16**, **17**) in 10 mL of concentrated sulfuric acid cooled at 0 °C, and was then allowed to rest at r.t. for 30 min. The resulting solution was treated with ice and concentrated NH_4OH until the pH was 8. The solid was collected by filtration and washed with water. Compounds (**20**, **34**, **35**) were purified by crystallization (*table I*). The mixtures obtained by diazotization of **4** and **17** respectively were separated by flash chromatography, eluting with ethyl acetate–diethylamine (8/1), to obtain the target compounds (**26**, **28** and **27**, **29**) (*table I*).

*4.1.10. General procedure for the preparation of piperazinyl-1,8-naphthyridine derivatives (**40–52**),*

A mixture of 1 mmole of the appropriate carbethoxypiperazinyl-1,8-naphthyridine derivative (**5**, **9**, **16**, **17**, **19**, **22**, **23**, **25**, **27**, **33–35**, **38**, **39**) in 10 mL of 10% of NaOH and 10 mL of ethanol was refluxed for 8 h. From the solution obtained, ethanol was removed under reduced pressure and the pH was adjusted to 7–8 by concentrated HCl. The acqueous solution was extracted three times with chloroform. The combined extracts were dried ($MgSO_4$) and evaporated to dryness under reduced pressure, obtaining the target compounds (**40–52**), which were purified by crystallization (*table I*).

4.2. Pharmacology

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

4.2.1. Vasorelaxing activity

To determine a possible vasodilator mechanism of action, the compounds were tested on isolated thoracic aortae of male normotensive Wistar rats (250–350 g), as previously described [6]. The rats were killed by cervical dislocation under light ether anaesthesia and bled. The aortae were immediately excised and freed from extraneous tissues, and the endothelium was removed by gently rubbing the intimal surface of the vessels. Aortic rings were suspended, under a preload of 2 g, in 10 mL organ baths containing Tyrode solution (composition of saline in mM: NaCl 136.8; KCl 2.95; $CaCl_2$ 1.80; $MgSO_4 \cdot 7H_2O$ 1.05; NaH_2PO_4 0.41; $NaHCO_3$ 11.9; glucose 5.5), thermostated at 37 °C and continuously bubbled with a mixture of O_2 (95%) and CO_2 (5%). Changes in tension were recorded by means of an isometric transducer (Basile mod. 7005), connected with a unirecord microdynamometer (Basile mod. 7050).

After an equilibration period of 45 min, the effective removal of the endothelial layer was investigated by the administration of acetylcholine (Ach, 5 μM) on norepinephrine (NE, 1 μM)-precontracted tissues. An Ach-induced relaxation <20% of the NE-induced contraction was considered to be representative of an acceptable lack of the endothelial layer, while the organs showing a relaxation ≥20% (i.e. a significant presence of the endothelium), were not used in the experimental procedures. Thirty to 40 min after confirmation of endothelium removal, the aortic preparations were contracted by treatment with a single concentration of KCl (20 mM) and when the contraction reached a stable plateau, three-fold increasing concentrations of the test compounds (10 nM–10 mM) were added cumulatively. Furthermore, for some selected compounds (**19**, **22**, **23**, **26**, **28**, **29**, **47**), the vasorelaxing activity was also evaluated on KCl 20 mM-contracted vessels, in the presence of the guanylate cyclase blocker ODQ (1 μM) or of the adenylate cyclase blocker SQ 22536 (0.1 mM). Finally, compounds **19**, **22** and **23** were also tested in the presence of the ATP-sensitive potassium channel blocker glybenclamide (1 μM).

Preliminary experiments showed that the KCl (20 mM)-induced contractions remained constant in a stable tonic state for at least 40 min. Norepinephrine hydrochloride (Sigma), acetylcholine chloride (Sigma), SQ 22536 (Alexis) and KCl were dissolved in bi-distilled water. ODQ (Alexis) was dissolved (1 mM) in ethanol and further diluted in bi-distilled water. Glybenclamide was dissolved (1 mM) in NaOH 0.1 N, by sonication, while all the synthesised derivatives were dissolved (10 mM) in HCl 0.1 N. All the further dilutions were performed in bi-distilled water. All the solutions were prepared immediately before the pharmacological experimental procedures. Previous experiments showed a complete ineffectiveness of the administration of the vehicle.

4.2.2. Data analysis

The efficacy of the vasorelaxing respons was expressed as the maximal relaxant effect (E_{max}), calculated as a percent of the contractile tone developed by the smooth muscle preparation, after the administration of KCl 20 mM.

The parameter of potency of the vasorelaxing effects was expressed as pIC_{50}, which is the negative logarithm of the vasodilator molar concentration determining a 50% reduction in the contractile tone induced by the contractile agent. The above parameters were calculated by means of non-linear regression analysis of the sigmoidal concentration-response curves (computer program: GRAPHPAD Prism).

All the results are expressed as means±S.E.M. of 4–6 experiments. Statistical comparison of experimental data was performed by two-tailed Student's *t*-test and ANOVA. A value of $P<0.05$ was considered significant.

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