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Synthesis of 1,8-naphthyridine derivatives: Potential antihypertensive agents – Part VII

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Abstract – A series of 2-(carbethoxypiperazinyl)- and 2-piperazinyl-1,8-naphthyridine derivatives, variously substituted, have been synthesized and pharmacologically investigated for their antihypertensive activity. Some of them exhibited a significant and prolonged decrease of the mean arterial pressure (MAP) on spontaneously hypertensive rats. For this series of compounds, on the basis of the pharmacological results obtained, no structure–activity relationship can be deduced at this time. Moreover, the most active and representative compounds **11b**, **12a** and **16b** were investigated by means of in vitro pharmacological functional studies and in vivo, as diuretic agents, to determine a possible mechanism of the antihypertensive activity, which is unknown for the moment. © Elsevier, Paris

antihypertensive derivative / piperazinyl / 1,8-naphthyridine derivative

1. Introduction

In a previous work [1] we have synthesized a series of 2-piperazinyl-1,8-naphthyridines **1** variously substituted, related to Prazosin **2** (*figure 1*). Some of them exhibited a marked antihypertensive activity on spontaneously hypertensive rats (SHR) and **1a** (R = CH_3O; R_1 = H) resulted as the most active compound of this series. Moreover the results from pharmacological testing on the compounds of this series revealed that the furoyl group generally reduced the antihypertensive activity.

The above-mentioned results prompted us to continue our chemical and pharmacological investigation on derivatives of 2-piperazinyl-1,8-naphthyridines in order to evaluate the effect of structural modifications on antihypertensive activity. On the basis of these considerations it appeared to be of interest to introduce different substituents on 1,8-naphthyridine nucleus, without preparing the corresponding furoyl derivatives at N-4 of piperazine ring.

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2. Chemistry

The 1,8-naphthyridines **3a** [2] and **3b** [3] (*figure 2*) were prepared in a different way by reaction of 2,6-diaminopyridine (2,6-DAP) with diethylmalonate in diphenyl ether and ethyl trifluoromethylacetoacetate in concentrated sulfuric acid, respectively. Compounds **3** were converted in good yield to the corresponding acetamido derivatives **4**, which, by treatment with phosphoryl chloride, gave the chloro derivatives **5**. The amino derivatives **6** were prepared by hydrolysis of **5** with diluted sulfuric acid and then converted by diazotization in trifluoroacetic acid to the corresponding hydroxynaphthyridines **7** (*figure 2, table I*).

The compounds **5** (*figure 3*) were allowed to react with carbethoxypiperazine (CEP) in toluene in sealed tube to give the carbethoxypiperazine derivatives **8**. The structure of **8a** was demonstrated by chemical evidence: in fact this compound was converted by catalytical hydrogenation to the known 7-acetamido-2-(4-carbethoxypiperazin-1-yl)-1,8-naphthyridine **9** [4]. The hydrolysis of **8a** with 10% hydrochloric acid give amino derivative **10a**. The bases **11** were instead obtained by alkaline hydrolysis of the compounds **8**. Under these conditions the piperazino derivative **11a**

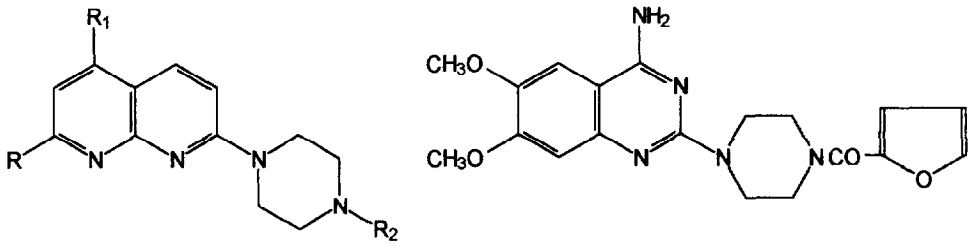

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Figure 1. 1,8-Naphthyridine derivatives and Prazosine.

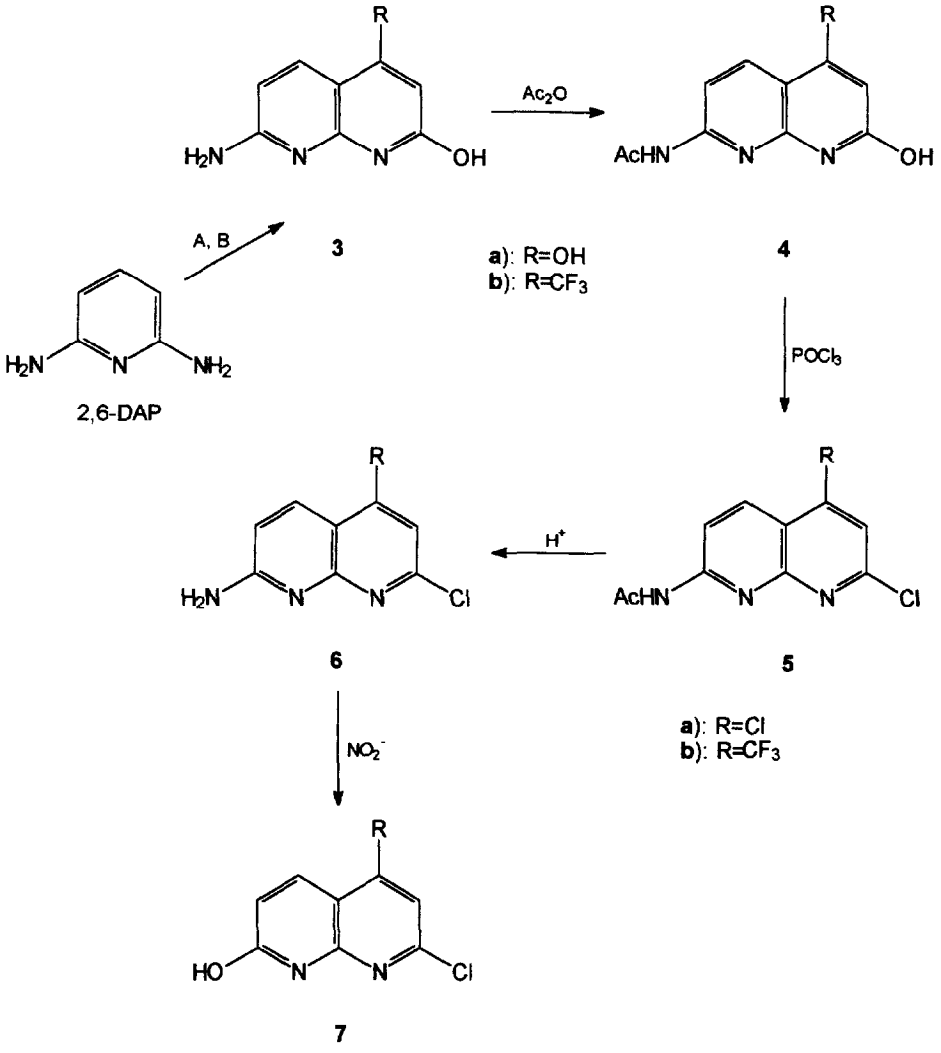
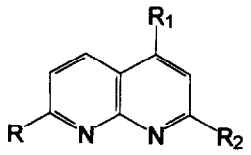


Figure 2. Reagents: A, ethyl malonate; B, ethyl trifluoroacetoacetate.

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



Compound	R	R_1	R_2	Yield (%)	M.p. (°C)	Recryst. solvent
3a [2]	NH_2	OH	OH	96	> 320	DMF
3b [3]	NH_2	CF_3	OH	91	> 320	DMF
4a [1]	NHAc	OH	OH	89	> 320	DMF
4b	NHAc	CF_3	OH	75	> 320	EtOH
5a	NHAc	Cl	Cl	78	228–230	Acetone
5b	NHAc	CF_3	Cl	76	214–215	Toluene
6a	NH_2	Cl	Cl	56	245–247	MeOH
6b	NH_2	CF_3	Cl	82	221–224	Toluene
7a	OH	C	Cl	83	> 320	MeOH
7b	OH	CF_3	Cl	96	174–175	$CHCl_3$
18	OH	CF_3	OCH_3	92	208–211	Toluene
19	Cl	CF_3	OCH_3	89	113–115	*i*-PrOH
23	NH_2	OH	CF_3	90	320–322	Ethylene glycol
24	NHAc	OH	CF_3	84	246–247	EtOH
25	NHAc	Cl	CF_3	79	242–244	Toluene
26	NH_2	Cl	CF_3	84	218–219	Benzene
27	OH	Cl	CF_3	96	171–172	Toluene
28	OH	OCH_3	CF_3	88	237–240	Toluene
29	Cl	OCH_3	CF_3	86	93–94	Petr. ether 100–140 °C

was prepared in poor yields. Therefore an alternative profitable procedure for the preparation of **11a** was chosen: **5a** was transformed in good yields in **11a**·2HCl via formylpiperazine derivative **12a** (*figure 3, table II*).

Carbethoxypiperazine derivatives **13** (*figure 4*) were prepared by reaction of hydroxy compounds **7** with CEP and then converted to the corresponding piperazine derivatives **14**. It was also of interest to attempt the preparation of the 7-methoxy substituted analogous of **1**. The reaction of **13** with phosphoryl-chloride gave compounds **15**. Compounds **15a** and **15b** were allowed to react with sodium methoxyde for 4 h and 24 h respectively in order to obtain the corresponding 7-methoxy derivatives **16**. Under these conditions **15a** was converted to **16a** (R_1 = $COOC_2H_5$), whereas **15b** was converted by transesterification to **16b** (R_1 = $COOCH_3$). Alkaline hydrolysis of **16** gave the corresponding piperazine derivatives **17** (*figure 4, table II*).

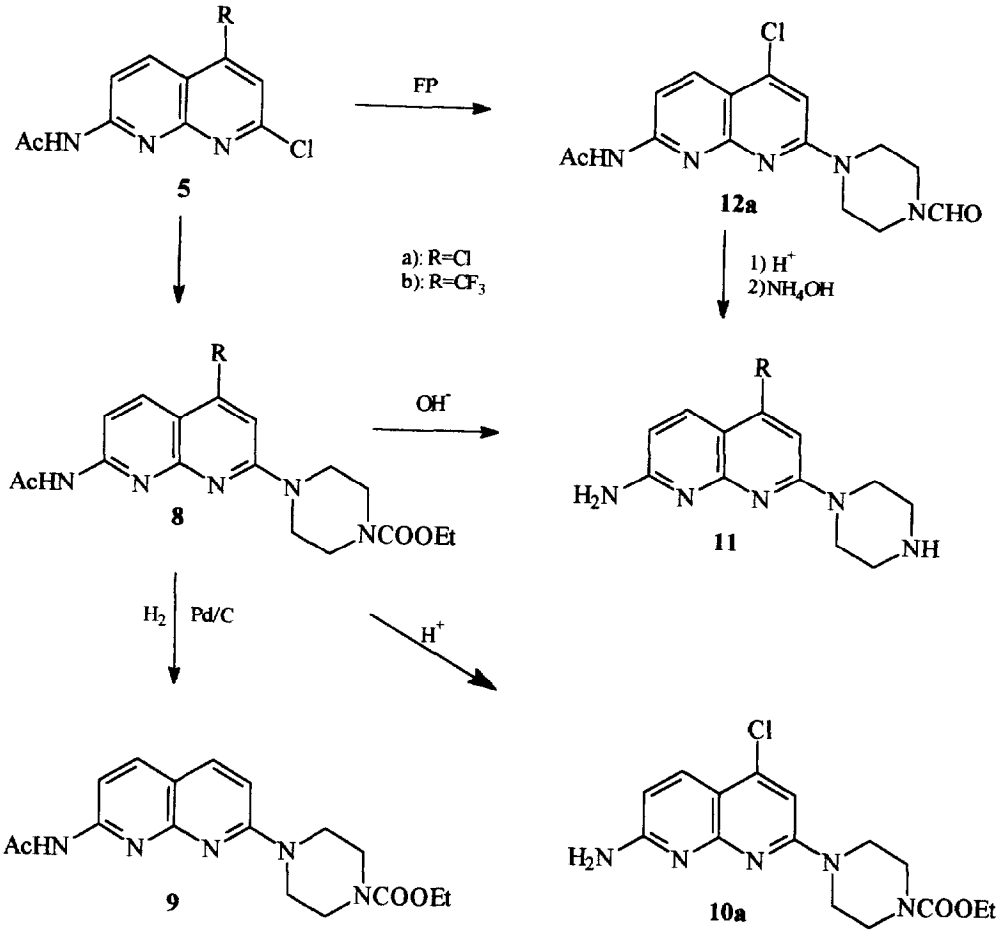


Figure 3. Reagents: FP, formylpiperazine; CEP, *N*-carbethoxypiperazine.

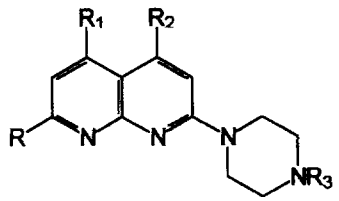
By reaction of **7b** (*figure 5*) with sodium methoxyde was obtained the methoxy derivative **18**, which by treatment with phosphoryl chloride at 70 °C for 30 min, gave **19** in good yield. Starting from the chloro compound **19** and treating with CEP, carbethoxy derivative **20** was prepared and then the compound **21**, isomer of **17b**, was obtained by alkaline hydrolysis of **20** (*figure 5, table I* and *II*).

To obtain a series of 2-trifluoro-methyl-1,8-naphthyridine derivatives the isomerization of pyridopyrimidine **22** [3] to 1,8-naphthyridine **23** (*figure 6*) was carried out in diphenyl ether under reflux condition. The corresponding acetyl derivative **24** was prepared and then allowed to react with phosphoryl chloride to give chloro derivative **25**, wich by hydrolysis with diluted sulfuric acid was converted to amino derivative **26**. Diazotization of this last compound in trifluoroacetic acid gave in good yield the hydroxy-1,8-naphthyridine **27** (*figure 6, table I*).

When the hydroxy derivative **27** (*figure 7*) was treated with sodium methoxyde **28** was obtained, which by reaction with phosphoryl chloride produced the corresponding chloro derivative **29**. Reaction of **29** with CEP gave the carbethoxypiperazine derivative **30**, which by alkaline hydrolysis gave the required compound **31** (*figure 7, table I* and *II*).

The assigned structures were fully confirmed by ^{1}H-NMR spectra (*table III*), IR and elemental analyses. The spectrum of **3a** shows two doublets at δ 6.3 and δ 7.8 due to H_6 and H_5 respectively and a singlet at δ 5.5 due to H_3 and the spectrum of **3b** shows two doublets at δ 6.5 and δ 7.7 due to H_6 and H_5 respectively and a singlet at δ 6.6 due to H_3. The ^{1}H-NMR spectra of piperazino derivatives show two multiplets ranging at δ 2.7–3.7 and δ 3.6–4.0, whereas the ^{1}H-NMR spectra of corresponding formyl and carbethoxypiperazino derivatives show a multiplet ranging at δ 3.4–3.7 due to the piperazino group.

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



Compound	R	R_1	R_2	R_3	Yield (%)	M.p. (°C)	Recryst. solvent
8a	NHAc	H	Cl	COOEt	64	278–279	MeOH
8b	NHAc	H	CF_3	COOEt	56	255–257	EtOH/H_2O
9	NHAc	H	H	COOEt	95	188–190	Benzene
10a	NH_2	H	Cl	COOEt	83	191–193	MeOH/H_2O
11a	NH_2	H	Cl	H	72	218–220	Benzene
11b	NH_2	H	CF_3	H	82	249–252	*i*-PrOH
12a	NHAc	H	Cl	CHO	65	287–290	EtOH
13a	OH	H	Cl	COOEt	61	258–261	AcOEt
13b	OH	H	CF_3	COOEt	80	190–193	Petr. ether[a]
14a	OH	H	Cl	H	79	237–239	Petr. ether[a]
14b	OH	H	CF_3	H	98	295–298	EtOH
15a	Cl	H	Cl	COOEt	90	148–150	EtOH
15b	Cl	H	CF_3	COOEt	98	157–158	Petr. ether[a]
16a	OCH_3	H	Cl	COOEt	85	140–143	Petr. ether[a]
16b	OCH_3	H	CF_3	COOMe	97	223–225	*i*-Pr. ether
17a	OCH_3	H	Cl	H	72	60–63	Petr. ether[a]
17b	OCH_3	H	CF_3	H	81	165–166	Petr. ether[a]
20	OCH_3	CF_3	H	COOEt	98	165–168	Cycloexane
21	OCH_3	CF_3	H	H	80	151–153	Cycloexane
30	CF_3	OCH_3	H	COOEt	95	188–190	Toluene
31	CF_3	OCH_3	H	H	64	119–122	Et_2O

[a]Petroleum ether 100–140 °C.

The ^{1}H-NMR spectrum of compound **12a** shows a singlet at δ 11.1 due to the formyl group. The ^{1}H-NMR spectra of methoxy derivatives show a singlet ranging at δ 4.1–4.3 due to the methoxy group.

The ^{1}H-NMR spectrum of **16a** show a triplet at δ 1.3 and a quartet at δ 4.2 due to carbethoxy group, whereas the ^{1}H-NMR spectrum of **16b** show a singlet at δ 3.8 due to carbomethoxy group.

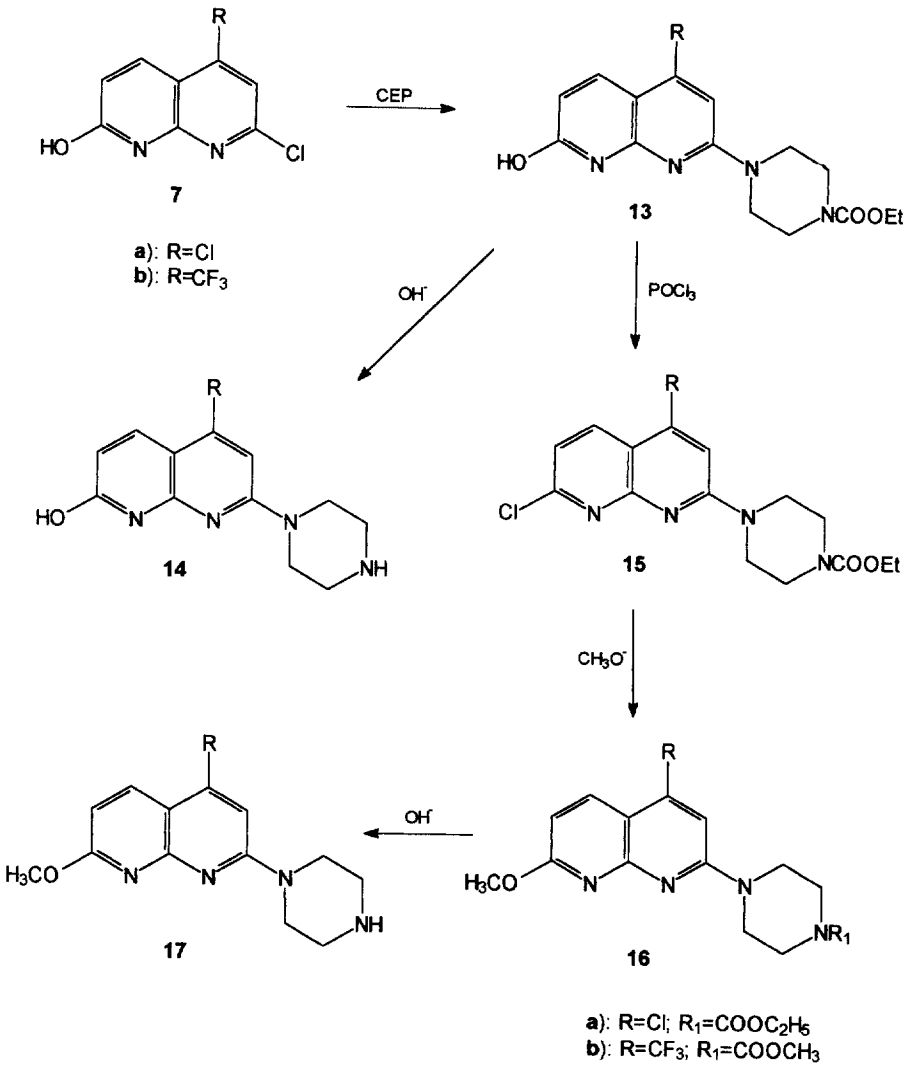


Figure 4. Reagent: CEP, *N*-carbethoxypiperazine.

In the ^{1}H-NMR spectra of 2- and 4-trifluoromethyl derivatives a long-range coupling of H_3 with fluorine is not observed, in the experimental conditions reported, whereas a narrow long-range coupling of H_5 with fluorine is present in the ^{1}H-NMR spectra of 4-trifluoromethyl derivatives.

3. Pharmacological results

3.1. Antihypertensive activity

Many examined compounds showed an interesting antihypertensive activity: the compounds **14b**, **15a**, **17a** and **31** determined a significant and prolonged decrease of the mean arterial pressure (MAP), while the compounds **8b** and **20** caused a marked, albeit transient, hypotensive effect, in the first few minutes following the administration. The compound **15a** showed both the profiles of activity, i.e. after an imediate strong hypotensive pick, a prolonged and significant antihypertensive effect could be observed. Finally, the strongest antihypertensive responses were evoked by the compounds **11b**, **12a** and **16b**. All the remaining molecules were not provided of a significant antihypertensive activity. The results are shown in *table IV*. The variations of MAP *vs* time, evoked by the compounds **12a**, **17b**, **15a** and **16a** are shown in *figure 8* and *9*.

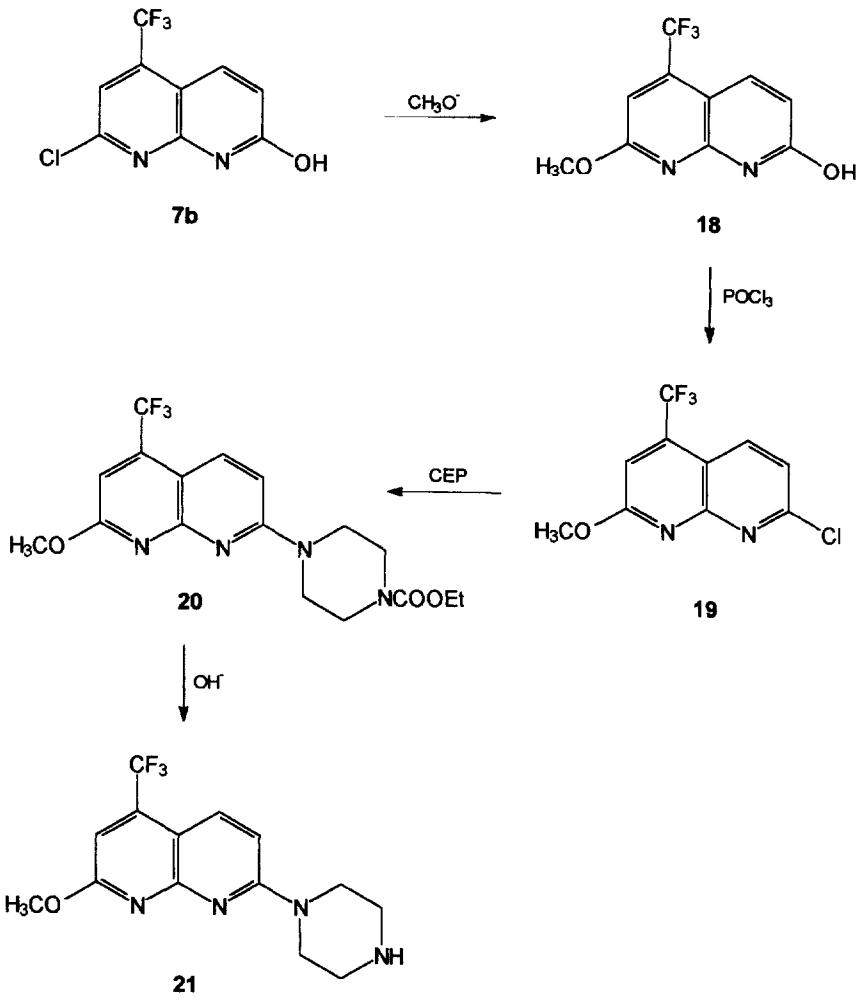


Figure 5. Reagent: CEP, *N*-carbethoxypiperazine.

3.2. Diuretic activity

The three selected compounds **11b**, **12a** and **16b** did not show any diuretic effect, while, on the contrary, they did show a significant antidiuretic activity, reducing the volumes of secreted urine by the treated rats. Furosemide significantly increased the diuresis. No significant differences of the volumes of the drunk water were observed, both in control and in treatment conditions. The results are shown in *table V* and in *figure 10*.

3.3. In vitro functional tests

All the selected compounds **11b**, **12a** and **16b** did not show any of the various evaluated possible mechanisms of action. A vasorelaxing activity was not observed both in NA- and in KCl-precontracted vessels, excluding an activity linked to an agonism for the receptors of endogenous vasodilators and excluding an action of different systems determining vascular smooth muscle relaxation, such as, for example, the increase of intracellular levels of cyclic nucleotides, the release of endothelial nitric oxide, the blocking of Ca^{++} channels or the opening of K^+ channels, the stimulation of vasorelaxing receptors.

All the compounds did not reduce the activity of the electrostimulated ileum, thus a possible α_2-agonism is not involved, while Clonidine determined a marked reduction of the pulse-induced smooth muscle contractions, until an almost complete abolition.

The evaluation of a possible antagonism toward α_1, β_1 and nicotinic receptors gave a negative response, as

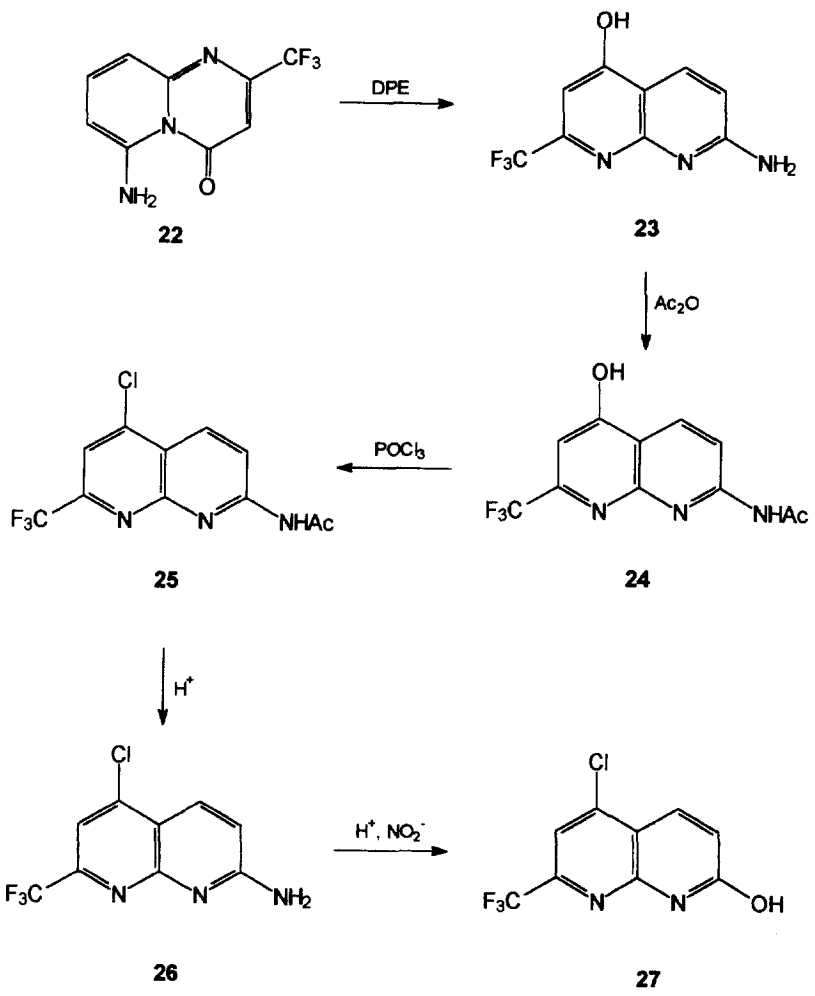


Figure 6. Reagent: DPE, diphenylether.

no inhibition of the responses evoked by the respective agonists was observed, while the α_1-antagonist Prazosine, the β-antagonist Propranolol and the nicotinic antagonist Hexamethonium significantly antagonized the responses induced respectively by NA on rat aorta, IPNA on guinea pig atria and nicotine on guinea pig ileum.

A possible ACE-inhibiting activity was also discarded, as on rat aortae the CRCs for AT-I were not changed by the selected compounds, while the CRCs for AT-I in the presence of Captopril were significantly shifted rightward with respect to the control CRCs, as the known ACE-inhibitor blocked the activity of the tissue enzyme and thus the conversion of AT-I to the more effective AT-II.

4. Conclusions

As shown in *table IV*, compounds **8**, **9**, **10a**, **11a**, **13**, **14a**, **15b** and **17b** were devoid of antihypertensive activity. Compounds **16a**, **20**, **21** and **30** showed a little antihypertensive activity, whereas compounds **11b**, **12a**, **14b**, **15a**, **16b**, **17a** and **31** showed the most antihypertensive properties, since their effect resulted strong and prolonged in time.

Among the checked compounds, the most interesting was the formylpiperazino derivative **12a**. The carbethoxypiperazino derivatives are generally weakly active or inactive, as already observed by us in a previous paper [1], with the exception of the compounds **15b** and **16b**. The piperazino derivatives

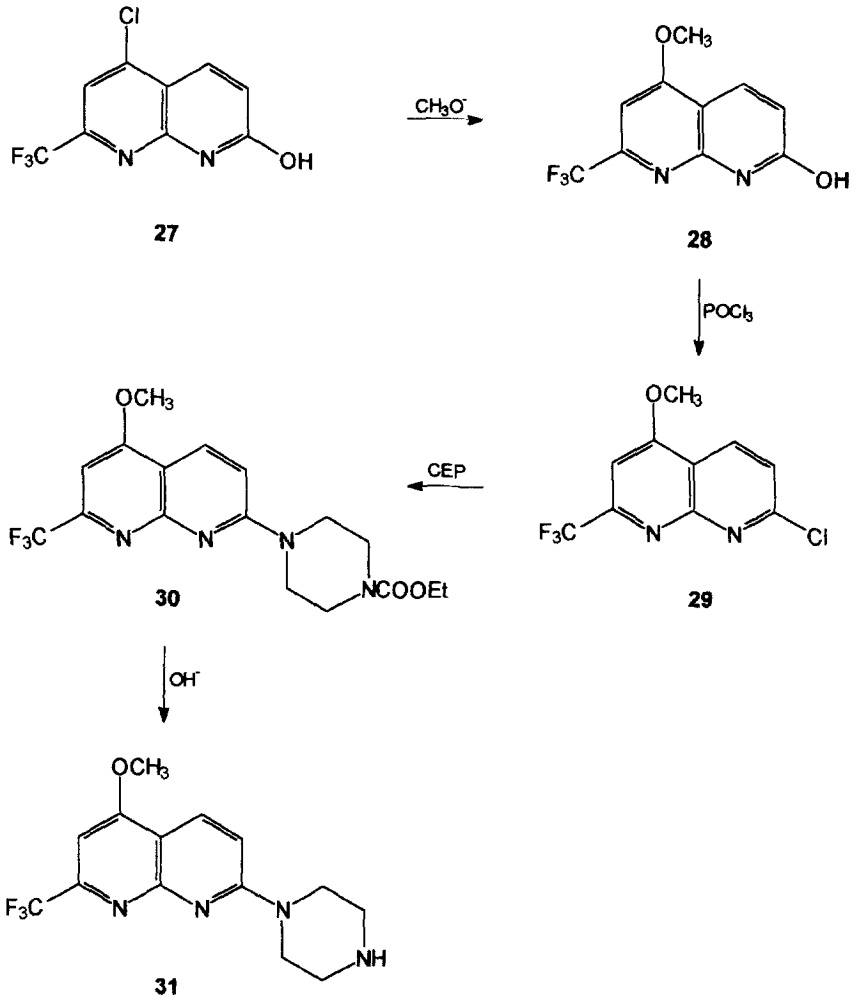


Figure 7. Reagent: CEP, *N*-carbethoxypiperazine.

are generally most active than the corresponding carbethoxypiperazino derivatives. The activity of these compounds does not seem to be related in a clear manner with the substituents present on the different positions of the 1,8-naphthyridine nucleus. In conclusion, on the basis of the above pharmacological results, no structure–activity relationship can be deduced at this time.

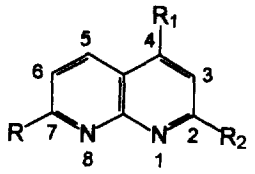
Regarding the possible mechanism of action, the performed functional studies did not permit to ascribe the tested compounds to a pharmacological category of antihypertensive drugs. The compounds were not direct vasodilators, because they did not relax the isolated precontracted vessels, excluding an action on vasorelaxing receptor systems or ion channels. Furthermore, they did not exhibit neither ACE-inhibitor effect, nor β_1- and α_1-blocking or α_2-stimulating activity, nor ganglioplegic effects. The compounds were not only devoid of diuretic activity, but they elicited a significant reduction of the secreted volume of urine, probably due to the lowering of the systemic blood pressure. Thus, a possible yet unknown central effect, different from an involvement of the α_2-adrenoceptor, can not be excluded.

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

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



Compound	H_3(s)	H_5(d)	H_6(d)	Pip(m)[a]	Others
3a	5.5	7.8	6.3		
3b	6.6	7.7	6.5		
4a	6.4	8.1	8.1		$COCH_3$ 2.2(s)
4b	7.6	8.2	8.2		$COCH_3$ 2.2(s)
5a	7.0	7.7	7.7		$COCH_3$ 2.0(s)
5b	7.7	8.8	8.5		$COCH_3$ 2.3(s)
6a	6.6	7.2	6.2		
6b	7.6	8.1	7.1		
7a	7.5	8.2	7.0		
7b	7.6	8.1	7.1		
8a	6.8	7.6	7.4	3.4	$COCH_3$ 2.0(s); C_2H_5 1.1(t), 3.7(q)
8b	7.5	8.2	8.2	3.6, 3.8	$COCH_3$ 2.1(s); C_2H_5 1.2(t), 4.1(q)
10a	6.6	7.5	6.3	3.4	C_2H_5 1.1(t), 3.8(q)
11a	6.9	7.8	6.6	2.7, 3.6	
11b	7.2	7.9	6.7	2.8, 3.6	
12a	7.5	8.2	8.2	3.6	$COCH_3$ 2.2(s); CHO 11.1(s)
13a	6.8	7.7	6.2	3.5	C_2H_5 1.2(t), 4.0(q)
13b	6.8	7.8	6.6	3.7	C_2H_5 1.3(t), 4.2(q)
14a	7.0	7.8	6.3	3.2, 3.9	
14b	7.2	7.8	6.4	3.3, 4.0	
15a	7.1	8.3	7.3	3.7	C_2H_5 1.3(t), 4.2(q)
15b	7.4	8.4	7.3	3.7, 3.9	C_2H_5 1.3(t), 4.2(q)
16a	7.6	8.1	6.8	3.7	C_2H_5 1.3(t), 4.2(q); OCH_3 4.1(s)
16b	7.1	8.1	6.8	3.7	CH_3 3.8(s); OCH_3 4.1(s)
17a	7.0	8.2	6.8	3.1, 3.8	OCH_3 4.1(s)
17b	7.1	8.1	6.8	3.0, 3.8	OCH_3 4.1(s)
18	7.0	8.0	6.7		OCH_3 4.1(s)
19	7.4	8.4	7.5		OCH_3 4.2(s)
20	7.0	8.1	6.9	3.7	C_2H_5 1.3(t), 4.2(q); OCH_3 4.1(s)
21	6.9	8.0	6.8	2.9, 3.7	OCH_3 4.1(s)
23	7.2	8.5	7.3		
24	7.0	8.6	8.3		$COCH_3$ 2.2(s)
25	7.9	8.8	8.8		$COCH_3$ 2.3(s)
26	7.7	8.3	7.2		
27	7.9	8.1	6.8		
28	7.0	8.1	6.8		OCH_3 4.1(s)
29	7.2	8.6	7.6		OCH_3 4.2(s)
30	6.9	8.3	7.0	3.6, 3.9	C_2H_5 1.2(t), 4.3(q); OCH_3 4.1(s)
31	6.9	8.2	7.0	2.9, 3.8	OCH_3 4.3(s)

[a]Pip, piperazine.

Table IV. Responses (expressed as variation % of the basal MAP) versus time (min), following the administration of the compounds (dose = 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 (min)								
		1	7.5	15	30	45	60	90	120	240
8a	156	0	3	3	10	8	6	9	2	2
8b	155	–43	–6	–4	–1	0	3	–1	0	1
9	178	–2	–5	–4	–2	1	0	1	5	3
10a	180	–5	–10	–9	–7	–4	–1	1	0	1
11a	167	–5	–10	–10	–9	3	4	2	1	2
11b	186	–26	–39	–46	–43	–32	–29	–26	–22	–3
12a	186	–65	–74	–73	–57	–52	–41	–19	–19	0
13a	180	–6	–10	–9	–7	–4	–1	1	0	1
13b	168	–1	4	4	5	4	5	2	4	1
14a	167	–5	–10	–10	–9	3	4	2	1	2
14b	167	–26	–31	–23	–21	–20	–14	–18	–11	–11
15a	150	–65	–13	–20	–17	–21	–23	–21	–10	–10
15b	180	–8	–13	–5	–7	–8	0	1	1	2
16a	179	–68	–13	–14	–4	0	2	–1	1	2
16b	175	–9	–25	–29	–29	–33	–38	–43	–50	–60
17a	154	–12	–32	–36	–22	–16	–23	–31	–18	–6
17b	174	0	1	–2	–6	–6	2	–5	–5	6
20	180	–51	–41	15	–11	–9	–1	2	3	6
21	196	–19	–18	–13	–8	2	0	–5	–6	1
30	196	–18	–21	–10	–5	0	–3	–6	–2	1
31	180	–20	–22	–20	–25	–17	–15	–8	–1	3

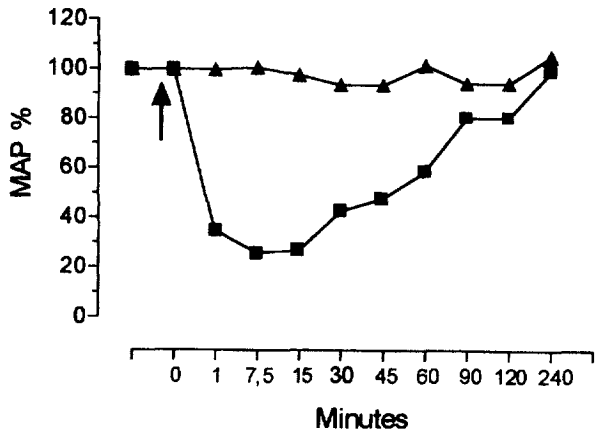


Figure 8. Variations of MAP (expressed as % of the basal MAP), following the administration (arrow) of the effective compound **12a** (squares) and of the ineffective compound **17b** (triangles). All the points represent the mean value of 4 experiments; all the values of SEM (not drawn for clarity) are < 10%.

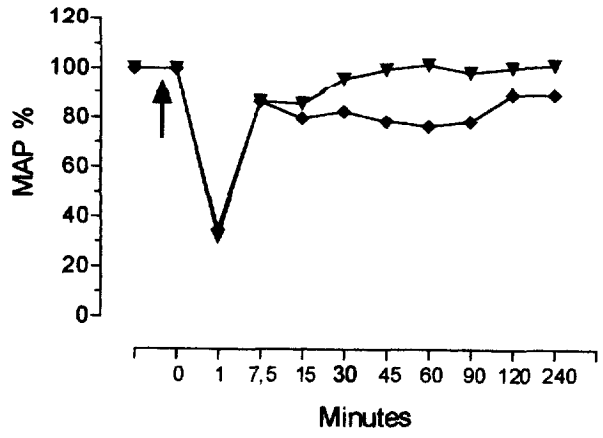


Figure 9. Variations of MAP (expressed as % of the basal MAP), following the administration (arrow) of the compound **15a** (diamonds) and of the compound **16a** (triangles), which evoked hypotensive picks, respectively followed and not followed by a further prolonged antihypertensive response. All the points represent the mean value of 4 experiments; all the values of SEM (not drawn for clarity) are < 10%.

Table V. The table shows the volumes of secreted urine (mL/24 h, ± S.E.M.), relative to the different groups of rats in control conditions and after treatment with the three selected compounds and Furosemide (n = 4/group). The asterisks indicate a significant difference from the respective control.

Compound	Control	Treated
16b	15 ± 3	6 ± 2*
11b	16 ± 2	7 ± 3*
12a	18 ± 3	7 ± 3 *
Furosemide	17 ± 2	28 ± 4*

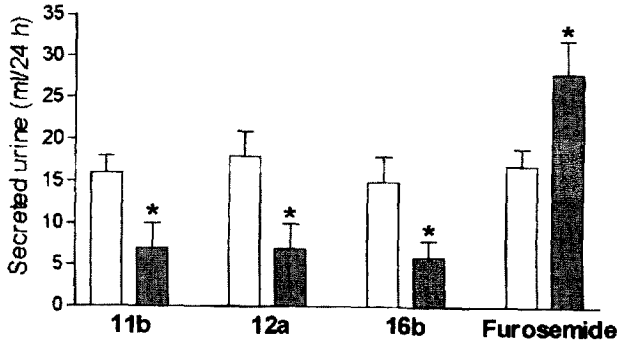


Figure 10. The histograms show the volumes of secreted urine (mL/24 h, S.E.M. is represented by the vertical bars), relative to the different groups of rats in control conditions (white columns) and after treatment with the three selected compounds and Furosemide (grey columns) (n = 4/group). The asterisks indicate a significant difference from the respective control.

spectra were measured with Perkin-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-2,4-dihydroxy-1,8-naphthyridine **3a** [2]*

A mixture of 2.0 mmol of 2,6-diaminopyridine (2,6-DAP) and 2.1 mmol of diethyl malonate in 30 mL of diphenyl ether was heated at 150 °C until to obtain a solution, and then kept for 30 min at 180 °C. After cooling, the solid was collected and washed with petroleum ether.

*5.1.2. 7-Amino-2-hydroxy-4-trifluoromethyl-1,8-naphthyridine **3b** [3]*

A mixture of 2.0 mmol of 2,6-DAP and 2.1 mmol of ethyl trifluoroacetoacetate was heated at 100 °C until the 2,6-DAP was completely dissolved. To the cooled solution (0 °C) were added dropwise 5.0 mL of concentrated sulfuric acid. After standing for 12 h at 60 °C the solution was poured into crushed ice and made basic with concentrated ammonium hydroxyde. The product was collected and washed with water.

*5.1.3. 7-Amino-4-hydroxy-2-trifluoromethyl-1,8-naphthyridine **23***

A solution of 5.0 mmol of 6-amino-2-trifluoromethyl-pyrido[1,2-*a*]pyrimidin-4-one **22** [3] in 10 mL of diphenyl ether was refluxed for 2 h. After cooling, the solid was collected and washed with petroleum ether to give **23**.

*5.1.4. General procedure for the preparation of acetamido-1,8-naphthyridine derivatives **4a** [2], **4b** and **24***

A suspension of 5.0 mmol of amino derivatives **3a** [2], **3b** or **23** in 10 mL of acetic anhydride was refluxed for 2 h. After cooling, the solid was collected and washed with water to give **4a** [2], **4b** and **24**.

*5.1.5. General procedure for the preparation of chloro-1,8-naphthyridine derivatives **5**, **15**, **19**, **25** and **29***

A mixture of 10 mL of phosphoryl chloride and 5.0 mmol of hydroxy-1,8-naphthyridine was heated in the case of **4**, **13** and **24** for 90 min at 100 °C and in the case of **18** and **28** for 30 min at 70 °C. After cooling, the solution was poured into crushed ice and treated with concentrated ammonium hydroxyde until pH 5 for the compound **15**, pH 8 for the compounds **19** and **29** and pH 9 for the compounds **5** and **25**. The solid was then collected and washed with water.

*5.1.6. General procedure for the preparation of amino-1,8-naphthyridine derivatives **6**, **10a** and **26***

A solution of 5.0 mmol of acetamido-1,8-naphthyridine derivatives **5**, **8a** or **25** 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 **6**, **10a** and **26**.

*5.1.7. General procedure for the preparation of hydroxy-1,8-naphthyridine derivatives **7** and **27***

To a cooled solution (0 °C) of 2.0 mmol of amino-1,8-naphthyridine derivatives in 10 mL of trifluoroacetic acid for **6** or sulfuric acid for **26**, was added 5.0 mmol of sodium nitrite in small amounts. 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 compounds **7** and **27** were collected by filtration and washed with water.

*5.1.8. General procedure for the preparation of 4-carbethoxy-piperazin-1-yl-1,8-naphthyridine derivatives **8**, **13**, **20**, **30** and of 4-formylpiperazin-1-yl-1,8-naphthyridine **12a***

A mixture of 10 mmol of **5**, **7**, **19** or **29**, 1.2 mmol of suitable piperazine derivative, 2.0 mL of triethyl amine was heated for 24 h at 100 °C in the case of compounds **8**, **20** and **30** and at 80 °C in the case of compound **12a** in sealed tube. After cooling the solvent was evaporated to dryness in vacuo, and the residue obtained was treated with water. The solid compounds **8**, **12a**, **13**, **20** and **30** was collected by filtration and washed with water.

*5.1.9. General procedure for the preparation of methoxy-1,8-naphthyridine derivatives **16**, **18** and **28***

To a solution of 50 mL of absolute methanol, in which 10 mmol of sodium metal were dissolved, 1.0 mmol of **15**, **7b**

or **27** was added, the mixture was refluxed for 4 h in the case compound **15a** and 24 h in the case compounds **7b**, **15b** and **27**. The methanol was evaporated to dryness in vacuo and in the case of **16** water was added, whereas in the case of **18** and **28** water was added and the pH of the mixture was adjusted to 4. Compounds **16**, **18** and **28** were then collected and washed with water.

*5.1.10. General procedure for the preparation of piperazin-1-yl-1,8-naphthyridine derivatives **11**, **14**, **17**, **21** and **31***

A suspension of 1.0 mmol of suitable 4-carbethoxypiperazinyl derivative, 15 mL of ethanol for the compounds **6** and **13** or 15 mL of methanol for the compounds **16**, **20** and **30** and 15 mL of 10% sodium hydroxide was refluxed for 8 h and the organic solvent was evaporated in vacuo. The products were then obtained by the following methods. In the case of **11b**, **17b**, **21** and **31** the solid was collected and washed with water. For the other compounds the pH of the aqueous solution was adjusted to 8 in the case of **11a** and **17a** or to 3 in the case of **14** and the mixture extracted with chloroform. The combined extracts were washed with water, dried (magnesium sulfate) and evaporated to dryness in vacuo to obtain the target compounds.

*5.1.11. 7-Amino-4-chloro-2-(piperazin-1-yl)-1,8-naphthyridine **11a***

A suspension of 1.0 mmol of **12a** in 10 mL of 10% hydrochloric acid was refluxed for 1 h and the solution evaporated to dryness in vacuo. The crude residue was then crystallized from methanol to give **11a**•2HCl.

The free base **11a** was obtained by treatment of **11a**•2HCl with 10% ammonium hydroxide. The solution was extracted with chloroform and the combined extracts evaporated to dryness in vacuo to give **11a**.

*5.1.12. 7-Acetamido-2-(4-carbethoxy piperazin-1-yl)-1,8-naphthyridine **9***

A stirred suspension of 1.0 mmol of **8a** in 25 mL of methanol was hydrogenated in presence of 5.0 mg of 10% palladium on charcoal for 12 h at room pressure and temperature. The mixture was filtered, the residue washed with hot methanol and the combined liquids evaporated to dryness at reduced pressure to give **9** [4].

5.2. Pharmacological methods

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

5.2.1. Antihypertensive activity

Adult male spontaneously hypertensive Wistar Kyoto rats (SHR) (250 ± 20 g), were anaesthetised with diethyl ether and implanted 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. Through a subcutaneous way, the catheters were exteriorized at the back of the neck and protected by spring wires. The arterial catheter was connected to a pressure transducer (Bentley-Trantec Basile mod. 800), and the blood pressure was recorded by a 2-channels recorder (Basile mod.Gemini 7070). Immediately after the 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-minute intervals, to avoid possible blood coagulation.

3–4 hours after the surgical protocol, the tested compounds were administered in bolus (30 mg/kg i.p.), in a volume of 0.5 mL to the conscious animals. The compounds were dissolved in physiological solution, containing tween 80 (10%) and dimethylsulfoxide (2%). The recording was performed for at least 4 h. Preliminary experiments showed that the administration of the vehicle (0.5 mL physiological solution, containing tween 80 10% and dimethylsulfoxide 2%) did not produce any effect. At the end of the experiments, the rats were killed by the administration i.v. of an overdose of Sodium penthobarbital.

5.2.2. Determination of the mechanism of action

The three representative antihypertensive compounds **11b**, **12a** and **16b** were investigated by means of in vitro pharmacological functional studies, to determine a possible vasodilator activity, an α_1-antagonism, an ACE-inhibitor action, a α_2-agonism, a β_2-agonism, or a nicotinic antagonism. Furthermore these three compounds were also tested in vivo, as diuretic agents.

5.2.3. In vitro pharmacological functional study

For the in vitro protocols, the compounds were dissolved (1 mM) in ethanol (96%) and further diluted in bi-distilled water.

5.2.4. Vasorelaxing activity

To determine a possible vasodilator mechanism of action, the compounds were tested on isolated aortae of male normotensive Wistar rats (250–350 g).

The rats were killed by cervical dislocation under light ether anaesthesia and bled. The aortae were immediately excised, freed of extraneous tissues and prepared as multiple-ring preparations [5]. Then the vessels 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; $MgSO_4$ $7H_2O$ 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 an unirecord microdynamometer (Basile mod. 7050).

After an equilibration period of 60 minutes, the endothelial integrity was confirmed by Acetylcholine (ACh) (55 μM)-induced relaxation of Norepinephrine (NE, 1 μM)-precontracted tissues. A relaxation ≥ 70% of the NE-induced contraction was considered representative of an acceptable presence of the endothelial layer, while the organs, showing a relaxation < 70%, were not used in the experimental procedures. 30–40 minutes after the confirmation of the endothelial integrity, the aortic preparations were contracted by treatment with a single concentration of NE (1 μM) or KCl (30 mM) and when the contraction reached a stable *plateau*, 3-fold increasing concentrations of the compounds (10 nM–10 mM) were added cumulatively.

Preliminary experiments showed that both the NE- and the KCl-induced contractions remained constant in a stable tonic state for at least 40 minutes.

5.2.5. α_1-antagonism

The tests were performed on aortae of male normotensive Wistar rats (250–350 g). The aortae were prepared as described in the above paragraph.

After the confirmation of the endothelial integrity and the equilibration time, a concentration–response curve (CRC) for NE was obtained cumulatively (0.1 nM–1 μM). The vessel

underwent an equilibration time (1 hour, wash-out at 15 min intervals), and then a second CRC for NE was obtained, in the presence of a reference concentration (3 μM) of the tested compounds, which was administered 15 min before the CRC. On parallel sets of experiments the known α_1-antagonist Prazosine (1 μM) was added, as reference antagonist. Previous experiments showed that the second CRC, obtained in the absence of any compound, was perfectly superimposable on the first one.

5.2.6. ACE-inhibitor activity

The tests were performed on aortae of male normotensive Wistar rats (250–350 g). The aortae were prepared as described in the above paragraph.

After the confirmation of the endothelial integrity and the equilibration time, control CRCs for Angiotensin I (AT-I) were obtained cumulatively (0.1 nM–1 μM). On parallel sets of experiments, the CRCs was carried out in the presence of a reference concentration (3 μM) of the compounds under test. In parallel experiments the CRCs for AT-I were obtained in the presence of Captopril (10 μM) [6].

5.2.7. β_1-antagonism

For the evaluation of a possible β_1-antagonism, the selected compounds were tested on spontaneously beating isolated atria of male Dunkin Hartley guinea pigs (weight 300–350 g), as also described in a previous work [7]. The animals were killed by cervical dislocation and bled, under light ether anaesthesia. The hearts were immediately excised and atria were separated from ventricular tissue and major blood vessels. The left atrium was sutured to a wire mounting rod fixed to the chamber of the isolated organ bath. The right atrium was connected by inextensive thread to the isometric force transducer (Basile mod. 7005) under a preload of 1 g. The atrial inotropic activity was recorded by a microdynamometer (Basile mod.7050). The bathing fluid was Tyrode saline, thermostated at 32 °C and gassed with O_2. The atria were left to equilibrate for 60 min, before starting the experimental protocol.

A first CRC to the β-agonist L-isoprenaline (IPNA, 0.1 nM–1 μM) was obtained with the method of the single concentration administration.

The β_1-antagonist activity was evaluated as the progressive reduction of the positive inotropic response to the repeated administrations of a chosen reference concentration (Ar) of the agonist IPNA, evoked by 10-fold increasing concentrations of the compound under test (10 nM–10 mM) or of the reference β-antagonist Propranolol (10 nM–10 mM). The Ar of IPNA was 100 nM.

5.2.8. α_2-agonism

For the evaluation of a possible α_2-agonism, the selected compounds were tested on the electrostimulated ileum of male Dunkin Hartley guinea pigs (weight 300–350 g), as previously described [8]. The animals were killed by cervical dislocation and bled, under light ether anaesthesia. Segments of ileum (2–3 cm in length) were excised (5–6 cm far from the ileo-cecal valve), freed from extraneous tissues and suspended between two platinum electrodes, under a preload of 0.5 g, in a 10 mL organ bath, containing Tyrode saline, thermostated at 37 °C and continuously bubbled with a mixture of O_2 (95%) and CO_2 (5%). Changes in isotonic tension were recorded by an isotonic transducer (Basile mod. 7006), connected to a microdynamometer (Basile mod.7050). After 30–40 minutes of equilibration time, the electrostimulation started (parameters: 0.1 Hz, 80 V, 1 ms of pulse width).

When the pulse-induced contracting spikes reached a stable constant width, 3-fold increasing concentrations (10 nM–10 mM) of the selected compounds were added cumulatively. On parallel sets of experiments the known α_2-agonist Clonidine was added cumulatively (0.1 nM–0.1 mM).

5.2.9. Nicotinic antagonism

The experiments were carried out on the ileum of male Dunkin Hartley guinea pigs (weight 300–350 g). The ileum was prepared as described in the above paragraph.

A first CRC for Nicotine (0.1 nM–1 mM) was obtained cumulatively. The nicotinic antagonism was evaluated as the progressive reductions of the contracting response to the repeated administrations of a chosen reference concentration (Ar) of the agonist Nicotine, evoked by 10-fold increasing concentrations of the compound under test (10 nM–10 mM) or of the reference antagonist Hexamethonium (10 nM–10 mM) [9]. The Ar of Nicotine was 0.1 μM.

5.2.10. Diuretic activity

Adult male spontaneously hypertensive rats (SHR) (250 ± 20 g), were individually housed in metabolic cages, with food and water ad libitum. The metabolic cages enabled to calculate exactly the volumes of drunken water and urine. After two days of stabilization time, on the third day (at 8.00 a.m.) the rats received the administration i.p. of the vehicle (0.5 mL of physiological solution, containing between 80 (10%) and dimethylsulfoxide 2%). On the fourth day (at 8.00 a.m.) the volumes of water and urine, respectively drunk and secreted in the last 24 hours, were calculated and represented the control parameters. Furthermore, on the fourth day (at 8.00 a.m.), the rats received the administration of the selected compounds (30 mg/kg i.p.), dissolved in 0.5 mL of physiological solution, containing between 80 (10%) and dimethylsulfoxide (2%). The fifth day (at 8.00 a.m.) the volumes of water and urine, respectively drunk and secreted in the last 24 h, were calculated and represented the parameters for the evaluation of the activity for the selected compounds.

A parallel group of rats were treated with the well-known diuretic drug Furosemide, following the same protocol.

5.2.11. Data analysis

The blood pressure parameters were recorded as systolic (SP) and diastolic (DP) pressure, and are shown as mean arterial pressure (MAP), calculated as DP + 2/3 (SP-DP).

The antihypertensive activity is shown as decrease %, relatively to the basal MAP. The time, in which the decrease of the MAP remained at a level > 10%, has been considered representative of the duration of the antihypertensive effect. The compounds, which did not produce a MAP decrease > 10%, were considered ineffective.

The significance of differences was evaluated by means of *Anova* and two-tail Student's *t* test. *P* values lower than 0.05 were considered significant.

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References

[1] Da Settimo A., Ferrarini P.L., Mori C., Primofiore G., Subissi A., Il Farmaco 41 (1986) 827–838 and notes reported therein.

[2] Lappin G.R., Petersen Q.R., Wheeler C.E., J. Org. Chem. 15 (1950) 377–378.
[3] Ferrarini P.L., Mori C., Primofiore G., Calzolari L., J. Heterocyclic Chem. 27 (1990) 881–886.
[4] Ferrarini P.L., Mori C., Primofiore G., J. Heterocyclic Chem. 23 (1986) 501–504.
[5] Calderone V., Martinotti E., Scatizzi R., Pellegrini A., Breschi M.C., J. Pharmacol. Toxicol. Meth. 35 (1996) 131–138.
[6] Kikta D.C., Fregly M.J., Hypertension 4 (1982) 118–124.
[7] Ferrarini P.L., Mori C., Badawneh M., Manera C., Saccomanni G., Calderone V., Scatizzi R., Barili P.L., Eur. J. Med. Chem. 32 (1997) 955–963.
[8] Balsamo A., Gentili D., Macchia M., Martinotti E., Rossello A., Scatizzi R., Eur. J. Med. Chem. 31 (1996) 713–716.
[9] Hayashi E., Yamada S., Mori M., Jpn. J. Pharmacol. 27 (1977) 659–665.