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Synthesis and antiplatelet activity of some 2,7-di(*N*-cycloamino)-3-phenyl-1,8-naphthyridine derivatives

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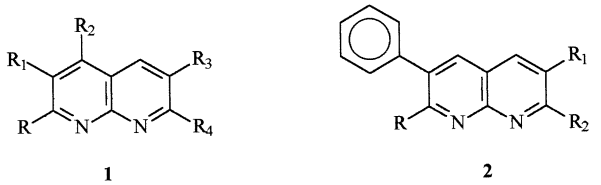
Abstract

Several 2,7-di(*N*-cycloamino)-3-phenyl-1,8-naphthyridine derivatives were synthesized and tested for their ability to inhibit human platelet aggregation in vitro induced by arachidonic acid, collagen and ADP. Only five compounds showed any appreciable activity, and the results of all the active derivatives were similar to those of papaverine in the test with arachidonic acid and collagen. Moreover, the most active compounds were investigated in the test with ADP and again showed a significant activity. The tested compounds that possessed the best activity were also shown to increase the c-AMP level significantly without involving the adenylyl cyclase system.

Keywords: 2,7-Di(*N*-cycloamino)-3-phenyl-1,8-naphthyridine; Inhibition of platelet aggregation; Human blood platelets; Morpholinyl; Piperidinyl; Piperazinyl

1. Introduction

For some time, we have been engaged in an effort to develop 1,8-naphthyridine derivatives, inhibitors of blood platelet aggregation induced by c-AMP phosphodiesterase, arachidonate and collagen as agents for the prevention and treatment of thrombotic and thromboembolic phenomena. We synthesized and tested for their ability to inhibit in vitro human platelet aggregation induced by arachidonate, collagen or ADP the 1,8-naphthyridines **1** [1–3] and the 3-phenyl-1,8-naphthyridines **2** [4] (Fig. 1), which carry piperidyl, piperazinyl or morpholinyl groups or an *N*-diethanol side-chain in the 2-, 7- and 2,7-positions. Many compounds showed an appreciable ability to inhibit the arachidonate- and collagen-induced aggregation of platelets, and the results of the most active derivatives were similar to those of papaverine in these tests. Among the tested compounds, only the 7-methoxy-2-morpholinyl-6-nitro-3-phenyl-1,8-naphthyridine **2a** showed a significant activity in the test with ADP.



1 2

Fig. 1. General structure of 1,8-naphthyridine derivatives with antiplatelet activity.

On the basis of these results, we decided to continue chemical and pharmacological investigation in the field of 3-phenyl-1,8-naphthyridine derivatives in order to evaluate the effects of the introduction of *N*-cycloamino groups in the 2,7-positions on antiplatelet activity.

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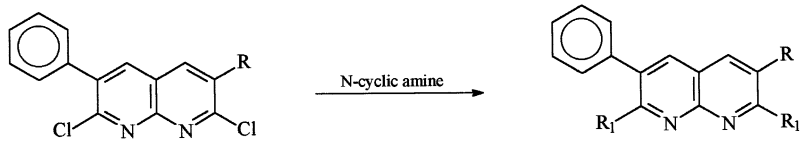


3

	R
3a	H
3b	NO_2

4-6

	R	R_1
4a	H	Cep
4b	NO_2	Cep
5a	H	Morph
5b	NO_2	Morph
6a	H	Pip
6b	NO_2	Pip

Scheme 1. Synthetic route to 2,7-di(*N*-cycloamino)- and 2,7-di(*N*-cycloamino)-6-nitro-1,8-naphthyridine derivatives. Cep = *N*-ethoxycarbonylpiperazin-1-yl, Morph = morpholin-1-yl, Pip = piperidin-1-yl.

2. Chemistry

As shown in Scheme 1, the known dichloro and dichloro nitro derivatives **3a**,**b** [3] were converted by reaction with the appropriate amine in a sealed tube at 140° to the corresponding 2,7-di(*N*-cycloamino) derivatives **4a**–**6a** and **4b**–**6b**, respectively (Tables 1 and 2). The treatment of the monochloro derivatives **7** [3,4], **8** [4] and **9** [4] with the appropriate *N*-six-membered cyclic amine, in the same manner used above, gave compounds **10**–**15**, substituted in the 2- and 7-positions with different *N*-cycloamino groups (Scheme 2, Tables 1 and 2). The nitro derivatives **4b**–**6b** and **10b**–**15b** were then converted by catalytic reduction in the presence of 10% palladium on charcoal, at room temperature and atmospheric pressure, to the corresponding amino derivatives **4c**–**6c** and **10c**–**15c** (Scheme 3, Tables 1 and 2). The piperazine derivatives **16a** and **17a**,**c**–**20a**,**c** were obtained by the treatment of the *N*-ethoxycarbonylpiperazine derivatives **4a**, **10a**,**c**–**12a**,**c** and **14a**,**c** with a 10% hydroalcoholic solution of sodium hydroxide, as illustrated in Scheme 4 (Tables 1 and 2).

3. Experimental

3.1. 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 E. Merck 0.2 mm precoated silica-gel glass plates (60 F_{254}) and the location of spots was detected by illumination with an UV lamp. Elemental analyses of all compounds synthesized for C, H and N were within $\pm$ 0.4% of theoretical values and were performed in our analytical laboratory.

3.1.1. General procedure for the preparation of the 2,7-di(N-cycloamino) derivatives *4a*,*b*–*6a*,*b* and *10a*,*b*–*15a*,*b*

A mixture of 1.5 mmol of the appropriate chloronaphthyridine **3a**,**b** [3], **7a** [3] **7b** [4], **8** or **9** [4] and 4.5 or 6.0 mmol of the appropriate amine for monochloro derivatives and for dichloro derivatives, respectively, was kept in a sealed tube at 140°C for 16 h. The crude residue was treated with water and the solid was collected by filtration, washed with water and purified by crystallization to obtain the di(*N*-cycloamino) derivatives **4a**,**b**–**6a**,**b** and **10a**,**b**–**15a**,**b** (Tables 1 and 2).

3.1.2. General procedure for the preparation of the 6-amino derivatives *4c*–*6c* and *10c*–*15c*

A solution of 1.0 mmol of the 6-nitro derivatives **4b**–**6b** or **10b**–**15b** in methanol was hydrogenated in the presence of 0.50 g of 10% palladium on charcoal at room temperature and atmospheric pressure for 3 h. The catalyst was filtered and the solvent was evaporated to dryness in vacuo. The crude residue was treated with water, made alkaline with 10% aqueous sodium hydroxide and then extracted twice with chloroform. The organic extracts combined were dried (magnesium sulfate) and evaporated to dryness in vacuo to give compounds **4c**–**6c** and **10c**–**15c**, which were purified by crystallization (Tables 1 and 2).

3.1.3. General procedure for the preparation of the 7-piperazin-1-yl derivatives *16a* and *17a*,*c*–*20a*,*c*

A suspension of 1.0 mmol of the appropriate *N*-ethoxycarbonylpiperazinyl derivatives **4a**, **10a**,**c**–**12a**,**c** or **14a**,**c** in 25 ml of ethanol and 25 ml of 10% aqueous sodium hydroxide was refluxed for 16 h and the organic solvent was evaporated in vacuo. The aqueous solution

was extracted with chloroform; the combined extracts were dried (magnesium sulfate) and evaporated to dryness in vacuo to give **16a** and **17a**,**c**–**20a**,**c**, which were purified by crystallization (Tables 1 and 2).

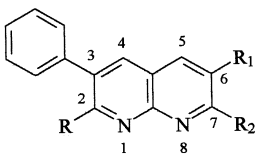
3.2. Pharmacological methods

Human blood samples were drawn from the anticubital vein and were anticoagulated with 3.8% sodium citrate (9:1 v/v). Platelet rich plasma (PRP) was prepared in accordance with the method described by Miceli et al [5]. The platelet count was adjusted to about 280 000 cell/μl.

Platelet aggregation was measured turbidimetrically in accordance with the method described by Born and Cross [6], using an aggregometer (Daichii model PA-3220).

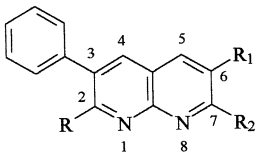
ADP (3.0 μM), arachidonic acid (AA) (0.7 mM) and collagen (5.0 μg/ml) were used as aggregating agents. Arachidonate sodium, ADP, papaverine, ASA, ibuprofen and indomethacin were provided by Sigma Chemicals, and collagen (from bovine tendon) was provided by Menarini Diagnostics.

Table 1
^{1}H NMR chemical shifts (δ ppm/TMS)



Compound	H_4 (s)	H_5	H_6 (d)	C_6H_5 (m)	 (m)	Others	Solv.
4a	7.62	7.84 (d)	6.87	7.42	3.62, 3.83	1.23 (t, CH_3); 4.13 (q, CH_2)	$CDCl_3$
4b	7.77	8.50 (s)		7.48	3.43, 3.60	1.30 (t, CH_3); 4.17 (q, CH_2)	$CDCl_3$
4c	7.18	7.61 (s)		7.41	3.33, 3.68	1.23 (t, CH_3); 4.13 (q, CH_2)	$CDCl_3$
5a	7.82	7.93 (d)	6.96	7.44	3.08, 3.53		DMSO
5b	7.64	8.40 (s)		7.43	3.56, 3.78		$CDCl_3$
5c	7.15	7.65 (s)		7.40	3.34, 3.47 3.68, 3.89	3.29 (brs, NH_2)	$CDCl_3$
6a	7.68	7.80 (d)	6.92	7.40	1.40, 1.55 3.03, 3.65		DMSO
6b	7.89	8.64 (s)		7.45	1.47, 1.63 3.26		DMSO
6c	7.18	7.67 (s)		7.40	1.45, 1.66 3.14	4.90 (brs, NH_2)	DMSO
10a	7.61	7.75 (d)	6.74	7.50	3.32, 3.79	1.23 (t, CH_3); 4.10 (q, CH_2)	$CDCl_3$
10b	7.65	8.40 (s)		7.43	3.38, 3.59 3.82	1.23 (t, CH_3); 4.10 (q, CH_2)	$CDCl_3$
10c	7.08	7.59 (s)		7.38	3.83, 3.38	1.23 (t, CH_3); 4.10 (q, CH_2)	$CDCl_3$
11a	7.57	7.67 (d)	6.78	7.39	1.68, 3.75 3.32	1.23 (t, CH_3); 4.10 (q, CH_2)	$CDCl_3$
11b	7.61	8.34 (s)		7.43	1.72, 3.37	1.23 (t, CH_3); 4.10 (q, CH_2)	$CDCl_3$
11c	7.03	7.56 (s)		7.32	1.69, 3.24 3.31	1.23 (t, CH_3); 4.10 (q, CH_2)	$CDCl_3$
12a	7.55	7.69 (d)	6.69	7.39	1.48, 3.34 3.65, 3.78	1.29 (t, CH_3); 4.17 (q, CH_2)	$CDCl_3$
12b	7.65	8.43 (s)		7.43	3.44, 3.57 3.61	1.28 (t, CH_3); 4.17 (q, CH_2)	$CDCl_3$
12c	7.09	7.58 (s)		7.40	3.21, 3.60	1.29 (t, CH_3); 4.17 (q, CH_2)	$CDCl_3$
13a	7.56	7.66 (d)	6.75	7.50	1.69, 3.27 3.62, 3.78		$CDCl_3$
13b	7.61	8.34 (s)		7.43	1.71, 3.43 3.56		$CDCl_3$
13c	7.15	7.72 (s)		7.40	1.63, 3.28 3.50	4.85 (brs, NH_2)	DMSO
14a	7.60	7.72 (d)	6.75	7.42	3.32, 3.67 3.76	1.29 (t, CH_3); 4.17 (q, CH_2)	$CDCl_3$
14b	7.59	8.42 (s)		7.43	1.50, 3.33 3.59	1.29 (t, CH_3); 4.16 (q, CH_2)	$CDCl_3$
14c	7.10	7.66 (s)		7.39	1.44, 3.17 3.32	1.29 (t, CH_3); 4.16 (q, CH_2)	$CDCl_3$
15a	7.55	7.69 (s)	6.68	7.39	1.49, 3.25 3.79		$CDCl_3$
15b	7.58	8.40 (s)		7.42	1.50, 3.33 3.56, 3.82		$CDCl_3$
15c	7.08	7.56 (s)		7.34	1.48, 3.19 3.42, 3.87		$CDCl_3$
16a	7.77	7.89 (d)	6.98	7.45	2.88, 3.15 3.70		DMSO
17a	7.60	7.72 (d)	6.72	7.40	2.94, 3.25 3.79		$CDCl_3$
17c	7.07	7.58 (s)		7.33	3.14, 3.26 3.58	2.19 (brs, NH); 3.90 (brs, NH_2)	$CDCl_3$
18a	7.54	7.64 (d)	6.78	7.44	1.68, 3.76 2.80, 3.24	1.87 (brs, NH)	$CDCl_3$
18c	7.07	7.60 (s)		7.42	1.71, 3.54 2.88, 3.25	2.08 (brs, NH)	$CDCl_3$
19a	7.78	7.88 (d)	6.98	7.43	2.85, 3.14 3.55	2.70 (brs, NH);	DMSO
19c	7.16	7.73 (s)		7.50	2.98, 3.23 3.54	1.87 (brs, NH)	$CDCl_3$
20a	7.54	7.66 (d)	6.70	7.31	1.48, 3.25 2.91, 3.75	1.87 (brs, NH)	$CDCl_3$
20c	7.23	7.78 (d)		7.45	1.48, 3.24 2.88, 3.18	5.03 (s, NH_2)	$CDCl_3$

Table 2
Physical data of 1,8-naphthyridine derivatives



Comp.	R	R_1	R_2	Yield (%)	M.p. (°C)	Recrystallization solvent
4a	Cep	H	Cep	91	233–235	petroleum ether 100–140°C
4b	Cep	NO_2	Cep	93	266–268	toluene
4c	Cep	NH_2	Cep	94	110–112	petroleum ether 100–140°C
5a	Morph	H	Morph	88	218–220	toluene
5b	Morph	NO_2	Morph	91	180–182	toluene
5c	Morph	NH_2	Morph	93	245–247	toluene
6a	Pip	H	Pip	88	154–156	petroleum ether 100–140°C
6b	Pip	NO_2	Pip	93	110–112	petroleum ether 100–140°C
6c	Pip	NH_2	Pip	95	135–137	toluene
10a	Cep	H	Morph	81	180–182	petroleum ether 100–140°C
10b	Cep	NO_2	Morph	96	115–117	petroleum ether 100–140°C
10c	Cep	NH_2	Morph	91	152–154	petroleum ether 100–140°C
11a	Cep	H	Pip	72	134–136	petroleum ether 100–140°C
11b	Cep	NO_2	Pip	96	110–112	petroleum ether 100–140°C
11c	Cep	NH_2	Pip	90	118–120	petroleum ether 100–140°C
12a	Morph	H	Cep	84	205–207	ethyl acetate
12b	Morph	NO_2	Cep	91	240–242	toluene
12c	Morph	NH_2	Cep	83	140–142	petroleum ether 100–140°C
13a	Morph	H	Pip	92	165–167	petroleum ether 100–140°C
13b	Morph	NO_2	Pip	79	204–206	toluene
13c	Morph	NH_2	Pip	83	143–145	petroleum ether 100–140°C
14a	Pip	H	Cep	89	119–121	toluene
14b	Pip	NO_2	Cep	86	210–212	petroleum ether 100–140°C
14c	Pip	NH_2	Cep	76	136–138	petroleum ether 100–140°C
15a	Pip	H	Morph	98	106–108	petroleum ether 100–140°C
15b	Pip	NO_2	Morph	84	112–114	petroleum ether 100–140°C
15c	Pip	NH_2	Morph	73	136–138	toluene
16a	Pipz	H	Pipz	90	178–180	toluene
17a	Pipz	H	Morph	82	218–220	petroleum ether 100–140°C
17c	Pipz	NH_2	Morph	87	132–134	petroleum ether 100–140°C
18a	Pipz	H	Pip	78	96–98	petroleum ether 100–140°C
18c	Pipz	NH_2	Pip	88	139–141	petroleum ether 100–140°C
19a	Morph	H	Pipz	97	210–212	petroleum ether 100–140°C
19c	Morph	NH_2	Pipz	87	128–130	toluene
20a	Pip	H	Pipz	85	110–112	petroleum ether 100–140°C
20c	Pip	NH_2	Pipz	68	132–134	toluene

Experiments were conducted by the following procedures. Substances at different concentrations, ranging from 10 to 0.1 μM, were added to PRP and incubated for 10 min at 37°C before the addition of the aggregating agent. To express the aggregation of platelets, the transmittance of PRP was set at 0% while the platelet poor plasma (PPP) was set at 100%.

The aggregation rate was also evaluated from the slope of the experimental plot of aggregation as a function of time.

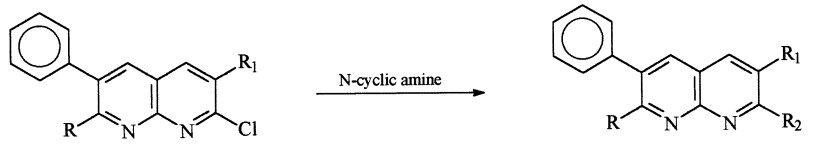
c-AMP was measured in intact platelets by the RIA method.

The test substances were dissolved in DMSO and the stock solution was diluted with water to obtain the experimental concentration. The DMSO solutions of compounds **5a**, **6a**, **10a**, **11a**–**c**, **12a**, **13a**, **14a**–**c**, **15a**,**b**, **17c**, **19c** and **20a** were diluted with water and a few drops of 0.1 M hydrochloric acid. The pH was then set to 7.4 with sodium hydrogen carbonate. Compounds **4c**, **5b**,**c**, **6b**, **10b**,**c**, **12b**,**c**, and **13b**,**c** were insoluble under these experimental conditions.

The final DMSO concentration was 0.5% v/v.

Control aggregation was studied in the presence of DMSO at the same concentration used for treated platelets.

Both adenylate cyclase and intracellular c-AMP levels were measured by the radioimmunoassay technique using commercially available tests (Rianen c-AMP ^{125}I-

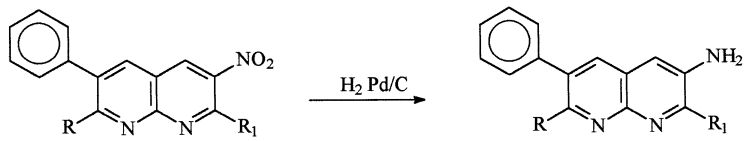


7-9 10-15

	R	R_1
7a	Cep	H
7b	Cep	NO_2
8a	Morph	H
8b	Morph	NO_2
9a	Pip	H
9b	Pip	NO_2

	R	R_1	R_2
10a	Cep	H	Morph
10b	Cep	NO_2	Morph
11a	Cep	H	Pip
11b	Cep	NO_2	Pip
12a	Morph	H	Cep
12b	Morph	NO_2	Cep
13a	Morph	H	Pip
13b	Morph	NO_2	Pip
14a	Pip	H	Cep
14b	Pip	NO_2	Cep
15a	Pip	H	Morph
15b	Pip	NO_2	Morph

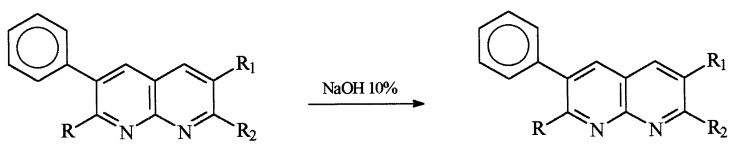
Scheme 2. Synthetic route to variously substituted 2,7-di(*N*-cycloamino)- and 2,7-di(*N*-cycloamino)-6-nitro-1,8-naphthyridine derivatives. Cep = *N*-ethoxycarbonylpiperazin-1-yl, Morph = morpholin-1-yl, Pip = piperidin-1-yl.



4b-6b, 10b-15b **4c-6c, 10c-15c**

	R	R_1
4b,c	Cep	Cep
5b,c	Morph	Morph
6b,c	Pip	Pip
10b,c	Cep	Morph
11b,c	Cep	Pip
12b,c	Morph	Cep
13b,c	Morph	Pip
14b,c	Pip	Cep
15b,c	Pip	Morph

Scheme 3. Reduction of 2,7-di(*N*-cycloamino)-6-nitro-1,8-naphthyridine derivatives. Cep = *N*-ethoxycarbonylpiperazin-1-yl, Morph = morpholin-1-yl, Pip = piperidin-1-yl.



4a, 10a,c-12a,c, 14a,c **16a, 17a,c-20a,c**

	R	R_1	R_2
4a	Cep	H	Cep
10a	Cep	H	Morph
10c	Cep	NH_2	Morph
11a	Cep	H	Pip
11c	Cep	NH_2	Pip
12a	Morph	H	Cep
12c	Morph	NH_2	Cep
14a	Pip	H	Cep
14c	Pip	NH_2	Cep

	R	R_1	R_2
16a	Pipz	H	Pipz
17a	Pipz	H	Morph
17c	Pipz	NH_2	Morph
18a	Pipz	H	Pip
18c	Pipz	NH_2	Pip
19a	Morph	H	Pipz
19c	Morph	NH_2	Pipz
20a	Pip	H	Pipz
20c	Pip	NH_2	Pipz

Scheme 4. Hydrolysis of *N*-ethoxycarbonylpiperazin-1-yl derivatives. Cep = *N*-ethoxycarbonylpiperazin-1-yl, Morph = morpholin-1-yl, Pip = piperidin-1-yl, Pipz = piperazin-1-yl.

RIA kit; ^{32}P-ATP; NEN-Du Pont). Adenylate cyclase was measured in platelet plasma membranes prepared in accordance with the method described by Kahn and Sinha [7] while c-AMP levels were measured in intact platelets. 200 ml of PRP was centrifuged at 200*g* for 30 min. The supernatant was gently decanted, and the soft pellet containing intact platelets was suspended in 12 ml of Tyrode buffer, pH 7.4; the final number of platelets was adjusted to $\approx 10^8$/ml. For each sample, 300-μl aliquots of this suspension were preincubated at 30°C with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (0.5 mM), both in the absence and in the presence of 1 mM EGTA. The compounds to be tested were added where required 10 min later, to a final volume of 500 μl. Incubation was stopped after 10 min by the addition of 1 ml of 3% perchloric acid. Samples were sonicated and centrifuged at 30 000*g* for 15 min. The supernatant was neutralized with an excess (about 100 mg) of $CaCO_3$.

The samples were then centrifuged twice at 30 000*g* for 15 min to remove the excess of $CaCO_3$, and 100 μl aliquots of the supernatant were assayed for their cyclic AMP. c-AMP was measured in triplicate determinations using the above-mentioned RIA kit.

4. Results and discussion

All the substances were subjected to preliminary screening to evaluate their effects at a fixed concentration (10 μM) on the platelet aggregation induced by 0.7 mM arachidonate (Table 3). On the basis of these results, the substances tested were subdivided into two different groups, in accordance with the inhibition values observed, which are expressed by a percentage: (A) from 0% to 50%, from inactive to moderately active; and (B) from 51% onward, really active.

We were unable to determine the efficacy of compounds **4c**, **5b**, **6b**,**c**, **10b**,**c**, **12b**,**c** and **13b**,**c**, because they could not be dissolved by the procedures used. Compounds **4a**,**b**, **5a**,**c**, **6a**, **10a**, **11a**,**b**, **14a**,**b**, **15a**–**c**, **16a**, **17c**, **18a**,**c**, **19a**,**c** and **20a**,**c** displayed a poor activity. The IC_{50} for the dose-dependent inhibition of platelet aggregation induced by arachidonate was evaluated for compounds **11c**, **12a**, **13a**, **14c** and **17a**, which exhibited a considerable activity in the preliminary screening. As shown in Table 4, all the compounds revealed a very low IC_{50}, ranging from 0.7 to 10.0 μM for both parameters measured (inhibition of maximum aggregation and inhibition of the speed of aggregation); these values were higher than those of the reference compounds.

With the aim of excluding a possible selective inhibition of the membrane enzyme phospholipase A_2, the inhibition of the aggregation induced by collagen at a concentration of 2.0 μg/ml was evaluated for compounds **11c**, **12a**, **13a**, **14c** and **17a**. The results shown in Table 4 confirmed high inhibitory activities for these compounds: in particular compound **17a** was extremely active ($IC_{50} < 0.1$ μM).

The collagen-induced aggregation made it possible to evaluate the latency time of aggregation, that is the time, expressed in seconds, between the addition of the agonist and the start of aggregation of platelets. This parameter expresses the delay of platelet aggregation activation mechanisms.

On the basis of results for this parameter, compared with a mean basal value of 72–85 s, all the compounds tested, **11c**, **12a**, **13a** and **17a**, delayed the start of aggregation by 310–360 s, evaluated at a concentration of 10 μM, and by a time lag ranging from 125 to 300 s at a concentration of 5 μM, and from 100 to 250 s at a concentration of 1 μM (Table 5).

To complete the study on the effects of these compounds on platelet aggregation, the dose–effect curve for compounds **11c**, **14c** and **17a** versus the major physiological agonist, ADP, was determined. The results obtained show that compounds **11c**, **14c** and **17a** (Table 6) exhibited a significant biological activity.

The study of intracellular events due to contact of intact platelets with the compounds **14c** and **17a** showed an increase in c-AMP levels, as shown in Table 7, independently of the activation of adenylate cyclase, whose activity was, on the contrary, reduced by the compounds tested (data not shown) and probably mediated by inhibition of phosphodiesterase [2,4].

Further studies on the mechanism of action of these compounds are in progress.

Only compounds **11c**, **12a**, **13a**, **14c** and **17a** showed a significant ability to inhibit the arachidonate- and collagen-induced aggregation of platelets, whereas several compounds were insoluble or showed a poor activity.

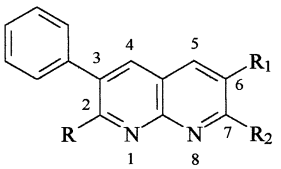
In a previous paper [4] we reported that some 2-*N*-cycloamino-3-phenyl-1,8-naphthyridine derivatives showed an appreciable ability to inhibit in vitro human platelet aggregation induced by arachidonate, collagen or ADP, but on the basis of the pharmacological results, no structure–activity relationship could be deduced. Hoping to obtain a structure–activity relationship, we also synthesized and tested this series of 2,7-di(*N*-cycloamino)-3-phenyl-1,8-naphthyridine derivatives.

The pharmacological results obtained for this last series of compounds have not fully made clear the role of the various substituents, also because in this series, many compounds are insoluble.

To draw a conclusion, we analyzed the pharmacological results of the 2-*N*-cycloamino- [4] and 2,7-di(*N*-cycloamino)-3-phenyl-1,8-naphthyridine derivatives.

The influence of the nitro group in position 6 of the 1,8-naphthyridine nucleus on the inhibition of the aggregation induced by arachidonate in these two series

Table 3
Preliminary screening for inhibition of arachidonate-induced (0.7 mM) platelet rich plasma (PRP) aggregation by compounds tested at a concentration of 10 μM



Compound	R	R_1	R_2	Solvent used	*A* [a]	*B* [a]
4a	Cep	H	Cep	DMSO	5 ± 2	7 ± 3
4b	Cep	NO_2	Cep	DMSO	3 ± 1	7 ± 3
4c	Cep	NH_2	Cep	no sol	n.t.	n.t.
5a	Morph	H	Morph	DMSO–HCl	18 ± 2	10 ± 2
5b	Morph	NO_2	Morph	no sol.	n.t.	n.t.
5c	Morph	NH_2	Morph	DMSO	18 ± 3	0
6a	Pip	H	Pip	DMSO–HCl	29 ± 3	11 ± 2
6b	Pip	NO_2	Pip	no sol.	n.t.	n.t.
6c	Pip	NH_2	Pip	no sol.	n.t.	n.t.
10a	Cep	H	Morph	DMSO–HCl	19 ± 2	4 ± 1
10b	Cep	NO_2	Morph	no sol.	n.t.	n.t.
10c	Cep	NH_2	Morph	no sol.	n.t.	n.t.
11a	Cep	H	Pip	DMSO–HCl	25 ± 3	26 ± 3
11b	Cep	NO_2	Pip	DMSO–HCl	20 ± 3	12 ± 4
11c	Cep	NH_2	Pip	DMSO–HCl	100 ± 0	100 ± 0
12a	Morph	H	Cep	DMSO–HCl	100 ± 0	100 ± 0
12b	Morph	NO_2	Cep	no sol.	n.t.	n.t.
12c	Morph	NH_2	Cep	no sol.	n.t.	n.t.
13a	Morph	H	Pip	DMSO–HCl	98 ± 5	98 ± 5
13b	Morph	NO_2	Pip	no sol.	n.t.	n.t.
13c	Morph	NH_2	Pip	no sol.	n.t.	n.t.
14a	Pip	H	Cep	DMSO–HCl	19 ± 4	5 ± 2
14b	Pip	NO_2	Cep	DMSO–HCl	22 ± 3	28 ± 6
14c	Pip	NH_2	Cep	DMSO–HCl	100 ± 0	100 ± 0
15a	Pip	H	Morph	DMSO–HCl	20 ± 2	8 ± 3
15b	Pip	NO_2	Morph	DMSO–HCl	28 ± 3	18 ± 5
15c	Pip	NH_2	Morph	DMSO	22 ± 4	27 ± 4
16a	Pipz	H	Pipz	DMSO	0	4 ± 2
17a	Pipz	H	Morph	DMSO	95 ± 6	85 ± 9
17c	Pipz	NH_2	Morph	DMSO–HCl	26 ± 4	28 ± 2
18a	Pipz	H	Pip	DMSO	17 ± 2	20 ± 2
18c	Pipz	NH_2	Pip	DMSO	20 ± 3	26 ± 2
19a	Morph	H	Pipz	DMSO	22 ± 5	26 ± 4
19c	Morph	NH_2	Pipz	DMSO	28 ± 3	24 ± 5
20a	Pip	H	Pipz	DMSO–HCl	28 ± 6	25 ± 5
20c	Pip	NH_2	Pipz	DMSO–HCl	24 ± 3	21 ± 3

[a] (*A*) = % Inhibition of maximal aggregation, (*B*) = % inhibition of aggregation rate. Values are mean ± SD of at least three independent experiments. n.t. = Not tested.

of compounds seems to be unimportant. Only one compound of the previous series showed an appreciable activity [4].

On the contrary, the amino group in position 6 of the heterocyclic ring seems to play a more important role, because it is altogether present in five compounds of the two series, which showed an appreciable activity.

The order of effectiveness of the substituents in position 2 seems to be morpholin-1-yl > piperidin-1-yl > piperazin-1-yl = 4-ethoxycarbonylpiperazin-1-yl.

The substitution of a chloro or methoxy group in position 7, which seems to favor an appreciable activity, as reported in a previous paper [4], with an *N*-cycloamino group, such as morpholin-1-yl, piperidin-1-yl or 4-ethoxycarbonylpiperazin-1-yl generally resulted in the maintenance or an increase of activity.

The compounds which present the same *N*-cycloamino groups in positions 2 and 7 showed unimportant activity.

In the collagen test, the compounds showed an activity similar to that obtained in the arachidonate test, as reported in previous papers [2,4].

Table 4
Inhibition of platelet rich plasma (PRP) aggregation induced by 0.7 mM arachidonic acid and 2.0 μg/ml collagen

Comp.	IC_{50} (μM) [a]			
	Arachidonic acid		Collagen	
	A	*B*	*A*	*B*
11c	4.6 ± 1.0	2.9 ± 1.0	2.2 ± 1.0	1.4 ± 0.5
12a	10.4 ± 2.0	6.9 ± 1.0	5.3 ± 1.2	5.7 ± 0.9
13a	4.3 ± 1.5	2.2 ± 1.0	3.8 ± 1.0	3.4 ± 0.8
14c	2.2 ± 1.0	0.8 ± 0.3	5.7 ± 2.1	5.9 ± 1.5
17a	0.7 ± 0.5	0.5 ± 0.3	0.08 ± 0.05	0.07 ± 0.03
ASA [b]	30.0 ± 2.0	36.0 ± 3.1	42.0 ± 5.6	54.0 ± 5.2
Pap [c]	16.0 ± 0.3	20.9 ± 2.0	66.0 ± 1.5	
Ibu [d]	4.2 ± 0.5		6.2 ± 0.4	
Indo	1.5 ± 0.5	1.8 ± 0.4	1.9 ± 0.6	1.6 ± 0.5

[a] IC_{50} of compounds calculated as % inhibition of maximal aggregation (*A*) and % of aggregation rate (*B*) induced by 0.7 mM arachidonic acid and 2.0 μg/ml collagen. Values are mean ± SE of at least three independent experiments.
[b] ASA: acetylsalicylic acid.
[c] Pap: papaverine.
[d] Ibu: ibuprofen. Value obtained from reference [8].

Table 5
Effects of compounds tested on latency time (s) of collagen-induced aggregation (2.0 μg/ml)

Comp.	Comp. dose (μM) [a]			
	10	5	1	0
11c	360″ ± 0	250″ ± 30	130″ ± 20	72″ ± 10
12a	310″ ± 30	125″ ± 20	100″ ± 15	85″ ± 10
13a	330″ ± 25	160″ ± 30	105″ ± 10	85″ ± 10
17a	360″ ± 0	300″ ± 40	250″ ± 25	72″ ± 10
Basal value	72–85″ ± 10			

[a] Values are mean ± SE of at least three independent experiments. The basal value is the mean of latency times of aggregation without the test compounds.

Table 6
IC_{50} (μM) of active compounds in ADP-induced platelet aggregation (3.0 μM)

Compound	IC_{50} [a]	
	Inhibition of maximal aggregation value	Inhibition of aggregation rate
11c	32.2 ± 8.0	31.8 ± 6.5
14c	27.2 ± 5.0	30.8 ± 6.3
17a	21.0 ± 5.5	19.7 ± 4.9
Pap [b]	121.0 ± 5.5	104.0 ± 6.1

[a] Values are mean ± SE of at least three independent experiments.
[b] Pap: papaverine.

Table 7
Percentage increase of c-AMP levels in intact platelets [a]

Compound	(μM)	Increase (%)
14c	10	21.0 ± 3.1
	20	55.0 ± 4.5
17a	10	85.0 ± 6.1
	20	104.0 ± 3.5

[a] A probability level of $P<0.05$ was considered statistically significant (Student's *t*-test). Values are mean ± SE of at least three independent experiments, each performed in triplicate. The basal value of c-AMP was16.15 ± 0.55 pmol × 10^8 cells.

The three compounds tested for their ability to inhibit the aggregation induced by ADP showed a significant activity.

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References

[1] P.L. Ferrarini, C. Mori, M. Criscuoli, Study of inhibition of platelet aggregation of 1,8-naphthyridine derivatives, Farmaco 44 (1989) 579–584.
[2] P.L. Ferrarini, C. Mori, M. Miceli, F. Franconi, Synthesis and antiplatelet activity of some 1,8-naphthyridine derivatives, Eur. J. Med. Chem. 29 (1994) 735–741.
[3] P.L. Ferrarini, C. Mori, M. Badawneh, C. Manera, A. Martinelli, M. Miceli, F. Romagnoli, G. Saccomanni, Unusual nitration of substituted 7-amino-1,8-naphthyridine in the synthesis of compounds with antiplatelet activity, J. Heterocyclic Chem. 34 (1997) 1501–1510.
[4] P.L. Ferrarini, C. Mori, M. Badawneh, F. Franconi, C. Manera, M. Miceli, G. Saccomanni, Synthesis and antiplatelet activity of some 3-phenyl-1,8-naphthyridine derivatives, Farmaco 55 (2000) 603–610 (and references therein).
[5] M. Miceli, F. Bennardini, A. Mattana, J. Covarrubias, G. Seghieri, F. Franconi, Taurine modifies platelet sensitivity to aspirin, indomethacin, sodium nitroprusside and papaverine, Platelets 3 (1992) 265–269.
[6] G.V.R. Born, M.J. Cross, Aggregation of blood platelets, J. Physiol. 168 (1963) 178–195.
[7] N.N. Kahn, A.K. Sinha, Inhibition of prostaglandin E1-induced activation of adenylate cyclase in human blood platelet membrane, Biochim. Biophys. Acta 972 (1988) 45–53.
[8] J.R. Vane, S.H. Ferreira, Handbook of Experimental Pharmacology, vol. 50/II, Springer, Berlin, 1979, p. 321 (Table 4).