ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 55 (2000) 603–610

IL FARMACO

Synthesis and antiplatelet activity of some 3-phenyl-1,8-naphthyridine derivatives

Pier Luigi Ferrarini [a,*], Claudio Mori [a], Muwaffag Badawneh [b], Flavia Franconi [c], Clementina Manera [a], Mauro Miceli [d], Giuseppe Saccomanni [a]

[a] *Dipartimento di Scienze Farmaceutiche, Università di Pisa, via Bonanno 6, 56126 Pisa, Italy*
[b] *Philadelphia University, P.O. Box 1101, Sweileh, Jordan*
[c] *Dipartimento del Farmaco, Università di Sassari, via Muroni 23A, 07100 Sassari, Italy*
[d] *Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Università di Pisa, sez. via Bonanno 6, 56126 Pisa, Italy*

Received 3 April 2000; accepted 31 July 2000

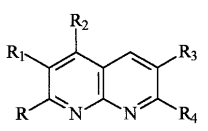
Abstract

A series of 2-cycloalkylamino-3-phenyl-1,8-naphthyridine derivatives, variously substituted in the 6- and 7-positions were synthesized and tested for their ability to inhibit human platelet aggregation in vitro induced by arachidonate, collagen and ADP. Compounds **5a**,**b**, **7a**,**b**, **8a** and **10c**,**d** showed a remarkable activity similar to that of indomethacin in the test with arachidonate and collagen. In the test with ADP only compound **8a** showed a significant activity. The presence of a morpholinyl or piperidinyl group in position 2 and of a chloro or methoxy group in position 7 of the 1,8-naphthyridine nucleus seem to favour a higher activity. However on the basis of the pharmacological results, no structure–activity relationship can be deduced. Compounds **5b** and **7b**, which possess the best activity in the arachidonate test, were also shown to increase the c-AMP level significantly, without involving the adenylyl cyclase system.

Keywords: 3-Phenyl-1,8-naphthyridine; Inhibition of platelet aggregation; Human blood platelets; Morpholinyl; Piperidinyl; Piperazinyl

1. Introduction

In the course of investigations on platelet aggregation inhibitors, some compounds carrying an *N*-ethanolamine or *N*-diethanolamine side chain attached to a heterocyclic nucleus were characterized for this activity in the 1970s. Among these, dipyridamole attracted the highest interest as a platelet aggregation inhibitor drug [1,2].



1

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

In consideration of the alleged effects on platelet aggregation of compounds bearing the 1,6-naphthyridine nucleus isoster of 1,8-naphthyridine [3], we studied some 2- or 4-*N*-diethanolamine-1,8-naphthyridine derivatives (Fig. 1). These compounds displayed a remarkable ability to inhibit aggregation of rabbit and rat platelets challenged with arachidonate, collagen or ADP [4].

We then decided to examine the correlation between dipyridamole and the 1,8-naphthyridine heterocyclic system. A new series of 1,8-naphthyridine derivatives carrying a piperidyl group or an *N*-diethanolamine side chain in the 2-, 7- or 2,7-positions, were therefore synthesized and tested for their ability to inhibit in vitro human platelet aggregation induced by arachidonate, collagen or ADP. Some compounds showed a remarkable activity in the test with arachidonate and collagen, whereas in the test with ADP the compounds tested did not show any significant activity [5].

Several 2-(dialkylamino)chromones and 4-(1-piperazinyl)coumarins, as well as their angular benzo-fused

* Corresponding author. Tel. +39-050-500209; fax: +39-050-40517.
E-mail address: ferrarini@farm.unipi.it (P.L. Ferrarini).

0014-827X/00/$ - see front matter
PII: S0014-827X(00)00085-9

benzopyranones and naphthopyranones [6], dialkylpyrimido[1,2-*a*]quinolin-1-ones and dialkylpyrimido[2,1-*a*]isoquinolin-4-ones [7] and some substituted pyridazinones [8] were prepared and tested in vitro for their inhibitory activity on human platelet aggregation.

In a recent paper, we reported the synthesis and the antiplatelet activity of 1,8-naphthyridines (**1**) (Fig. 1), carrying piperidyl, carbethoxypiperazinyl or morpholinyl groups in the 2- or 7-position. Some compounds were tested for their ability to inhibit the arachidonate- and collagen-induced aggregation of human platelets. Only compound **1a** (R = morpholinyl, $R_1 = R_3 = H$, $R_2 = Cl$, $R_4 = OH$) revealed an interesting activity: the IC_{50} values (μM), calculated as the concentration which reduced by 50% both inhibition of maximal aggregation induced by 0.7 mM arachidonate in human platelets in rich plasma, and inhibition of aggregation rate, are 2.0 ± 1.0 and 0.3 ± 0.2 respectively [9].

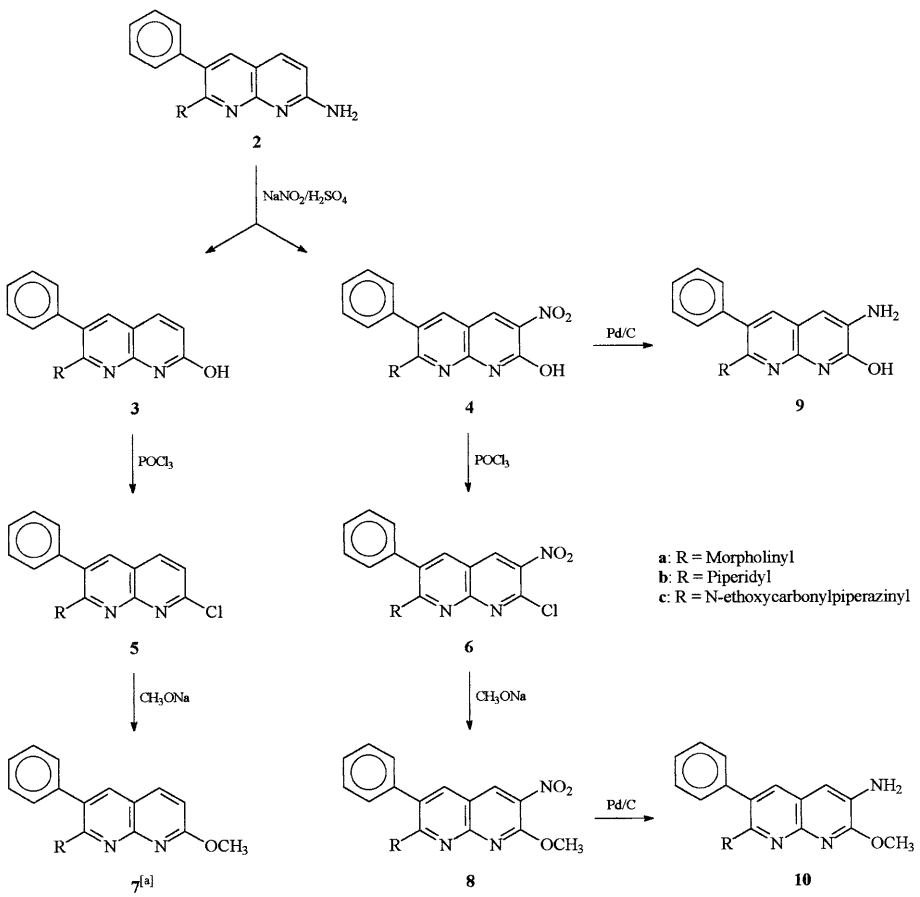
The compounds reported [1–9] carry various substituents on the heterocyclic ring, such as dialkylamino, piperidyl, piperazinyl, morpholinyl or phenyl groups.

In light of these considerations, we decided to continue our chemical and pharmacological investigations in this field, in order to evaluate the effects of structural modification on antiplatelet activity. A series of derivatives of 3-phenyl-1,8-naphthyridine carrying a morpholinyl, piperazinyl or piperidyl group in the 2-position were consequently prepared and tested in vitro for their inhibitory activity on human platelet aggregation.

2. Chemistry

As illustrated in Scheme 1, the known 1,8-naphthyridine derivatives **2** were converted to the starting compounds **3** and **4** by diazotization, as reported in a previous paper [9].

The 2-hydroxy-1,8-naphthyridines **3** and **4** were heated at 90°C with phosphoryl chloride to give the corresponding 2-chloro derivatives **5** and **6**, which, when refluxed for 4 h with sodium methoxyde in methanol, gave the 2-methoxyderivatives **7a**,**b** and **8** (Scheme 1, Tables 1 and 2). Compound **5c** was refluxed



[a]**7c**: R= N-methoxycarbonylpiperazinyl

Scheme 1. Synthetic route to 1,8-naphthyridine derivatives variously substituted in the 6- and 7-position.

with sodium methoxide in methanol for 12 h to give the 7-methoxy-3-phenyl-2-(4-methoxycarbonylpiperazin-1-yl)-1,8-naphthyridine (**7c**). Selective reduction of nitro derivatives **4** and **8** was performed in methanol, in the presence of Pd/C as the catalyst, to give the corresponding amino derivatives **9** and **10** (Scheme 1). Compounds **2d**, **3d**, **7d**, **9d** and **10d** were then obtained by alkaline hydrolysis of the compounds **2c** [9], **3c** [9], **7c**, **9c** and **10c** respectively (Scheme 2, Tables 1 and 2).

3. Experimental

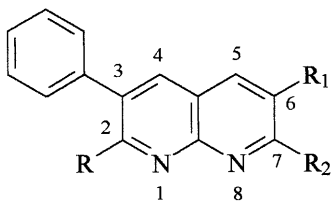
3.1. Chemistry

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 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 compounds synthesized for C, H and N were within $\pm 0.4\%$ of theoretical values and were performed by our analytical laboratory.

3.1.1. General procedure for the preparation of 2-chloro derivatives **5** and **6**

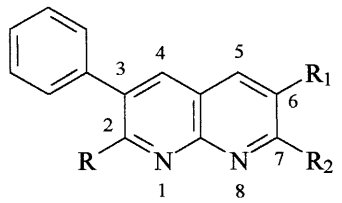
A suspension of 1.0 g of 2-hydroxy derivatives **3** [9], **4** [9] or **12** in 10 ml of phosphoryl chloride was heated at 90°C for 45 min and, after cooling, crushed ice was added. The solution was then made basic with concentrated ammonium hydroxide (pH $\cong$ 8) and the solid was collected by filtration, washed with water and purified by crystallization to give **5** and **6** (Tables 1 and 2).

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



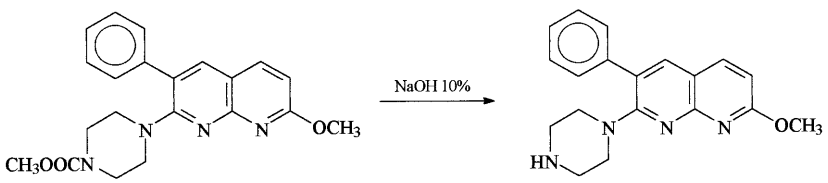
Comp.	H_4 (s)	H_5	H_6 (d)	C_6H_5 (m)	OCH_3 (s)	 (m)	Others	Solvent
2d	7.67	7.77(d)	6.59	7.56		2.83, 3.30	1.84 (brs,NH); 5.07 (brs,NH_2)	$CDCl_3$
3d	7.71	7.72(d)	6.22	7.47		2.55, 3.06		DMSO
5a	7.75	7.90(d)	7.20	7.48		3.30, 3.55		$CDCl_3$
5b	8.04	8.22(d)	7.33	7.48		1.48, 3.13		DMSO
5c	8.22	8.32(d)	7.35	7.50		3.25	1.31 (t,CH_3); 4.00 (q,CH_2)	DMSO
6a	7.80	8.55(s)		7.46		3.55		$CDCl_3$
6b	8.08	8.90(s)		7.51		1.48, 3.33		DMSO
6c	7.93	8.67(s)		7.56		3.50	1.23 (t,CH_3); 4.13 (q,CH_2)	$CDCl_3$
7a	7.98	8.10(d)	6.82	7.51	3.98	3.16, 3.52		DMSO
7b	7.91	8.05(d)	6.80	7.50	3.98	1.45, 3.11		DMSO
7c	8.06	8.15(d)	6.88	7.60	3.99	3.25	3.58 (s,$COOCH_3$)	$CDCl_3$
7d	7.90	8.28(d)	6.83	7.52	3.98	2.72, 3.10		DMSO
8a	8.12	8.93(s)		7.50	4.09	3.30, 3.50		DMSO
8b	8.05	8.90(s)		7.43	4.02	1.43, 3.27		DMSO
8c	7.89	8.67(s)		7.57	4.03	3.40	1.26 (t,CH_3); 3.90 (q,CH_2)	DMSO
9a	6.79	7.51(s)		7.47		3.17, 3.63	6.54 (brs,NH_2)	$CDCl_3$
9b	6.24	6.69(s)		7.36		1.46, 3.06	6.43 (brs,NH_2)	$CDCl_3$
9c	6.61	7.52(s)		7.41		3.25	1.21 (t,CH_3); 4.02 (q,CH_2); 5.21 (brs,NH_2)	DMSO
9d	6.68	7.22(s)		7.40		2.79, 3.05	2.15 (brs,NH); 4.23 (brs,NH_2)	$CDCl_3$
10a	7.00	7.57(s)		7.40	4.19	3.19, 3.56		$CDCl_3$
10b	7.08	7.49(s)		7.39	4.17	1.41, 3.28		$CDCl_3$
10c	7.01	7.59(s)		7.42	4.20	3.27	1.23 (t,CH_3); 4.14 (q,CH_2); 3.90 (brs,NH_2)	$CDCl_3$
10d	6.99	7.57(s)		7.33	4.20	2.83, 3.13	1.82 (brs,NH)	$CDCl_3$

Table 2
Physical data of 1,8-naphthyridine derivatives [a]

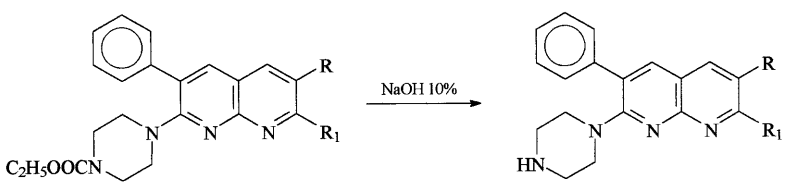


Comp.	R	R_1	R_2	Yield (%)	M.p. (°C)	Recrystallization solvent
2d	Pipz	H	NH_2	48	246–248	benzene
3d	Pipz	H	OH	61	208–210	petr. ether 100–140°C
5a	Morph	H	Cl	84	205–207	petr. ether 100–140°C
5b	Pip	H	Cl	76	170–172	petr. ether 100–140°C
5c	Cep	H	Cl	68	154–156	petr. ether 100–140°C
6a	Morph	NO_2	Cl	83	158–160	petr. ether 100–140°C
6b	Pip	NO_2	Cl	82	162–164	$EtOH/H_2O$
6c	Cep	NO_2	Cl	79	218–220	petr. ether 100–140°C
7a	Morph	H	OCH_3	77	128–130	petr. ether 100–140°C
7b	Pip	H	OCH_3	75	168–170	petr. ether 100–140°C
7c	Cmp	H	OCH_3	68	188–190	benzene
7d	Pipz	H	OCH_3	75	240–242	petr. ether 100–140°C
8a	Morph	NO_2	OCH_3	69	190–192	petr. ether 100–140°C
8b	Pip	NO_2	OCH_3	72	148–150	petr. ether 100–140°C
8c	Cep	NO_2	OCH_3	82	208–210	petr. ether 100–140°C
9a	Morph	NH_2	OH	85	204–206	toluene
9b	Pip	NH_2	OH	86	165–167	toluene
9c	Cep	NH_2	OH	71	220–222	toluene
9d	Pipz	NH_2	OH	67	140–142	petr. ether 100–140°C
10a	Morph	NH_2	OCH_3	85	130–132	petr. ether 100–140°C
10b	Pip	NH_2	OCH_3	70	182–184	toluene
10c	Cep	NH_2	OCH_3	79	173–175	toluene
10d	Pipz	NH_2	OCH_3	70	120–122	petr. ether 100–140°C

[a] Cep, *N*-ethoxycarbonylpiperazinyl; Cmp, *N*-methoxycarbonylpiperazinyl; Morph, morpholinyl; Pip, piperidinyl; Pipz, piperazinyl.



7c **7d**



2c,3c,9c,10c **2d,3d,9d,10d**

2: R= H ; R_1= NH_2
3: R= H ; R_1= OH
9: R= NH_2 ; R_1= OH
10: R= NH_2 ; R_1= OCH_3

Scheme 2. Hydrolysis of methoxy- and ethoxycarbonylpiperazino derivatives.

*3.1.2. General procedure for the preparation of 2-methoxy derivatives **7** and **8***

A solution of 10 mmol of freshly prepared sodium methoxide and 1.0 mmol of the 2-chloro derivatives **5** or **6** in 10 ml of anhydrous methanol was refluxed for 4 h for **5a**,**b** and **6** or 12 h for **5c** and the reaction mixture was evaporated to dryness in vacuo. The crude residue was treated with water and neutralized with 10% hydrochloric acid, and the solid precipitate, collected by filtration, was purified by crystallization to obtain **7** and **8** (Tables 1 and 2).

*3.1.3. General procedure for the preparation of 3-amino derivatives **9** and **10***

A solution of 1.1 mmol of 3-nitro derivatives **4** or **8** in glacial acetic acid was hydrogenated in the presence of 0.03 g of 10% palladium on charcoal at room temperature and at atmospheric pressure for 3 h. The catalyst was filtered and the solvent evaporated to dryness in vacuo to give compounds **9** and **10**, which were purified by crystallization (Tables 1 and 2).

*3.1.4. General procedure for the preparation of piperazin-7-yl-1,8-naphthyridine derivatives **2d**, **3d**, **7d**, **9d** and **10d***

A suspension of 1.0 mmol of the appropriate *N*-ethoxycarbonylpiperazinyl derivatives **2c** [9], **3c** [9], **9c**, **10c** or *N*-methoxycarbonylpiperazinyl **7c**, 15 ml of ethanol and 15 ml of 10% sodium hydroxide was refluxed for 16 h and the organic solvent was evaporated in vacuo. The aqueous solution was extracted with chloroform, dried ($MgSO_4$) and evaporated to dryness in vacuo to give compounds **2d**, **3d**, **7d**, **9d** and **10d**, 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. [10]. 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 [11], using an aggregometer (Daichii model PA-3220).

ADP (3.0 μM), arachidonate sodium (0.7 mM) and collagen (2.0 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.

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 itself 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.

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 **2d**, **3d**, **5a**–**c**, **6a**,**c**, **7a**–**c**, **8a**, and **10b**,**c** 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 **6b**, **8b**,**c** and **9b**–**d**, 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 level were measured by a radioimmunoassay technique using commercially available tests (Rianen c-AMP ^{125}I-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 [12], 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 ca. 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 the presence of 1 mM EGTA. 10 min later, the compounds to be tested were added where required 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

To evaluate the antiplatelet properties, the substances were subjected to a preliminary screening estimating the effects of a fixed concentration (10 μM) on the platelet aggregation induced by 0.7 mM arachidonate (Table 3). It was thus possible to determine the inhibition of the maximum aggregation due to the agonist, and the speed of aggregation or 'slope' which gives the amount of platelets aggregating in the time unit, whose levels are expressed as a percentage.

On the basis of these results, the substances tested were subdivided into two different groups, in accordance with the inhibition values observed: from 0 to 50%, from inactive to moderately active; from 51% onward, really active

Compounds **2d**, **3d**, **5c**, **6a**,**c**, **7c**,**d**, **9a** and **10b** displayed a poor activity, while we were unable to state the efficacy of compounds **6b**, **8b**,**c** and **9b**–**d**, because they could not be dissolved by the procedures used. The IC_{50} for the dose-dependent inhibition of platelet aggregation induced by arachidonate was evaluated for compounds **5a**,**b**, **7a**,**b**, **8a**, **10a**,**c** and **10d**. As shown in Table 4, all the compounds exhibited a very low IC_{50}, ranging from 1.0 to 6.0 μM for both parameters, similar to values of the reference compounds, with the exception of **10a** for the maximal aggregation.

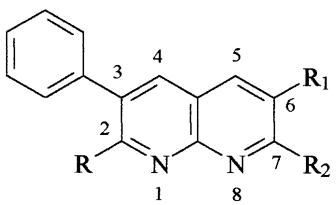
With the aim of excluding a possible selective inhibition of the membrane enzyme phospholipase A_2, the inhibition of the aggregation induced by collagen at a concentration of 2.0 μg/ml was evaluated for compounds **5a**, **7a**,**b** and **8a**, which were active towards arachidonate. The results obtained confirmed high inhibitory activities for these compounds (IC_{50} ranging from 0.6 to 6.0 μM), as shown in Table 4, similar to the values seen in the arachidonate patterns.

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 s, we can state that all the compounds tested, **5a**, **7a**,**b** and **8a**, delayed the start of aggregation by 360 s evaluated at a concentration of 10 μM and by a time lag ranging from 168 to 177 s at the 5.0 μM concentration (Table 5).

To complete the study on the effects of these compounds on platelet aggregation, the dose/effect curve towards the major physiological agonist ADP was plotted. The results obtained show that only compound **8a** exhibited a significant biological activity ($IC_{50} = 23.0 \pm$

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



Comp.	R	R_1	R_2	Solvent used	A	B
2d	Pipz	H	NH_2	DMSO–HCl	3 ± 1	5 ± 2
3d	Pipz	H	OH	DMSO–HCl	19 ± 4	15 ± 6
5a	Morph	H	Cl	DMSO–HCl	99 ± 3	100 ± 0
5b	Pip	H	Cl	DMSO–HCl	97 ± 3	100 ± 0
5c	Cep	H	Cl	DMSO–HCl	10 ± 2	27 ± 7
6a	Morph	NO_2	Cl	DMSO–HCl	8 ± 3	0
6b	Pip	NO_2	Cl	not sol.	n.t.	n.t.
6c	Cep	NO_2	Cl	DMSO–HCl	9 ± 3	12 ± 4
7a	Morph	H	OCH_3	DMSO–HCl	95 ± 4	100 ± 0
7b	Pip	H	OCH_3	DMSO–HCl	95 ± 5	94 ± 5
7c	Cmp	H	OCH_3	DMSO–HCl	0	2 ± 1
7d	Pipz	H	OCH_3	DMSO	4 ± 2	6 ± 3
8a	Morph	NO_2	OCH_3	DMSO–HCl	93 ± 6	100 ± 0
8b	Pip	NO_2	OCH_3	not sol.	n.t.	n.t.
8c	Cep	NO_2	OCH_3	not sol.	n.t.	n.t.
9a	Morph	NH_2	OH	DMSO	25 ± 3	28 ± 6
9b	Pip	NH_2	OH	not sol.	n.t.	n.t.
9c	Cep	NH_2	OH	not sol.	n.t.	n.t.
9d	Pipz	NH_2	OH	not sol.	n.t.	n.t.
10a	Morph	NH_2	OCH_3	DMSO	58 ± 5	42 ± 6
10b	Pip	NH_2	OCH_3	DMSO–HCl	12 ± 3	18 ± 4
10c	Cep	NH_2	OCH_3	DMSO–HCl	65 ± 7	60 ± 5
10d	Pipz	NH_2	OCH_3	DMSO	89 ± 6	82 ± 9

[a] A, % inhibition of maximal aggregation; B, % inhibition of aggregation rate. Values are means ± SD of at least three independent experiments. n.t. not tested.

Table 4
Inhibition of platelet rich plasma (PRP) aggregation induced by 0.7 mM arachidonate and 2.0 μg/ml collagen [a]

Compound	IC_{50} (μM)			
	Arachidonate		Collagen	
	A	B	A	B
5a	3.4 ± 1.2	2.0 ± 0.5	5.8 ± 1.5	4.5 ± 1.0
5b	1.3 ± 0.8	1.4 ± 1.0	n.t.	n.t.
7a	5.5 ± 1.5	5.0 ± 1.0	3.2 ± 1.5	0.6 ± 0.2
7b	1.8 ± 1.0	1.2 ± 1.0	5.7 ± 2.1	5.9 ± 1.5
8a	6.0 ± 1.0	6.1 ± 1.5	3.5 ± 2.0	1.3 ± 0.7
10a	31.7 ± 2.2	6.3 ± 1.5	n.t.	n.t.
10c	5.7 ± 2.0	2.9 ± 1.5	n.t.	n.t.
10d	2.3 ± 1.2	1.0 ± 0.5	n.t.	n.t.
ASA	30.0 ± 2.0	36.0 ± 3.1	42.0 ± 5.6	54.0 ± 5.2
Pap	16.0 ± 0.3	20.9 ± 2.0	66.0 ± 1.5	
Ibu [b]	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 arachidonate and 2.0 μg/ml collagen. Values are means ± SE of at least three independent experiments. nt, not tested; ASA, acetylsalicylic acid; Pap, papaverine; Ibu, ibuprofen; Indo, indomethacin.
[b] Value obtained from [13].

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

Comp.	10 μM	5 μM
5a	360 ± 0	170 ± 12
7a	360 ± 0	177 ± 15
7b	360 ± 0	170 ± 10
8a	360 ± 0	168 ± 15
Basal value	72 ± 7	

[a] Values are means ± SE from 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) [a]

Comp.	IC_{50}	
	Inhibition of maximal aggregation value	Inhibition of aggregation rate
5a	>50	>50
5b	40.0 ± 5.5	38.5 ± 6.2
7b	>50	>50
8a	23.0 ± 7.0	21.4 ± 6.7
Pap	121.0 ± 5.5	104.0 ± 6.1

[a] Values are means ± SE from at least three independent experiments. Pap, papaverine.

7.0 μM), while the remaining substances exhibited a lower inhibitory activity (Table 6), which does not support a probable mechanism of action in which we can accept the selective inhibition of phosphodiesterase by these compounds, as reported in a previous paper for similar compounds [5].

All the compounds synthesized generally showed a poor solubility. In particular the presence of the substituent in position 6 leads to a lowering of the solubility, and consequently some compounds of this class were not tested.

The activity of the compounds tested does not seem to be clearly related to the substituent present in the different positions of the 3-phenyl-1,8-naphthyridine nucleus, as reported in previous papers [5,9].

However, on the basis of the above pharmacological results, it is interesting to point out that in the arachidonate test, the most effective substituents in position 2 of the 1,8-naphthyridine ring system seem to be the morpholinyl and piperidyl groups.

The influence of the amino or nitro group, in position 6 of the heterocyclic ring, on the inhibition of aggregation induced by arachidonate in these series of compounds is not clear at this time, most probably because of the limited pharmacological data obtained.

When an amino or hydroxy group was introduced into position 7 of the 1,8-naphthyridine nucleus, compounds were obtained that show an unimportant activity (3–25% of inhibition at a concentration of 10 μM), as reported in a previous paper [9]. On the contrary, substituents such as a chloro or methoxy group, in the same position, seem to favour an increase in activity. All the compounds that show a remarkable activity present a chloro or methoxy group in position 7.

In the collagen test, the compounds tested showed an activity similar to that obtained in the arachidonate test, as reported in a previous paper [5].

Four compounds were also tested for their ability to inhibit the aggregation induced by ADP: only one compound showed a significant activity.

In conclusion, on the basis of the above pharmacological results, no structure–activity relationship can be deduced, at this time.

Regarding the possible inhibitory action, the functional studies performed do not permit us, at this time, to assign to the compounds tested a certain mechanism of action. The study of intracellular events due to contact of intact platelets with compounds **5b** and **7b** showed a significant 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.

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

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

Comp.	(μM)	% Increase
5b	10	25.0 ± 5.1
	20	85.0 ± 4.5
7b	10	75.0 ± 7.1
	20	84.0 ± 3.5

[a] A probability level of $P < 0.05$ was considered statistically significant (Students' t-test). Values are means $\pm$ SE from at least three independent experiments, each performed in triplicate. The basal value of c-AMP was 16.15 ± 0.55 pmol $\times 10^8$ cells.

Acknowledgements

We thank Dr Fabio Naro, Istituto di Istologia Medica, Università La Sapienza, Roma, for radioimmunoassay measurements. This work was supported by a grant from the Ministero dell'Università e della Ricerca Scientifica (40%).

References

[1] L.J. Czuba, Annual Reports in Medicinal Chemistry, vol. 6, Academic Press, NY, 1970, pp. 64–66.
[2] L. Caprino, F. Borrelli, R. Falchetti, Effect of 4,5-diphenyl-2-bis-(2-hydroxyethyl)aminoxazol (Ditazol) on platelet aggregation, adhesiveness and bleeding time, Arzneimittel-Forsch. 23 (1973) 1277–1283.
[3] G. Satzinger, J. Hartenstein, K. Mannardt, J. Kleinschroth, M. Herrmann, E. Fritschi, H.D. Tauschel, B. Wagner, G. Wolf, 1,6-Naphthyridinone derivatives and their use in the treatment of vascular diseases, Ger Offen. DE 3,327,650; C.A. 103: 71301e (1985).
[4] P.L. Ferrarini, C. Mori, M. Criscuoli, Study of inhibition of platelet aggregation of 1,8-naphthyridine derivatives, Farmaco 44 (1989) 579–584.
[5] 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.
[6] M. Di Braccio, G. Roma, G. Leoncini, M. Poggi, Pyran derivatives XIX. (dialkylamino) substituted 1-benzopyranones and naphthopyranones with platelet antiaggregating activity, Farmaco 50 (1995) 703–711.
[7] M. Di Braccio, G. Roma, G. Leoncini, 1,2-Fused pyrimidines VII. 3-(dialkylamino)-1H-pyrimido[1,2*a*]quinolin-1-ones and 2-(dialkylamino)-4H-pyrimido[2,1*a*]isoquinolin-4-ones as antiplatelet compounds, Eur. J. Med. Chem. 30 (1995) 27–38.
[8] V. Dal Piaz, G. Ciciani, M.P. Giovannoni, Synthesis of 4,5-functionalized-2-methyl-6-(substituted aryl)-3(2*H*)-pyridazinones: a new group of potent platelet aggregation inhibitors, Farmaco 52 (1997) 173–178.
[9] 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.
[10] M. Miceli, F. Bennardini, A. Mattana, J. Covarrubias, G. Seghieri, F. Franconi, Taurine modifies platelet sensivity to aspirin, indomethacin, sodium nitroprusside and papaverine, Platelets 3 (1992) 265–269.
[11] G.V.R. Born, M.J. Cross, Aggregation of blood platelets, J. Physiol. 168 (1963) 178–195.
[12] 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.
[13] J.R. Vane, S.H. Ferreira, Handbook of Experimental Pharmacology, vol. 50/II, Springer, Berlin, 1979.