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1,8-Naphthyridines IX. Potent anti-inflammatory and/or analgesic activity of a new group of substituted 5-amino[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamides, of some their Mannich base derivatives and of one novel substituted 5-amino-10-oxo-10*H*-pyrimido[1,2-a][1,8]naphthyridine-6-carboxamide derivative

Mario Di Braccio [a, *], Giancarlo Grossi [a, *], Silvana Alfei [b], Vigilio Ballabeni [c], Massimiliano Tognolini [c], Lisa Flammini [c], Carmine Giorgio [c], Simona Bertoni [c], Elisabetta Barocelli [c]

[a] *Dipartimento di Farmacia, Sezione di Chimica del Farmaco e del Prodotto Cosmetico, Università di Genova, Viale Benedetto XV, 3, I-16132 Genova, Genova, Italy*
[b] *Dipartimento di Farmacia, Sezione di Chimica e Tecnologie Farmaceutiche e Alimentari, Università di Genova, Via Brigata Salerno, 13, I-16147 Genova, Italy*
[c] *Dipartimento di Farmacia, Università di Parma, Parco Area delle Scienze 27/A, I-43124 Parma, Italy*

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ABSTRACT

A new group of 5-(alkylamino)-9-isopropyl[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine derivatives bearing a CONHR group at the 6-position (**1c**–**g**), designed to obtain new effective analgesic and/or anti-inflammatory agents, were synthesized and tested along with three new 9-alkyl-5-(4-alkyl-1-piperazinyl)-*N*,*N*-diethyl [1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamides (**2b**–**d**). Besides, a new class of analogues of compounds **1** and **2**, bearing a Mannich base moiety at the 9-position (**12a**–**d**), as well as the novel *N*,*N*-diethyl-5-(isobutylamino)-8-methyl-10-oxo-10*H*-pyrimido[1,2-*a*][1,8]naphthyridine-6-carboxamide (**15**) were prepared and tested. Compounds **1c**–**g** exhibited very interesting anti-inflammatory properties in rats, whereas compounds **2b**–**d** and **15** proved to be endowed with prevalent analgesic activity frequently associated with sedative effects in mice. On the contrary, the Mannich bases **12a**–**d** resulted inactive. The most effective (80% inhibition of oedema) and potent (threshold dose 1.6 mg kg^{-1} with 31% inhibition of oedema) anti-inflammatory compound **1d** did not show gastrolesive effects following 100 mg kg^{-1} oral administration in rats.

1. Introduction

On the basis of the pharmacological data concerning different series of original tricyclic compounds published in previous papers [1–5], a number of 5-amino[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamides proved to be potent analgesic and/or anti-inflammatory agents, depending on the substituents present in the common scaffold. Namely, through experimental findings obtained in rodents we were able to identify the 5-isobutylamino compounds **1a** [1] and **1b** [5] as effective anti-inflammatory agents, whereas the 5-(4-methyl-1-piperazinyl)derivative **2a** [3], in addition to a moderate anti-inflammatory activity, exhibited very potent analgesic properties: all these triazolonaphthyridine derivatives were devoid of acute gastrolesivity [4,5].

Compounds **1a** and **2a**, labelled as NF 161 and NF 177, respectively, were subjected to an in vitro study which showed that their anti-inflammatory activity reasonably derives from inhibitory effects on adhesion, evoked by several pro-inflammatory stimuli, of human polymorphonuclear cells (PMNs) to human umbilical vascular endothelial cells (HUVEC), as well as from their inhibition of superoxide anion production and of myeloperoxidase release, without involving the cyclooxygenase (COX) inhibition, in accordance with the absence of acute gastrolesivity [6]. This pharmacological profile was interesting since it positively responds to the

* Corresponding authors.
E-mail addresses: dbraccio@unige.it (M. Di Braccio), grossig@unige.it (G. Grossi).

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requirement of a reduced gastrolesivity that has been pursued, during the past decade, through the development of various structural classes of NSAIDs with improved gastric tolerability mainly thanks to their ability to selectively inhibit COX-2 isoenzyme [7].

In the present work we further investigate the structure–activity relationship within the 5-amino substituted [1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamide class through fine chemical modulations of the two more interesting molecules synthesized by us within these studies: **1b** (which proved to be an anti-inflammatory agent better than **1a** [5]) and the best analgesic compound **2a** (Fig. 1). With this purpose, three subset of new compounds related to compounds **1b** and **2a** were developed and tested for biological activity (see Scheme 1). The first group (compounds **1c**–**g**) includes analogues of **1b** in which the 9-isopropyl group is maintained on the triazole ring, while the alkyl groups both on the 5-amino substituent and on the 6-carboxamide function are properly varied; the second subset (compounds **2b**–**d**) consists of close structural analogues of the 5-(4-methyl-1-piperazinyl)derivative **2a**. A third subseries of compounds (**12a**–**d**) is characterized by a Mannich base moiety inserted in the triazole ring in place of 9-alkyl group, while at the positions 5 and 6 of the [1,2,4]triazolo[4,3-*a*][1,8]naphthyridine scaffold are maintained the substituents which previously have displayed the best pharmacological results, i.e. the 5-isopropylamino or 5-(4-methyl-1-piperazinyl) substituents and the *N,N*-diethyl 6-carboxamide group, respectively. Furthermore a single variation of the heterocyclic ring fused with the 1,8-naphthyridine system was examined by synthesizing the *N,N*-diethyl-5-(isobutylamino)-8-methyl-10-oxo-10*H*-pyrimido[1,2-a][1,8]naphthyridine-6-carboxamide **15**, with the aim to evaluate the effects of such molecular change on the anti-inflammatory and/or analgesic activities.

All the compounds were studied in vivo for their anti-inflammatory (in rat paw oedema test) and analgesic (in mice writhing test) activities. The molecules endowed with a statistically significant antinociceptive effect were tested also for the inhibition of the spontaneous locomotor activity in mice to ascertain the presence of sedative effects confounding writhing test data. For the compound **1d**, which proved to be the most interesting one among all new synthesized compounds because of its marked and potent anti-inflammatory activity without any acute gastrolesivity in rats, the in vitro antiplatelet activity was also determined as well as ability to inhibit NO_2^- production in cell cultures treated with lipopolysaccharides (LPS).

2. Chemistry

In Scheme 1 are reported the synthetic routes to target compounds **1c**–**g**, **2b**–**d**, **12a**–**d**, and **15**. Thus, the reaction of ester **3** [8] heated in a closed vessel (160 °C, 16 h) with an ethanol solution of suitable primary amines gave good yields of 1,2-dihydro-4-hydroxy-2-oxo-1,8-naphthyridine-3-carboxamides **4a**,**c**,**d**, which in turn were converted into 2,4-dichloroderivatives **5a**,**c**,**d**, by reaction with $POCl_3$ at reflux for 1 h (compounds **5a**,**c**) or with the Vilsmeier reagent in refluxing chloroform (2h) (compound **5d**). From the subsequent cyclocondensation of the dichloroderivatives **5a**,**c**,**d** with isobutyrohydrazide (Dowtherm A, 160 °C, 30 min), the 5-chloro-9-isopropyl[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamide derivatives **6a**,**c**,**d** were obtained in moderate yields along with small amounts of the corresponding 5-hydroxy analogues **7a**,**c**,**d**, very likely derived through hydrolysis of 5-chloroderivatives **6a**,**c**,**d** in the reaction medium (the *N*-ethyl carboxamide derivatives **4b**, **5b** and **6b** have been previously described by us [4]). As a last step, the treatment of **6a**–**d** with an excess of isopropylamine (compounds **1c**,**d**,**f**,**g**) or 3-pentylamine (compound **1e**) (ethanol solution, closed vessel, 140 °C, 16 h) afforded high yields of the desired compounds **1c**–**g**.

The reaction of 9-alkyl-5-chloro-*N,N*-diethyl[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamides **8a** [4] or **8b** [3] with suitable 1-alkylpiperazines in dimethyl sulfoxide (130 °C, 2 h) afforded the corresponding 5-(4-alkyl-1-piperazinyl)derivatives **2b**–**d** in good yields.

On the other hand, the reaction of morpholine or 1-methylpiperazine with ethyl bromoacetate (molar ratio 2:1, dry benzene solution, room temperature) gave the corresponding ethyl (dialkylamino)acetates, which were evaporated to dryness and directly treated with an equimolar amount of hydrazine hydrate (ethanol solution, closed vessel, 120 °C, 8 h) to yield the desired (dialkylamino)acetic acid hydrazides **9a**,**b** (overall yields 52–58%). The subsequent cyclocondensation of hydrazides **9a**,**b** with the 2,4-dichloro-*N,N*-diethyl-1,8-naphthyridine-3-carboxamide **10** [9] (Dowtherm A, 160 °C, 90 min), gave moderate yields of 9-(dialkylamino)methyl-5-chloro-*N,N*-diethyl[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamide derivatives **11a**,**b**, which in turn, by reaction with an excess of isopropylamine (ethanol solution, closed vessel, 140 °C, 16 h) or 1-methylpiperazine (dimethyl sulfoxide solution, 130 °C, 2 h), gave good yields of desired compounds **12a**,**c** or **12b**,**d**, respectively.

Finally, an ethanolic solution of 2-chloro-*N,N*-diethyl-4-(isobutylamino)-1,8-naphthyridine-3-carboxamide **13** [10] was treated with an excess of 30% aqueous ammonia (closed vessel, 130 °C, 24 h) to give the corresponding 2-aminoderivative **14** in high yield. This one was cyclocondensed with an excess of ethyl acetoacetate in the presence of a catalytic amount of monohydrate *p*-toluenesulfonic acid (Dowtherm A, 180 °C, 20 h) to afford the expected *N,N*-diethyl-5-(isobutylamino)-8-methyl-10-oxo-10*H*-pyrimido[1,2-*a*][1,8]naphthyridine-6-carboxamide **15** in rather low yield (30%).

The structures attributed to the compounds described in this paper are consistent with the results of elemental analyses and IR, ^{1}H NMR and ^{13}C NMR spectral data (see Section 5.1.). In the ^{1}H NMR

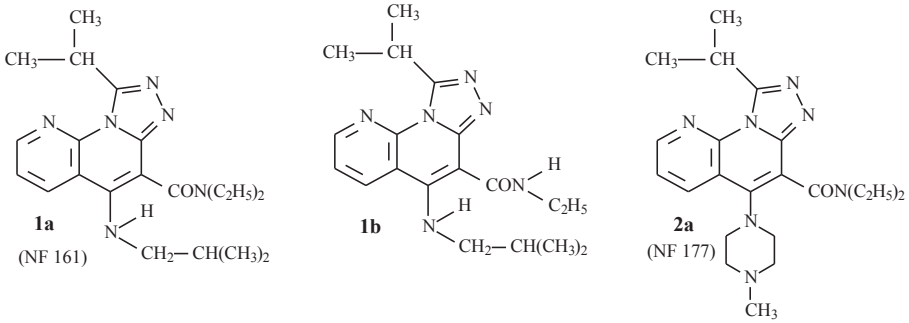
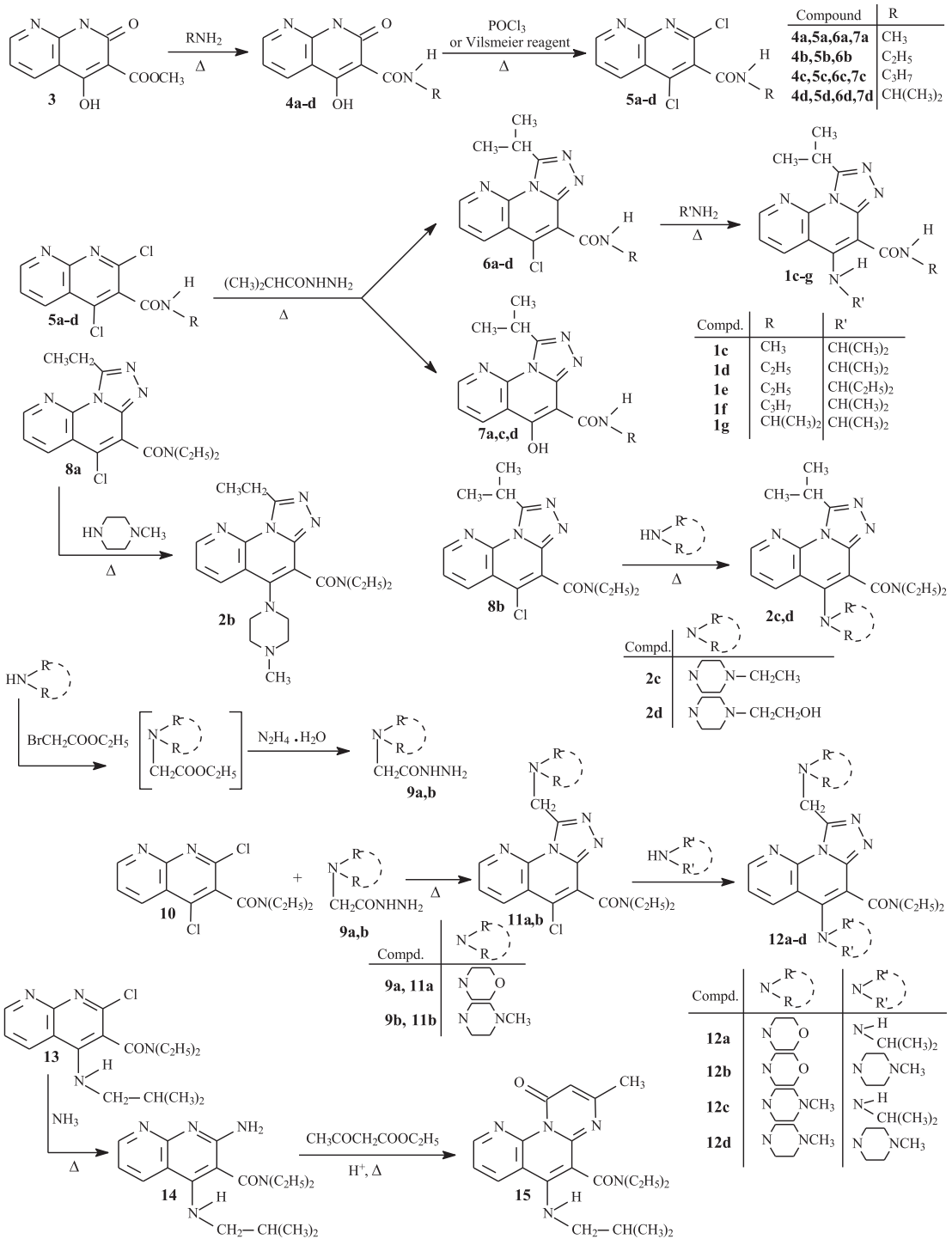


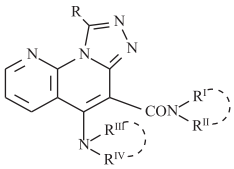
Fig. 1. Structures of the substituted 5-amino[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamides **1a**, **1b** and **2a**, taken as lead compounds.



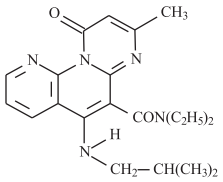
Scheme 1. Synthetic routes to the [1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamide derivatives **1c**–**g**, **2b**–**d**, **12a**–**d** and to the *N*,*N*-diethyl-5-(isobutylamino)-8-methyl-10-oxo-10*H*-pyrimido[1,2-*a*][1,8]naphthyridine-6-carboxamide **15**.

spectra ($CDCl_3$) of diethylamide derivatives **2b**–**d**, **12a**–**d**, **14** and **15**, as previously observed in the case of analogous compounds [11], the complex pattern of CH_2 signals indicates that the methylene protons are diastereotopic, thus suggesting the chirality of these molecules (atropisomerism due to the hindered rotation around the $-CONEt_2$ bond because of the presence of an adjacent secondary or tertiary alkylamino group). Particular evidence of this situation is given by the ^1H NMR spectra of the Mannich bases **12a**–**d** in which the 9-CH_2 signals appear as an AB system ($J = 14.2$ Hz) whose centres are situated in the ranges δ 4.47–4.52 ppm and 4.55–4.63 ppm, respectively (compounds **12b**–**d**), whereas for **12a** the AB system is centred at δ 4.84 ppm and 4.92 ppm. On the other hand, in the case of less hindered 5-chloroderivatives **11a**,**b** the 9-CH_2 protons are almost isochronous and the 9-CH_2 signal appears as a broad singlet at δ 4.80 ppm (compound **11a**) or a sharp singlet at δ 4.63 ppm (compound **11b**), respectively.

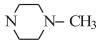
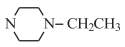
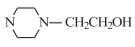
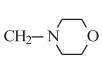
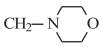
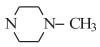
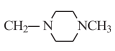
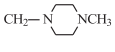
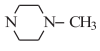
Table 1
Structures and pharmacological data of compounds **1c–g**, **2b–c**, **12b–c** and **15**.



1c-g 2b-d 12a-d



15

Compd.	R	$N(R^{I})(R^{II})$	$N(R^{III})(R^{IV})$	Dose (mg kg^{-1} oral)	Analgesic activity[a] (% inhibition)	Anti-inflammatory activity[b] (% inhibition)	Spontaneous mice locomotor activity[c]
1c	$CH(CH_3)_2$	$NH(CH_3)$	$NH(CH(CH_3)_2)$	100	67*[d]	50*	Sedation
				50	11	3	
1d	$CH(CH_3)_2$	$NH(C_2H_5)$	$NH(CH(CH_3)_2)$	100	66*	80**	Sedation
				50	64**[d]	72**	
				25	15	57**	
				12.5	–	54**	
				6.25	–	40*	
				3.12	–	31*	
				1.6	–	31*	
				0.8	–	0	
1e	$CH(CH_3)_2$	$NH(C_2H_5)$	$NH(CH(C_2H_5)_2)$	100	34	60**	
				50	41	35*	
				25	0	30*	
				12.5	–	12	
1f	$CH(CH_3)_2$	$NH(C_3H_7)$	$NH(CH(CH_3)_2)$	100	0	72**	
				50	0	63*	
				25	–	70**	
				12.5	–	44**	
				6.25	–	19	
1g	$CH(CH_3)_2$	$NH(CH(CH_3)_2)$	$NH(CH(CH_3)_2)$	100	0	34**	
				50	–	33*	
				25	–	9	
2b	C_2H_5	$N(C_2H_5)_2$		100	95**	39**	Sedation
				50	81*	29*	Sedation
				25	10	22	
2c	$CH(CH_3)_2$	$N(C_2H_5)_2$		100	100**	31*	Sedation
				50	74**	35*	Sedation
				25	72**	36**	Sedation
				12.5	50*	31*	Sedation
				6.25	0	8	
2d	$CH(CH_3)_2$	$N(C_2H_5)_2$		100	85**	0	Sedation
				50	96**	–	Sedation
				25	48*	–	Sedation
				12.5	13	–	
12a		$N(C_2H_5)_2$	$NH(CH(CH_3)_2)$	100	0	0	
12b		$N(C_2H_5)_2$		100	37	8	
12c		$N(C_2H_5)_2$	$NH(CH(CH_3)_2)$	100	0	13	
12d		$N(C_2H_5)_2$		100	0	0	

(*continued on next page*)

Table 1 (*continued*)

Compd.	R			Dose (mg kg^{-1} oral)	Analgesic activity[a] (% inhibition)	Anti-inflammatory activity[b] (% inhibition)	Spontaneous mice locomotor activity[c]
15				100	71**[d]	15	
				50	54**		
				25	0		
Indomethacin				10	84**[e]	51**[e]	

[a] Acetic acid induced writhing in mice.
[b] Carrageenan induced rat paw oedema.
[c] Measured as the distance travelled in 30 min.
[d] *$P < 0.05$,**$P < 0.01$ significance as compared to controls (Student's *t*-test).
[e] Data from Ref. [2].

3. Results and discussion

The analgesic and anti-inflammatory activities of compounds under study (Table 1) have been evaluated in vivo at the initial dose of 100 mg kg^{-1} oral. The compounds exhibiting a statistically significant activity at this dose were further tested at doses decreasing by a factor of two, until statistically significant activity was no longer observed.

In the subset of molecules structurally related to the compound **1b** the prevalence of anti-inflammatory activity over the analgesic effect was clearly detected. In particular, derivative **1d** proved to possess very potent anti-inflammatory activity in the carrageenan-induced paw oedema assay in rats showing a threshold dose of 1.6 mg kg^{-1} oral (about 30% inhibition; $P < 0.05$). Compound **1d** proved to be also the most effective compound within this series in counteracting the oedematous response caused by carrageenan injection, showing a 80% inhibition at 100 mg kg^{-1} and being its 12.5 mg kg^{-1} oral dose quite equiactive with conventional NSAID indomethacin 10 mg kg^{-1} (Table 1). Compound **1d**, at the highest dose tested (100 mg kg^{-1}os) proved to be completely devoid of acute gastrolesivity in rats (0/8 rats with gastric lesions) showing a more favourable tolerability profile than indomethacin (8/8 rats with gastric lesions). It is noteworthy that the substitution of isobutylamino moiety with isopropylamino group in position 5 of the [1,2,4]triazolo[4,3-*a*][1,8]naphthyridine structure yielded the compound **1d** more potent and effective than the parent compound **1b** (Fig. 1) [5] and of the analogue **1a** (Fig. 1) [1,6] previously described as interesting anti-inflammatory agents.

Compound **1d** inhibited in vitro platelet aggregation induced in guinea pig platelet rich plasma (PRP) by arachidonic acid, U46619 [a synthetic analogue of Thromboxane A2 (TXA_2)] and ADP, showing a non acetylsalicylic acid-like potency profile (Table 2). Such results suggest for **1d** antiplatelet activity also a COX-independent mechanism consistent with the findings reported for the analogue **1a** (NF 161) when studied on prostaglandin E2 (PGE_2) and prostacyclin (PGI_2) production by human umbilical vascular endothelial cells (HUVEC) in response to arachidonic acid [6]. Further in vitro data show that compound **1d**, at variance with dexamethasone, does not inhibit LPS-induced nitrite production in the murine macrophage cell line J774, suggesting no interference with inducible nitric oxide synthase (iNOS) pro-inflammatory activity. Indeed, compared with cells treated with LPS in absence of any pharmacological treatment (0.78 ± 0.05 μg mg^{-1} protein), conventional anti-inflammatory drug dexamethasone (1 μM), as expected, significantly inhibited nitrite production (0.39 ± 0.09 μg mg^{-1} protein; −50%; $P < 0.01$), whereas compound **1d** (100 μM) slightly reduced nitrite levels (0.69 ± 0.07 μg mg^{-1} protein; −12%; $P > 0.05$).

As regards the analogues of the lead compound **2a**, all the compounds showed an apparent analgesic activity in writhing test prevailing over the anti-inflammatory activity which was exhibited only by compounds **2c** and **2b** (Table 1). Actually, mice treated with antinociceptive doses of the compounds showed reduced spontaneous locomotor activity with respect to control mice when evaluated by video-analysis (14.9 ± 5.0 m 30 min^{-1} and 16.8 ± 3.6 m 30 min^{-1} respectively travelled by treated animals compared to 26.3 ± 3.5 m 30 min^{-1} for control animals), suggesting the occurrence of confounding sedative effect. Indeed, the fine modulation of the 4-methyl-1-piperazinyl moiety of **2a** performed in derivatives **2c** and **2d** as well as the replacement of 9-isopropyl substituent with a 9-ethyl group (compound **2b**) produced a fall in analgesic activity with respect to the parent compound **2a** that had proved to possess antinociceptive effect at non-sedative doses [3,4].

Unfortunately, the Mannich bases **12a**–**d** were completely inactive as anti-inflammatory or antinociceptive agents up to 100 mg kg^{-1} oral dose.

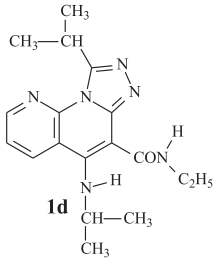
Finally, the *N,N*-diethyl-5-(isobutylamino)-8-methyl-10-oxo-10*H*-pyrimido[1,2-*a*][1,8]naphthyridine-6-carboxamide **15** did not exhibit any appreciable anti-inflammatory activity, whereas showed a certain antinociceptive property being significantly active (54% inhibition in the writhing test in mice, $P < 0.01$) down to the 50 mg kg^{-1} dose without any sedative effect.

4. Conclusions

Taking into account the pharmacological data of the new compounds **1c**–**g**, **2b**–**d**, **12a**–**d** and **15** described in the present paper (Tables 1 and 2), the following conclusions can be drawn.

- The overall good anti-inflammatory activities of compounds **1c**–**g** confirms that the replacement of the 6-CON(C_2H_5)$_2$ substituent of the lead compound **1a**, with a properly monoalkyl substituted 6-carboxamide group afforded very interesting anti-inflammatory agents. The best *N*-alkyl substituents of the carboxamide group were C_2H_5 or C_3H_7 (compounds **1d**,**e**,**f**), whereas the short methyl group (compound **1c**) or the branched isopropyl group (compound **1g**) afforded negative results.
- Maintaining fixed the 6-CONHC_2H_5 and 9-*i*-C_3H_7 substituents, the 5-alkylamino group which proved to be the most effective for the anti-inflammatory activity was the isopropylamino group (compound **1d**), clearly better than the isobutylamino (lead compound **1b** [5]) or 3-pentylamino (compound **1e**) groups.
- The most active compound (**1d**) was a very potent anti-inflammatory agent, which exhibited oedema inhibition values of 80% ($P < 0.01$) and 72% ($P < 0.01$) at the 100 mg kg^{-1} and

Table 2
In vitro inhibitory activity of *N*-ethyl-9-isopropyl-5-(isopropylamino)[1,2,4]triazolo[4,3-*a*][1,8]-naphthyridine-6-carboxamide **1d** on platelet aggregation induced in guinea pig PRP by ADP, arachidonic acid and U46619. Acetylsalicylic acid (ASA) was used as reference drug.



Compound	IC_{50} (μM) ± SD[a]		
	ADP (5.0 μM)	Arachidonic acid (100.0 μM)	U46619 (2.0 μM)
1d	281 ± 24	24 ± 10	89 ± 21
ASA	>500	45 ± 15	>500

[a] IC_{50} = compound concentration which inhibits platelet aggregation by 50%.

50 mg kg^{-1} doses, respectively and it proved to be significantly active down to the 1.6 mg kg^{-1} threshold dose (inhibition 31%, $P < 0.05$). It also showed significant analgesic activity (without sedative effect) only at 50 mg kg^{-1} dose (inhibition 64%, $P < 0.01$). It is noteworthy that **1d** was devoid of gastrolesive effects at the anti-inflammatory dose equiactive with ulcerogenic dose of indomethacin.

- Compound **1d** exhibited also in vitro activity against the platelet aggregation induced in guinea pig PRP by ADP, arachidonic acid and U46619: it is noteworthy to point out that its antiplatelet profile, compared with that of acetylsalicylic acid, suggests additional mode of action other than COX-inhibition, thus confirming previous studies on analogous compounds [6]. The occurrence of non COX-dependent mechanisms could be an interesting feature since also the use of the newer NSAIDs, namely the selective COX-2 inhibitors, has been associated with increased cardiovascular risks raising a question on their safety (withdrawal of rofecoxib and valdecoxib from the market) [12]. In vitro data showing the ineffectiveness of **1d** in reducing nitrite production in LPS-treated murine macrophages seem to rule out that the inhibition of iNOS pro-inflammatory pathway could be one of these additional mechanisms which remain to be clarified.
- The 5-(4-alkyl-1-piperazinyl)derivatives **2b**–**d**, designed as analogues of lead compound **2a** [3,4], exhibited the expected good antinociceptive properties in writhing test in mice, with the 9-isopropyl substituted compounds **2c**,**d** being apparently the most potent analgesic agents. Moreover, it should be emphasized that all these antinociceptive effects were accompanied by a marked sedation which could strongly confound the correct evaluation of analgesic activity.
- The replacement of the 9-alkyl substituent with a Mannich base moiety (compounds **12a**–**d**) resulted in a nearly complete loss of the analgesic/anti-inflammatory properties.
- Inserting a dihydropyrimidinone moiety in place of the triazole ring in a molecule which maintained the same 5-amino and 6-carboxamide groups of lead compound **1a**, the new compound **15** was obtained which exhibited marked, although not potent, analgesic activity (inhibition 71%, $P < 0.01$ and 54%, $P < 0.01$, at the 100 mg kg^{-1} and 50 mg kg^{-1} doses, respectively). On the contrary, compound **15** was nearly inactive in the Winter's assay for anti-inflammatory activity.

5. Experimental protocols

5.1. Chemistry

Melting points were determined using a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer "Spectrum One" spectrophotometer (abbreviations relative to IR bands: br = broad, s = strong, w = weak, sh = shoulder). ^{1}H NMR spectra were recorded partly on a Varian Gemini 200 (200 MHz) spectrometer and partly on a Bruker WM-300 (300 MHz) spectrometer; chemical shifts (δ) are reported in ppm using tetramethylsilane as an internal reference ($\delta = 0$). Spin multiplicities are given as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet). *J* values for H-2, H-3, H-4 signals (dd) of all triazolonaphthyridine derivatives: $J_{2,3} = J_{3,2} = 4.7$ Hz, $J_{2,4} = J_{4,2} = 1.7$ Hz, $J_{3,4} = J_{4,3} = 8.1$ Hz. ^{13}C NMR spectra were acquired on a Bruker DPX spectrometer at 75.5 MHz with tetramethylsilane as internal reference. Analyses were performed by the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, Universita' di Genova. Thin layer chromatograms were run on Merck silica gel 60 F254 precoated plastic sheets (layer thickness 0.2 mm). Column chromatography was performed using Carlo Erba silica gel (0.05–0.20 mm) or Carlo Erba neutral aluminium oxide (Brockmann activity I).

5.1.1. *N*-substituted-1,2-dihydro-4-hydroxy-2-oxo-1,8-naphthyridine-3-carboxamides ***4a***,***c***,***d***

A mixture of 25.0 mmol (5.50 g) of ester **3** [8], 50 mL of ethanol and an excess (10 mL) of propylamine or isopropylamine (compounds **4c** and **4d**, respectively) or 25 mL of 33% methylamine in absolute ethanol (compounds **4a**) was heated in a closed vessel at 150 °C for 16 h. After cooling the resulting suspension was concentrated at reduced pressure. The suspended solid was collected by filtration, washed with a little acetone and dried to give the nearly pure compound **4** which was then crystallized from the proper solvent. According to this procedure the following compounds were obtained:

*5.1.1.1. 1,2-Dihydro-4-hydroxy-N-methyl-2-oxo-1,8-naphthyridine-3-carboxamide (**4a**).* The reaction of **3** with methylamine yielded 5.10 g (93%) of **4a**, whitish needles, m.p. 288–289 °C dec, after crystallization from ethanol. It was impossible to record the ^{1}H NMR spectrum of **4a** due to its insolubility in all conventional solvents. IR (KBr): 3250–2400 (OH + NH), 1667 s and 1617 s (CO), 1572 s, br cm^{-1}. Anal. Calcd for $C_{10}H_9N_3O_3$ (219.20): C, 54.79; H, 4.14; N, 19.17. Found: C, 55.05; H, 4.26; N, 19.03.

*5.1.1.2. 1,2-Dihydro-4-hydroxy-N-propyl-2-oxo-1,8-naphthyridine-3-carboxamide (**4c**).* The reaction of **3** with propylamine yielded 5.75 g (93%) of **4c**, whitish needles, m.p. 232–233 °C, after crystallization from ethanol (Lit. [13], no m.p. reported). IR (KBr): 3250–2400 (OH + NH), 1660 s and 1614 s (CO), 1566 s, br cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 0.93 (t, J = 7.3 Hz, 3H, $CONHCH_2CH_2CH_3$), 1.59 (m, 2H, $CONHCH_2CH_2CH_3$), 3.34 (m, 2H, $CONHCH_2CH_2CH_3$; t, $J = 7.3$ Hz, after treatment with D_2O), 7.35 (dd, $J_{6,5} = 8$ Hz, $J_{6,7} = 4.8$ Hz, 1H, H-6), 8.36 (dd, $J_{5,6} = 8$ Hz, $J_{5,7} = 1.6$ Hz, 1H, H-5), 8.69 (dd, $J_{7,6} = 4.8$ Hz, $J_{7,5} = 1.6$ Hz, 1H, H-7), 10.24 (broad s, 1H, $CONHCH_2CH_2CH_3$; disappeared with D_2O), 12.27 (s, 1H, 1-NH; disappeared with D_2O); (4-OH signal was not detectable). Anal. Calcd for $C_{12}H_{13}N_3O_3$ (247.25): C, 58.29; H, 5.30; N, 16.99. Found: C, 58.07; H, 5.45; N, 17.12.

*5.1.1.3. 1,2-Dihydro-4-hydroxy-N-isopropyl-2-oxo-1,8-naphthyridine-3-carboxamide (**4d**).* The reaction of **3** with isopropylamine yielded 5.56 g (90%) of **4d**, whitish needles, m.p.

238–239 °C, after crystallization from acetone (Lit. [14], no m.p. reported). IR (KBr): 3250–2450 (OH + NH), 1658 s and 1616 s (CO), 1558 s, br cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 1.24 [d, J = 6.6 Hz, 6H, CONHCH(CH_3)$_2$], 4.11 [m, 1H, CONHCH(CH$_3$)$_2$], 7.35 (dd, $J_{6,5}$ = 8 Hz, $J_{6,7}$ = 4.8 Hz, 1H, H-6), 8.35 (dd, $J_{5,6}$ = 8 Hz, $J_{5,7}$ = 1.6 Hz, 1H, H-5), 8.69 (dd, $J_{7,6}$ = 4.8 Hz, $J_{7,5}$ = 1.6 Hz, 1H, H-7), 10.15 [d, J = 6.8 Hz, 1H, CONHCH(CH$_3$)$_2$; disappeared with D_2O], 12.28 (s, 1H, 1-NH; disappeared with D_2O); (4-OH signal was not detectable). ^{13}C NMR (75.5 MHz, DMSO-d_6, ppm): δ 22.06, 40.81, 95.99, 109.85, 118.73, 133.35, 133.38, 154.00, 163.29, 169.45, 172.12. Anal. Calcd for $C_{12}H_{13}N_3O_3$ (247.25): C, 58.29; H, 5.30; N, 16.99. Found: C, 58.37; H, 5.51; N, 16.63.

5.1.2. *N-substituted-2,4-dichloro-1,8-naphthyridine-3-carboxamides **5a**,**c***

A mixture of 5.0 g of **4a** (22.81 mmol) or **4c** (20.22 mmol) and 50 mL of $POCl_3$ was stirred at 110 °C for 1 h. The excess of $POCl_3$ was removed by heating at reduced pressure and the residue was dissolved in warm water; the resulting dark solution (300 mL) was cooled, carefully treated with $NaHCO_3$ up to pH 7; CH_2Cl_2 (200 mL) was added and the mixture was stirred at room temperature for 30 min, decolourized with charcoal, filtered and transferred in a separatory funnel. The organic layer was collected and the aqueous one was exhaustively extracted with CH_2Cl_2. The combined extracts (dried over anhydrous Na_2SO_4 and evaporated to dryness at reduced pressure) afforded a thick oil which was chromatographed on a silica gel column, eluting first with the mixture CH_2Cl_2–EtOAc (2:1) to discard some impurities, then with the mixture CH_2Cl_2–EtOAc–acetone (2:1:1) to recover the proper compound **5**. From the eluate collected, after removal of solvents, the following compounds were obtained:

5.1.2.1. *2,4-Dichloro-N-methyl-1,8-naphthyridine-3-carboxamide (**5a**).*

The reaction carried out with **4a** gave 3.05 g (52%) of **5a**, white crystals, m.p. 231–232 °C, after crystallization from acetone/petroleum ether. IR (KBr): 3262 (NH), 1649 s (CO), 1589, 1548 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 2.87 (d, J = 4.7 Hz, 3H, CONHCH_3; s, after treatment with D_2O), 7.89 (dd, $J_{6,5}$ = 8 Hz, $J_{6,7}$ = 4.8 Hz, 1H, H-6), 8.74 (dd, $J_{5,6}$ = 8 Hz, $J_{5,7}$ = 1.6 Hz, 1H, H-5), 8.80 (broad q, 1H, CONHCH$_3$; disappeared with D_2O), 9.24 (dd, $J_{7,6}$ = 4.8 Hz, $J_{7,5}$ = 1.6 Hz, 1H, H-7). ^{13}C NMR (75.5 MHz, DMSO-d_6, ppm): δ 25.89, 119.87, 124.50, 131.08, 134.24, 140.79, 149.01, 153.51, 155.96, 162.55. Anal. Calcd for $C_{10}H_7Cl_2N_3O$ (256.09): C, 46.90; H, 2.76; N, 16.41. Found: C, 46.91; H, 2.77; N, 16.32.

5.1.2.2. *2,4-Dichloro-N-propyl-1,8-naphthyridine-3-carboxamide (**5c**).*

The reaction carried out with **4c** gave 3.16 g (55%) of **5c**, as an off white resinous solid; white needles, m.p. 118–119 °C, after crystallization from EtOAc/petroleum ether. IR (KBr): 3301 (NH), 1657 s (CO), 1595 w, 1579, 1554 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.06 (t, J = 7.3 Hz, 3H, CONHCH$_2$CH$_2$CH_3), 1.74 (m, 2H, CONHCH$_2$CH_2CH$_3$), 3.51 (m, 2H, CONHCH_2CH$_2$CH$_3$; t, J = 7.3 Hz, after treatment with D_2O), 7.44 (t, J = 7.3 Hz, 1H, CONHCH$_2$CH$_2$CH$_3$; disappeared with D_2O), 7.51 (dd, $J_{6,5}$ = 8 Hz, $J_{6,7}$ = 4.8 Hz, 1H, H-6), 8.28 (dd, $J_{5,6}$ = 8 Hz, $J_{5,7}$ = 1.6 Hz, 1H, H-5), 8.95 (dd, $J_{7,6}$ = 4.8 Hz, $J_{7,5}$ = 1.6 Hz, 1H, H-7). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 11.55, 22.56, 41.94, 119.72, 123.43, 131.15, 134.16, 141.33, 150.62, 153.60, 155.16, 162.64. Anal. Calcd for $C_{12}H_{11}Cl_2N_3O$ (284.14): C, 50.72; H, 3.90; N, 14.79. Found: C, 50.96; H, 3.74; N, 14.62.

5.1.3. *2,4-Dichloro-N-isopropyl-1,8-naphthyridine-3-carboxamide (**5d**)*

To a cooled amount of 300 mmol (21.93 g) of DMF were added dropwise 300 mmol (27.5 mL) of $POCl_3$ and the mixture was stirred at room temperature for 15 min; a suspension of 15.0 mmol (3.71 g) of **4d** in $CHCl_3$ (150 mL) was then added and the resulting mixture was refluxed for 2 h. The final mixture was poured onto ice-water: after hydrolysis of excess of $POCl_3$, the mixture was carefully alkalized with Na_2CO_3, then exhaustively extracted with CH_2Cl_2. The combined extracts (dried over anhydrous Na_2SO_4 and evaporated to dryness at reduced pressure) afforded a thick oil which was chromatographed on a silica gel column, eluting first with the mixture CH_2Cl_2–EtOAc (3:1) to discard several impurities, then with the mixture CH_2Cl_2–EtOAc (1:2) to recover **5d** as a whitish solid (2.09 g, 49%); white needles, m.p. 182–184 °C, after crystallization from EtOAc/petroleum ether. IR (KBr): 3264 (NH), 1644 s (CO), 1586, 1547 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 1.37 [d, J = 6.6 Hz, 6H, CONHCH(CH_3)$_2$], 4.39 [m, 1H, CONHCH(CH$_3$)$_2$], 6.79 [d, J = 6.8 Hz, 1H, CONHCH(CH$_3$)$_2$; disappeared with D_2O], 7.58 (dd, $J_{6,5}$ = 8 Hz, $J_{6,7}$ = 4.8 Hz, 1H, H-6), 8.42 (dd, $J_{5,6}$ = 8 Hz, $J_{5,7}$ = 1.6 Hz, 1H, H-5), 9.07 (dd, $J_{7,6}$ = 4.8 Hz, $J_{7,5}$ = 1.6 Hz, 1H, H-7). Anal. Calcd for $C_{12}H_{11}Cl_2N_3O$ (284.14): C, 50.72; H, 3.90; N, 14.79. Found: C, 50.59; H, 4.09; N, 14.96.

5.1.4. *N-substituted-5-chloro-9-isopropyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamides **6a**,**c**,**d** and N-substituted-5-hydroxy-9-isopropyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamides **7a**,**c**,**d***

A mixture of 8.0 mmol of **5a** (2.05 g) or **5c** (2.27 g) or **5d** (2.27 g), 12.0 mmol (1.23 g) of isobutyrohydrazide and 10 mL of Dowtherm A was stirred at 160 °C for 30 min. After cooling, 10% aqueous Na_2CO_3 (50 mL) and CH_2Cl_2 (50 mL) were added and the mixture was further stirred at room temperature for 30 min. After discarding some insoluble impurities by filtration, the mixture was transferred in a separatory funnel, then the organic layer was collected and the aqueous one was exhaustively extracted with CH_2Cl_2. The combined extracts were dried (anhydrous Na_2SO_4) then evaporated to dryness at reduced pressure, and the residue was chromatographed on a silica gel column eluting first with CH_2Cl_2 to remove Dowtherm A. Then the column was eluted with the mixture CH_2Cl_2–EtOAc (1:1), affording small amounts of the 5-hydroxyderivatives **7**; the subsequent elution with the mixture CH_2Cl_2–EtOAc–acetone (2:1:1) gave the desired compounds **6**. According to this procedure, there were obtained the following compounds:

5.1.4.1. *5-Chloro-9-Isopropyl-N-methyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**6a**) and 5-hydroxy-9-isopropyl-N-methyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**7a**).*

The reaction carried out with **5a** afforded:

*Compound **7a***: 0.09 g, (3.9%); white needles, m.p. 265–266 °C, after crystallization from acetone. ^1H NMR was not recorded because of the insolubility of **7a** in all conventional solvents. IR (KBr): 3170 br (NH + OH), 1643 s (CO), 1612 cm^{-1}. Anal. Calcd for $C_{14}H_{15}N_5O_2$ (285.31): C, 58.94; H, 5.30; N, 24.55. Found: C, 59.06; H, 5.48; N, 24.61.

*Compound **6a***: 0.66 g, (27%); white crystals, m.p. 261–263 °C, after crystallization from EtOAc. IR (KBr): 3284 (NH), 1655 s (CO), 1608 w, 1591 w cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.50 [d, J = 6.8 Hz, 6H, 9-CH(CH_3)$_2$], 3.12 (d, J = 4.8 Hz, 3H, CONHCH_3; s, after treatment with D_2O), 4.44 [m, 1H, 9-CH(CH$_3$)$_2$], 7.65 (dd, 1H, H-3), 8.74–8.81 (m, 2H, H-2,4), 9.04 (broad s, 1H, CONHCH$_3$; disappeared with D_2O). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 21.00, 26.75, 28.10, 118.53, 119.98, 122.51, 133.97, 136.62, 142.76, 148.24, 150.23, 157.25, 161.75. Anal. Calcd. for $C_{14}H_{14}ClN_5O$ (303.75): C, 55.36; H, 4.65; N, 23.06. Found: C, 55.39; H, 4.54; N, 23.34.

5.1.4.2. *5-Chloro-9-Isopropyl-N-propyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**6c**) and 5-hydroxy-9-isopropyl-N-propyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**7c**).*

The reaction carried out with **5c** afforded:

Compound ***7c***: 0.11 g, (4.4%); white needles, m.p. 236–237 °C, after crystallization from acetone. IR (KBr): 3200 br (NH + OH), 1658 sh and 1632 s (CO), 1605 w, 1571 w, 1543 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 0.95 (t, J = 7.3 Hz, 3H, $CONHCH_2CH_2CH_3$), 1.42 [d, J = 6.8 Hz, 6H, 9-$CH(CH_3)_2$], 1.55 (m, 2H, $CONHCH_2CH_2CH_3$), 3.55 (m, 2H, $CONHCH_2CH_2CH_3$; t, J = 7.3 Hz, after treatment with D_2O), 4.41 [m, 1H, 9-$CH(CH_3)_2$], 7.74 (dd, 1H, H-3), 8.73 (dd, 1H, H-4), 8.86 (dd, 1H, H-2), 10.19 (broad t, 1H, $CONHCH_2CH_2CH_3$; disappeared with D_2O), 13.84 (broad s, 1H, OH; disappeared with D_2O). Anal. Calcd for $C_{16}H_{19}N_5O_2$ (313.36): C, 61.33; H, 6.11; N, 22.35. Found: C, 61.18; H, 6.23; N, 22.49.

Compound ***6c***: 1.20 g (45%); white crystals, m.p. 215–216 °C, after crystallization from EtOAc/petroleum ether. IR (KBr): 3278 (NH), 1656 s (CO), 1606 w, 1590 w, 1571 w cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.07 (t, J = 7.3 Hz, 3H, $CONHCH_2CH_2CH_3$), 1.54 [d, J = 6.8 Hz, 6H, 9-$CH(CH_3)_2$], 1.75 (m, 2H, $CONHCH_2CH_2CH_3$), 3.54 (m, 2H, $CONHCH_2CH_2CH_3$; t, J = 7.3 Hz, after treatment with D_2O), 4.47 [m, 1H, 9-$CH(CH_3)_2$], 7.65 (dd, 1H, H-3), 8.74–8.81 (m, 2H, H-2,4), 8.96 (broad s, 1H, $CONHCH_2CH_2CH_3$; disappeared with D_2O). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 11.72, 21.04, 22.57, 28.13, 41.94, 118.52, 120.09, 122.49, 133.87, 136.57, 142.75, 148.32, 150.21, 157.26, 161.05. Anal. Calcd. for $C_{16}H_{18}ClN_5O$ (331.80): C, 57.92; H, 5.47; N, 21.11. Found: C, 57.66; H, 5.55; N, 20.93.

*5.1.4.3. 5-Chloro-N-9-diisopropyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**6d**) and 5-hydroxy-N-9-diisopropyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**7d**).* The reaction carried out with **5d** afforded:

Compound ***7d***: 0.10 g (4%); white needles, m.p. 284–285 °C, after crystallization from acetone. IR (KBr): 3167 br (NH + OH), 1639 s (CO), 1611, 1557, 1541 w cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6, ppm): δ 1.23 [d, J = 6.8 Hz, 6H, $CONHCH(CH_3)_2$], 1.43 [d, J = 6.8 Hz, 6H, 9-$CH(CH_3)_2$], 4.15 [m, 1H, $CONHCH(CH_3)_2$], 4.41 [m, 1H, 9-$CH(CH_3)_2$], 7.78 (dd, 1H, H-3), 8.73 (dd, 1H, H-4), 8.89 (dd, 1H, H-2), 10.15 [broad d, 1H, $CONHCH(CH_3)_2$; disappeared with D_2O], 13.30 (broad s, 1H, OH; disappeared with D_2O). Anal. Calcd for $C_{16}H_{19}N_5O_2$ (313.36): C, 61.33; H, 6.11; N, 22.35. Found: C, 61.20; H, 6.35; N, 22.30.

Compound ***6d***: 1.04 g, (39%); white crystals, m.p. 258–260 °C, after crystallization from EtOAc. IR (KBr): 3265 (NH), 1672 sh and 1650 s (CO), 1602 w, 1588 w, 1564 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 1.37 [d, J = 6.8 Hz, 6H, $CONHCH(CH_3)_2$], 1.59 [d, J = 6.8 Hz, 6H, 9-$CH(CH_3)_2$], 4.40 [m, 1H, $CONHCH(CH_3)_2$], 4.54 [m, 1H, 9-$CH(CH_3)_2$], 7.65 (dd, 1H, H-3), 8.70–8.95 [m, 3H, H-2,4 + $CONHCH(CH_3)_2$; m, 2H after treatment with D_2O]. Anal. Calcd. for $C_{16}H_{18}ClN_5O$ (331.80): C, 57.92; H, 5.47; N, 21.11. Found: C, 58.08; H, 5.30; N, 21.19.

*5.1.5. General procedure for the synthesis of compounds **1c–g***

A mixture of 2.0 mmol of the proper 5-chloroderivative **6**, 25 mL of ethanol and an excess (5 mL) of the proper alkylamine was heated in a closed vessel at 140 °C for 16 h. After cooling the resulting yellow fluorescent solution was evaporated to dryness in vacuo and the residue was partitioned between CH_2Cl_2 (100 mL) and 5% aqueous $NaHCO_3$ (50 mL); the organic layer was collected and the aqueous one was further extracted twice with CH_2Cl_2. The combined organic extracts were dried (anhydrous Na_2SO_4) then evaporated to dryness at reduced pressure, and the residue was chromatographed on a silica gel column eluting with the mixture CH_2Cl_2–EtOAc (1:1) to afford the pure compounds **1c–g** as thick oils which were treated with a small amount of petroleum ether to give yellow crystalline solids, which then were crystallized from an appropriate solvent or solvent mixture. The following compounds were prepared in this way:

*5.1.5.1. 9-Isopropyl-5-(isopropylamino)-N-methyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**1c**).* The reaction carried out with **6a** (0.61 g) and isopropylamine yielded **1c** (0.57 g, 87%); yellow needles, m.p. 161–163 °C, after crystallization from diisopropyl ether. IR (KBr): 3290 br (NH), 1642 s (CO), 1616, 1558 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.33 [d, J = 6.3 Hz, 6H, $HNCH(CH_3)_2$], 1.55 [d, J = 6.8 Hz, 6H, 9-$CH(CH_3)_2$], 3.04 (d, J = 4.7 Hz, 3H, $CONHCH_3$, s after treatment with D_2O), 3.95 [m, 1H, $HNCH(CH_3)_2$], 4.43 [m, 1H, 9-$CH(CH_3)_2$], 7.48 (dd, 1H, H-3), 8.45 (dd, 1H, H-4), 8.73 (dd, 1H, H-2), 9.96 [d, 9.7 Hz, 1H, $HNCH(CH_3)_2$; disappeared with D_2O], 10.57 (broad s, 1H, $CONHCH_3$, disappeared with D_2O). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 21.13, 24.28, 26.08, 28.09, 51.12, 98.94, 115.92, 120.60, 136.62, 144.04, 149.84, 150.03, 152.90, 155.54, 167.55. Anal. Calcd. for $C_{17}H_{22}N_6O$ (326.40): C, 62.56; H, 6.79; N, 25.75. Found: C, 62.40; H, 6.91; N, 25.50.

*5.1.5.2. N-Ethyl-9-isopropyl-5-(isopropylamino)[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**1d**).* The reaction carried out with **6b** [4] (0.64 g) and isopropylamine yielded **1d** (0.58 g, 85%); yellow needles, m.p. 148–149 °C, after crystallization from diisopropyl ether. IR (KBr): 3220 br (NH), 1641 s (CO), 1616, 1556 s cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.30–1.34 [d + t, 6H + 3H, $HNCH(CH_3)_2$ + $CONHCH_2CH_3$], 1.55 [d, J = 6.8 Hz, 6H, 9-$CH(CH_3)_2$], 3.52 (m, 2H, $CONHCH_2CH_3$; q, after treatment with D_2O), 3.95 [m, 1H, $HNCH(CH_3)_2$], 4.43 [m, 1H, 9-$CH(CH_3)_2$], 7.48 (dd, 1H, H-3), 8.45 (dd, 1H, H-4), 8.73 (dd, 1H, H-2), 9.97 [d, J = 9.8 Hz, 1H, $HNCH(CH_3)_2$; disappeared with D_2O], 10.62 (broad s, 1H, $CONHCH_2CH_3$, disappeared with D_2O). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 14.62, 21.14, 24.27, 28.09, 34.38, 51.16, 99.06, 115.96, 120.61, 136.60, 144.03, 149.82, 150.11, 152.99, 155.54, 166.72. Anal. Calcd. for $C_{18}H_{24}N_6O$ (340.43): C, 63.50; H, 7.11; N, 24.69. Found: C, 63.31; H, 7.38; N, 24.59.

*5.1.5.3. N-Ethyl-5-[(1-ethylpropyl)amino]-9-isopropyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**1e**).* The reaction carried out with **6b** [4] (0.64 g) and 3-pentylamine gave **1e** (0.62 g, 84%); yellow needles, m.p. 92.5–94 °C, after crystallization from petroleum ether. IR (KBr): 3235 br (NH), 1630 s (CO), 1606, 1556 s cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 0.99 [t, J = 7.4 Hz, 6H, $HNCH(CH_2CH_3)_2$], 1.33 (t, J = 7.2 Hz, 3H, $CONHCH_2CH_3$), 1.55–1.85 [m, 4H, $HNCH(CH_2CH_3)_2$], 1.59 [d, J = 6.8 Hz, 6H, 9-$CH(CH_3)_2$], 3.45–3.80 [m, 2H + 1H, $CONHCH_2CH_3$ + $HNCH(CH_2CH_3)_2$], 4.50 [m, 1H, 9-$CH(CH_3)_2$], 7.53 (dd, 1H, H-3), 8.54 (dd, 1H, H-4), 8.75 (dd, 1H, H-2), 10.35 [d, J = 9.8 Hz, 1H, $HNCH(CH_2CH_3)_2$; disappeared with D_2O], 10.53 (broad s, 1H, $CONHCH_2CH_3$, disappeared with D_2O). Anal. Calcd. for $C_{20}H_{28}N_6O$ (368.47): C, 65.19; H, 7.66; N, 22.81. Found: C, 65.20; H, 7.77; N, 23.07.

*5.1.5.4. 9-Isopropyl-5-(isopropylamino)-N-propyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**1f**).* The reaction carried out with **6c** (0.66 g) and isopropylamine yielded **1f** (0.64 g, 90%); yellow needles, m.p. 145–146 °C, after crystallization from diisopropyl ether/petroleum ether. IR (KBr): 3225 br (NH), 1639 s (CO), 1613, 1558 s cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$,ppm): δ 1.05 (t, J = 7.3 Hz, 3H, $CONHCH_2CH_2CH_3$), 1.33 [d, J = 6.3 Hz, 6H, $HNCH(CH_3)_2$], 1.55 [d, J = 6.8 Hz, 6H, 9-$CH(CH_3)_2$], 1.72 (m, 2H, $CONHCH_2CH_2CH_3$), 3.46 (m, 2H, $CONHCH_2CH_2CH_3$; t, J = 7.3 Hz, after treatment with D_2O), 3.94 [m, 1H, $HNCH(CH_3)_2$], 4.44 [m, 1H, 9-$CH(CH_3)_2$], 7.48 (dd, 1H, H-3), 8.45 (dd, 1H, H-4), 8.72 (dd, 1H, H-2), 9.97 [d, J = 9.9 Hz, 1H, $HNCH(CH_3)_2$; disappeared with D_2O], 10.65 (broad s, 1H, $CONHCH_2CH_2CH_3$, disappeared with D_2O). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 11.87, 21.14, 22.63, 24.26, 28.09, 41.33, 51.16, 99.18, 115.97, 120.62, 136.60, 144.04, 149.81, 150.12, 152.98, 155.54, 166.84. Anal. Calcd. for $C_{19}H_{26}N_6O$ (354.46): C, 64.38; H, 7.39; N, 23.71. Found: C, 64.20; H, 7.55; N, 23.60.

*5.1.5.5. N-9-diisopropyl-5-(isopropylamino)[1,2,4]triazolo[4,3-a][1,8] naphthyridine-6-carboxamide (**1g**).* The reaction carried out with **6d** (0.66 g) and isopropylamine yielded **1g** (0.61 g, 86%); yellow needles, m.p. 152–154 °C, after crystallization from diisopropyl ether/petroleum ether. IR (KBr): 3211 br (NH), 1622 s (CO), 1575, 1558 s cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 1.34 [2 d overlapped, $J = 6.3$ Hz, 6H + 6H, HNCH(CH_3)$_2$ + CONHCH(CH_3)$_2$], 1.57 [d, $J = 6.8$ Hz, 6H, 9-CH(CH_3)$_2$], 3.95 [m, 1H, HNC*H*(CH_3)$_2$], 4.31 [m, 1H, CONHC*H*(CH_3)$_2$], 4.45 [m, 1H, 9-C*H*(CH_3)$_2$], 7.50 (dd, 1H, H-3), 8.47 (dd, 1H, H-4), 8.75 (dd, 1H, H-2), 10.21 [broad s, 1H, *H*NCH(CH_3)$_2$; disappeared with D_2O], 10.42 (broad s, 1H, CON*H*CH(CH_3)$_2$, disappeared with D_2O). Anal. Calcd. for $C_{19}H_{26}N_6O$ (354.46): C, 64.38; H, 7.39; N, 23.71. Found: C, 64.14; H, 7.56; N, 23.84.

*5.1.6. General procedure for the synthesis of compounds **2b–d***

A mixture of 3.5 mmol of chloroderivative **8a** [4] or **8b** [3], 5 mL of dimethyl sulfoxide and an excess (5 mL) of the proper 1-alkylpiperazine was heated at 130 °C for 2 h, with stirring. Then the resulting solution was poured into water and the mixture was exhaustively extracted with CH_2Cl_2. The combined extracts were dried (anhydrous Na_2SO_4), then concentrated to a small volume at reduced pressure, and finally chromatographed on a silica gel column eluting first with the mixture CH_2Cl_2–EtOAc (1:1) to remove impurities and small amounts of starting compounds. The subsequent elution with the mixture EtOAc–acetone–MeOH (9:9:2) gave solid residues which were taken up in diethyl ether and filtered to give pure compounds **2b–d** as off white solids which were then crystallized from the proper solvents. The following compounds were so obtained:

*5.1.6.1. N,N-9-triethyl-5-(4-methyl-1-piperazinyl)[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**2b**).* The reaction carried out with **8a** [4] (1.16 g) and 1-methylpiperazine yielded **2b** (1.17 g, 84%); white crystals, m.p. 194–195 °C, after crystallization from EtOAc. IR (KBr): 1631 s (CO), 1598, 1582, 1550 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.23 and 1.35 [2 t, $J = 7.2$ Hz, 3H + 3H, N(CH_2CH_3)$_2$], 1.50 (t, $J = 7.4$ Hz, 3H, 9-CH_2CH_3), 2.39 (s, 3H, N–CH_3), 2.70–3.90 [m, 14H, piperazine CH_2's + 9-CH_2CH_3 + N(CH_2CH_3)$_2$], 7.50 (dd, 1H, H-3), 8.43 (dd, 1H, H-4), 8.69 (dd, 1H, H-2). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 11.98, 12.28, 13.57, 22.41, 38.75, 43.77, 46.30, 49.00–52.00 (very broad signal), 55.61, 116.29, 118.27, 121.29, 134.87, 143.66, 144.18, 148.49, 148.60, 152.42, 164.22. Anal. Calcd. for $C_{21}H_{29}N_7O$ (395.51): C, 63.77; H, 7.39; N, 24.79. Found: C, 63.66; H, 7.60; N, 24.97.

*5.1.6.2. N,N-diethyl-5-(4-ethyl-1-piperazinyl)-9-isopropyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**2c**).* The reaction carried out with **8b** [3] (1.21 g) and 1-ethylpiperazine yielded **2c** (1.20 g, 81%); white crystals, m.p. 183–184 °C, after crystallization from EtOAc/diisopropyl ether. IR (KBr): 1634 s (CO), 1604, 1587 w, 1557 w cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.14 (t, $J = 7.2$ Hz, 3H, N–CH_2CH_3), 1.23 and 1.36 [2 t, $J = 7.2$ Hz, 3H + 3H, N(CH_2CH_3)$_2$], 1.49 and 1.58 [2 d, $J = 6.8$ Hz, 3H + 3H, 9-CH(CH_3)$_2$], 2.30–3.74 [m, 12H, piperazine CH_2's + N(CH_2CH_3)$_2$], 2.52 (q, $J = 7.2$ Hz, 2H, N–CH_2CH_3), 4.43 [m, 1H, 9-C*H*(CH_3)$_2$], 7.48 (dd, 1H, H-3), 8.43 (dd, 1H, H-4), 8.68 (dd, 1H, H-2). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 12.02, 12.26, 13.57, 20.78, 21.35, 27.98, 38.76, 43.78, 52.45, 53.33, 53.36, 116.16, 118.40, 121.14, 134.91, 143.82, 144.32, 148.41, 148.45, 156.15, 164.29. Anal. Calcd. for $C_{23}H_{33}N_7O$ (423.56): C, 65.22; H, 7.85; N, 23.15. Found: C, 65.25; H, 7.88; N, 22.98.

*5.1.6.3. N,N-diethyl-5-[(4-(2-hydroxyethyl)-1-piperazinyl)]-9-isopropyl[1,2,4]triazolo[4,3-a][1,8]-naphthyridine-6-carboxamide (**2d**).* The reaction carried out with **8b** [3] (1.21 g) and 1-(2-hydroxyethyl)piperazine yielded **2d** (1.33 g, 86%); white crystals, m.p. 180–182 °C, after crystallization from EtOAc/petroleum ether. IR (KBr): 3343 br (OH), 1635 s (CO), 1605, 1589 w, 1556 w cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 1.25 and 1.38 [2 t, $J = 7.2$ Hz, 3H + 3H, N(CH_2CH_3)$_2$], 1.51 and 1.59 [2 d, $J = 6.8$ Hz, 3H + 3H, 9-CH(CH_3)$_2$], 2.40–3.90 [m, 17H, piperazine CH_2's + N(CH_2CH_3)$_2$ + N–CH_2CH_2OH + OH; m, 16H after treatment with D_2O], 4.45 [m, 1H, 9-C*H*(CH_3)$_2$], 7.55 (dd, 1H, H-3), 8.48 (dd, 1H, H-4), 8.74 (dd, 1H, H-2). Anal. Calcd. for $C_{23}H_{33}N_7O_2$ (439.56): C, 62.85; H, 7.57; N, 22.31. Found: C, 62.57; H, 7.82; N, 22.01.

*5.1.7. Synthesis of (dialkylamino)acetic acid hydrazides **9a,b***

Ethyl bromoacetate (8.35 g, 50 mmol) was added dropwise to a cooled solution of 100 mmol of morpholine (8.71 g) or 1-methylpiperazine (10.02 g) in dry benzene (20 mL) and the mixture was stirred at room temperature for 3 h. The white precipitate (dialkylamine hydrobromide) was then removed by filtration and the filtrate, containing the ethyl (dialkylamino)acetate, was evaporated to dryness in vacuo. To the resulting oil, dissolved in EtOH (50 mL), hydrazine hydrate (2.50 g, 50 mmol) was added and the mixture was heated in closed vessel at 120 °C for 8 h. After removal of solvent, the residue was treated with a little petroleum ether to give the nearly pure hydrazides as white solids.

*5.1.7.1. 4-Morpholinylacetic acid hydrazide (**9a**).* Yield: 4.15 g (52%) m.p. 99–101 °C (Lit. [15] m.p. 103–104 °C).

*5.1.7.2. 4-Methyl-1-piperazinylacetic acid hydrazide (**9b**).* Yield: 5.00 g (58%) m.p. 86–87 °C (Lit. [16] m.p. 87–89 °C).

*5.1.8General procedure for the synthesis of 9-[(dialkylamino) methyl]-5-chloro-N,N-diethyl[1,2,4]triazolo[4,3-a][1,8] naphthyridine-6-carboxamides **11a,b***

The mixture of 2,4-dichloro-*N,N*-diethyl-1,8-naphthyridine-3-carboxamide **10** [9] (2.98 g, 10.0 mmol) and 4-morpholinylacetic acid hydrazide **9a** (2.39 g, 15.0 mmol) or 4-methyl-1-piperazinylacetic acid hydrazide **9b** (2.58 g, 15.0 mmol) and Dowtherm A (10 mL) was stirred at 160 °C for 1.5 h.

After cooling, 10% aqueous Na_2CO_3 (50 mL) and CH_2Cl_2 (50 mL) were added and the mixture was further stirred at room temperature for 30 min. After discarding some insoluble impurities by filtration, the mixture was transferred in a separatory funnel, then the organic layer was collected and the aqueous one was exhaustively extracted with CH_2Cl_2. The combined extracts were dried (anhydrous Na_2SO_4) then evaporated to dryness at reduced pressure, and the residue was chromatographed on a silica gel column eluting first with CH_2Cl_2 to remove Dowtherm A. Then the column was eluted with EtOAc to remove small amounts of starting compound **10** and some impurities; the subsequent elution with the mixture EtOAc–acetone–MeOH (9:9:2) gave oily residues from which, after treatment with a small amount of diisopropyl ether, pure compounds **11a,b** separated out as pale pink crystalline solids, which then were crystallized from an appropriate solvent or solvent mixture. According to this procedure, there were obtained the following compounds:

*5.1.8.1. 5-Chloro-N,N-diethyl-9-(4-morpholinylmethyl)[1,2,4]triazolo [4,3-a][1,8]naphthyridine-6-carboxamide (**11a**).* Obtained from reaction carried out with 4-morpholinylacetic acid hydrazide **9a** (1.58 g, 39%); white crystals, m.p. 161–163 °C, after crystallization from EtOAc/petroleum ether. IR (KBr): 1643 s (CO), 1599, 1585, 1555 w cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 1.20 and 1.41 [2 t, $J = 7.2$ Hz, 3H + 3H, N(CH_2CH_3)$_2$], 2.99 (broad s, 4H, morpholine NCH_2's), 3.33 [q, $J = 7.2$ Hz, 2H, 2H of N(CH_2CH_3)$_2$], 3.55–3.97 [m, 6H, morpholine OCH_2's + 2H of N(CH_2CH_3)$_2$], 4.80 (broad s, 2H, 9-CH_2N), 7.69 (dd, 1H, H-3), 8.61 (dd, 1H, H-4), 8.82 (dd, 1H, H-2).

Anal. Calcd. for $C_{19}H_{23}ClN_6O_2$ (402.88): C, 56.64; H, 5.75; N, 20.86. Found: C, 56.33; H, 5.59; N, 20.81.

*5.1.8.2. 5-Chloro-N,N-diethyl-9-[(4-methyl-1-piperazinyl)methyl][1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**11b**).* Obtained from reaction carried out with 4-methyl-1-piperazinylacetic acid hydrazide **9b** (1.46 g, 35%); white crystals, m.p. 106–107.5 °C, after crystallization from diisopropyl ether. IR (KBr): 1634 s (CO), 1600 w, 1584 w, 1556 w cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 1.17 and 1.38 [2 t, J = 7.2 Hz, 3H + 3H, $N(CH_2CH_3)_2$], 2.38 (s, 3H, N–CH_3), 2.61 and 2.95 (2 broad s, 4H + 4H, piperazine CH_2's), 3.30 [q, J = 7.2 Hz, 2H, 2H of $N(CH_2CH_3)_2$], 3.52–3.95 [m, 2H, 2H of $N(CH_2CH_3)_2$], 4.62 (s, 2H, 9-CH_2N), 7.65 (dd, 1H, H-3), 8.57 (dd, 1H, H-4), 8.79 (dd, 1H, H-2). Anal. Calcd. for $C_{19}H_{23}ClN_6O_2$ (415.93): C, 57.76; H, 6.30; N, 23.57. Found: C, 57.52; H, 6.57; N, 23.52.

*5.1.9. General procedure for the synthesis of compounds **12a,c***

A mixture of 3.0 mmol of the 5-chloroderivatives **11a** or **11b**, 25 mL of ethanol and an excess (5 mL) of isopropylamine was heated in a closed vessel at 140 °C for 16 h. After cooling the resulting solution was evaporated to dryness in vacuo and the residue was partitioned between CH_2Cl_2 (100 mL) and 5% aqueous $NaHCO_3$ (50 mL); the organic layer was collected and the aqueous one was further extracted twice with CH_2Cl_2. The combined organic extracts were dried (anhydrous Na_2SO_4) then evaporated to dryness at reduced pressure, and the oily residue was taken up in a small amount of EtOAc/diisopropyl ether and let to stand at room temperature until the sufficiently pure compound **12a** or **12c**, respectively separated out as yellowish solid which were then crystallized from the proper solvents. The following compounds were prepared in this way:

*5.1.9.1. N,N-diethyl-5-(isopropylamino-9-(4-morpholinylmethyl))[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**12a**).* The reaction carried out with **11a** (1.21 g) yielded **12a** (1.18 g, 92%); pale yellow crystals, m.p. 211–213 °C, after crystallization from EtOAc. IR (KBr): 3370 (NH), 1635 s (CO), 1601 s, 1558 w, 1532 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 1.14–1.43 [m, 12H, $N(CH_2CH_3)_2$ + $HNCH(CH_3)_2$], 2.76–4.00 [m, 13H, morpholine CH_2's + $N(CH_2CH_3)_2$ + $HNCH(CH_3)_2$], 4.43 [d, J = 9.8 Hz, 1H, $HNCH(CH_3)_2$; disappeared with D_2O], 4.84 and 4.91 (AB system, J = 14.2 Hz, 1H + 1H, 9-CH_2N), 7.56 (dd, 1H, H-3), 8.36 (dd, 1H, H-4), 8.72 (dd, 1H, H-2). Anal. Calcd. for $C_{22}H_{31}N_7O_2$ (425.53): C, 62.10; H, 7.34; N, 23.04. Found: C, 61.79; H, 7.56; N, 22.76.

*5.1.9.2. N,N-diethyl-5-(isopropylamino)-9-[(4-methyl-1-piperazinyl)methyl][1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**12c**).* The reaction carried out with **11b** (1.25 g) yielded **12c** (1.04 g, 79%); pale yellow crystals, m.p. 192–194 °C, after crystallization from EtOAc. IR (KBr): 3365 (NH), 1627 s (CO), 1600 s, 1545 s, cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.19 and 1.34 [2 t, J = 7.2 Hz, 3H + 3H, $N(CH_2CH_3)_2$], 1.22 and 1.29 [2 d, J = 6.3 Hz, 3H + 3H, $HNCH(CH_3)_2$], 2.11–3.90 [m, 13H, piperazine CH_2's + $N(CH_2CH_3)_2$ + $HNCH(CH_3)_2$], 2.28 (s, 3H, N–CH_3), 4.40 [d, J = 10.3 Hz, 1H, $HNCH(CH_3)_2$; disappeared with D_2O], 4.51 and 4.55 (AB system, J = 14.2 Hz, 1H + 1H, 9-CH_2N), 7.46 (dd, 1H, H-3), 8.30 (dd, 1H, H-4), 8.67 (dd, 1H, H-2). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 12.71, 13.85, 24.07, 24.14, 39.12, 43.64, 45.95, 49.12, 53.25, 54.23, 54.92, 107.85, 116.61, 121.06, 133.78, 141.11, 143.70, 146.99, 148.50, 148.58, 164.69. Anal. Calcd. for $C_{23}H_{34}N_8O$ (438.58): C, 62.99; H, 7.81; N, 25.55. Found: C, 63.04; H, 7.89; N, 25.23.

*5.1.10. General procedure for the synthesis of compounds **12b,d***

A mixture of 3.0 mmol of chloroderivative **11a** or **11b**, 5 mL of dimethyl sulfoxide and an excess (5 mL) of 1-methylpiperazine was heated at 130 °C for 2 h, with stirring. The resulting solution was then poured into water and the mixture was exhaustively extracted with CH_2Cl_2. The combined extracts were dried (anhydrous Na_2SO_4) and evaporated to dryness in vacuo to give thick oily residues from which the pure compound **12b** or **12d**, respectively, was obtained as described below:

*5.1.10.1. N,N-diethyl-5-(4-methyl-1-piperazinyl)-9-(4-morpholinylmethyl)[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**12b**).* The oily residue derived from the reaction carried out with **11a** (1.21 g) was taken up in a mixture EtOAc/Et_2O/petroleum ether and let to stand at room temperature until the sufficiently pure **12b**·0.5H_2O separated out as an off white solid (1.09 g, 76%); pale pink crystals, m.p. 182–184 °C, after crystallization from EtOAc/diisopropyl ether. IR (KBr): 3578 (crystallization water), 1634 s (CO), 1602, 1583, 1552 w cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.22 and 1.35 [2 t, J = 7.2 Hz, 3H + 3H, $N(CH_2CH_3)_2$], 2.39 (s, 3H, N–CH_3), 2.70–3.90 [m, 20H, piperazine CH_2's + morpholine CH_2's + $N(CH_2CH_3)_2$], 4.47 and 4.63 (AB system, J = 14.2 Hz, 1H + 1H, 9-CH_2N), 7.52 (dd, 1H, H-3), 8.44 (dd, 1H, H-4), 8.68 (dd, 1H, H-2). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 12.72, 13.96, 39.29, 44.20, 44.26, 46.71, 54.09, 55.02, 56.01, 67.40, 116.45, 118.88, 121.95, 135.47, 144.46, 144.67, 147.72, 148.96, 149.22, 164.52. Anal. Calcd. for $C_{24}H_{34}N_8O_2$·0.5H_2O (475.60): C, 60.61; H, 7.42; N, 23.56. Found: C, 60.27; H, 7.62; N, 23.26.

*5.1.10.2. N,N-diethyl-5-(4-methyl-1-piperazinyl)-9-[(4-methyl-1-piperazinyl)methyl][1,2,4]tria-zolo[4,3-a][1,8]naphthyridine-6-carboxamide (**12d**).* The oily residue derived from the reaction carried out with **11b** (1.25 g) was chromatographed on a neutral aluminium oxide column, eluting with the mixture CH_2Cl_2/petroleum ether/MeOH (6:6:1). The eluate collected, after removal of solvents, afforded a thick oil from which the pure compound **12d** separated out as a solid after treatment with a small amount of diisopropyl ether (0.84 g, 58%); pale pink crystals, m.p. 142–143 °C, after crystallization from EtOAc/petroleum ether. IR (KBr): 1636 s (CO), 1605, 1586 w, 1555 w cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.22 and 1.35 [2 t, J = 7.2 Hz, 3H + 3H, $N(CH_2CH_3)_2$], 2.27 and 2.39 (2 s, 3H + 3H, 2 N–CH_3), 2.30–3.90 [m, 20H, 2 piperazine CH_2's + $N(CH_2CH_3)_2$], 4.52 and 4.59 (AB system, J = 14.2 Hz, 1H + 1H, 9-CH_2N), 7.51 (dd, 1H, H-3), 8.43 (dd, 1H, H-4), 8.68 (dd, 1H, H-2). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 12.30, 13.54, 38.82, 43.80, 45.99, 46.33, 50.00–52.00 (very broad signal), 53.23, 54.11, 54.96, 55.60, 115.97, 118.42, 121.49, 134.99, 144.02, 144.14, 147.58, 148.55, 148.74, 164.11. Anal. Calcd. for $C_{25}H_{37}N_9O$ (479.63): C, 62.61; H, 7.78; N, 26.28. Found: C, 62.92; H, 7.64; N, 26.06.

*5.1.11. 2-Amino-N,N-diethyl-4-(isobutylamino)-1,8-naphthyridine-3-carboxamide (**14**)*

A mixture of 2-chloro-*N,N*-diethyl-4-(isobutylamino)-1,8-naphthyridine-3-carboxamide **13** [10] (3.35 g, 10.0 mmol), ethanol (50 mL) and 30% aqueous NH_3 (25 mL) was heated in a closed vessel at 130 °C for 24 h. After cooling, the mixture was concentrated in vacuo to remove ethanol, then was diluted with water (150 mL) and exhaustively extracted with $CHCl_3$. The combined extracts were dried (anhydrous Na_2SO_4), then evaporated to dryness at reduced pressure to give a white solid which was taken up in a small amount of Et_2O and filtered obtaining pure compound **14** (2.40 g, 76%), white crystals, m.p. 251–252 °C dec., after crystallization from acetone/EtOAc. IR (KBr): 3467, 3353 and 3264 (NH_2 + NH), 1627 (CO), 1604, 1553 s, 1527 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 0.80–1.35 [m, 6H, $N(CH_2CH_3)_2$], 0.99 [d, J = 6.6 Hz, 6H, $HNCH_2CH(CH_3)_2$], 1.90 [m, 1H, $HNCH_2CH(CH_3)_2$], 3.00–3.90 [m, 6H, $N(CH_2CH_3)_2$ + $HNCH_2CH(CH_3)_2$], 4.85 [broad t, 1H, $HNCH_2CH(CH_3)_2$; disappeared with D_2O], 5.23 (broad s, 2H, NH_2;

disappeared with D_2O), 7.15 (dd, $J_{6,5} = 8.2$ Hz, $J_{6,7} = 4.3$ Hz, 1H, H-6), 8,02 (dd, $J_{5,6} = 8.2$ Hz, $J_{5,7} = 1.8$ Hz, 1H, H-5), 8.78 (dd, $J_{7,6} = 4.3$ Hz, $J_{7,5} = 1.8$ Hz, 1H, H-7). Anal. Calcd for $C_{17}H_{25}N_5O$ (315.42): C, 64.74; H, 7.99; N, 22.20. Found: C, 64.41; H, 7.97; N, 22.27.

5.1.12. *N,N-diethyl-5-(isobutylamino)-8-methyl-10-oxo-10H-pyrimido[1,2-a][1,8]naphthyridine-6-carboxamide (**15**)*

A mixture of compound **14** (1.89 g, 6.0 mmol), an excess of ethyl acetoacetate (5 mL), Dowtherm A (5 mL) and monohydrate *p*-toluenesulfonic acid (0.20 g) was heated at 180 °C for 20 h with stirring. After cooling, the mixture was partitioned between CH_2Cl_2 (100 mL) and 10% aqueous Na_2CO_3 (50 mL); the organic layer was collected and the aqueous one was further extracted twice with CH_2Cl_2. The combined organic extracts were dried (anhydrous Na_2SO_4) then evaporated to dryness at reduced pressure. The dark liquid residue was chromatographed on a silica gel column eluting first with CH_2Cl_2 to remove Dowtherm A, then with EtOAc to remove the excess ethyl acetoacetate and impurities and finally with the mixture EtOAc–THF (1:1) to recover compound **15**. The eluate collected, after removal of solvents, afforded a brown thick oil from which, after addition of a small amount of Et_2O, the sufficiently pure compound **15** separated out as a yellowish solid (0.69 g, 30%); yellow crystals, m.p. 210–211 °C, after crystallization from EtOAc. IR (KBr): 3317 (NH), 1674 s (10-CO), 1620 (amide CO), 1602, 1552 w cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.02 [d, $J = 6.6$ Hz, 6H, $HNCH_2CH(CH_3)_2$], 1.13 and 1.30 [2 t, $J = 7.2$ Hz, 3H + 3H, $N(CH_2CH_3)_2$], 2.00 [m, 1H, $HNCH_2CH(CH_3)_2$], 2.23 (s, 3H, 8-CH_3), 3.06 and 3.42 [2 m, 1H + 1H, $HNCH_2CH(CH_3)_2$], 3.29 and 3.49–3.72 [2 m, 2H + 2H, $N(CH_2CH_3)_2$], 5.51 [broad s, 1H, $HNCH_2CH(CH_3)_2$; disappeared with D_2O], 6.10 (s, 1H, H-9), 7.31 (m, 1H, H-3), 8.14 (m, 1H, H-4), 8.73 (m, 1H, H-2). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 11.92, 13.45, 20.40, 23.72, 29.26, 38.83, 43.35, 52.75, 106.11, 106.24, 115.07, 121.39, 130.89, 144.31, 146.40, 148.49, 151.03, 161.77, 162.78, 166.73. Anal. Calcd. for $C_{21}H_{27}N_5O_2$ (381.48): C, 66.12; H, 7.13; N, 18.36. Found: C, 66.04; H, 7.30; N, 18.41.

5.2. Pharmacology

Wistar rats (250–300 g) and Swiss mice (25–35 g) of either sex were used. The test compounds were suspended in 0.5% methylcellulose and administered at the initial dose of 100 mg kg^{-1} by oral gavage (1 mL 100 g^{-1} body weight) in animals fasted overnight. Compounds that exhibited a statistically significant effect at this dose were further tested at doses decreasing by a factor of two. In these experimental tests for the evaluation of analgesic and anti-inflammatory activity, indomethacin (10 mg kg^{-1} oral dose) was used as reference drug. The same dose of indomethacin was used in rats to investigate the acute gastrolesivity while in the same test the compound **1d** was studied at the oral dose of 100 mg kg^{-1}. Groups of 8–10 animals were used and control animals were orally treated with an equivalent volume of vehicle alone.

All procedures and animal care were in accordance with the European Communities Council Directive (86/609/EEC) and were approved by the Veterinarian Department of the Italian Ministry of Health (DL 116/92).

5.2.1. *Anti-inflammatory activity*

Anti-inflammatory activity was studied by inducing paw oedema according to Winter's method [17]. 1% Carrageenan (0.1 mL) (Sigma Aldrich, Milan, Italy) was injected subcutaneously into the plantar surface of the rat hind paw 1 h after the oral administration of the test compounds or indomethacin. Control group received 0.5% methylcellulose. Paw volume was determined immediately after the injection of phlogogen agent and again 3 h later by means of a plethysmometer [Mod. 7140, Basile, Comerio (VA), Italy]. Oedema values, obtained in the control group, were considered arbitrarily as 100; the percentage of inhibition was calculated from the difference in the swelling between the treated and the control group.

5.2.2. *Analgesic activity*

Antinociceptive activity was studied by writhing test [18], through the intraperitoneal injection of 0.2 mL/mouse of acetic acid solution (0.6%) (Sigma Aldrich, Milan, Italy) 1 h after oral treatment with the test drugs or indomethacin. Control group received 0.5% methylcellulose. Complete extension of either hind limb was regarded as a writhing response. The number of writhes of each mouse was counted over a period of 30 min after the injection of the noxious agent. The percentage of inhibition was calculated from the difference in the writhing response between the treated and the control group.

5.2.3. *Spontaneous locomotor activity*

Locomotor activity was measured by means of a black and white video tracking system (Any-maze, Stoelting Co, Wood Dale, IL) connected to a PC and operating on the principle of contrast [19]. The mouse to be tracked is placed against a contrasting background within individual 21 × 21 × 30 cm open field (arena), the apparatus assigns an X–Y coordinate pair to the centroid of the subject and the X–Y coordinates are continuously monitored and compared to detect motion. The locomotor activity was measured, after oral administration of the compounds under study or vehicle, as the distance (metres) travelled in 30 min (a measure of general ambulation), recorded by the videocamera and analysed with the video-tracking system that considers an animal inside a zone whenever its centre point is within it. All experiments were conducted from 9.00 to 13.00. For every compound under study, the occurrence of statistically significant differences in the distance travelled between the treated and the control group was indicated as “sedation” in Table 1.

5.2.4. *Gastrolesivity*

The acute gastrolesivity of compound **1d** (100 mg kg^{-1}) or indomethacin (10 mg kg^{-1}) was evaluated by examining the stomachs excised 5 h after oral administration in rats. The stomachs, fixed in 2% formalin (injection of 6 mL per stomach), were opened along the greater curvature and mounted over a flat surface. The stomachs were examined with a stereomicroscope (M8 Wild Heerbrung, Switzerland) by an observer unaware of the treatment the rats received. Acute gastrolesivity was expressed as the number of animals with gastric damage over the number of treated animals.

5.2.5. *In vitro antiplatelet activity*

Guinea pig blood was obtained by cardiac puncture after CO_2 euthanasia and collected in plastic tubes in presence of sodium citrate 3.8% (1 part citrate: 9 parts blood). After centrifugation for 15 min at 180 *g* to obtain platelet rich plasma (PRP), the remaining blood was spinned 10 min at 2000 *g* to obtain platelet poor plasma (PPP). Platelet aggregation was performed in the PAP4D aggregometer (BioData corp, USA) at 37 °C under continuous stirring (1000 rpm), following Born's turbidimetric method [20]. Aggregation was recorded as the percent change in light transmission: the baseline was set using PRP and maximal transmission using PPP. PRP was preincubated at 37 °C for 5 min with solvent (dimethyl sulfoxide, at the maximal final concentration 0.5%), compound **1d** or acetylsalicylic acid (ASA) before addition of platelet aggregatory agent. Maximal aggregation was induced stimulating platelets with 2 μM U46619, 5 μM ADP or 100 μM arachidonic acid. Tests were performed within 3 h to avoid platelet inactivation. The effects of

1d and acetylsalicylic acid were assessed as percent inhibition compared with control sample. DMSO 0.5% did not interfere with platelet aggregation.

5.2.6. Cell cultures and nitrite determination

The murine monocyte/macrophage cell line J774 was obtained from ATCC (Manassas, USA). J774 cell line was grown in Dulbecco's modified Eagle's medium (DMEM – Gibco Laboratories, Grand Island, NY) and cultured at 37 °C in humidified 5% CO_2/95% air. Culture medium was supplemented with 10% foetal bovine serum and 2 mM glutamine. The cells were plated onto 24-well culture plates at a density of 2.5×10^5 mL^{-1} and allowed to adhere overnight. Thereafter, medium was replaced with fresh medium. J774 macrophages produced NO in response to LPS (30 μg mL^{-1}) (Sigma–Aldrich, St. Louis, MO) and compound **1d** (100 μM) was added to cells at the same time as LPS stimulation. Dexamethasone (Sigma–Aldrich, St. Louis, MO), used as reference anti-inflammatory drug, was added at 1 μM in cells stimulated with LPS. Supernatants were collected after 6 h and stored at −20 °C.

As an indicator of NO production, the nitrite concentration in the culture medium was determined using the Griess reagent as described previously by Green et al. [21]. The culture supernatant (100 μL) was mixed with the Griess reagent (100 μL, 10 g L^{-1} sulfanilamide, 1 g L^{-1} *N*-1-naphthylethylenediamine) for 10 min, after which the chromophoric azo-derivative molecule's absorbance was measured in a microplate reader at 540 nm. Fresh culture medium was used as the blank in all experiments. A range of dilutions of sodium nitrite was used to construct a standard curve in order to determine the amount of nitrite in each sample.

5.2.7. Data analysis

Results were expressed as mean ± SEM. Differences between treated and control groups were determined by Student's *t*-test ($^*P < 0.05$ or $^{**}P < 0.01$ being considered as statistically significant or highly significant, respectively).

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