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Original article

1,8-Naphthyridines VI. Synthesis and anti-inflammatory activity of 5-(alkylamino)-*N*,*N*-diethyl[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamides with a new substitution pattern on the triazole ring

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Abstract

On the basis of the good anti-inflammatory properties shown by the 9-alkyl-*N*,*N*-dialkyl-5-(alkylamino)[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamides **1**, a series of analogues of such compounds, in which the 9-alkyl substituent was replaced by an ester or amide group (compounds **3a–i**), was prepared and tested (inhibition of carrageenan-induced paw edema in the rat). Also two 5-(*N*-alkyl,*N*-acylamino) derivatives (compounds **4a**,**b**) were synthesized and evaluated for the same purpose. Even though the general trend for these new [1,2,4]triazolo[4,3-*a*][1,8]naphthyridine derivatives was a decrease in activity compared with compounds **1**, some of the new synthesized compounds exhibited still good anti-inflammatory properties.

Keywords: [1,2,4]Triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamide derivatives; Anti-inflammatory activity

1. Introduction

We previously described the synthesis of a series of new original anti-inflammatory agents having the 5-(monoalkylamino)[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamide structure **1** [1].

Fourteen out of the twenty-four compounds prepared exhibited a statistically significant anti-inflammatory activity, when tested for the inhibitory properties against the carrageenan-induced paw edema in the rat. The best results were obtained when $R = C_2H_5$, $R' = C_2H_5$ or i-C_4H_9 and $R'' = C_2H_5$ or i-C_3H_7. In particular, compounds **1c** ($R = C_2H_5$, $R' = C_2H_5$, $R'' = i$-C_3H_7) and **1n** ($R = C_2H_5$, $R' = i$-C_4H_9, $R'' = i$-C_3H_7) resulted the most effective anti-inflammatory agents (edema inhibition = 61% at 25 mg/kg p.o.) [1].

More recently, we have prepared and tested a new series of [1,2,4]triazolo[4,3-*a*][1,8]naphthyridine derivatives **2** analogue to compounds **1** in which the monoalkylamino group at position 5 was replaced by a dialkylamino one. In this series, on the basis of the pharmacological results reported above for compounds **1**, the substituents at positions 6 and 9 were always $CON(C_2H_5)_2$ and i-C_3H_7, respectively [2].

The pharmacological results afforded by compounds **2** were satisfactory. Several members of this series displayed a fairly good anti-inflammatory activity, even though inferior to that of the most active compounds **1**; in addition, some derivatives showed very interesting analgesic properties (acetic acid induced writhing test in mice). In particular, compound **2h** (Fig. 1) exhibited a very potent analgesic activity (85% inhibition of the acetic acid induced writhing in the mouse down to 3.12 mg/kg dose) [2].

On this basis, in order to explore the potentiality of this new class of analgesic/anti-inflammatory agents, we have considered

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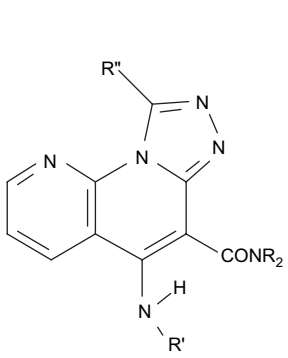
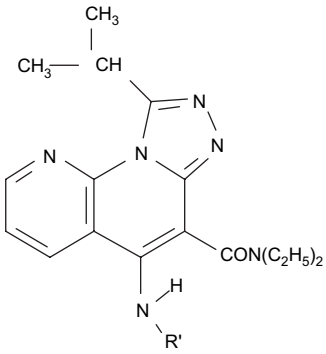
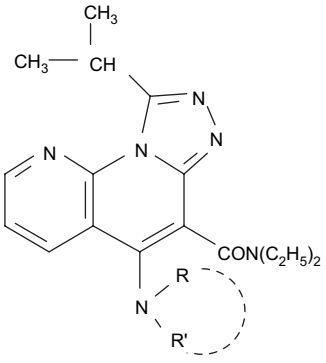


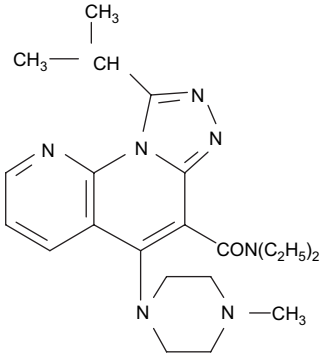
1



1c : R' = C_2H_5
1n : R' = *i*-C_4H_9



2



2h

Fig. 1. Structures of 9-alkyl-*N*,*N*-dialkyl-5-(alkylamino)[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamides **1** (e.g. **1c**,**n**) and 5-(dialkylamino)-*N*,*N*-diethyl-9-isopropyl[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamides **2** (e.g. **2h**).

interesting to change the type of substitution at the position 9 of the triazolonaphthyridine scaffold by introducing in this position an ester or a substituted amide group directly or through a one carbon spacer. This project bases its rationale on the well known importance of the COOH and related groups in the structures of the most known NSAIDs. Therefore we carried out the synthesis of new compounds, represented by the general structure **3** (Fig. 2), where the amide group in position 6 was always $CON(C_2H_5)_2$, and the amino groups in position 5 were $NH-C_2H_5$, $NH-C_4H_9$, or $NH-i-C_4H_9$.

We also prepared compounds **4a** and **4b**, in which an amide functionality was obtained by involving the 5-amino group of two of the most active compounds of the series **1**.

2. Chemistry

The synthetic strategy used to prepare compounds **3** consisted in introducing the ester or amide moieties into proper bifunctional hydrazides (compounds **6a–c**, **8a–c**, or **10a**,**b**) (Scheme 1), which were in turn cyclocondensed with suitable 1,8-naphthyridine derivatives (compounds **11a**,**b** or **12**) to give, in one or two steps, the desired compounds **3** (Scheme 2).

The preparation of ester-hydrazides **6a–c** was performed using a method analogous to that employed by O'Callaghan [3] for the synthesis of compound **6b**, with work-up variations to get an easier recovery of desired hydrazides. Thus, a suitable diester (diethyl oxalate **5a**, diethyl malonate **5b**, or diethyl 2-methylmalonate **5c**) was treated with hydrazine hydrate in ethanol at room temperature overnight, using a 3:1 molar excess of ester versus hydrazine to limit, as far as possible, the bishydrazide formation. After removal of the solvent, a little amount of insoluble bishydrazide was removed by filtration and the desired compounds **6a–c** were separated from the excess ester through partition between water and diethyl ether, throwing away the organic phase and collecting the aqueous one. After removal of the water at reduced pressure, compounds **6a–c** were obtained in moderate yields (27–36%).

The reaction of ethyl 2-chloro-2-oxoacetate with an excess of suitable amines (in dichloromethane solution, room temperature, 30 min) afforded the corresponding ethyl *N*,*N*-dialkyloxamates **7a–c**, which were in turn treated with hydrazine hydrate in refluxing ethanol (2 h) to give the desired *N*,*N*-dialkyl-2-hydrazino-2-oxoacetamides **8a–c** (yields: 75–87%); similarly, the *N*-substituted ethyl malonamates **9a** [4] or **9b** [5], treated with hydrazine hydrate in

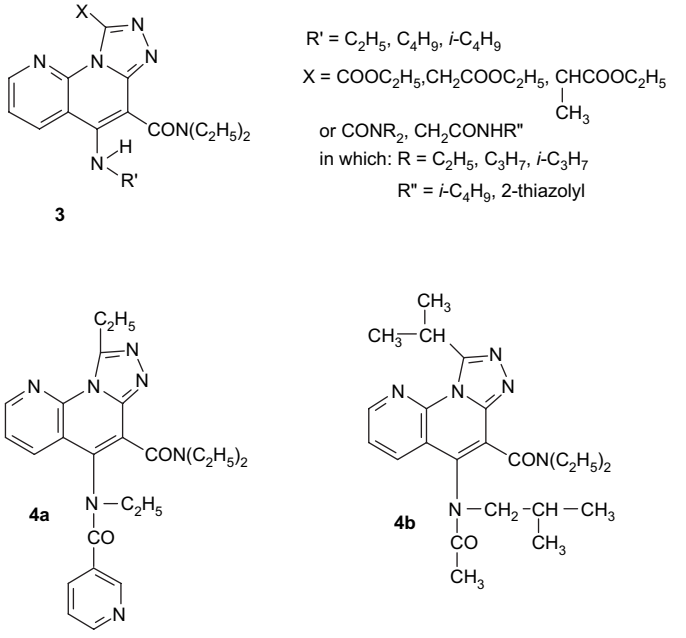
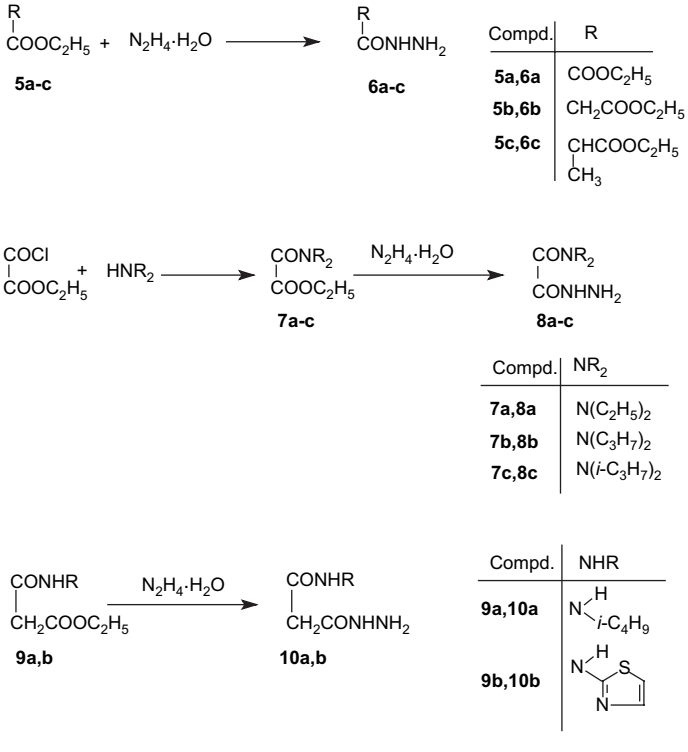


Fig. 2. Structures of compounds **3** and **4a**,**b**.

refluxing ethanol (2 h), afforded the corresponding *N*-substituted 3-hydrazino-3-oxopropanamides **10a**,**b** (yields: 60–74%), respectively (Scheme 1).

The cyclocondensation of **11a** [6] or **11b** [7] with ester-hydrazides **6a–c** in excess (Dowtherm A, 150 °C, 20 min) gave fairly good yields (41–62%) of compounds **3a–d**. Since in the case of amide-hydrazides **8a–c** and **10a**,**b** the cyclocondensation afforded unsatisfactory yields of the expected compounds, these bifunctional hydrazides were more conveniently treated with **12** [6] to give the intermediate chloroderivatives **13a–e**. The experimental conditions of this step were properly optimized, depending on the kind of hydrazide: heating in Dowtherm A at 150 °C for short time (20–45 min) in the case of preparation of **13a**,**b**,**d** or for 2 h in the case of the sterically hindered compound **13c**; heating in a mixture of Dowtherm A–pyridine (1:1) to solubilize **10b** at 160 °C for 1.5 h, in the case of preparation of **13e**. The yields ranged from 52–64% (**13a**,**b**,**d**) to 15–19% (**13e**,**c**), respectively. The subsequent reaction of chloroderivatives **13a–e** with *n*-butylamine or isobutylamine [excess amine in ethanol, 130 °C in closed vessel for 8 h (preparation of **3e–g**), or excess amine in dimethyl sulphoxide at 130 °C for 3 h (preparation of **3h**,**i**)] afforded the desired compounds **3e–i** in good yields (73–89%).



Scheme 1. Synthesis of bifunctional hydrazides **6a–c**, **8a–c** and **10a**,**b**.

Finally, the reaction of **1b** [1] with nicotinoyl chloride hydrochloride, in anhydrous toluene, in the presence of triethylamine (120 °C, 3 h) afforded **4a** in moderate yield (40%), while the treatment of **1n** [1] with acetic anhydride at 140 °C for 8 h gave a good yield (85%) of **4b** (Scheme 2).

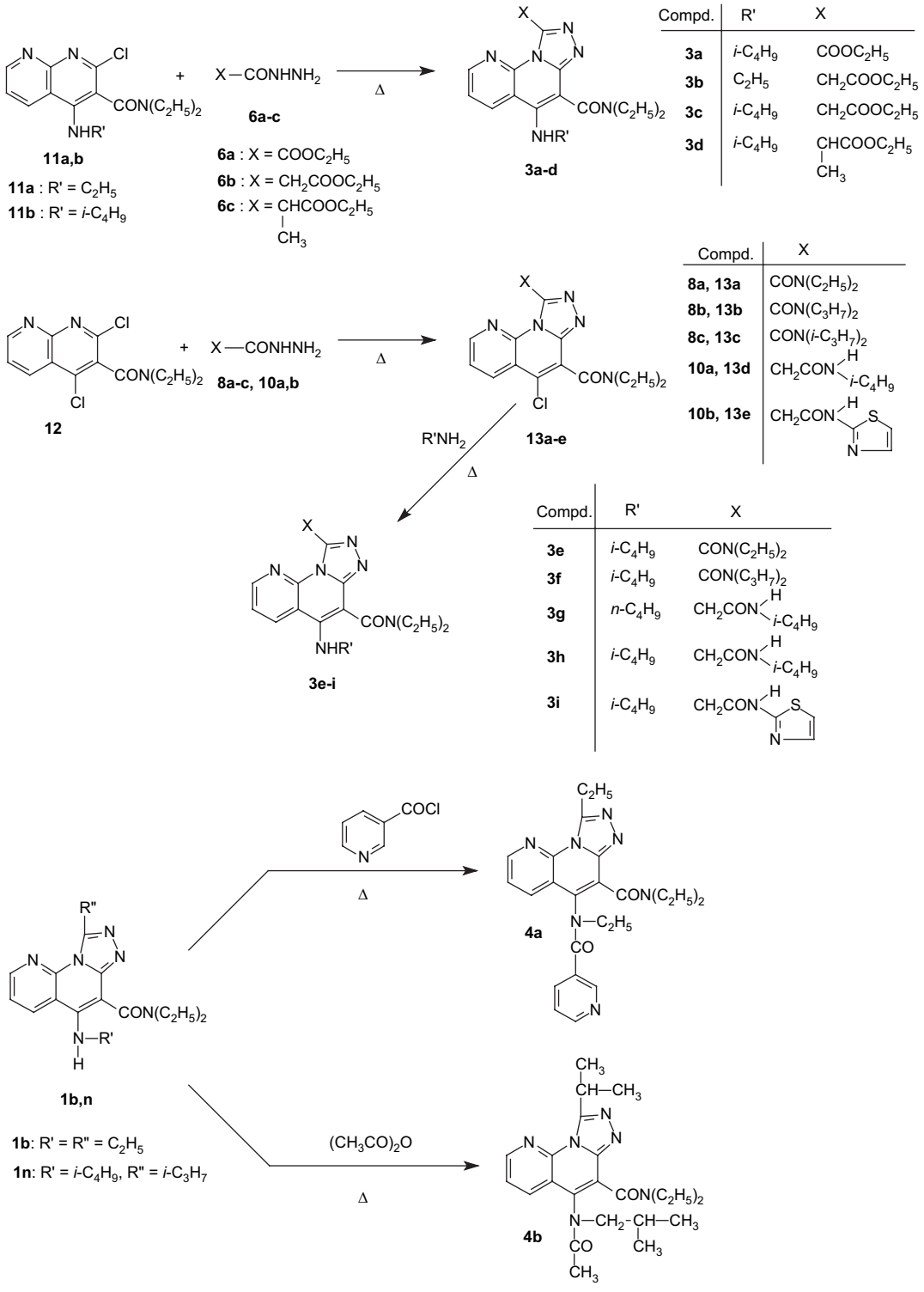
The structures attributed to the compounds described in this paper are consistent with the results of elemental analyses and IR and ^{1}H NMR spectral data (see Section 5).

In the ^{1}H NMR spectra ($CDCl_3$) of compounds **3a–i**, **13a–e**, **4a**,**b**, as previously observed in the case of compounds **1** [1] and **2** [2], 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 C-6–$CONEt_2$ bond). Particular evidence of this situation is given by the ^{1}H NMR spectra of 9-acetate and 9-acetamide derivatives (compounds **3b**,**c** and **3g–i**, **13d**,**e**, respectively) in which the 9-CH_2 signals appear as an AB system ($J = 18$ Hz) whose centers are situated in the ranges δ 4.40–4.48 and 4.48–4.66, respectively (5-amino-derivatives **3b**,**c**,**g**,**h**), whereas for 5-chloroderivatives **13d**,**e** the AB system is centered at δ 4.68 and 4.74 or 5.00 and 5.10, respectively. On the other hand, in the case of **3i** the 9-CH_2 protons are almost isochronous and the AB pattern appears as a nearly singlet at δ 4.88.

3. Pharmacological results and discussion

Compounds **3a–i**, **4a**,**b** and **13** were tested in vivo for their anti-inflammatory activity (carrageenan-induced paw edema test in the rat). All the compounds were administered orally and assayed at the initial dose of 200 mg/kg. Compounds which exhibited a statistically significant activity at this dose were further tested at doses decreasing by a factor of two. The results of the pharmacological evaluation are listed in Table 1.

All the tested compounds did not induce direct signs of toxicity or mortality in the animals subjected to experiment. Four of the twelve tested compounds showed a statistically significant anti-inflammatory activity in carrageenan-induced paw edema test in the rat. At the 200 mg/kg dose the degree of protection was 65% for **3c**, 60% for **3e**, 51% for **4a** and 35% for

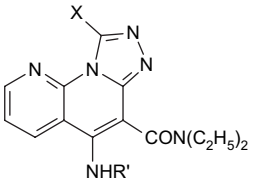


Scheme 2. Synthetic routes to compounds **3a–i** and **4a**,**b**.

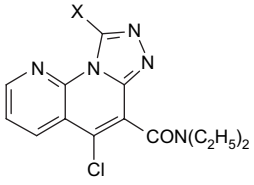
3f. The most active compound was **3c** that produced a statistically significant inhibition of the carrageenan-induced paw edema also at the 100 mg/kg dose (35%), whereas the inhibition was not statistically significant at 50 mg/kg (21%). The other three compounds did not display a statistically significant activity when tested at the 100 mg/kg dose. From the above pharmacological results the following remarks can be made.

- With compounds bearing an ester group in position 9 the best result was shown by the ethyl 9-acetate **3c** [good and statistically significant inhibition of paw edema at 200 mg/kg

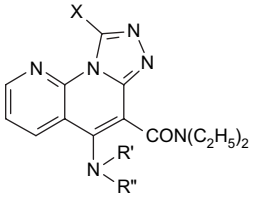
Table 1
Anti-inflammatory activity of compounds **3a–i**, **13c** and **4a**,**b**



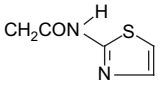
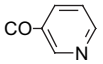
3a-i



13c



4a,b

Compound	R′	X	R″	Dose (mg/kg p.o.)	Anti-inflammatory activity in rat[a]	
					Edema (μL) (mean ± SD)	Inhibition (%)
3a	*i*-C_4H_9	$COOC_2H_5$	–	200	233 ± 560	20
3b	C_2H_5	$CH_2COOC_2H_5$	–	200	348 ± 61	0
3c	*i*-C_4H_9	$CH_2COOC_2H_5$	–	200	102 ± 45	65**
				100	190 ± 46	35*
				50	228 ± 24	21
3d	*i*-C_4H_9	$CH(CH_3)COOC_2H_5$	–	200	254 ± 42	12
3e	*i*-C_4H_9	$CON(C_2H_5)_2$	–	200	117 ± 31	60**
				100	204 ± 32	30
3f	*i*-C_4H_9	$CON(C_3H_7)_2$	–	200	190 ± 40	35*
3g	*n*-C_4H_9	CH_2CONH-*i*-C_4H_9	–	200	350 ± 38	0
3h	*i*-C_4H_9	CH_2CONH-*i*-C_4H_9	–	200	239 ± 44	18
3i	*i*-C_4H_9		–	200	238 ± 45	18
4a	C_2H_5	C_2H_5		200	143 ± 57	51**
				100	199 ± 30	31
4b	*i*-C_4H_9	*i*-C_3H_7	$COCH_3$	200	276 ± 164	5
13c	–	$CON(i\text{-}C_3H_7)_2$	–	200	204 ± 49	30
Indomethacin				6	47 ± 27	92**

$^{**}P < 0.01$, $^{*}P < 0.05$ (Student *t*-test versus controls).
[a] Carrageenan paw edema test (control value: 290 ± 93 μL).

(65%), partially maintained at 100 mg/kg (35%)]. The ethyl 9-carboxylate **3a** and ethyl 9-(2-propanoate) **3d** did not show any significant activity even at 200 mg/kg. Surprisingly, when the 5-(isobutylamino) group in the ethyl 9-acetate **3c** was replaced with the 5-(ethylamino) one (compound **3b**), a complete disappearance of the activity was observed. This result is in contrast with what previously observed [1] for compounds of type **1** in which the 5-(ethylamino) derivative **1c** (Fig. 1) was as active as the corresponding 5-(isobutylamino) analogue **1n**.

- In compounds bearing an amide group in position 9 the best results were obtained with the 9-carboxamide derivatives **3e** and **3f**. The edema inhibition at 200 mg/kg was good and statistically significant (60%) for the *N*,*N*-diethyl derivative **3e**, whereas the *N*,*N*-dipropyl analogue **3f** resulted only moderately active (35%). Also the *N*,*N*-diisopropyl 5-chloro-9-carboxamide **13c** showed a similar level of edema inhibition (30%), even if not statistically significant. The *N*-monosubstituted 9-acetamides **3g–i** resulted almost inactive.
- As far as the amides **4a** and **4b** are concerned, the nicotinoyl derivative **4a** showed fairly good anti-inflammatory properties [51% of edema inhibition at 200 mg/kg and 31% (not statistically significant) at 100 mg/kg], whereas the acetyl derivative **4b** was nearly inactive.

4. Conclusions

Compounds **3a–i**, **4a**,**b** and **13a–e** are novel chemically interesting [1,2,4]triazolo[4,3-*a*][1,8]naphthyridine derivatives in which a second acyl moiety has been introduced in addition to the pre-existent *N*,*N*-diethyl 6-carboxamide group. Contrary to our expectations, the introduction of an additional acyl group did not improve the anti-inflammatory properties of the new synthesized compounds. Nevertheless, some representative members of the series (**3c**,**e**,**f** and **4a**) still maintain a good 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: s = strong, br = broad, w = weak, sh = shoulder). ^{1}H NMR spectra were recorded on a Varian Gemini 200 (200 MHz) spectrometer, using $(CH_3)_4Si$ as an internal reference ($\delta = 0$), and chemical shifts are reported in parts per million. Spin multiplicities are given as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet). Analyses of all new compounds (Table 2) were within ±0.4% of the theoretical values and were performed by the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, Università di Genova. Thin layer chromatograms were run on Merck silica gel 60 F_{254} 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. General procedure for the synthesis of compounds **6a–c**

A mixture of 0.36 mol of the proper diester [diethyl oxalate (54.60 g), diethyl malonate (57.60 g) or diethyl 2-methylmalonate (62.64 g)], 0.12 mol (6.00 g) of hydrazine hydrate and 40 mL of ethanol was stirred at room temperature overnight. The final mixture was concentrated at reduced pressure and cooled at 4 °C so that the bishydrazide separated out as a white solid. This solid was removed by filtration and the filtered solution was transferred into a separatory funnel and partitioned between water and diethyl ether. The aqueous phase was collected whereas the organic layer containing the excess diester was extracted twice with water and discarded. The entire aqueous phase was then evaporated to dryness at reduced pressure affording a sticky residue from which, after addition of a little methanol/diethyl ether (1:1), the desired monohydrazide separated out as a white solid which was recovered by filtration and dried.

According to this procedure the following compounds were prepared.

*5.1.1.1. Ethyl 2-hydrazino-2-oxoacetate (**6a**).* Yield 4.52 g (29%), white needles, m.p. 50–52 °C (lit. [8]: m.p. 51–53 °C).

*5.1.1.2. Ethyl 3-hydrazino-3-oxopropanoate (**6b**).* Yield 6.23 g (36%), white needles, m.p. 72–74 °C (lit. [3]: m.p. 68–69 °C).

*5.1.1.3. Ethyl 3-hydrazino-2-methyl-3-oxopropanoate (**6c**).* Yield 5.12 g (27%), white needles, m.p. 86–87 °C. ^{1}H NMR ($CDCl_3$): δ 1.20 (t, 3H, CH_2CH_3), 1.37 (d, 3H, $CHCH_3$), 3.22 (q, 1H, $CHCH_3$), 3.60–4.00 (broad signal, 2H, $CONHNH_2$; disappeared with D_2O), 4.11 (q, 2H, CH_2CH_3), 7.74 (br s, 1H, $CONHNH_2$; disappeared with D_2O). IR (KBr): 3288 and 3212 (NH), 1733 (ester CO), 1648 s (hydrazide CO), 1544, 1459 cm^{-1}.

5.1.2. General procedure for the synthesis of *N*,*N*-dialkyl-2-hydrazino-2-oxoacetamides **8a–c**

A one-pot procedure was used starting from ethyl 2-chloro-2-oxoacetate and proper dialkylamines to give the intermediate ethyl *N*,*N*-dialkyloxamates **7a–c**, which in turn were treated with hydrazine hydrate to give the desired *N*,*N*-dialkyl-2-hydrazino-2-oxoacetamides **8a–c**. Thus, to an ice-cooled solution of 0.10 mol of the proper dialkylamine [diethylamine (7.30 g), dipropylamine (10.10 g) or diisopropylamine (10.10 g)] in 20 mL of dichloromethane was carefully added 0.04 mol (5.44 g) of ethyl 2-chloro-2-oxoacetate diluted in 20 mL of dichloromethane: an exothermic reaction evolving white fumes occurred. The mixture was stirred at

Table 2
Elemental analyses of compounds **6a–c**, **10a,b**, **3a–i**, **13a–e** and **4a,b**

Compound	Molecular formula	Analysis (calcd./found)			
		C%	H%	N%	S%
6a	$C_4H_8N_2O_3$	36.36, 36.15	6.10, 6.30	21.20, 21.44	–
6b	$C_5H_{10}N_2O_3$	41.09, 41.31	6.90, 7.06	19.17, 18.92	–
6c	$C_6H_{12}N_2O_3$	44.99, 45.25	7.55, 7.80	17.49, 17.21	–
10a	$C_7H_{15}N_3O_2$	48.54, 48.31	8.73, 8.87	24.26, 24.04	–
10b	$C_6H_8N_4O_2S$	35.99, 36.26	4.03, 4.21	27.98, 27.64	16.01, 15.80
3a	$C_{21}H_{28}N_6O_3$	61.15, 60.80	6.84, 6.92	20.37, 20.60	–
3b	$C_{20}H_{26}N_6O_3$	60.29, 60.29	6.58, 6.79	21.09, 21.10	–
3c	$C_{22}H_{30}N_6O_3$	61.95, 60.78	7.09, 7.40	19.70, 19.50	–
3d	$C_{23}H_{32}N_6O_3$	62.71, 63.08	7.32, 7.43	19.08, 19.39	–
3e	$C_{23}H_{33}N_7O_2$	62.85, 62.62	7.57, 7.51	22.31, 22.35	–
3f	$C_{25}H_{37}N_7O_2$	64.21, 64.06	7.98, 8.20	20.97, 20.82	–
3g	$C_{24}H_{35}N_7O_2$	63.55, 63.81	7.78, 7.92	21.62, 21.48	–
3h	$C_{24}H_{35}N_7O_2$	63.55, 63.53	7.78, 7.85	21.62, 21.77	–
3i	$C_{23}H_{28}N_8O_2S$	57.48, 57.10	5.87, 6.06	23.32, 23.12	6.67, 6.35
13a	$C_{19}H_{23}ClN_6O_2$	56.64, 56.42	5.75, 5.67	20.86, 20.78	–
13b	$C_{21}H_{27}ClN_6O_2$	58.53, 58.56	6.32, 5.97	19.50, 19.34	–
13c	$C_{21}H_{27}ClN_6O_2$	58.53, 58.65	6.32, 6.27	19.50, 19.57	–
13d	$C_{20}H_{25}ClN_6O_2$	57.62, 57.66	6.04, 6.34	20.16, 19.82	–
13e	$C_{19}H_{18}ClN_7O_2S$	51.41, 51.43	4.09, 4.38	22.09, 21.85	7.22, 7.43
4a	$C_{24}H_{27}N_7O_2$	64.70, 65.05	6.11, 6.25	22.01, 21.73	–
4b	$C_{23}H_{32}N_6O_2$	65.05, 64.96	7.60, 7.38	19.80, 19.79	–

room temperature for 30 min, then poured into cold water (150 mL) and, after careful addition of $NaHCO_3$ up to pH 7, was kept stirring at room temperature for further 30 min. The organic layer was then collected and the aqueous one was extracted twice with dichloromethane. The combined organic phase was then dried (Na_2SO_4) and evaporated to dryness at reduced pressure to give the desired ethyl *N*,*N*-dialkyloxamates **7a–c** as colourless oils. To these oils, dissolved in ethanol (15 mL), 0.04 mol (2.00 g) of hydrazine hydrate was added and the mixture was refluxed for 2 h with stirring. The resulting colourless solutions were then evaporated to dryness at reduced pressure to give the crude compounds **8a–c** as waxy oils which were used in the subsequent synthetic step without further purification.

According to this procedure the following compounds were prepared.

*5.1.2.1. N,N-Diethyl-2-hydrazino-2-oxoacetamide (**8a**).* Yield 5.54 g (87%) (lit. [9]: m.p. 83–85 °C).

*5.1.2.2. 2-Hydrazino-2-oxo-N,N-dipropylacetamide (**8b**).* Yield 5.90 g (79%).

*5.1.2.3. 2-Hydrazino-N,N-diisopropyl-2-oxoacetamide (**8c**).* Yield 5.60 g (75%).

*5.1.3. General procedure for the synthesis of N-substituted 3-hydrazino-3-oxopropanamides **10a**,**b***

A mixture of 15 mmol of the proper *N*-substituted ethyl malonamate **9a** or **9b**, 15 mmol (0.75 g) of hydrazine hydrate and 100 mL of ethanol was refluxed for 2 h with stirring. From the resulting colourless solution, compounds **10a**,**b** were obtained as described below.

*5.1.3.1. 3-Hydrazino-N-isobutyl-3-oxopropanamide **10a**.* The solution derived from reaction carried out with 2.80 g of *N*-isobutyl ethyl malonamate **9a** (prepared through the reaction of ethyl 3-chloro-3-oxopropanoate with excess isobutylamine in dichloromethane at room temperature; also a new preparation has been reported [4]) was concentrated at reduced pressure and diluted with a little petroleum ether so that **10a** separated out as a white solid (1.56 g, 60%), m.p. 71–72 °C, after crystallization from diisopropyl ether.

*5.1.3.2. 3-Hydrazino-3-oxo-N-(2-thiazolyl)propanamide **10b**.* The solution derived from reaction carried out with 3.21 g of *N*-(2-thiazolyl) ethyl malonamate **9b** [5] was cooled down to room temperature so that **10b** crystallized as white needles (2.23 g, 74%), m.p. 198–199 °C, after recrystallization from ethanol (lit. [10]: m.p. 200 °C).

*5.1.4. General procedure for the synthesis of compounds **3a–d***

A mixture of 5.0 mmol of compound **11a** [6] or **11b** [7], 10.0 mmol of the proper ester-hydrazide **6a**, **6b** or **6c** and 6 mL of Dowtherm A was stirred at 150 °C for 20 min. After cooling, dichloromethane (100 mL) and 10% aqueous Na_2CO_3 (50 mL) were added to the mixture which was stirred for 30 min at room temperature. The mixture was filtered to remove a reddish insoluble solid, then transferred into a separatory funnel. The organic layer was collected and the aqueous one was extracted twice with dichloromethane. The combined organic extracts (dried over Na_2SO_4 and decolourized with charcoal) were evaporated to dryness at reduced pressure to give an orange liquid which was chromatographed on an aluminium oxide column, eluting with dichloromethane until Dowtherm A was completely removed. The desired compounds **3a–d** were then recovered eluting with tetrahydrofuran. The eluate collected, after removal of solvent, afforded a pale orange thick oil from which compounds **3a–d** were obtained as reported below.

*5.1.4.1. Ethyl 6-[(diethylamino)carbonyl]-5-(isobutylamino)[1,2,4]triazolo[4,3-a][1,8]naphthridine-9-carboxylate **3a**.* The solid residue derived from reaction carried out with 1.67 g of **11b** [7] and 1.30 g of ethyl 2-hydrazino-2-oxoacetate **6a**, was taken up in a little diethyl ether and filtered to afford the pure **3a** (0.85 g, 41%), whitish solid, m.p. 217–218 °C, after crystallization from ethyl acetate with charcoal. ^{1}H NMR ($CDCl_3$): δ 1.01 and 1.03 [2d, 3H + 3H, $NCH_2CH(CH_3)_2$], 1.23 and 1.35 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.47 (t, 3H, $COOCH_2CH_3$), 2.16 [m, 1H, $NCH_2CH(CH_3)_2$], 2.67 [m, 1H, 1H of $NCH_2CH(CH_3)_2$], 3.27–3.57 [m, 4H, 3H of $N(CH_2CH_3)_2$ + 1H of $NCH_2CH(CH_3)_2$], 4.08 [m, 1H, 1H of $N(CH_2CH_3)_2$], 4.58 (q, 2H, $COOCH_2CH_3$), 6.75–6.89 [m, 2H, H-3 + NH; dd (δ = 6.83), $J_{3,4}$ = 8.3 Hz, $J_{3,2}$ = 4.6 Hz, 1H, H-3, after treatment with D_2O], 8.13 (dd, $J_{4,3}$ = 8.3 Hz, $J_{4,2}$ = 1.5 Hz, 1H, H-4), 8.40 (dd, $J_{2,3}$ = 4,6 Hz, $J_{2,4}$ = 1.5 Hz, 1H, H-2). IR (KBr): 3264 (NH), 1744 (ester CO), 1604 s (amide CO), 1545, 1463 cm^{-1}.

*5.1.4.2. Ethyl [6-[(diethylamino)carbonyl]-5-(ethylamino)[1,2,4]triazolo[4,3-a][1,8]naphthyridin-9-yl]acetate **3b**.* The solid residue obtained from reaction carried out with 1.53 g of **11a** [6] and 1.44 g of ethyl 3-hydrazino-3-oxopropanoate **6b**, was treated with a little diethyl ether so that **3b** separated out as a crystalline solid (1.12 g, 56%), whitish crystals, m.p. 152.5–153 °C, after crystallization from ethyl acetate/petroleum ether with charcoal. ^{1}H NMR ($CDCl_3$): δ 1.03–1.49 [m, 12H, $N(CH_2CH_3)_2$ + 5-NCH_2CH_3 + $COOCH_2CH_3$], 3.12 (m, 1H, 1H of 5-NCH_2CH_3) 3.33–3.62 [m, 4H, 3H of $N(CH_2CH_3)_2$ + 1H of 5-NCH_2CH_3], 3.96 [m, 1H, 1H of $N(CH_2CH_3)_2$], 4.13 (q, 2H, $COOCH_2CH_3$), 4.44 and 4.52 (AB system, J = 18 Hz, 1H + 1H, 9-CH_2COO), 5.90 (br s, 1H, NH; disappeared with D_2O), 7.04 (dd, $J_{3,4}$ = 8.3 Hz, $J_{3,2}$ = 4.6 Hz, 1H, H-3), 8.18 (dd, $J_{4,3}$ = 8.3 Hz, $J_{4,2}$ = 1.5 Hz, 1H, H-4), 8.32 (dd, $J_{2,3}$ = 4.6 Hz, $J_{2,4}$ = 1.5 Hz, 1H, H-2). IR (KBr): 3293 (NH), 1743 (ester CO), 1607 s (amide CO), 1547, 1476 cm^{-1}.

*5.1.4.3. Ethyl [6-[(diethylamino)carbonyl]-5-(isobutylamino)[1,2,4]triazolo[4,3-a][1,8]naphthyridin-9-yl]acetate **3c**.* The solid residue obtained from reaction carried out with 1.67 g of **11b** [7] and 1.44 g of ethyl 3-hydrazino-3-oxopropanoate

6b, was taken up in a little diethyl ether to give pure **3c** (1.33 g, 62%), whitish crystals, m.p. 199–200 °C, after crystallization from ethyl acetate/petroleum ether with charcoal. ^{1}H NMR ($CDCl_3$): δ 1.01 and 1.03 [2d, 3H + 3H, $NCH_2CH(CH_3)_2$], 1.15 and 1.17 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.31 (t, 3H, $COOCH_2CH_3$), 2.04 [m, 1H, $NCH_2CH(CH_3)_2$], 2.72 [m, 1H, 1H of $NCH_2CH(CH_3)_2$], 3.22–3.51 [m, 4H, 3H of $N(CH_2CH_3)_2$ + 1H of $NCH_2CH(CH_3)_2$], 3.91–4.18 [m, 1H, 1H of $N(CH_2CH_3)_2$], 4.11 (q, 2H, $COOCH_2CH_3$), 4.40 and 4.48 (AB system, J = 18 Hz, 1H + 1H, 9-CH_2COO), 6.30 (br s, 1H, NH; disappeared with D_2O), 6.96 (dd, $J_{3,4}$ = 8.3 Hz, $J_{3,2}$ = 4.6 Hz, 1H, H-3), 8.17 (dd, $J_{4,3}$ = 8.3 Hz, $J_{4,2}$ = 1.5 Hz, 1H, H-4), 8.24 (dd, $J_{2,3}$ = 4.6 Hz, $J_{2,4}$ = 1.5 Hz, 1H, H-2). IR (KBr): 3257 (NH), 1743 (ester CO), 1608 s (amide CO), 1548, 1469 cm^{-1}.

*5.1.4.4. Ethyl 2-[6-[(diethylamino)carbonyl]-5-(isobutylamino)[1,2,4]triazolo[4,3-a][1,8]naphthyridin-9-yl]propanoate **3d**.* The solid residue obtained from reaction carried out with 1.67 g of **11b** [7] and 1.58 g of ethyl 3-hydrazino-2-methyl-3-oxopropanoate **6c**, was taken up in a little diethyl ether and filtered to give pure **3d** (1.00 g, 45%), whitish crystals, m.p. 192–193 °C, after crystallization from ethyl acetate/diisopropyl ether with charcoal. ^{1}H NMR ($CDCl_3$): δ 0.90–1.30 [m, 12H, $N(CH_2CH_3)_2 + NCH_2CH(CH_3)_2$], 1.35 [t, 3H, $COOCH_2CH_3$], 1.85–2.16 [m, 1H, $NCH_2CH(CH_3)_2$], 1.95 [d, 3H, 9-$CH(CH_3)COOCH_2CH_3$], 2.70 [m, 1H, 1H of $NCH_2CH(CH_3)_2$], 3.20–3.60 [m, 4H, 3H of $N(CH_2CH_3)_2$ + 1H of $NCH_2CH(CH_3)_2$], 3.80–4.21 [m, 1H, 1H of $N(CH_2CH_3)_2$], 4.05 [q, 2H, $COOCH_2CH_3$], 4.68 [q, 1H, 9-$CH(CH_3)COOCH_2CH_3$], 6.49 (near d, 1H, NH; disappeared with D_2O), 6.88 (dd, $J_{3,4}$ = 8.3 Hz, $J_{3,2}$ = 4.6 Hz, 1H, H-3), 8.04–8.28 (m, 2H, H-2,4). IR (KBr): 3277 (NH), 1736 (ester CO), 1608 s (amide CO), 1542, 1463 cm^{-1}.

*5.1.5. General procedure for the synthesis of 9-substituted 5-chloro-N,N-diethyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamides **13a–d***

A mixture of 5.0 mmol (1.49 g) of dichloroderivative **12** [6], 8.0 mmol of the proper amide-hydrazide **8a–c** or **10a** and 6 mL of Dowtherm A was stirred at 150 °C for the time reported below for each case. After cooling, dichloromethane (100 mL) and 10% aqueous Na_2CO_3 (50 mL) were added to the mixture which was stirred for 30 min at room temperature. The mixture was filtered to remove a reddish insoluble solid, then was transferred into a separatory funnel. The organic layer was collected and the aqueous one was extracted twice with dichloromethane. The combined organic extracts (dried over Na_2SO_4 and decolourized with charcoal) were evaporated to dryness at reduced pressure to give an orange liquid which was chromatographed on a silica gel column. The elution was carried out first with dichloromethane to remove the Dowtherm A, then with dichloromethane/ethyl acetate (1:1) to remove a small amount of starting compound **12** and finally with ethyl acetate to recover compounds **13a–d**. The eluate collected, after removal of solvent, afforded a pale orange thick oil from which compounds **13a–d** were obtained as reported below.

*5.1.5.1. 5-Chloro-N,N,N′,N′-tetraethyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6,9-dicarboxamide **13a**.* The pink solid residue obtained from reaction carried out (20 min) with 1.27 g of *N*,*N*-diethyl-2-hydrazino-2-oxoacetamide **8a**, was taken up in a little diethyl ether/petroleum ether and filtered to obtain pure **13a** (1.05 g, 52%), white crystals, m.p. 198–199 °C, after crystallization from ethyl acetate/petroleum ether with charcoal. ^{1}H NMR ($CDCl_3$): δ 1.16, 1.20, 1.40 and 1.43 [4t, 3H + 3H + 3H + 3H, 6-CON$(CH_2CH_3)_2$ + 9-CON$(CH_2CH_3)_2$], 3.09–3.42, 3.46–3.71 and 3.77–4.04 [3m, 4H + 2H + 2H, 6-CON$(CH_2CH_3)_2$ + 9-CON$(CH_2CH_3)_2$], 7.68 (dd, $J_{3,4}$ = 8.1 Hz, $J_{3,2}$ = 4.8 Hz, 1H, H-3), 8.58 (dd, $J_{4,3}$ = 8.1 Hz, $J_{4,2}$ = 1.6 Hz, 1H, H-4), 8.74 (dd, $J_{2,3}$ = 4.8 Hz, $J_{2,4}$ = 1.6 Hz, 1H, H-2). IR (KBr): 1647 s, br (CO), 1602, 1587 w, 1556 w, 1521 w, 1508 cm^{-1}.

*5.1.5.2. 5-Chloro-N^6,N^6-diethyl-N^9,N^9-dipropyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6,9-dicarboxamide **13b**.* The thick oily residue obtained from reaction carried out (20 min) with 1.50 g of *N*,*N*-dipropyl-2-hydrazino-2-oxoacetamide **8b**, was dissolved in a little diethyl ether and allowed to stand until pure **13b** separated out as a pink crystalline solid (1.38 g, 64%); after crystallization from ethyl acetate/petroleum ether with charcoal, white crystals (m.p. 172–173 °C) were obtained. ^{1}H NMR ($CDCl_3$): δ 0.77 [t, 3H, 3H of $N(CH_2CH_2CH_3)_2$], 1.12 and 1.21 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.41 [t, 3H, 3H of $N(CH_2CH_2CH_3)_2$], 1.48–2.00 [m, 4H, $N(CH_2CH_2CH_3)_2$], 2.96–3.94 [m, 8H, $N(CH_2CH_3)_2 + N(CH_2CH_2CH_3)_2$], 7.67 (dd, $J_{3,4}$ = 8.1 Hz, $J_{3,2}$ = 4.8 Hz, 1H, H-3), 8.58 (dd, $J_{4,3}$ = 8.1 Hz, $J_{4,2}$ = 1.6 Hz, 1H, H-4), 8.75 (dd, $J_{2,3}$ = 4.8 Hz, $J_{2,4}$ = 1.6 Hz, 1H, H-2). IR (KBr): 1643 s, br (CO), 1603, 1588 w, 1557 w, 1520 w, 1505 cm^{-1}.

*5.1.5.3. 5-Chloro-N^6,N^6-diethyl-N^9,N^9-diisopropyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6,9-dicarboxamide **13c**.* The thick oily residue obtained from reaction carried out (2 h) with 1.50 g of *N*,*N*-diisopropyl-2-hydrazino-2-oxoacetamide **8c**, was dissolved in a little diethyl ether and allowed to stand until pure **13c** separated out as a pink crystalline solid (0.40 g, 19%); after crystallization from ethyl acetate with charcoal, white crystals (m.p. 236–237 °C) were obtained. ^{1}H NMR ($CDCl_3$): δ 1.07 and 1.20 [2d, 3H + 3H, $NCH(CH_3)_2$], 1.21 and 1.41 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.67 and 1.74 [2d, 3H + 3H, $NCH(CH_3)_2$], 3.35 [q, 2H, 2H of $N(CH_2CH_3)_2$], 3.52–3.97 [m, 4H, 2H of $N(CH_2CH_3)_2 + NCH(CH_3)_2 + NCH(CH_3)_2$], 7.67 (dd, $J_{3,4}$ = 8.1 Hz, $J_{3,2}$ = 4.8 Hz, 1H, H-3), 8.56 (dd, $J_{4,3}$ = 8.1 Hz, $J_{4,2}$ = 1.6 Hz, 1H, H-4), 8.74 (dd, $J_{2,3}$ = 4.8 Hz, $J_{2,4}$ = 1.6 Hz, 1H, H-2). IR (KBr): 1647 s (CO), 1599, 1582 w, 1551 w, 1516 w, 1501 cm^{-1}.

*5.1.5.4. 5-Chloro-N,N-diethyl-9-[2-(isobutylamino)-2-oxoethyl][1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide **13d**.* The thick oily residue obtained from reaction carried out (45 min) with 1.39 g of *N*-isobutyl-3-hydrazino-3-oxopropanamide **10a**,

was taken up in a little diethyl ether and diisopropyl ether and kept at 4 °C so that pure **13d** separated out as a pink-orange crystalline solid (1.19 g, 57%); after crystallization from ethyl acetate/petroleum ether with charcoal, a whitish crystalline solid (m.p. 185–186 °C) was obtained. ^{1}H NMR ($CDCl_3$): δ 0.86 and 0.87 [2d, 3H + 3H, $NCH_2CH(CH_3)_2$], 1.14 and 1.36 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.76 [m, 1H, $NCH_2CH(CH_3)_2$], 3.08 [t, 2H, $NCH_2CH(CH_3)_2$], 3.28 [q, 2H, 2H of $N(CH_2CH_3)_2$], 3.56 and 3.84 [2m, 1H + 1H, 2H of $N(CH_2CH_3)_2$], 4.68 and 4.74 (AB system, J = 18 Hz, 1H + 1H, 9-CH_2CON), 7.29 (br s, 1H, NH; disappeared with D_2O), 7.60 (dd, $J_{3,4}$ = 8.1 Hz, $J_{3,2}$ = 4.8 Hz, 1H, H-3), 8.51 (dd, $J_{4,3}$ = 8.1 Hz, $J_{4,2}$ = 1.6 Hz, 1H, H-4), 8.70 (dd, $J_{2,3}$ = 4.8 Hz, $J_{2,4}$ = 1.6 Hz, 1H, H-2). IR (KBr): 3339 (NH), 1690 and 1682 sh (secondary amide CO), 1634 s (tertiary amide CO), 1601 w, 1588 w, 1548, 1483 cm^{-1}.

5.1.6. 5-Chloro-N,N-diethyl-9-[2-oxo-2-(2-thiazolylamino) ethyl][1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide **13e**

A mixture of 9.0 mmol (2.68 g) of dichloroderivative **12** [6], 11.0 mmol (2.20 g) of 3-hydrazino-3-oxo-*N*-(2-thiazolyl)-propanamide **10b**, 20 mL of Dowtherm A and 20 mL of dry pyridine was stirred at 160 °C for 1.5 h. After cooling, 150 mL of diethyl ether and 150 mL of aqueous 6 N HCl were added and the mixture was stirred at room temperature for 30 min. The acidic aqueous phase was collected whereas the ethereal one was extracted with aqueous 6 N HCl, then discarded. The entire aqueous phase was cooled with ice, treated with concentrated aqueous ammonia up to pH 9 and finally extracted with chloroform. The organic phase, dried over Na_2SO_4 and evaporated to dryness at reduced pressure, afforded a dark oil which was subjected to column chromatography on a silica gel column. The elution was carried out first with ethyl acetate to remove some impurities, then with the mixture dichloromethane/acetone (1:1) to recover **13e**. The eluate collected, after removal of solvents, gave a solid residue from which, by adding a little ethyl acetate, pure compound **13e** (0.60 g, 15%) separated out as a whitish crystalline solid, m.p. 262–263.5 °C, after crystallization from dichloromethane/ethyl acetate. ^{1}H NMR ($CDCl_3$): δ 1.21 and 1.41 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 3.36 [q, 2H, 2H of $N(CH_2CH_3)_2$], 3.63 and 3.88 [2m, 1H + 1H, 2H of $N(CH_2CH_3)_2$], 5.00 and 5.10 (AB system, J = 18 Hz, 1H + 1H, 9-CH_2CON), 6.99 (d, J = 3.6 Hz, 1H, thiazolyl H-5′), 7.51 (d, J = 3.6 Hz, 1H, thiazolyl H-4′), 7.63 (dd, $J_{3,4}$ = 8.1 Hz, $J_{3,2}$ = 4.8 Hz, 1H, H-3), 8.58 (dd, $J_{4,3}$ = 8.1 Hz, $J_{4,2}$ = 1.6 Hz, 1H, H-4), 8.69 (dd, $J_{2,3}$ = 4.8 Hz, $J_{2,4}$ = 1.6 Hz, 1H, H-2), 12.28 (br s, 1H, NH; disappeared with D_2O). IR (KBr): 3190 (NH), 1686 (secondary amide CO), 1627 s (tertiary amide CO), 1602, 1587 w, 1556 s, 1482 cm^{-1}.

5.1.7. General procedure for the synthesis of 9-substituted 5-(alkylamino)-N,N-diethyl[1,2,4]triazolo[4,3-a][1,8] naphthyridine-6-carboxamides **3e–i**

Compounds **3e–g**: A mixture of 3.0 mmol of the proper compound **13**, 7 mL of isobutylamine or *n*-butylamine and 15 mL of ethanol was heated in a closed vessel at 130 °C for 8 h. After cooling, the resulting solution was evaporated to dryness at reduced pressure and the residue was partitioned between dichloromethane and aqueous 5% $NaHCO_3$; the organic phase was then collected and the aqueous one was further extracted twice with dichloromethane. The combined organic extracts (dried over Na_2SO_4), after removal of solvent, afforded an oily or nearly solid residue from which compounds **3e–g** were recovered as described below for each case.

Compounds **3h,i**: A mixture of 3.0 mmol of the proper compound **13**, 7 mL of isobutylamine and 7 mL of dimethyl sulphoxide was heated at 130 °C for 3 h. The resulting solution was poured into ice-water and the mixture was subjected to an exhaustive extraction with chloroform. The combined organic extracts (dried over Na_2SO_4), after removal of solvent, afforded an oily or nearly solid residue from which compounds **3h,i** were recovered as described below for each case.

According to the above procedures the following compounds were prepared.

*5.1.7.1. N,N,N′,N′-Tetraethyl-5-(isobutylamino)[1,2,4]triazolo [4,3-a][1,8]naphthyridine-6,9-dicarboxamide **3e***. The oily residue derived from the reaction carried out with **13a** (1.21 g) and isobutylamine was treated with a little diethyl ether so that pure compound **3e** separated out as a crystalline solid (1.17 g, 89%); whitish crystals, m.p. 193–194 °C, after crystallization from ethyl acetate/petroleum ether. ^{1}H NMR ($CDCl_3$): δ 1.04 [d, 6H, $NCH_2CH(CH_3)_2$], 1.13, 1.21, 1.35 and 1.39 [4t, 3H + 3H + 3H + 3H, 6-$CON(CH_2CH_3)_2$ + 9-$CON(CH_2CH_3)_2$], 2.12 [m, 1H, $NCH_2CH(CH_3)_2$], 2.90 [m, 1H, 1H of $NCH_2CH(CH_3)_2$], 3.19 [q, 2H, 2H of 9-$CON(CH_2CH_3)_2$], 3.35–3.56, 3.60–3.77 and 3.95 [3m, 4H + 2H + 1H, 4H of 6-$CON(CH_2CH_3)_2$ + 2H of 9-CON(-$CH_2CH_3)_2$ + 1H of $NCH_2CH(CH_3)_2$], 5.46 (br s, 1H, NH; disappeared with D_2O), 7.11 (dd, $J_{3,4}$ = 8.3 Hz, $J_{3,2}$ = 4.6 Hz, 1H, H-3), 8.21 (dd, $J_{4,3}$ = 8.3 Hz, $J_{4,2}$ = 1.5 Hz, 1H, H-4), 8.56 (dd, $J_{2,3}$ = 4.6 Hz, $J_{2,4}$ = 1.5 Hz, 1H, H-2). IR (KBr): 3325 (NH), 1627 s (CO), 1606, 1540, 1505 w cm^{-1}.

*5.1.7.2. N^6,N^6-Diethyl-5-(isobutylamino)-N^9,N^9-dipropyl[1,2,4] triazolo[4,3-a][1,8]naphthyridine-6,9-dicarboxamide **3f***. The oily residue obtained from the reaction carried out with **13b** (1.29 g) and isobutylamine were treated with a little diethyl ether so that pure compound **3f** separated out as a crystalline solid (1.04 g, 74%); whitish crystals, m.p. 177–178 °C, after crystallization from ethyl acetate/diisopropyl ether with charcoal. ^{1}H NMR ($CDCl_3$): δ 0.72 [t, 3H, 3H of $N(CH_2CH_2CH_3)_2$], 1.04 [d, 6H, $NCH_2CH(CH_3)_2$], 1.10 and 1.16 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.35 [t, 3H, 3H of $N(CH_2CH_2CH_3)_2$], 1.50–1.73 and 1.76–1.94 [2m, 2H + 2H, $N(CH_2CH_2CH_3)_2$], 2.10 [m, 1H, $NCH_2CH(CH_3)_2$], 2.92 [m, 1H, 1H of $NCH_2CH(CH_3)_2$], 3.12 [t, 2H, 2H of $N(CH_2CH_2CH_3)_2$], 3.32–3.71 [m, 6H, 3H of $N(CH_2CH_3)_2$ + 2H of $N(CH_2CH_2CH_3)_2$ + 1H of $NCH_2CH(CH_3)_2$], 3.95 [m, 1H, 1H of $N(CH_2CH_3)_2$], 5.33 (br s, 1H, NH; disappeared with D_2O), 7.14 (dd, $J_{3,4}$ = 8.3 Hz, $J_{3,2}$ = 4.6 Hz, 1H, H-3), 8.20 (dd, $J_{4,3}$ = 8.3 Hz, $J_{4,2}$ = 1.5 Hz, 1H, H-4), 8.57 (dd,

$J_{2,3} = 4.6$ Hz, $J_{2,4} = 1.5$ Hz, 1H, H-2). IR (KBr): 3318 (NH), 1637 s (CO), 1608, 1536 cm^{-1}.

*5.1.7.3. 5-(Butylamino)-N,N-diethyl-9-[2-(isobutylamino)-2-oxoethyl][1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide **3g***. The nearly solid residue obtained from the reaction carried out with **13d** (1.25 g) and *n*-butylamine were taken up in a little diethyl ether and the crystalline solid that separated out was recovered by filtration to give pure **3g** (1.08 g, 79%); whitish crystals, m.p. 213–214 °C, after crystallization from ethyl acetate with charcoal. ^{1}H NMR ($CDCl_3$): δ 0.86–1.08 [m, 9H, $NCH_2CH(CH_3)_2 + NCH_2CH_2CH_2CH_3$], 1.18 and 1.34 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.48 (m, 2H, $NCH_2CH_2CH_2CH_3$), 1.71–1.95 [m, 3H, $NCH_2CH_2CH_2CH_3 + NCH_2CH(CH_3)_2$], 2.95 [m, 1H, 1H of $NCH_2CH(CH_3)_2$], 3.14 (t, 2H, $NCH_2CH_2CH_2CH_3$), 3.30–3.52 [m, 4H, 3H of $N(CH_2CH_3)_2$ + 1H of $NCH_2CH(CH_3)_2$], 4.00 [m, 1H, 1H of $N(CH_2CH_3)_2$], 4.47 and 4.63 (AB system, $J = 18$ Hz, 1H + 1H, 9-CH_2CON), 6.38 (near d, 1H, 5-*NH*CH_2; disappeared with D_2O), 6.98 (dd, $J_{3,4} = 8.3$ Hz, $J_{3,2} = 4.6$ Hz, 1H, H-3), 7.93 (near t, 1H, CO*NH*CH_2; disappeared with D_2O), 8.17 (dd, $J_{4,3} = 8.3$ Hz, $J_{4,2} = 1.5$ Hz, 1H, H-4), 8.26 (dd, $J_{2,3} = 4.6$ Hz, $J_{2,4} = 1.5$ Hz, 1H, H-2). IR (KBr): 3329 br (NH), 1685 sh and 1671 (secondary amide CO), 1607 s (tertiary amide CO), 1548, 1463 cm^{-1}.

*5.1.7.4. N,N-Diethyl-5-(isobutylamino)-9-[2-(isobutylamino)-2-oxoethyl][1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide **3h***. The oily residue obtained from the reaction carried out with **13d** (1.25 g) and isobutylamine were subjected to chromatography on a silica gel column eluting first with acetone to remove a little amount of starting compound **13d**, then with the mixture dichloromethane/methanol (9:1) to recover **3h**. The eluate collected, after removal of solvent gave an oil that was treated with a little ethyl ether to afford pure **3h** (1.09 g, 80%); whitish crystals, m.p. 194–195 °C, after crystallization from ethyl acetate with charcoal. ^{1}H NMR ($CDCl_3$): δ 0.85–1.15 [m, 12H, 5-$NCH_2CH(CH_3)_2$ + CON$HCH_2CH(CH_3)_2$], 1.19 and 1.33 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.85 and 2.09 [2m, 1H + 1H, 5-$NCH_2CH(CH_3)_2$ + $CONHCH_2CH(CH_3)_2$], 2.70 [m, 1H, 1H of 5-$NCH_2CH(CH_3)_2$], 3.15 [t, 2H, $CONHCH_2CH(CH_3)_2$], 3.24–3.52 [m, 4H, 3H of $N(CH_2CH_3)_2$ + 1H of 5-$NCH_2CH(CH_3)_2$], 4.04 [m, 1H, 1H of $N(CH_2CH_3)_2$], 4.48 and 4.66 (AB system, $J = 18$ Hz, 1H + 1H, 9-CH_2CON), 6.40 (near d, 1H, 5-*NH*CH_2; disappeared with D_2O), 6.99 (dd, $J_{3,4} = 8.3$ Hz, $J_{3,2} = 4.6$ Hz, 1H, H-3), 7.87 (near t, 1H, CO*NH*CH_2; disappeared with D_2O), 8.19 (dd, $J_{4,3} = 8.3$ Hz, $J_{4,2} = 1.5$ Hz, 1H, H-4), 8.26 (dd, $J_{2,3} = 4.6$ Hz, $J_{2,4} = 1.5$ Hz, 1H, H-2). IR (KBr): 3271 br (NH), 1684 sh and 1665 (secondary amide CO), 1607 s (tertiary amide CO), 1548, 1466 cm^{-1}.

*5.1.7.5. N,N-Diethyl-5-(isobutylamino)-9-[2-oxo-2-(2-thiazolylamino)ethyl][1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide **3i***. The oily residue obtained from the reaction carried out with **13e** (1.33 g) and isobutylamine were subjected to chromatography on a silica gel column. The elution was carried out first with ethyl acetate to remove a little amount of starting compound **13e**, then with acetone to recover **3i**. The eluate collected, after removal of solvent afforded a yellowish solid that was taken up in a little diethyl ether and filtered to yield pure **3i** (1.05 g, 73%); pale-yellow crystals, m.p. 184–186 °C dec, after crystallization from ethyl acetate/diisopropyl ether with charcoal. ^{1}H NMR ($CDCl_3$): δ 1.06 [d, 6H, $NCH_2CH(CH_3)_2$], 1.17 and 1.34 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 2.11 [m, 1H, $NCH_2CH(CH_3)_2$], 2.68 [m, 1H, 1H of $NCH_2CH(CH_3)_2$], 3.22–3.54 [m, 4H, 3H of $N(CH_2CH_3)_2$ + 1H of $NCH_2CH(CH_3)_2$], 4.05 [m, 1H, 1H of $N(CH_2CH_3)_2$], 4.88 (near s, 2H, 9-CH_2CON), 6.67 (br s, 1H, 5-*NH*CH_2; disappeared with D_2O), 6.80–6.93 (m, 1H, H-3), 6.85 (d, $J = 3.6$ Hz, 1H, thiazolyl H-5′), 7.37 (d, $J = 3.6$ Hz, 1H, thiazolyl H-4′), 8.09–8.27 (m, 2H, H-2,4), 12.10 (br s, 1H, CO*NH*; disappeared with D_2O). IR (KBr): 3263 and 3204 (NH), 1697 w and 1667 w (secondary amide CO), 1607 s (tertiary amide CO), 1552, 1471 cm^{-1}.

*5.1.8. N,N,9-Triethyl-5-[ethyl(pyridin-3-ylcarbonyl)amino][1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide **4a***

A mixture of 4.0 mmol (1.30 g) of **1b** [1], 20.0 mmol (3.54 g) of nicotinoyl chloride hydrochloride, 50 mL of anhydrous toluene and 20 mL of triethylamine was heated at 120 °C for 3 h, with stirring. The mixture was then evaporated to dryness at reduced pressure and the residue partitioned between dichloromethane and 10% aqueous Na_2CO_3. The organic phase was collected and the aqueous one was extracted twice with dichloromethane. The combined organic extracts, dried over Na_2SO_4 and evaporated to dryness at reduced pressure, afforded a thick oil which was chromatographed on an aluminium oxide column. The elution was performed first with dichloromethane to remove some impurities, then with the mixture dichloromethane/triethylamine (9:1) to recover **4a**. The eluate collected, after removal of solvents, gave a thick oil from which, after addition of a little diethyl ether and standing, pure **4a** separated out as a pink solid (0.71 g, 40%); whitish crystals, m.p. 203–204 °C, after crystallization from ethyl acetate/petroleum ether. ^{1}H NMR ($CDCl_3$): δ 1.11 and 1.20–1.65 [t + m, 3H + 9H, $N(CH_2CH_3)_2$ + 5-NCH_2CH_3 + 9-CH_2CH_3], 3.11–4.20 and 4.42–4.65 [2m, 7H + 1H, $N(CH_2CH_3)_2$ + 5-NCH_2CH_3 + 9-CH_2CH_3], 6.90–7.05 and 7.40–8.95 (2m, 7H, H-2,3,4 + pyridyl H's). IR (KBr): 1645 s (CO), 1611, 1587, 1562 w, 1482 w cm^{-1}.

*5.1.9. 5-[Acetyl(isobutyl)amino]-N,N-diethyl-9-isopropyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide **4b***

A mixture of 3.0 mmol (1.15 g) of **1n** [1] and 10 mL of acetic anhydride was stirred at 140 °C for 8 h. The mixture was then poured into ice-water and the resulting solution was treated with $NaHCO_3$ up to pH 7, then exhaustively extracted with chloroform. The thick oil obtained from the combined extracts (dried over Na_2SO_4 and evaporated to dryness at reduced pressure) was chromatographed on an aluminium oxide

column. The elution was carried out first with dichloromethane to remove some impurities, then with ethyl acetate to recover **4b**. From the eluate collected, after removal of solvent, a thick oil was obtained from which, after addition of a little diisopropyl ether, pure **4b** separated out as a white solid (1.08 g, 85%), m.p. 137–138 °C, after crystallization from diisopropyl ether. ^{1}H NMR ($CDCl_3$): δ 0.80–0.95, 1.00–1.15, 1.25–1.40 and 1.52–1.90 [4m, 19H, 5-$NCH_2CH(CH_3)_2$ + $N(CH_2CH_3)_2$ + 9-$CH(CH_3)_2$ + 5-$NCH_2CH(CH_3)_2$], 2.00 (s, 3H, $COCH_3$), 3.05–4.10 [m, 6H, $N(CH_2CH_3)_2$ + 5-$NCH_2CH(CH_3)_2$], 4.54 [m, 1H, 9-$CH(CH_3)_2$], 7.61 (dd, $J_{3,4}$ = 8.3 Hz, $J_{3,2}$ = 4.6 Hz, 1H, H-3), 8.17 (dd, $J_{4,3}$ = 8.3 Hz, $J_{4,2}$ = 1.5 Hz, 1H, H-4), 8.82 (dd, $J_{2,3}$ = 4.6 Hz, $J_{2,4}$ = 1.5 Hz, 1H, H-2). IR (KBr): 1671 (CO), 1632 s (CO), 1609, 1587, 1558 w, 1514 w cm^{-1}.

5.2. Pharmacology

The carrageenan-induced paw edema test [11] was performed in groups of five male Sprague-Dawley rats (120–150 g). Animals were purchased from Harlan (Correzzana, Italy) and allowed to acclimate in our animal facility for one week before use. They were housed in an air-conditioned room (22 ± 2 °C, relative humidity 50 ± 10%) with a 12-h light/dark cycle, kept on bedding chips (Harlan), and had free access to tap water and rat chow (TRM, Harlan). Rats were fasted for 15 h before treatment, but had always free access to water.

5.2.1. Anti-inflammatory activity

All compounds were administered orally by gastric intubation, as finely homogenized suspension in 0.5% carboxymethylcellulose (1 mL/100 g body weight), at the initial dose of 200 mg/kg. The compounds which exhibited at this dose a statistically significant activity were further tested at doses decreasing by a factor of two. Controls received the same volume of the vehicle.

Sixty minutes after the administration of the test compound, 0.1 mL of a 1% carrageenan solution in saline was injected into the plantar surface of the right hind paw of each rat. Paw volume, as determined by measuring the amount of water displaced after immersing the paw to the level of the lateral malleolus, was recorded immediately after the carrageenan injection, and 2 h later. The difference between the two values was taken as edema volume. The percent inhibition of the edema in treated rats as compared to controls was calculated. Indomethacin (6 mg/kg p.o.) was used as positive control.

5.2.2. Data analysis

Results were calculated as mean ± SD. 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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