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Original article

1,8-Naphthyridines VII. New substituted 5-amino[1,2,4]triazolo [4,3-*a*][1,8]naphthyridine-6-carboxamides and their isosteric analogues, exhibiting notable anti-inflammatory and/or analgesic activities, but no acute gastrolesivity

Giorgio Roma [a,*], Giancarlo Grossi [a], Mario Di Braccio [a], Daniela Piras [a], Vigilio Ballabeni [b], Massimiliano Tognolini [b], Simona Bertoni [b], Elisabetta Barocelli [b]

[a] *Dipartimento di Scienze Farmaceutiche, Università di Genova, Viale Benedetto XV, 3, I-16132 Genova, Italy*
[b] *Dipartimento di Scienze Farmacologiche, Biologiche e Chimiche Applicate, Università di Parma, Viale G.P. Usberti 27/A, I-43100 Parma, Italy*

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Abstract

The [1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamide derivatives 5-amino (**2**) or 5-alkoxy (**3**) substituted and the 5-amino[1,2,4]triazolo[4,3-*a*]quinoline-4-carboxamide derivatives (**4**), designed to obtain new effective analgesic and/or anti-inflammatory agents were synthesized. Ten compounds **2** and **4** showed an interesting analgesic activity: the most potent ones are **2j** (36% inhibition, $P < 0.05$) and **4b** (77% inhibition, $P < 0.01$) at 6.25 and 25 mg kg^{-1} doses, respectively. Compounds **2i–l** and **4c** showed notable anti-inflammatory properties: the most potent ones are **2i** (68% inhibition, $P < 0.01$) and **2l** (42% inhibition, $P < 0.05$) at 12.5 and 6.25 mg kg^{-1} doses, respectively. The replacement in compounds **2** of the *N*-substituted 5-amino substituents with similar alkoxy groups usually afforded less active compounds **3**.

Keywords: [1,2,4]Triazolo[4,3-*a*][1,8]naphthyridines; Isosteric analogues; Anti-inflammatory; Analgesic; Gastrolesivity; Structure–activity relationships

1. Introduction

In the course of our studies aimed at obtaining new anti-inflammatory and analgesic agents devoid of undesirable side effects, properly substituted 4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-5-amines **1** [1,2] and 5-amino[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamides **2** [3,4] proved to be the most interesting compounds, being both endowed with appreciable anti-inflammatory and analgesic properties and devoid of acute gastrolesivity and significant acute toxicity (Fig. 1).

However, the 1,8-naphthyridine tricyclic derivatives **2** appear to be clearly more effective than the 1,5-benzodiazepine derivatives **1**, not only due to their notably higher anti-inflammatory activity (carrageenan-induced rat paw edema test), but also due to their more potent analgesic properties (acetic acid induced writhing test in mice) [3,4], depending on the substituents. It must also be observed that, on the whole, compounds **2** show notable potency difference between their analgesic and anti-inflammatory activities [4]: such results suggest that different mechanisms of action support their analgesic and anti-inflammatory properties, respectively. The activities of the most potent anti-inflammatory (**2a**,**b**) [3] and analgesic (**2c**) [4] compounds previously described by us are reported in Table 1.

The results of a recent in vitro study clearly indicate that the anti-inflammatory activity of compounds **2a**,**c** (chosen as examples) reasonably derives from the anti-adhesive effects of these compounds and from their inhibition of superoxide anion production and of myeloperoxidase release, without

* Corresponding author. Tel.: +39 10 353 8374; fax: +39 10 353 8358.
E-mail address: roma@unige.it (G. Roma).

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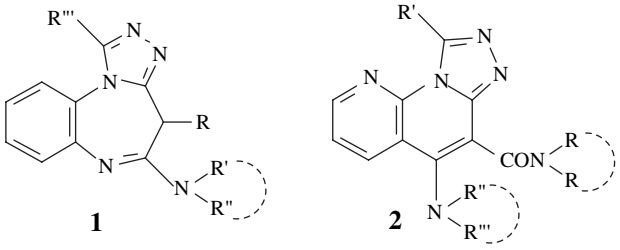


Fig. 1. Structures of the substituted 4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-5-amines **1** and 5-amino[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamides **2**.

involving the cyclooxygenase (COX) inhibition (in accordance with the absence of acute gastrolesivity) [5].

On the basis of the very interesting pharmacological properties and the structural features of compounds **2a–c**, we have now designed and synthesized several novel properly substituted compounds **2** (**2d–o**), their 5-alkoxy substituted analogues **3a–f**, and the [1,2,4]triazolo[4,3-*a*]quinoline derivatives **4a–d**, whose tricyclic system is isosteric to that of compounds **2** and **3**. All these compounds have been tested for their anti-inflammatory and analgesic properties, in order to further investigate the structure–activity relationships (SAR) in this structural–pharmacological field and possibly to obtain new potent anti-inflammatory and/or analgesic agents. For the effective compounds the acute gastrolesivity, which is the most frequent harmful side effect in NSAIDs, was also evaluated.

Furthermore, the compounds showing interesting and statistically significant antinociceptive activity in the writhing test were also tested for their inhibitory properties on spontaneous mice locomotor activity, in order to verify the occurrence of confounding CNS depressant properties of these compounds.

2. Chemistry

The [1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamide derivatives **2d–o** and **3a–f** were prepared through the synthetic routes reported in Scheme 1. Thus, the methyl 1,2-dihydro-4-hydroxy-2-oxo-1,8-naphthyridine-3-carboxylate **5** [6] was heated in a closed vessel (150 °C) with an ethanol solution of excess ethylamine or pyrrolidine to give high yields of the corresponding amides **6a** (95%) or **6b** (93%), respectively, which were then heated in refluxing phosphorus oxychloride, affording good yields of the corresponding 2,4-dichloroderivatives **7a**,**b**. From the subsequent cyclocondensation of dichloroderivatives **7a**, **7b**, **and 7c** [7] with proper hydrazides (Dowtherm A, 160 °C) the novel 5-chloro[1,2,4]-triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamide derivatives **8a–c**, respectively, were obtained in moderate yields. Finally, the treatment of the proper 5-chloroderivatives **8a–c**, **8d** [4], or **8e**,**f** [8] with an excess of suitable amines afforded rather high yields of the desired compounds **2d–k**,**m–o**. This reaction was carried out under two different heating conditions, depending on the use of primary amines (120 °C in closed vessel, compounds **2h–k**,**n**) or substituted piperazines (130 °C, compounds **2d–g**,**m**,**o**). The 5-(dialkylamino)derivative **2l** was obtained by methylation of the previously described compound **2b** [3], using iodomethane in 2-butanone at reflux in the presence of anhydrous K_2CO_3–KOH.

The treatment of the 5-chloroderivatives **8e** [8] or **8d** [4] with a mild excess of the suitable sodium alkoxides, in anhydrous tetrahydrofuran at reflux, gave good yields of the 5-alkoxyderivatives **3a** or **3b–f**, respectively.

The substituted 5-amino[1,2,4]triazolo[4,3-*a*]quinoline-4-carboxamides **4a–d** were obtained by the four-step procedure described in Scheme 2, starting from ethyl 1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxylate **9** [9] which was treated with excess diethylamine in ethanol solution (closed vessel, 150 °C) to give the corresponding diethylamide **10** (65% yield). By heating compound **10** in refluxing $POCl_3$, a mixture of the 4-chloroderivative **11** (12%) and 2,4-dichloroderivative **12** (67%) was obtained, which were easily separated by column chromatography. Compound **11** was then converted into the 2,4-dichloroderivative **12** (82%) by subsequent treatment with refluxing $POCl_3$. The cyclocondensation of 2,4-dichloroderivative **12** with formic hydrazide or isobutyrohydrazide (Dowtherm A, 150 °C) afforded the 5-chloro[1,2,4]triazolo[4,3-*a*]quinoline-4-carboxamide derivative **13a** or **13b**, respectively, in low yields. Finally, the treatment of **13a** or **13b** with excess 1-methylpiperazine (140 °C) afforded the corresponding compound **4a** (69%) or **4b** (52%), whereas the reaction of **13b** with ethylamine or isobutylamine (160 °C, in a closed vessel) gave compound **4c** (84%) or **4d** (80%), respectively.

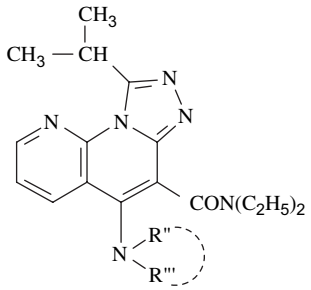
The results of elemental analyses, IR and ^{1}H NMR spectral data completely agree with the structures attributed to the compounds described in this paper (see Section 5.1 and Table 3), and the spectral data are consistent with those previously reported by us for analogous compounds [3,4].

3. Results and discussion

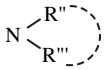
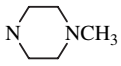
Compounds **2d–o**, **3a–f** and **4a–d** were tested in vivo for their anti-inflammatory and analgesic activities at the initial dose of 100 mg kg^{-1}. The compounds exhibiting a statistically significant activity at this dose were further tested at doses decreasing by a factor of two, until statistically significant activity was no longer observed. For the compounds showing statistically significant analgesic activity their effect on spontaneous locomotor activity in mice (at properly chosen doses) was evaluated after oral administration. The corresponding pharmacological results are listed in Table 2. The compounds provided with statistically significant anti-inflammatory and/or analgesic activities were also tested for their acute gastrolesivity in rats (at 200 mg kg^{-1} os dose): for all the compounds tested no acute gastrolesive effect was detected in each of the 8 treated rats (0/8), whereas indomethacin caused gastric ulcers in 7 of the 8 treated rats (7/8) at 10 mg kg^{-1} os dose [4] (see Section 5.2).

Many novel compounds **2** exhibited interesting activities. For instance, compounds **2i–k**,**l** showed statistically significant anti-inflammatory activity down to 12.5 and 6.25 mg kg^{-1}

Table 1
Structures and pharmacological data of the most potent anti-inflammatory (**2a**,**b**) and analgesic (**2c**) agents previously described by us



2a-c

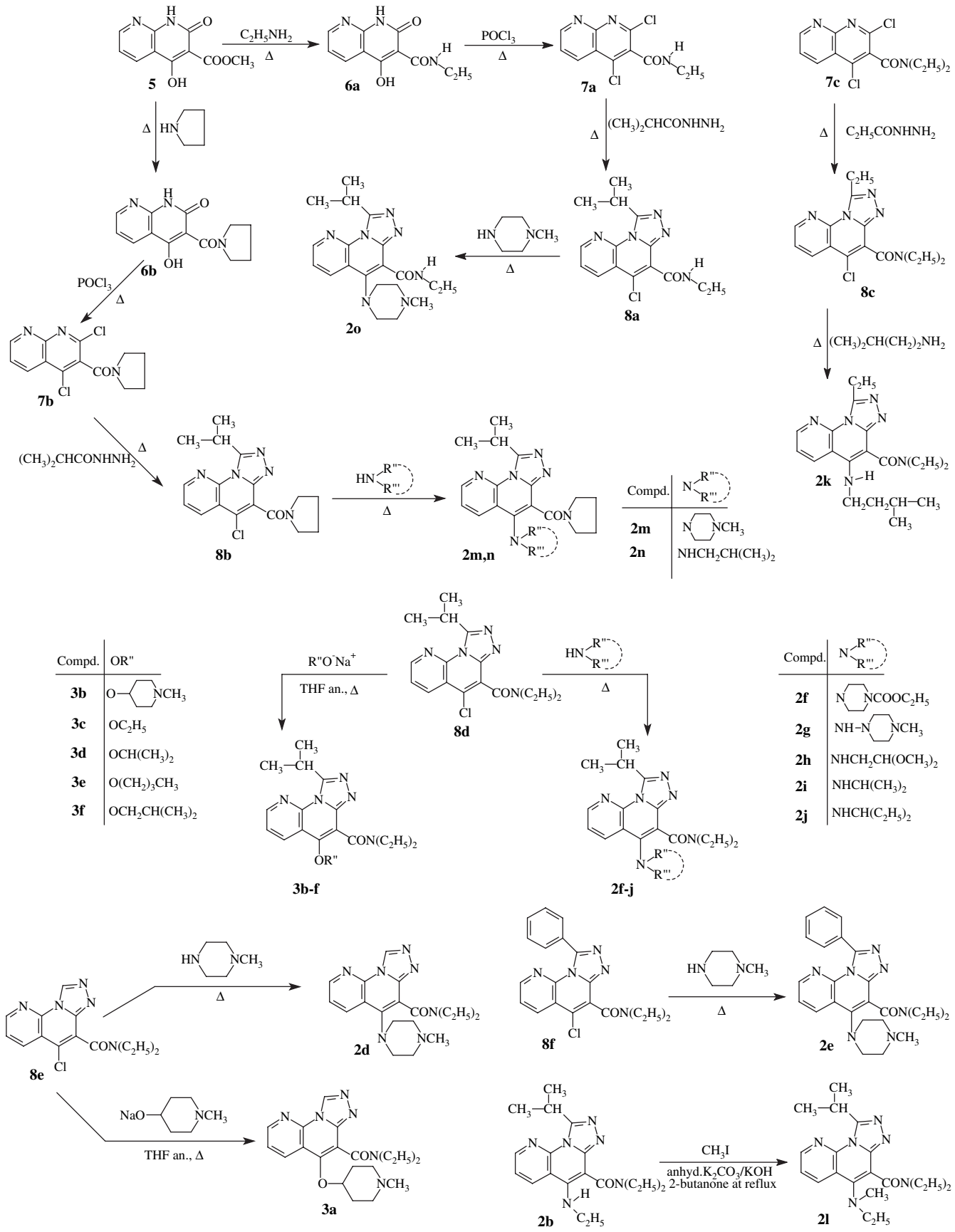
Compound		Dose (mg kg^{-1} os)	Analgesic activity[a,e] (% inhibition)	Anti-inflammatory activity[b,f] (% inhibition)	Spontaneous mice locomotor activity[c,e] (% inhibition)	Acute gastrolesivity in rats[d,e]
2a	NH–*i*-C_4H_9	200	100**[g]	74**		
		100	100**	70**	18	0/8
		50	33	66**		
		25	–	61**		
		12.5	–	50**		
		6.25	–	27*		
		3.12	–	15		
2b	NH–C_2H_5	200	100**	65**		
		100	100**	63**	9	0/8
		50	20	63**		
		25	–	61**		
		12.5	–	35**		
		6.25	–	33*		
		3.12	–	13		
2c		200	86**	34**		
		100	88**	23	94**	0/8
		50	85**	–	100**[h]	
		25	90**	–	99**[h]	
		12.5	92**	–	74**[h]	
		6.25	83**	–	19[h]	
		3.12	85**	–	3	
		1.6	39*	–	–	
		0.8	3	–	–	

[a] Acetic acid induced writhing in mice.
[b] Carrageenan-induced rat paw edema.
[c] Activity counted in 30 min in mice.
[d] Number of rats showing gastric lesions.
[e] Data from Ref. [4].
[f] Data from Ref. [3] for **2a**,**b** and from Ref. [4] for **2c**.
[g] $*P < 0.05$, $**P < 0.01$ significance as compared to controls (Student's *t*-test).
[h] Unpublished data.

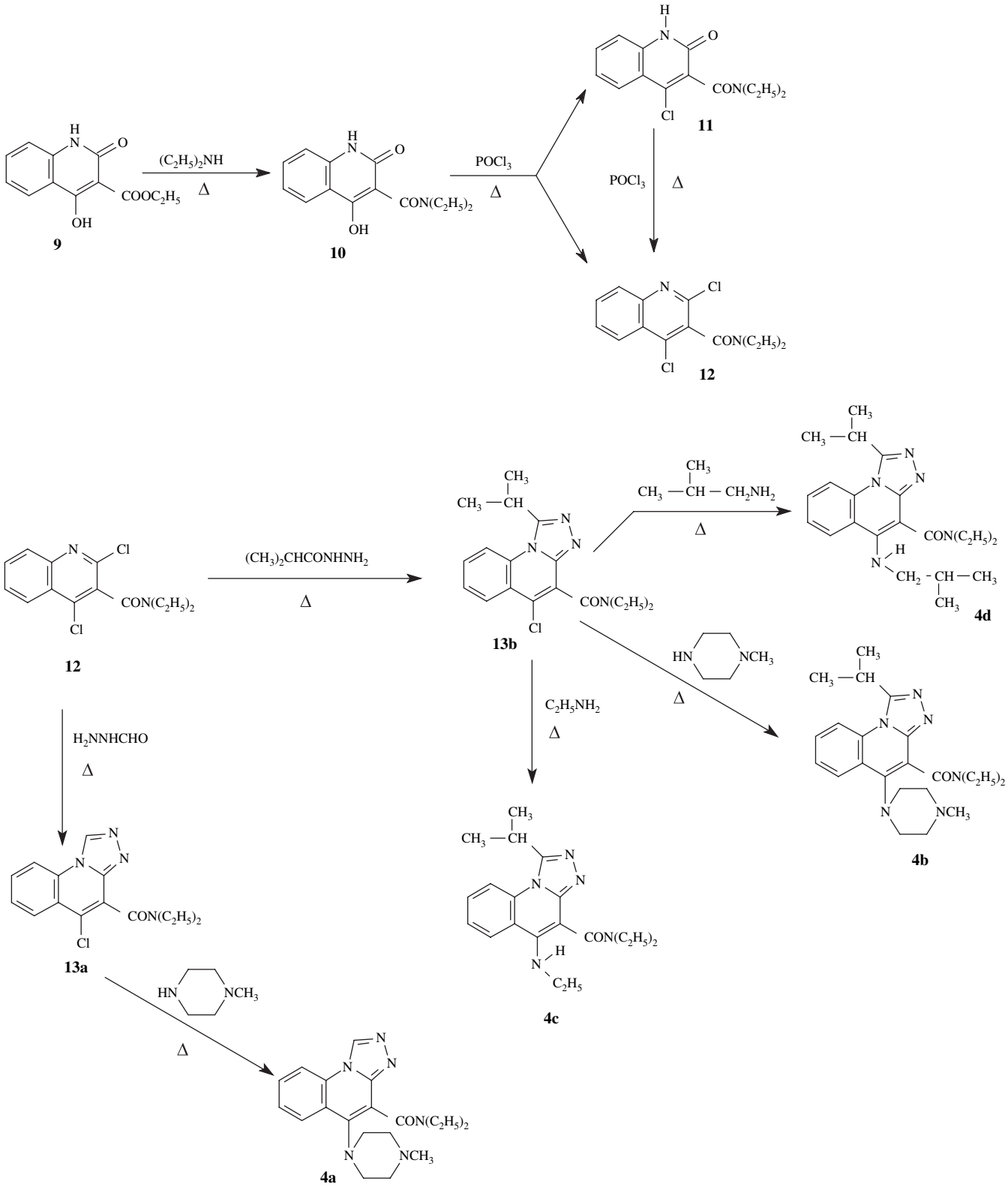
doses, respectively (carrageenan-induced rat paw edema assay). In particular, compound **2i**, at 12.5 mg kg^{-1} dose (68% edema inhibition, $P < 0.01$), and compound **2l**, at 6.25 mg kg^{-1} dose (42% edema inhibition, $P < 0.05$), respectively, appear a little more potent than the lead compound **2a** at the same doses (50%, $P < 0.01$, and 27%, $P < 0.05$ edema inhibition, respectively) (Table 1). It is worth noting that no compound of this group caused acute gastric damage in rats even when orally administered at 200 mg kg^{-1} dose.

On the other hand, compounds **2e**,**f**,**h**–**l**,**n**,**o** at 100 mg kg^{-1} dose (inhibition range 47–100%) and seven of them also at 50 mg kg^{-1} dose (inhibition range 54–75%) showed statistically significant antinociceptive activity in the acetic acid induced writhing test in mice. Compound **2j**, the most potent one as analgesic agent, produced an interesting statistically significant protection down to the 6.25 mg kg^{-1} dose (36%, $P < 0.05$), but was found to be clearly less potent than the lead compound **2c** [4] (Table 1). It must be observed that, in the range of the doses tested, several compounds sometimes notably inhibited the spontaneous locomotor activity of the treated mice, thus exhibiting sedative effects confounding the analgesia study.

Only two compounds (**2h**,**n**) out of nine demonstrated statistically significant antinociceptive activity, not associated with significant sedative effects, at 100 mg kg^{-1} dose: 47%



Scheme 1. Synthetic routes to the [1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamide derivatives **2d–o** and **3a–f**.

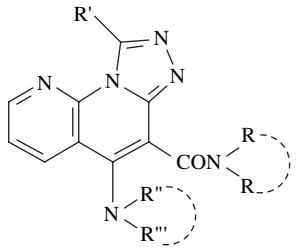


Scheme 2. Synthetic route to the 5-amino[1,2,4]triazolo[4,3-*a*]quinoline-4-carboxamide derivatives **4a**–**d**.

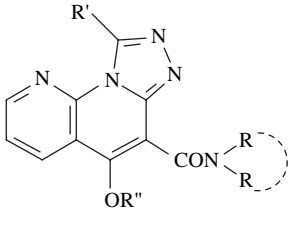
($P < 0.05$) and 60% ($P < 0.01$) reduction of writhe number, respectively. It is also interesting to point out that compound **2l**, at 50 mg kg^{-1} dose, showed a notable and statistically significant antinociceptive activity (59%, $P < 0.01$), but not significant and very low (22%) inhibition of spontaneous locomotor activity in mice.

Only three (**3a**,**b**,**e**) of the substituted 5-alkoxy[1,2,4]-triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamides **3** herein

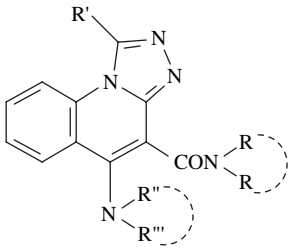
Table 2
Structures and pharmacological data of compounds **2d–o**, **3a–f**, and **4a–d**



2d-o



3a-f



4a-d

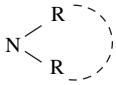
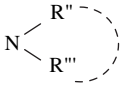
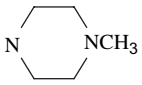
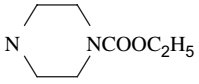
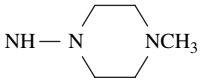
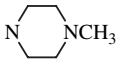
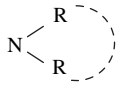
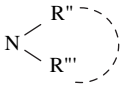
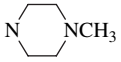
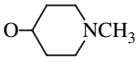
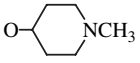
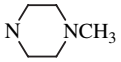
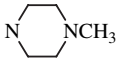
Compound		R′		OR″	Dose (mg kg^{-1} os)	Analgesic activity[a] (% inhibition)	Anti-inflammatory activity[b] (% inhibition)	Spontaneous mice locomotor activity[c] (% inhibition)
2d	$N(C_2H_5)_2$	H		–	100	14	0	–
2e	$N(C_2H_5)_2$	C_6H_5		–	100	100**[d]	0	72**
					50	62*	–	62**
					25	15	–	–
2f	$N(C_2H_5)_2$	$CH(CH_3)_2$		–	100	100**	0	83**
					50	75**	–	69**
					25	53*	–	96**
					12.5	29	–	–
2g	$N(C_2H_5)_2$	$CH(CH_3)_2$		–	100	17	0	–
2h	$N(C_2H_5)_2$	$CH(CH_3)_2$	$NH-CH_2CH(OCH_3)_2$	–	100	47*	0	0
					50	8	–	–
					25	–	–	–
2i	$N(C_2H_5)_2$	$CH(CH_3)_2$	$NH-CH(CH_3)_2$	–	100	83**	81**	98**
					50	54**	75**	64*
					25	33*	75**	26
					12.5	0	68**	0
					6.25	–	15	–
2j	$N(C_2H_5)_2$	$CH(CH_3)_2$	$NH-CH(C_2H_5)_2$	–	100	91**	50**	97**
					50	75**	47**	84**
					25	58**	47**	70**
					12.5	49*	45*	92**
					6.25	36*	11	81**
					3.12	18	–	–
2k	$N(C_2H_5)_2$	C_2H_5	$NH-(CH_2)_2CH(CH_3)_2$	–	100	98**	41**	95**
					50	75**	41**	76**
					25	15	43*	–
					12.5	–	29*	–
					6.25	–	0	–
2l	$N(C_2H_5)_2$	$CH(CH_3)_2$		–	100	81**	71**	53*
					50	59**	67**	22
					25	17	65**	–
					12.5	–	51*	–
					6.25	–	42*	–
					3.12	–	0	–
2m		$CH(CH_3)_2$		–	100	39	0	–
2n		$CH(CH_3)_2$	$NH-CH_2CH(CH_3)_2$	–	100	60**	0	5
					50	64*	–	–
					25	29	–	–

Table 2 (*continued*)

Compound		R′		OR″	Dose (mg kg^{-1} os)	Analgesic activity[a] (% inhibition)	Anti-inflammatory activity[b] (% inhibition)	Spontaneous mice locomotor activity[c] (% inhibition)
2o	$NH-C_2H_5$	$CH(CH_3)_2$		–	100 50	79** 24	0 –	80** –
3a	$N(C_2H_5)_2$	H	–		100 50	45* 20	0 –	55* –
3b	$N(C_2H_5)_2$	$CH(CH_3)_2$	–		100 50	87** 35	18 –	65* –
3c	$N(C_2H_5)_2$	$CH(CH_3)_2$	–	OC_2H_5	100	22	10	–
3d	$N(C_2H_5)_2$	$CH(CH_3)_2$	–	$OCH(CH_3)_2$	100 50	11 –	24* 0	– –
3e	$N(C_2H_5)_2$	$CH(CH_3)_2$	–	$O(CH_2)_3CH_3$	100 50	40* 14	3 –	76* 21
3f	$N(C_2H_5)_2$	$CH(CH_3)_2$	–	$OCH_2CH(CH_3)_2$	100	26	0	–
4a	$N(C_2H_5)_2$	H		–	100 50 25	55** 58* 28	0 – –	0 – –
4b	$N(C_2H_5)_2$	$CH(CH_3)_2$		–	100 50 25 12.5	100** 100** 77** 14	34* 0 – –	54** 48** 21 –
4c	$N(C_2H_5)_2$	$CH(CH_3)_2$	$NH-C_2H_5$	–	100 50 25 12.5	91** 43* 12 –	42** 43** 24* 6	39* 2 – –
4d	$N(C_2H_5)_2$	$CH(CH_3)_2$	$NH-CH_2CH(CH_3)_2$	–	100 50	88** 29	0 –	14 –
Indomethacin					10	84**[e]	51**[e]	–
Diazepam					10	–	–	98**[f]

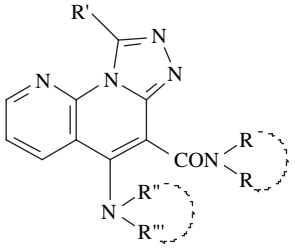
[a] Acetic acid induced writhing in mice.
[b] Carrageenan-induced rat paw edema.
[c] Activity counted in 30 min in mice.
[d] $*P < 0.05$, $**P < 0.01$ significance as compared to controls (Student's *t*-test).
[e] Data from Ref. [2].
[f] Data from Ref. [4].

described exhibited a statistically significant antinociceptive activity (inhibition range 40–87%, at 100 mg kg^{-1} dose), always coupled with sedation (inhibition range of spontaneous mice locomotor activity 55–76%, $P < 0.05$), whereas no compound **3** afforded anti-inflammatory activity except for the weakly active compound **3d** (24% edema inhibition, $P < 0.05$) at the 100 mg kg^{-1} dose.

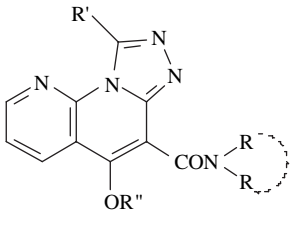
The *N*,*N*-diethyl[1,2,4]triazolo[4,3-*a*]quinoline-4-carboxamide derivatives **4a–d** showed an, on the whole, interesting, statistically significant antinociceptive activity (writhing test), frequently also at not sedative doses (Table 2): in particular, the most potent compound **4b** produced a 77% writhe number reduction ($P < 0.01$) at a dose (25 mg kg^{-1}) not significantly effective for the spontaneous locomotor activity inhibition in mice. As regards the anti-inflammatory activity of compounds **4a–d**, the only interesting, but weakly potent, compound was **4c** which showed statistically significant activity down to 25 mg kg^{-1} dose (maximal inhibition 43%, $P < 0.01$, at 50 mg kg^{-1} dose). As for the tested compounds **2** and **3**, no acute gastrolesive effect was detected in rats after the oral administration of compounds **4a–d** at 200 mg kg^{-1} dose.

In order to investigate the mechanism underlying the antinociceptive activity of the compounds under study, the most active one, compound **2c**, was tested (writhing test) after pretreatment of mice with selective antagonists of different receptors involved in pain modulation. Compound **2c** was tested at the lowest dose (3.12 mg kg^{-1}) producing the same effect (85% inhibition, $P < 0.01$) shown at 200 mg kg^{-1} dose (see Table 1). The ineffectiveness of Naloxone [10], Atropine [11], Mecamylamine [12], or Methysergide [13] in counteracting the analgesic effect of compound **2c** led us to rule out any involvement of μ-opioid, muscarinic, nicotinic and serotonine receptors in the **2c** antinociceptive activity.

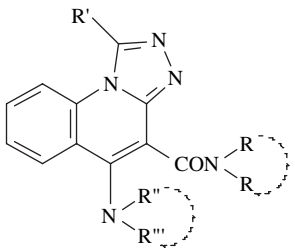
Table 3
Physical and chemical data of compounds **2d–o**, **3a–f**, **4a–d**



2d-o



3a-f



4a-d

Compound[a]	Yield (%)	Solvent[b] m.p. (°C)	Molecular formula[c]	IR[d] (cm^{-1})	^{1}H NMR[e] (δ, ppm)
2d	85	174–175 (A)	$C_{19}H_{25}N_7O$	1640 s (CO), 1602, 1583, 1549, 1508 w	1.14 and 1.28 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 2.15–3.57 [m, 11H, piperazine CH_2's + 3H of $N(CH_2CH_3)_2$], 2.31 (s, 3H, NCH_3), 3.80 [m, 1H, 1H of $N(CH_2CH_3)_2$], 7.40 (dd, 1H, H-3), 8.36 (dd, 1H, H-4), 8.61 (dd, 1H, H-2), 9.35 (s, 1H, H-9)
2e	70	227–228 (B)	$C_{25}H_{29}N_7O$	1636 s (CO), 1600, 1585, 1550, 1507 w	1.19 and 1.31 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 2.20–3.63 [m, 11H, piperazine CH_2's + 3H of $N(CH_2CH_3)_2$], 2.35 (s, 3H, NCH_3), 3.80 [m, 1H, 1H of $N(CH_2CH_3)_2$], 7.27–7.50 (m, 4H, H-3 + 3H of C_6H_5), 7.70–7.85 (m, 2H, 2H of C_6H_5), 8.30–8.41 (m, 2H, H-2,4)
2f	75	167–168 (C)	$C_{24}H_{33}N_7O_3$	1704 (carbamate CO), 1628 s (amide CO), 1602, 1587, 1555, 1513 w	1.11–1.33 [m, 9H, $N(CH_2CH_3)_2 + COOCH_2CH_3$], 1.37 and 1.50 [2d, 3H + 3H, 9-$CH(CH_3)_2$], 2.80–3.70 [m, 11H, piperazine CH_2's + 3H of $N(CH_2CH_3)_2$], 4.10 [q + m, 3H, $COOCH_2CH_3$ + 1H of $N(CH_2CH_3)_2$], 4.37 [m, 1H, 9-$CH(CH_3)_2$], 7.55 (dd, 1H, H-3), 8.37 (dd, 1H, H-4), 8.63 (dd, 1H, H-2)
2g	44	231–232 (B)	$C_{22}H_{32}N_8O$	3220 br (NH), 1606 s (CO), 1559, 1519	1.06 and 1.29 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.39 and 1.47 [2d, 3H + 3H, 9-$CH(CH_3)_2$], 2.21 (s, 3H, NCH_3), 2.27–3.49 [m, 11H, piperazine CH_2's + 3H of $N(CH_2CH_3)_2$], 3.77 [m, 1H, 1H of $N(CH_2CH_3)_2$], 4.31 [m, 1H, 9-$CH(CH_3)_2$], 5.56[f] (s, 1H, NH), 7.36 (dd, 1H, H-3), 8.50 (dd, 1H, H-4), 8.58 (dd, 1H, H-2)
2h	65	161–162 (B)	$C_{21}H_{30}N_6O_3$	3265 (NH), 1609 s (CO), 1551, 1523	1.11 and 1.32 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.40 and 1.47 [2d, 3H + 3H, 9-$CH(CH_3)_2$], 3.08–3.80 [m, 6H, $N(CH_2CH_3)_2 + NCH_2CH(OCH_3)_2$], 3.35 and 3.38 [2s, 3H + 3H, $NCH_2CH(OCH_3)_2$], 4.20 [m, 1H, 9-$CH(CH_3)_2$], 4.60 [t, 1H, $NCH_2CH(OCH_3)_2$], 5.40[f] (near t, 1H, NH), 7.18 (dd, 1H, H-3), 8.13 (dd, 1H, H-4), 8.46 (dd, 1H, H-2)
2i	90	209–210 (B)	$C_{20}H_{28}N_6O$	3363 (NH), 1638 s (CO), 1604 s, 1532	1.03–1.35 [m, 12H, $N(CH_2CH_3)_2 + NCH(CH_3)_2$], 1.43 and 1.50 [2d, 3H + 3H, 9-$CH(CH_3)_2$], 3.13–3.85 [m, 5H, $N(CH_2CH_3)_2 + NCH(CH_3)_2$], 4.23[f] (d, 1H, NH), 4.33 [m, 1H, 9-$CH(CH_3)_2$], 7.39 (dd, 1H, H-3), 8.23 (dd, 1H, H-4), 8.61 (dd, 1H, H-2)
2j	87	149–150 (C)	$C_{22}H_{32}N_6O$	3362 (NH), 1604 s, br (CO), 1531	0.83 and 0.92 [2t, 3H + 3H, $NCH(CH_2CH_3)_2$], 1.11 and 1.26 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.35–1.77 [m, 4H, $NCH(CH_2CH_3)_2$], 1.41 and 1.48 [2d, 3H + 3H, 9-$CH(CH_3)_2$], 3.10–3.70 [m, 5H, $N(CH_2CH_3)_2 + NCH(CH_2CH_3)_2$], 4.31 [m, 1H, 9-$CH(CH_3)_2$], 4.35[f] (d, 1H, NH), 7.38 (dd, 1H, H-3), 8.23 (dd, 1H, H-4), 8.59 (dd, 1H, H-2)
2k	73	185–187 (D)	$C_{21}H_{30}N_6O$	3265 (NH), 1603 s (CO), 1549, 1520 w	0.88 and 0.91 [2 overlapped d, 6H, $NCH_2CH_2CH(CH_3)_2$], 1.11 and 1.28 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.42 [t, 3H, 9-CH_2CH_3], 1.58–1.77 [m, 3H, $NCH_2CH_2CH(CH_3)_2$], 2.88 [m, 1H, 1H of $NCH_2CH_2CH(CH_3)_2$], 3.21–3.55 [m, 6H, 3H of $N(CH_2CH_3)_2$ + 9-CH_2CH_3 + 1H of $NCH_2CH_2CH(CH_3)_2$], 3.85 [m, 1H, 1H of $N(CH_2CH_3)_2$], 6.35[f] (d, 1H, NH), 6.80 (dd, 1H, H-3), 8.04 (dd, 1H, H-4), 8.19 (dd, 1H, H-2)

Table 3 (*continued*)

Compound[a]	Yield (%)	Solvent[b] m.p. (°C)	Molecular formula[c]	IR[d] (cm^{-1})	^1H NMR[e] (δ, ppm)
2l	69	161–162 (A)	$C_{20}H_{28}N_6O$	1630 s (CO), 1599, 1582, 1549	1.09–1.32 [m, 9H, $NCH_2CH_3 + N(CH_2CH_3)_2$], 1.42 and 1.50 [2d, 3H + 3H, 9-$CH(CH_3)_2$], 2.83 (s, 3H, NCH_3), 3.00–3.78 [m, 6H, $NCH_2CH_3 + N(CH_2CH_3)_2$], 4.36 [m, 1H, 9-$CH(CH_3)_2$], 7.21 (dd, 1H, H-3), 8.34 (dd, 1H, H-4), 8.60 (dd, 1H, H-2)
2m	84	211–212 (B)	$C_{22}H_{29}N_7O$	1637 s (CO), 1601, 1584, 1550, 1518 w	1.41 and 1.48 [2d, 3H + 3H, 9-$CH(CH_3)_2$], 1.70–2.08 (m, 4H, pyrrolidine β-CH_2's), 2.20–3.90 (m, 12H, pyrrolidine α-CH_2's + piperazine CH_2's), 2.33 (s, 3H, NCH_3), 4.36 [m, 1H, 9-$CH(CH_3)_2$], 7.41 (dd, 1H, H-3), 8.35 (dd, 1H, H-4), 8.60 (dd, 1H, H-2)
2n	88	233–235 (B)	$C_{21}H_{28}N_6O$	3283 br (NH), 1606 s (CO), 1552, 1521 sh	0.92 [d, 6H, $NCH_2CH(CH_3)_2$], 1.25–1.63 [m, 6H, 9-$CH(CH_3)_2$], 1.70–2.08 [m, 5H, pyrrolidine β-CH_2's + $NCH_2CH(CH_3)_2$], 2.45–3.90 [m, 6H, pyrrolidine α-CH_2's + $NCH_2CH(CH_3)_2$], 4.09 [m, 1H, 9-$CH(CH_3)_2$], 6.43[f] (near t, 1H, NH), 6.93 (dd, 1H, H-3), 8.20 (dd, 1H, H-4), 8.27 (dd, 1H, H-2)
2o	82	231–232 (B)	$C_{20}H_{27}N_7O$	3251 (NH), 1661 s (CO), 1602, 1586, 1553, 1535, 1515	1.27 (t, 3H, $HNCH_2CH_3$), 1.35 [d, 6H, 9-$CH(CH_3)_2$], 2.31 (s, 3H, NCH_3), 2.56 and 3.28 (2m, 4H + 4H, piperazine CH_2's), 3.51 (m, 2H, $HNCH_2CH_3$; q after treatment with D_2O), 4.09 [m, 1H, 9-$CH(CH_3)_2$], 7.43 (dd, 1H, H-3), 8.18[f] (near t, 1H, NH), 8.41 (dd, 1H, H-4), 8.56 (dd, 1H, H-2)
3a	72	206–208 (B)	$C_{20}H_{26}N_6O_2$	1628 s (CO), 1607, 1593 sh, 1558, 1517 w	1.06 and 1.29 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.75–2.45 (m, 6H, 6H of piperidine), 2.27 (s, 3H, NCH_3), 2.64–2.93 (m, 2H, 2H of piperidine), 3.12–3.57 [m, 3H, 3H of $N(CH_2CH_3)_2$], 3.84 [m, 1H, 1H of $N(CH_2CH_3)_2$], 4.60 (m, 1H, piperidine OCH), 7.51 (dd, 1H, H-3), 8.42 (dd, 1H, H-4), 8.67 (dd, 1H, H-2), 9.40 (s, 1H, H-9)
3b	72	143–145 (A)	$C_{23}H_{32}N_6O_2$	1638 s (CO), 1606, 1593 sh, 1558, 1516 w	1.07 and 1.28 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.42 and 1.50 [2d, 3H + 3H, 9-$CH(CH_3)_2$], 1.70–2.35 (m, 6H, 6H of piperidine), 2.21 (s, 3H, NCH_3), 2.55–2.80 (m, 2H, 2H of piperidine), 3.12–3.35 and 3.43–3.80 [2m, 2H + 2H, $N(CH_2CH_3)_2$], 4.36 [m, 1H, 9-$CH(CH_3)_2$], 4.56 (m, 1H, piperidine OCH), 7.44 (dd, 1H, H-3), 8.39 (dd, 1H, H-4), 8.64 (dd, 1H, H-2)
3c	83	124–125 (E)	$C_{19}H_{25}N_5O_2$	1635 s (CO), 1608, 1594, 1561 w	1.19 and 1.36 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.50 (t, 3H, OCH_2CH_3), 1.52 and 1.60 [2d, 3H + 3H, 9-$CH(CH_3)_2$], 3.30–3.49 and 3.55–3.87 [2m, 2H + 2H, $N(CH_2CH_3)_2$], 4.25–4.65 [m, 3H, OCH_2CH_3 + 9-$CH(CH_3)_2$], 7.54 (dd, 1H, H-3), 8.48 (dd, 1H, H-4), 8.74 (dd, 1H, H-2)
3d	75	153–154 (F)	$C_{20}H_{27}N_5O_2$	1636 s (CO), 1608, 1590, 1559, 1516 w	1.16 and 1.36 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.38 and 1.44 [2d, 3H + 3H, $OCH(CH_3)_2$], 1.52 and 1.60 [2d, 3H + 3H, 9-$CH(CH_3)_2$], 3.20–3.45 and 3.50–3.85 [2m, 2H + 2H, $N(CH_2CH_3)_2$], 4.46 [m, 1H, 9-$CH(CH_3)_2$], 4.95 [m, 1H, $OCH(CH_3)_2$], 7.53 (dd, 1H, H-3), 8.48 (dd, 1H, H-4), 8.73 (dd, 1H, H-2)
3e	77	111–112 (D)	$C_{21}H_{29}N_5O_2$	1639 s (CO), 1607, 1592 sh, 1560, 1516	0.98 (t, 3H, $OCH_2CH_2CH_2CH_3$), 1.16 and 1.32 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.37–1.61 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.48 and 1.56 [2d, 3H + 3H, 9-$CH(CH_3)_2$], 1.71–1.93 (m, 2H, $OCH_2CH_2CH_2CH_3$), 3.20–3.86 [m, 4H, $N(CH_2CH_3)_2$], 4.12–4.55 [m, 3H, $OCH_2CH_2CH_2CH_3$ + 9-$CH(CH_3)_2$], 7.50 (dd, 1H, H-3), 8.44 (dd, 1H, H-4), 8.70 (dd, 1H, H-2)
3f	78	160–161 (G)	$C_{21}H_{29}N_5O_2$	1639 s (CO), 1612 s, 1594, 1560, 1517	1.08 [d, 6H, $OCH_2CH(CH_3)_2$], 1.19 and 1.34 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.50 and 1.58 [2d, 3H + 3H, 9-$CH(CH_3)_2$], 2.19 [m, 1H, $OCH_2CH(CH_3)_2$], 3.30–3.88 [m, 4H, $N(CH_2CH_3)_2$], 3.96–4.10 and 4.23–4.36 (2m, 1H + 1H, $OCH_2CH(CH_3)_2$], 4.43 [m, 1H, 9-$CH(CH_3)_2$], 7.51 (dd, 1H, H-3), 8.47 (dd, 1H, H-4), 8.72 (dd, 1H, H-2)

(*continued on next page*)

Table 3 (*continued*)

Compound[a]	Yield (%)	Solvent[b] m.p. (°C)	Molecular formula[c]	IR[d] (cm^{-1})	^1H NMR[e] (δ, ppm)
4a	69	233–234 (B)	$C_{20}H_{26}N_6O$	1617 s (CO), 1598, 1559, 1517 w	1.12 and 1.27 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 2.17–3.56 [m, 11H, piperazine CH_2's + 3H of $N(CH_2CH_3)_2$], 2.34 (s, 3H, NCH_3), 3.67–3.90 [m, 1H, 1H of $N(CH_2CH_3)_2$], 7.45 and 7.61 (2 near t, 1H + 1H, H-7,8), 7.85 and 8.08 (2 near d, 1H + 1H, H-6,9), 9.07 (s, 1H, H-1)
4b	52	145–147 (G)	$C_{23}H_{32}N_6O$	1635 s (CO), 1598, 1559, 1524 w	1.14 and 1.29 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.49 and 1.58 [2d, 3H + 3H, 1-$CH(CH_3)_2$], 2.04–3.78 [m, 13H, piperazine CH_2's + $N(CH_2CH_3)_2$ + 1-$CH(CH_3)_2$], 2.36 (s, 3H, NCH_3), 7.46 and 7.60 (2 near t, 1H + 1H, H-7,8), 8.08 and 8.15 (2 near d, 1H + 1H, H-6,9)
4c	84	159–160 (C)	$C_{20}H_{27}N_5O$	3281 (NH), 1610 s, br (CO), 1563 sh, 1547, 1504 w	1.09 and 1.27 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.30 (t, 3H, $HNCH_2CH_3$), 1.46 and 1.55 [2d, 3H + 3H, 1-$CH(CH_3)_2$], 3.00–3.78 [m, 7H, $HNCH_2CH_3$ + $N(CH_2CH_3)_2$ + 1-$CH(CH_3)_2$], 5.21[f] (br s, 1H, NH), 7.17 and 7.41 (2 near t, 1H + 1H, H-7,8), 7.84 (near d, 2H, H-6,9)
4d	80	125–126 (H)	$C_{22}H_{31}N_5O$	3270 (NH), 1612 s, br (CO), 1546	0.96 [d, 6H, $NCH_2CH(CH_3)_2$], 1.10 and 1.26 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.45 and 1.55 [2d, 3H + 3H, 1-$CH(CH_3)_2$], 1.97 [m, 1H, $NCH_2CH(CH_3)_2$], 2.82 [m, 1H, 1H of $NCH_2CH(CH_3)_2$], 3.17–3.87 [m, 6H, $N(CH_2CH_3)_2$ + 1H of $NCH_2CH(CH_3)_2$ + 1-$CH(CH_3)_2$], 5.35[f] (br s, 1H, NH), 7.16 and 7.40 (2 near t, 1H + 1H, H-7,8), 7.84 (near d, 2H, H-6,9)

[a] For the substituents, see Table 2.
[b] Crystallization solvent: A = diisopropyl ether, B = ethyl acetate, C = ethyl acetate–diisopropyl ether, D = ethyl acetate–petroleum ether, E = dichloromethane–petroleum ether, F = dichloromethane–diisopropyl ether, G = diethyl ether–petroleum ether, H = diethyl ether–diisopropyl ether.
[c] Anal. C, H, N.
[d] In KBr pellets. Abbreviations: s = strong, w = weak, br = broad, sh = shoulder.
[e] In $CDCl_3$ solutions. Abbreviations: s = singlet, br s = broad singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet. 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.
[f] Disappeared with D_2O.

As we reported above (Table 1), **2a**, **and 2c** proved to be, respectively, the most potent anti-inflammatory and the most potent analgesic compounds **2** previously synthesized by us [3,4]. The remarkable difference between the activities of these two compounds clearly depends on the structural diversity of their selectively effective 5-amino substituents, whereas the 6-(diethylcarbamoyl) and 9-isopropyl substituents resulted the most effective ones for both the activities of compounds **2** [3,4]. The pharmacological results now exhibited by the novel compounds **2d–o** (Table 2) are in accordance with the above conclusions. For instance, the replacement of the 6-$CON(C_2H_5)_2$ substituent with its cyclic analogue 6-CON  , in the very potent analgesic agent **2c** (Table 1), afforded compound **2m** (Table 2) completely devoid of statistically significant analgesic activity; by performing the same replacement in compound **2a** (Table 1), its notably potent anti-inflammatory activity was eliminated (compound **2n**, Table 2). Similarly, as regards the 9-substituent, the phenyl substituted compound **2e** (Table 2) has shown an analgesic potency notably lower than that of its 9-isopropyl substituted analogue **2c**.

On the other hand, the replacement, in the reference compound **2c** [4], of the 5-(4-methyl-1-piperazinyl) substituent (the most effective one for the analgesic potency) with similar groups has afforded the less active compounds **2f**,**g**, among which **2f** (whose 5-substituent is a 4-substituted-1-piperazinyl group) exhibits a lower but interesting analgesic activity. Also in the case of the anti-inflammatory reference compound **2a** [3], the replacement of its 5-(isobutylamino) substituent with the (2-dimethoxyethyl)amino group (whose two oxygen atoms are potential hydrogen bond acceptors) gave compound **2h** (Table 2) completely devoid of anti-inflammatory properties.

Anyway, by properly modifying the 5-(isobutylamino) substituent of compound **2a**, we have now obtained a group of new, very interesting anti-inflammatory agents showing no acute gastrolesivity in rats (compounds **2i**,**j**,**l**), the most potent of which and of all the compounds **2** previously synthesized by us [3,4] is the 5-(isopropylamino) substituted compound **2i**, that at 12.5 mg kg^{-1} dose exhibited a 68% ($P < 0.01$) inhibition of the carrageenan-induced rat paw edema (indomethacin: 51% inhibition, $P < 0.01$, at 10 mg kg^{-1} dose) (Table 2).

The *N*,*N*-diethyl-9-isopropyl[1,2,4]triazolo[4,3-*a*][1,8]-naphthyridine-6-carboxamide derivatives **2c**,**b**,**i**,**a**, and their corresponding compounds **3b**,**c**,**d**,**f**, respectively, differ only in their 5-substituents which are *N*-substituted amino groups or similarly *O*-substituted alkoxy groups, respectively. Both the analgesic activity of compound **3b** and the anti-inflammatory activity of compounds **3c**,**d**,**f** (Table 2) are remarkably less potent than those exhibited by the corresponding compounds **2c** and **2b**,**i**,**a** (Tables 1 and 2), respectively (see in particular the anti-inflammatory activity data of compounds **3c**,**f**).

The above structural and pharmacological data seem to clearly indicate that it is harmful to replace the 5-amino substituent of compounds **2** with a 5-alkoxy one.

The molecular modification of compounds **2a**, **2b**, and **2c**, taken as leads in this study, has also concerned the synthesis of their identically substituted isosteric analogues [1,2,4]triazolo[4,3-*a*]quinoline-4-carboxamide derivatives **4d**, **4c**, and **4b** respectively. In this case, the analgesic activity of compound **4b** is very interesting, although less potent than that of **2c**, and also **4c** is rather active. As regards the anti-inflammatory properties, compound **4c** exhibited an interesting activity, but notably less potent than that of the corresponding lead compound **2b**, whereas compound **4d**, isosteric analogue of the very potent anti-inflammatory agent **2a**, did not show any activity (Tables 1 and 2).

4. Conclusions

On the basis of the pharmacological data of compounds **2d–o**, **3a–f**, and **4a–d**, reported in Table 2, the following conclusions can be drawn.

- The herein described compounds **2i**,**l** showed a remarkable anti-inflammatory activity (Table 2), slightly more potent than that of the lead compounds **2a**,**b** (Table 1), whereas compounds **2f**,**i** and particularly the potent compound **2j** showed notable analgesic activity (Table 2), but proved to be clearly less potent than **2c** (Table 1). On the basis of the very satisfactory pharmacological properties (among which the complete absence of acute gastrolesivity) on the whole shown by compounds **2** previously [3,4] and now synthesized by us, we regard it reasonable to consider this one as a very interesting new structural class of potent anti-inflammatory and/or analgesic agents, devoid of COX inhibitory properties [5].
- The pharmacological data shown by compounds **3a–f** (Table 2) clearly indicate that the 5-alkoxy substituents are notably less effective than the *N*-alkyl substituted 5-amino substituents for the anti-inflammatory and/or analgesic activities of the 5-substituted [1,2,4]triazolo[4,3-*a*][1,8] naphthyridine-6-carboxamide derivatives.
- On the other hand, the first interesting pharmacological results afforded by the 5-amino[1,2,4]triazolo[4,3-*a*]quinoline-4-carboxamide derivatives **4a–d** now tested induce us to pursue this study.

As reported above, the mechanism of action underlying the anti-inflammatory activity of compounds **2** involves their anti-adhesive effects, as well as their inhibition of superoxide anion production and of myeloperoxidase release, but not the COX inhibition [5]. As regards the mechanism of action related to the analgesic activity of compounds **2**, the further results now obtained for compound **2c** seem to exclude the involvement in its analgesic activity of the μ-opioid, muscarinic, nicotinic and serotonine receptors, and also of a generic sedative effect.

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 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), br s (broad singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet). Analyses of all new compounds, indicated by the symbols of the elements, 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. N-Substituted 1,2-dihydro-4-hydroxy-2-oxo-1, 8-naphthyridine-3-carboxamides (**6a**,**b**)

A mixture of 25.0 mmol (5.50 g) of ester **5** [6], 50 mL of ethanol and an excess (10 mL) of the proper amine was heated in a closed vessel at 150 °C for 16 h. After cooling the resulting suspension was evaporated to dryness at reduced pressure. The residue so obtained was taken up in water and carefully treated with 2 N HCl down to pH 5: the solid that separated out was collected by filtration, washed with water and dried to give the nearly pure compound **6** which was then crystallized from ethanol. According to this procedure the following compounds were obtained.

*5.1.1.1. N-Ethyl-1,2-dihydro-4-hydroxy-2-oxo-1,8-naphthyridine-3-carboxamide (**6a**).* The reaction of **5** with ethylamine yielded 5.54 g (95%) of **6a** [14], whitish needles, m.p. 250–252 °C. ^{1}H NMR (DMSO-d_6): δ 1.14 (t, 3H, HNCH$_2$*CH*$_3$), 3.37 (m, 2H, HN*CH*$_2$CH$_3$; q, after treatment with D_2O), 7.31 (dd, $J_{6,5} = 8$ Hz, $J_{6,7} = 4.8$ Hz, 1H, H-6), 8.32 (dd, $J_{5,6} = 8$ Hz, $J_{5,7} = 1.6$ Hz, 1H, H-5), 8.65 (dd, $J_{7,6} = 4.8$ Hz, $J_{7,5} = 1.6$ Hz, 1H, H-7), 10.13 (near t, 1H, *H*NCH$_2$CH$_3$; disappeared with D_2O), 12.21 (s, 1H, 1-NH; disappeared with D_2O); (4-OH signal was not detectable); IR (KBr): 3200–2550 (OH + NH), 1663 s (2-CO), 1614 s (amide CO), 1574 s, br cm^{-1}. Anal. ($C_{11}H_{11}N_3O_3$) C, H, N.

*5.1.1.2. 4-Hydroxy-3-(pyrrolidin-1-ylcarbonyl)-1,8-naphthyridin-2(1H)-one (**6b**).* The reaction of **5** with pyrrolidine yielded 6.03 g (93%) of **6b**, white needles, m.p. 262–265 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.77 (m, 4H, pyrrolidine β-CH$_2$′s), 3.30 (m, 4H, pyrrolidine α-CH$_2$′s), 7.22 (dd, $J_{6,5} = 8$ Hz, $J_{6,7} = 4.8$ Hz, 1H, H-6), 8.24 (dd, $J_{5,6} = 8$ Hz, $J_{5,7} = 1.6$ Hz, 1H, H-5), 8.51 (dd, $J_{7,6} = 4.8$ Hz, $J_{7,5} = 1.6$ Hz, 1H, H-7), 11.84 (s, 1H, 1-NH; disappeared

with D_2O); (4-OH signal was not detectable); IR (KBr): 3200–2400 s (OH + NH), 1635 s, br (CO), 1580 s, br cm^{-1}. Anal. ($C_{13}H_{13}N_3O_3$) C, H, N.

5.1.2. N-Substituted 2,4-dichloro-1,8-naphthyridine-3-carboxamides (**7a**,**b**)

A mixture of 5.0 g of **6a** (21.44 mmol) or **6b** (19.28 mmol) and 50 mL of $POCl_3$ was stirred at 110 °C for 1 h. The excess $POCl_3$ was removed by heating at reduced pressure and the residue was dissolved in warm water; the resulting solution was cooled, carefully treated with $NaHCO_3$ up to pH 7, and finally exhaustively extracted with dichloromethane. 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 with dichloromethane–ethyl acetate (1:1). From the eluate collected, after removal of solvents, the following compounds were obtained.

*5.1.2.1. 2,4-Dichloro-N-ethyl-1,8-naphthyridine-3-carboxamide (**7a**).* The reaction carried out with **6a** gave 3.59 g (62%) of **7a**, white crystals, m.p. 132–133.5 °C, after crystallization from ethyl acetate. ^1H NMR ($CDCl_3$): δ 1.28 (t, 3H, $HNCH_2CH_3$), 3.53 (m, 2H, $HNCH_2CH_3$; q, after treatment with D_2O), 7.05 (near t, 1H, $HNCH_2CH_3$; disappeared with D_2O), 7.47 (dd, $J_{6,5} = 8.4$ Hz, $J_{6,7} = 4.2$ Hz, 1H, H-6), 8.28 (dd, $J_{5,6} = 8.4$ Hz, $J_{5,7} = 2$ Hz, 1H, H-5), 8.93 (dd, $J_{7,6} = 4.2$ Hz, $J_{7,5} = 2$ Hz, 1H, H-7); IR (KBr): 3312 s (NH), 1668 s (CO), 1598 w, 1576, 1553, 1529 cm^{-1}. Anal. ($C_{11}H_9Cl_2N_3O$) C, H, N.

*5.1.2.2. (2,4-Dichloro-1,8-naphthyridin-3-yl)(1-pyrrolidinyl)-methanone (**7b**).* The reaction carried out with **6b** gave 3.20 g (56%) of **7b**, white crystals, m.p. 149–151 °C, after crystallization from ethyl acetate–petroleum ether. ^1H NMR ($CDCl_3$): δ 1.66–2.15 (m, 4H, pyrrolidine β-CH_2's), 3.02–3.30 (m, 2H, 2H of pyrrolidine α-CH_2's), 3.69 (t, 2H, 2H of pyrrolidine α-CH_2's), 7.59 (dd, $J_{6,5} = 8.4$ Hz, $J_{6,7} = 4.2$ Hz, 1H, H-6), 8.53 (dd, $J_{5,6} = 8.4$ Hz, $J_{5,7} = 2$ Hz, 1H, H-5), 9.12 (dd, $J_{7,6} = 4.2$ Hz, $J_{7,5} = 2$ Hz, 1H, H-7); IR (KBr): 1634 s (CO), 1596, 1573, 1542 cm^{-1}. Anal. ($C_{13}H_{11}Cl_2N_3O$) C, H, N.

5.1.3. N-Substituted 5-chloro[1,2,4]triazolo-[4,3-a][1,8]naphthyridine-6-carboxamides (**8a–c**)

A mixture of 6.0 mmol of **7a** (1.62 g) or **7b** (1.78 g), 9.0 mmol (0.92 g) of isobutyrohydrazide and 10 mL of Dowtherm A (in the case of **8a** or **8b**), or a mixture of 6.0 mmol of **7c** [7] (1.79 g), 9.0 mmol (0.79 g) of propionic hydrazide and 10 mL of Dowtherm A (in the case of **8c**), were stirred at 160 °C for 30 min (1 h in the case of preparation of **8c**). After cooling, 10% aqueous Na_2CO_3 (50 mL) and dichloromethane (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 dichloromethane. The combined organic phases were dried (anhydrous Na_2SO_4), then evaporated to dryness at reduced pressure, and the residue was subjected to column chromatography (silica gel for **8a**,**b**; neutral aluminium oxide for **8c**), in all cases eluting first with dichloromethane to remove Dowtherm A. The reaction products were then recovered eluting with ethyl acetate (compound **8a**,**c**) or with the mixture ethyl acetate–tetrahydrofuran (1:1) (compound **8b**). The eluate collected, after removal of solvents, gave an oily or solid residue from which compounds **8a–c** were obtained as described below.

*5.1.3.1. 5-Chloro-N-ethyl-9-isopropyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**8a**).* The solid residue obtained from the reaction performed with **7a** was taken up in a little diethyl ether and filtered to give the nearly pure **8a** as a whitish crystalline solid (0.65 g, 34%); white crystals, m.p. 223–224 °C, after crystallization from ethyl acetate. ^1H NMR ($CDCl_3$): δ 1.26 (t, 3H, $HNCH_2CH_3$), 1.47 [d, 6H, 9-$CH(CH_3)_2$], 3.52 (m, 2H, $HNCH_2CH_3$; q, after treatment with D_2O), 4.43 [m, 1H, 9-$CH(CH_3)_2$], 7.57 (dd, $J_{3,4} = 8.1$ Hz, $J_{3,2} = 4.7$ Hz, 1H, H-3), 8.65–8.75 (m, 2H, H-2,4), 8.87 (br s, 1H, NH; disappeared with D_2O); IR (KBr): 3255 (NH), 1667 s (CO), 1601, 1586, 1559, 1520 cm^{-1}. Anal. ($C_{15}H_{16}ClN_5O$) C, H, N.

*5.1.3.2. (5-Chloro-9-isopropyl[1,2,4]triazolo[4,3-a][1,8]naphthyridin-6-yl)(1-pyrrolidinyl)methanone (**8b**).* The oily residue derived from the reaction carried out with **6a**, treated with a little diisopropyl ether, afforded pure **8b** as whitish solid (0.70 g, 34%); m.p. 206–208 °C after crystallization from ethyl acetate–diisopropyl ether. ^1H NMR ($CDCl_3$): δ 1.45 and 1.51 [2d, 3H + 3H, 9-$CH(CH_3)_2$], 1.70–2.11 (m, 4H, pyrrolidine β-CH_2's), 3.13–3.45 and 3.60–3.91 (2 m, 2H + 2H, pyrrolidine α-CH_2's), 4.42 [m, 1H, 9-$CH(CH_3)_2$], 7.55 (dd, $J_{3,4} = 8.1$ Hz, $J_{3,2} = 4.7$ Hz, 1H, H-3), 8.48 (dd, $J_{4,3} = 8.1$ Hz, $J_{4,2} = 1.7$ Hz, 1H, H-4), 8.71 (dd, $J_{2,3} = 4.7$ Hz, $J_{2,4} = 1.7$ Hz, 1H, H-2); IR (KBr): 1651 s (CO), 1602, 1590, 1559, 1519 cm^{-1}. Anal. ($C_{17}H_{18}ClN_5O$) C, H, N.

*5.1.3.3. 5-Chloro-N,N-9-triethyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**8c**).* The nearly solid residue obtained from the reaction performed with **7c** and propionic hydrazide was taken up in a little diethyl ether and filtered to give pure **8c** as a whitish crystalline solid (0.86 g, 43%); white crystals, m.p. 176–177 °C, after crystallization from ethyl acetate. ^1H NMR ($CDCl_3$): δ 1.10 and 1.31 [2t, 3H + 3H, $N(CH_2CH_3)_2$], 1.45 (t, 3H, 9-CH_2CH_3), 3.24 [q, 2H, 2H of $N(CH_2CH_3)_2$], 3.40–3.93 [m, 4H, 2H of $N(CH_2CH_3)_2$ + 9-CH_2CH_3], 7.56 (dd, $J_{3,4} = 8.1$ Hz, $J_{3,2} = 4.7$ Hz, 1H, H-3), 8.48 (dd, $J_{4,3} = 8.1$ Hz, $J_{4,2} = 1.7$ Hz, 1H, H-4), 8.70 (dd, $J_{2,3} = 4.7$ Hz, $J_{2,4} = 1.7$ Hz, 1H, H-2); IR (KBr): 1639 s (CO), 1599, 1586, 1556, 1519 w cm^{-1}. Anal. ($C_{16}H_{18}ClN_5O$) C, H, N.

5.1.4. Synthesis of the N-substituted 5-amino[1,2,4]triazolo-[4,3-a][1,8]naphthyridine-6-carboxamide derivatives **2d–k**,**m–o**

Compounds **2d–k**,**m–o** were prepared by the reaction of the new 5-chloroderivatives **8a–c** or the previously described

8d–f with an excess of the proper amine through the below described experimental procedures, depending on the amine used.

*5.1.4.1. Compounds **2h–k,n**.* A mixture of 5.0 mmol of **8b** (1.72 g) (reaction with isobutylamine to give **2n**), or **8c** (1.66 g) (reaction with isopentylamine to give **2k**), or **8d** [4] (1.73 g) (reactions with 2,2-dimethoxyethylamine, isopropylamine or 3-pentylamine to give **2h**, **2i**, or **2j**, respectively), 5 mL of the proper amine and 25 mL of anhydrous ethanol was heated at 120 °C in a closed vessel for 14 h. After cooling, the resulting solution was evaporated to dryness at reduced pressure and the residue was partitioned between 10% aqueous Na_2CO_3 (100 mL) and dichloromethane (100 mL). The organic layer was collected and the aqueous one was further extracted twice with dichloromethane. The combined organic phases were dried (anhydrous Na_2SO_4) and then evaporated to dryness at reduced pressure to give a thick oil which was chromatographed on a silica gel column eluting first with ethyl acetate to discard a little amount of starting compound and impurities, then with tetrahydrofuran to recover compounds **2**. The residues obtained from the fractions collected (after removal of solvent), treated with a little diethyl ether, afforded pure compounds **2h–k,n** which were crystallized from the suitable solvents.

*5.1.4.2. Compounds **2d–g,m,o**.* A mixture of 5.0 mmol of **8a** (1.59 g), or **8b** (1.72 g), or **8e** [8] (1.52 g), or **8f** [8] (1.90 g) (reactions with 1-methylpiperazine to give **2o**, **2m**, **2d**, or **2e**, respectively), or 5.0 mmol of **8d** [4] (1.73 g) (reactions with ethyl piperazine-1-carboxylate or 1-amino-4-methylpiperazine to give **2f** or **2g**, respectively), 5 mL of the proper amine and 5 mL of dimethyl sulphoxide was stirred at 130 °C for 2 h (compounds **2d,f,m**) or 4 h (compounds **2e,g**) or 5 h (compound **2o**). The solution obtained was then poured into water (200 mL) and the resulting emulsion was exhaustively extracted with dichloromethane. The combined extracts, dried (anhydrous Na_2SO_4) and evaporated to dryness in vacuo, afforded an oily or nearly solid residue from which, after treatment with a little diethyl ether (compounds **2d–g,m**) or ethyl acetate (compound **2o**) and standing, the expected compound **2** separated out as a pink-orange solid. After crystallization from the proper solvent with charcoal, white or whitish crystals were obtained.

Data for compounds **2d–k,m–o** are reported in Table 3.

*5.1.5. N,N-Diethyl-5-(N-ethyl-N-methylamino)-9-isopropyl-[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide (**2l**)*

To the suspension of 3.0 mmol (1.06 g) of **2b** [3] in 80 mL of 2-butanone, 24 mmol (1.34 g) of finely powdered KOH mixed with 2.0 g of anhydrous K_2CO_3 were added, then the mixture was refluxed (80 °C) for 5 min. A solution of 4.0 mmol (0.25 mL) of iodomethane in 10 mL of 2-butanone was then slowly added and the resulting mixture was further refluxed for 1 h with stirring. After cooling, the solvent was removed in vacuo and the residue was partitioned between water (200 mL) and dichloromethane (200 mL). The organic layer was collected and the aqueous one was further extracted twice with dichloromethane. The combined organic phases were dried (anhydrous Na_2SO_4) and then evaporated to dryness in vacuo to give an oil which was chromatographed on a silica gel column eluting first with ethyl acetate. The first fractions of this eluate, containing some impurities, were discarded. Compound **2l** was then recovered by eluting with the mixture ethyl acetate–acetone (4:1). The eluate collected, evaporated to dryness in vacuo, afforded an oil from which, after treatment with a little diisopropyl ether, pure compound **2l** separated out as pale yellow solid.

Data for compound **2l** are reported in Table 3.

*5.1.6. General procedure for the synthesis of 5-alkoxy-N,N-diethyl[1,2,4]triazolo[4,3-a][1,8]naphthyridine-6-carboxamide **3a** and its 9-isopropyl analogues **3b–f***

Sodium (0.14 g, 6 mmol) was dissolved in 5 mL of proper anhydrous alcohol (ethanol for **3c**, isopropanol for **3d**, *n*-butanol for **3e**, isobutanol for **3f**) or in 3 mL of 1-methyl-4-piperidinol (preparation of **3a,b**). When a limpid solution was obtained (in the case of butanols and 1-methyl-4-piperidinol, the reaction was carried out at 100 °C to hasten the dissolution of sodium), the alcohol was removed in vacuo: the resulting solid or resinous (in the case of 1-methyl-4-piperidinol) residue was taken up in 25 mL of anhydrous tetrahydrofuran, then 4.0 mmol of 5-chloroderivative **8e** [8] (1.21 g, preparation of **3a**) or **8d** [4] (1.38 g, preparation of **3b–f**) were added to the mixture. The resulting turbid solution was refluxed (80 °C) for 1 h with stirring. After cooling, the solvent was removed at reduced pressure and the residue was partitioned between water (100 mL) and dichloromethane (100 mL). The organic layer was collected and the aqueous one was further extracted twice with dichloromethane. The combined organic phases were washed with water, dried (anhydrous Na_2SO_4), and then evaporated to dryness in vacuo to give oily residues from which, after treatment with a little diisopropyl ether or petroleum ether, the pure compounds **3a–f** separated out as crystalline whitish solids that were crystallized from the proper solvents.

Data for compounds **3a–f** are reported in Table 3.

*5.1.7. N,N-Diethyl-1,2-dihydro-4-hydroxy-2-oxoquinoline-3-carboxamide (**10**)*

A mixture of 25.0 mmol (5.83 g) of ester **9** [9], 50 mL of ethanol and an excess (10 mL) of diethylamine was heated in a closed vessel at 150 °C for 16 h. After cooling, the resulting suspension was evaporated to dryness at reduced pressure. The residue so obtained was taken up in water and carefully treated with 2 N HCl down to pH 5; the resulting emulsion was exhaustively extracted with chloroform and the combined extracts were dried (Na_2SO_4) and evaporated to dryness at reduced pressure. The solid residue obtained was taken up in a little ethyl acetate and filtered to give pure **10** (4.25 g, 65%), white crystals, m.p. 238–240 °C, after crystallization

from ethanol. ^{1}H NMR ($CDCl_3$): δ 1.31 [near t, 6H, N(CH_2CH_3)$_2$], 3.34–3.70 [m, 4H, N(CH_2CH_3)$_2$], 7.10–7.42, 7.61 and 8.13 (3m, 2H + 1H + 1H, H-6,7,8,9), 10.57 (br s, 1H, 1-NH; disappeared with D_2O); (4-OH signal was not detectable); IR (KBr): 3220–2500 (OH + NH), 1651 s (2-CO), 1608 (amide CO), 1583 br, 1496 cm^{-1}. Anal. ($C_{14}H_{16}N_2O_3$) C, H, N.

5.1.8. 4-Chloro-N,N-diethyl-1,2-dihydro-2-oxoquinoline-3-carboxamide (**11**) and 2,4-dichloro-N,N-diethylquinoline-3-carboxamide (**12**)

A mixture of 5.0 g (19.21 mmol) of **10** and 50 mL of $POCl_3$ was stirred at 110 °C for 2 h. The excess $POCl_3$ was removed by heating at reduced pressure and the residue was dissolved in warm water; the resulting solution was cooled, carefully treated with $NaHCO_3$ up to pH 7, and finally exhaustively extracted with dichloromethane. 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 with dichloromethane–ethyl acetate (1:1). The eluate collected, after removal of solvents, gave a solid residue which was taken up in a little petroleum ether and filtered to yield 3.81 g (67%) of pure **12**, white crystals, m.p. 120–121 °C, after crystallization from diisopropyl ether. ^{1}H NMR ($CDCl_3$): δ 1.09 and 1.26 [2t, 3H + 3H, N(CH_2CH_3)$_2$], 3.14 [q, 2H, 2H of N(CH_2CH_3)$_2$], 3.60 [m, 2H, 2H of N(CH_2CH_3)$_2$], 7.63 and 7.76 (2 near t, 1H + 1H, H-6,7), 7.98 and 8.15 (2 near d, 1H + 1H, H-5,8); IR (KBr): 1633 s (CO), 1575, 1557 cm^{-1}. Anal. ($C_{14}H_{14}Cl_2N_2O$) C, H, N.

Chromatography was pursued eluting with the mixture ethyl acetate–tetrahydrofuran (1:1): the fraction collected, after removal of solvents, afforded pure compound **11** as a white solid (0.64 g, 12%), m.p. 188–190 °C, after crystallization from ethyl acetate. ^{1}H NMR ($CDCl_3$): δ 1.11 and 1.27 [2t, 3H + 3H, N(CH_2CH_3)$_2$], 3.27 [q, 2H, 2H of N(CH_2CH_3)$_2$], 3.48 and 3.74 [2m, 1H + 1H, 2H of N(CH_2CH_3)$_2$], 7.24 and 7.51 (2 near t, 1H + 1H, H-6,7), 7.39 and 7.90 (2 near d, 1H + 1H, H-5,8), 12.75 (br s, 1H, 1-NH; disappeared with D_2O); IR (KBr): 3200–2400 (NH), 1650 (2-CO), 1634 s (amide CO), 1596, 1562 sh, 1500 cm^{-1}. Anal. ($C_{14}H_{15}ClN_2O_2$) C, H, N.

5.1.9. Chlorination of compound **11** to compound **12**

A mixture of 3.0 g (10.76 mmol) of **11** and 30 mL of $POCl_3$ was stirred at 110 °C for 2 h. After a work-up identical to the one described above in Section 5.1.8, 2.63 g (82%) of pure dichloroderivative **12** was obtained.

5.1.10. 5-Chloro-N,N-diethyl[1,2,4]triazolo-[4,3-a]quinoline-4-carboxamide derivatives **13a**,**b**

In the case of preparation of **13a**, a mixture of 8.0 mmol of **12** (2.38 g), 16.0 mmol of formic hydrazide (0.96 g) and 10 mL of Dowtherm A was heated at 180 °C for 3.5 h, with stirring; in the case of preparation of **13b**, a mixture of 8.0 mmol of **12** (2.38 g), 12.0 mmol of isobutyrohydrazide (1.22 g) and 10 mL of Dowtherm A was stirred at 180 °C for 1.5 h. In both the cases, after cooling, 10% aqueous Na_2CO_3 (50 mL) and dichloromethane (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 dichloromethane. The combined organic phases were dried (anhydrous Na_2SO_4), then evaporated to dryness at reduced pressure, and the resulting residue was subjected to chromatography on a silica gel column, eluting first with the mixture dichloromethane–petroleum ether (1:1) in order to remove Dowtherm A. The subsequent elution with the mixture dichloromethane–ethyl acetate (4:1) allowed the recovery of significant amounts of starting compound **12** (0.79 g or 0.71 g in the case of preparations of **13a** or **13b**, respectively). Finally, compound **13a** or **13b** was obtained eluting with the mixture ethyl acetate–tetrahydrofuran (1:1) [in the case of preparation of **13a**, the first fractions of this latter eluate (containing a small amount of an unidentified compound) were discarded]. The eluate collected, after removal of solvents, gave an oily or solid residue from which compounds **13a**, or **13b** was obtained as described below.

*5.1.10.1. 5-Chloro-N,N-diethyl[1,2,4]triazolo[4,3-a]quinoline-4-carboxamide (**13a**).* The solid residue obtained from the reaction performed with formic hydrazide was taken up in a little diethyl ether and filtered to give the nearly pure **13a** as a whitish crystalline solid (0.60 g, 25%); white crystals, m.p. 201–203 °C, after crystallization from ethyl acetate. ^{1}H NMR ($CDCl_3$): δ 1.09 and 1.31 [2t, 3H + 3H, N(CH_2CH_3)$_2$], 3.23 [q, 2H, 2H of N(CH_2CH_3)$_2$], 3.52 and 3.80 [2m, 1H + 1H, 2H of N(CH_2CH_3)$_2$], 7.59 and 7.73 (2 near t, 1H + 1H, H-7,8), 7.97 and 8.20 (2 near d, 1H + 1H, H-6,9), 9.22 (s, 1H, H-1); IR (KBr): 1630 s (CO), 1596, 1566, 1524 w cm^{-1}. Anal. ($C_{15}H_{15}ClN_4O$) C, H, N.

*5.1.10.2. 5-Chloro-N,N-diethyl-1-isopropyl[1,2,4]triazolo[4,3-a]quinoline-4-carboxamide (**13b**).* The residue obtained from the reaction performed with isobutyrohydrazide was treated with a little diisopropyl ether to afford the nearly pure **13b** as a whitish crystalline solid (0.91 g, 33%); white crystals, m.p. 166.5–168 °C, after crystallization from ethyl acetate–diisopropyl ether. ^{1}H NMR ($CDCl_3$): δ 1.09 and 1.30 [2t, 3H + 3H, N(CH_2CH_3)$_2$], 1.53 and 1.59 [2d, 3H + 3H, 1-CH(CH_3)$_2$], 3.22 [q, 2H, 2H of N(CH_2CH_3)$_2$], 3.48–3.89 [m, 3H, 2H of N(CH_2CH_3)$_2$ + 1-CH(CH_3)$_2$], 7.60 and 7.71 (2 near t, 1H + 1H, H-7,8), 8.15 and 8.23 (2 near d, 1H + 1H, H-6,9); IR (KBr): 1643 s (CO), 1601, 1567, 1530 w cm^{-1}. Anal. ($C_{18}H_{21}ClN_4O$) C, H, N.

5.1.11. Synthesis of substituted 5-amino[1,2,4]triazolo[4,3-a]quinoline-4-carboxamide derivatives **4a–d**

Compounds **4a–d** were prepared by the reaction of the 5-chloroderivatives **13a**,**b** with an excess of the proper amine

through the below described experimental procedures, depending on the amine used.

*5.1.11.1. N,N-Diethyl-5-(4-methyl-1-piperazinyl)[1,2,4]triazolo[4,3-a]quinoline-4-carboxamide (**4a**) and its 1-isopropyl derivative (**4b**).* A mixture of 5.0 mmol of **13a** (1.59 g, preparation of **4a**) or **13b** (1.72 g, preparation of **4b**), 5 mL of 1-methylpiperazine and 5 mL of dimethyl sulphoxide was stirred at 140 °C for 3 h. The solution obtained was then poured into water (200 mL) and the resulting emulsion was exhaustively extracted with dichloromethane. The combined extracts, dried (anhydrous Na_2SO_4) and evaporated to dryness in vacuo, afforded an oily residue which was chromatographed on a silica gel column, eluting first with mixture ethyl acetate–tetrahydrofuran (1:1) to remove impurities and a little amount of starting compound, then with the mixture dichloromethane–triethylamine (9:1) (compound **4a**) or dichloromethane–petroleum ether–triethylamine (6:3:1) (compound **4b**). After removal of solvents from the eluates collected, **4a** was obtained as whitish solid which was crystallized from ethyl acetate, whereas the oily **4b** was precipitated as maleate (white crystals, m.p. 218–220 °C) by treatment with a solution of maleic acid in anhydrous ethanol. From an analytical sample of this maleate, the free base **4b** was obtained (after treatment with aqueous $NaHCO_3$ and extraction with chloroform) as colourless thick oil which, after a long standing at room temperature, became an amorphous white solid that was crystallized from diethyl ether–petroleum ether.

*5.1.11.2. N,N-Diethyl-5-(ethylamino)-1-isopropyl[1,2,4]triazolo[4,3-a]quinoline-4-carboxamide (**4c**) and its 5-(isobutylamino) analogue (**4d**).* A mixture of 5.0 mmol of **13b** (1.72 g), 5 mL of ethylamine (**4c**) or isobutylamine (**4d**), and 25 mL of anhydrous ethanol was heated at 160 °C in a closed vessel for 14 h. After cooling, the resulting solution was evaporated to dryness at reduced pressure and the residue was partitioned between 10% aqueous Na_2CO_3 (100 mL) and dichloromethane (100 mL). The organic layer was collected and the aqueous one was further extracted twice with dichloromethane. The combined organic phases were dried (anhydrous Na_2SO_4), then evaporated to dryness at reduced pressure, to give a thick oil from which nearly pure compound **4d** separated out as a whitish solid by simple addition of a little diethyl ether–petroleum ether, whereas, in the case of **4c**, a chromatography on a silica gel column, eluting with the mixture dichloromethane–petroleum ether–triethylamine (5:5:1), was necessary to obtain pure **4c**.

Data for compounds **4a–d** are reported in Table 3.

5.2. Pharmacology

Wistar rats (250–300 g) and Swiss mice (25–35 g) of either sex were used. During the night preceding each experiment they were kept in proper cages to prevent coprophagy at constant environmental conditions (21 ± 1 °C; 40 ± 5% relative humidity).

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 active at this dose were further tested at doses decreasing by a factor of 2. Control animals were orally treated with an equivalent volume of vehicle alone. Groups of 8–10 animals were used. The ethical guidelines for investigation of experimental pain in conscious animals were followed and all the tests were carried out according to the EEC ethical regulation (EEC Council 86/609; D.L. 27/01/1992, No. 116).

5.2.1. Anti-inflammatory activity

Anti-inflammatory activity was studied by inducing paw edema according to Winter's method [15]. Carrageenan (1%, 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 compound. 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). Edema 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 [16], 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. 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 groups.

To investigate the possible mechanism underlying the antinociceptive effect demonstrated by compound **2c**, different groups of mice were pretreated with Naloxone (1 mg kg^{-1} i.p.), Atropine (5 mg kg^{-1} i.p.), Mecamylamine (1 mg kg^{-1} i.p.) and Methysergide (1 mg kg^{-1} s.c.) 10 min before compound **2c** (3.12 mg kg^{-1}) or vehicle oral administration. All the drugs were purchased from Sigma–Aldrich, Milan, Italy.

5.2.3. Spontaneous locomotor activity

Locomotor activity was measured by means of an activity cage (height 35 cm, width 23 cm, depth 19 cm, Model 7401, Basile, Comerio (VA), Italy) where the bridges the animals make or break with their paws produce random configurations which are converted into pulses. After oral administration of the compounds under study or vehicle, mice were placed singularly into the activity cage and locomotor activity was automatically recorded at time intervals of 5 min for 90 min. Mice spontaneous motility was evaluated considering the number of pulses recorded in the final 30 min period. All experiments were conducted from 9:00 to 13:00. The percentage of inhibition was calculated from the difference in the number of pulses between the treated and the control groups.

5.2.4. Gastrolesivity

The acute gastrolesivity of the test compounds was evaluated by examining the stomachs excised 5 h after oral administration of the drugs (200 mg kg^{-1}) 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. Data analysis

Results were expressed as means ± 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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