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Short communication

Synthesis and pharmacological study of ethyl 1-methyl-5-(substituted 3,4-dihydro-4-oxoquinazolin-3-yl)-1*H*-pyrazole-4-acetates

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Abstract – Several new ethyl 1-methyl-5-(substituted 3,4-dihydro-4-oxoquinazolin-3-yl)-1*H*-pyrazole-4-acetates **2**, substituted at 2 and, alternatively at, 6, 7 or 8 positions of the quinazolinone nucleus, were synthesised. The compounds were screened for their analgesic and antiinflammatory activities, acute toxicity and ulcerogenic effect. Substitution in the benzene moiety of the quinazolinone ring did not show any advantage for the analgesic activity, whereas it improved in some cases the antiinflammatory activity. Some compounds showed appreciable antiinflammatory activity and, at the same time, very low ulcerogenic index. © 2001 Éditions scientifiques et médicales Elsevier SAS

ethyl 1-methyl-5-(substituted 3,4-dihydro-4-oxoquinazolin-3-yl)-1*H*-pyrazole-4-acetates / antiinflammatory activity / analgesic activity / ulcerogenic proprieties

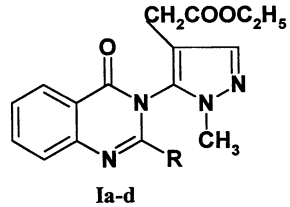
1. Introduction

Our research group has long been interested in the chemistry and pharmacology of 4(3*H*)-quinazolinones bearing a heterocyclic nucleus such as pyrazole, isoxazole and indazole. These compounds showed analgesic, antiinflammatory [1–6] and antineoplastic [7–9] activities.

Among the pyrazole derivatives, some have the structure of type **I** bearing the ethoxycarbonylmethyl group at the C-4 position of the pyrazole nucleus, presenting the structural feature of the heteroarylalkanoic esters (see *figure 1*). These compounds showed remarkable analgesic activity associated with appreciable antiexudative properties and very reduced ulcerogenic effects and systemic toxicity [5].

It is reported that heteroarylalkanoic esters could afford in vivo the related acids [10] which are a well-established class of non-steroidal antiinflammatory agents (NSADs), therapeutically useful in the treatment of acute as well as chronic inflammatory conditions [10].

In particular, Lonazolac-Ca, the active principle of the antiinflammatory and antirheumatic agent Irritren®, is the calcium salt of 1-phenyl-3-(*p*-chlorophenyl)-1*H*-pyrazole-4-acetic acid [11–14]. It



Ia-d

	a	b	c	d
R	H	CH_3	C_2H_5	C_6H_5

Figure 1. Structure of compounds **Ia**–**d**.

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shows a very good proportion of clinical efficacy and gastroduodenal side effects.

In order to improve the properties of the 5, 6, 7 and 8 unsubstituted quinazolinones (**I**), we prepared a new series of compounds which are substituted on the quinazolinone nucleus with groups that have similar lipophylic character but exert opposite electronic effects.

2. Chemistry

The synthesis of the quinazoline derivatives **2d–p** was carried out following *figure 2.*

The amino derivatives **1a–c** [15] were reacted with ethyl orthoacetates to produce the desired quinazolinones **2d–p**.

The structures of compounds **2** were assigned on the basis of analytical as well as spectroscopic data. The IR spectra exhibited bands in the 1670–1750 cm^{-1} region, due to the endocyclic and ester carbonyl groups. The ^{1}H-NMR spectra indicated hindered internal rotation about the bond that links the pyrazole and quinazolinone nuclei. In fact, when the substituent R is a methyl, ethyl or phenyl group, the spectra exhibited, for the methylene group of the acetate moiety, a double doublet in the 3.21–3.54 δ region with J values typical for a geminal coupling. The spectrum of compounds **2f,l,p** showed also the signals for the methylene portion of the ethyl group bonded at the 2 position of the quinazolinone ring. They appeared as two multiplets in the 2.27–2.59 δ region, due to geminal and vicinal coupling.

3. Pharmacology

All the new quinazolinones were screened in order to evaluate their analgesic and antiinflammatory activities, behavioural effect, acute toxicity and, lastly, ulcerogenic potential. Indomethacin (INDO) and phenylbutazone (PBZ) were tested in all the assays as reference standards.

4. Results and discussion

Pharmacological data are reported in *table I.*

4.1. Behavioural effects and acute toxicity in mice

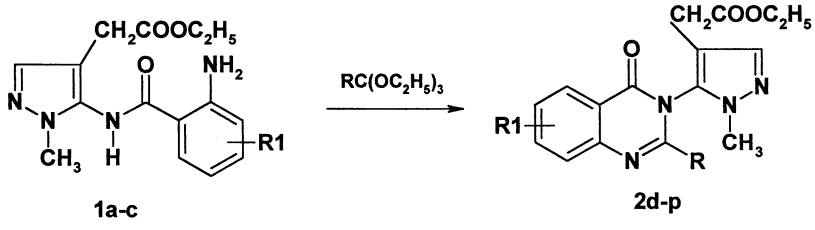
In mice, the test compounds did not induce remarkable behaviour modifications according to Irwin's assay at the dose of 700 mg kg^{-1} p.o. and 500 mg kg^{-1} i.p., whereas slight and transient sedation was the only effect noted after 1000 mg kg^{-1} p.o. and 800 mg kg^{-1} i.p.

Only compounds **2d** and **2g** showed marked sedation about 15 min after administration for a period of 1–3 h. Death generally occurred at 1–3 h postdrug in 40–60% of the animals.

The surviving mice appeared normal and remained so throughout the 7-days observation period.

4.2. Analgesic activity

In a phenylbenzoquinone-induced writhing test in mice, compounds **2f,m,p** were quite more active than phenylbutazone, whereas compounds **2d,e,l,o** were as active as, or slightly more active than, phenylbutazone.



1	a	b	c
R_1	4-Cl	5-Cl	3-CH_3

2	d	e	f	g	h	i	l	m	n	o	p
R	H	CH_3	C_2H_5	C_6H_5	H	CH_3	C_2H_5	C_6H_5	H	CH_3	C_2H_5
R_1	6-Cl	6-Cl	6-Cl	6-Cl	7-Cl	7-Cl	7-Cl	7-Cl	8-CH_3	8-CH_3	8-CH_3

Figure 2. Synthetic pathway of compounds **2d–p**.

4.3. Antiinflammatory activity

In the acetic acid peritonitis assay, the quinazolinones **2d**,**e**,**g**,**l**,**m**,**n**,**p** exhibited a remarkable antiexudative action which was, in the case of **2g**, fourfold higher than that of phenylbutazone but lower than that of indomethacin.

In the rat paw oedema test, compounds **2d**,**g** showed fair activity; however, they were less active than phenylbutazone.

4.4. Ulcerogenic activity

The most active compounds showed very low harmful effects on the stomach at the tested dose of 300 mg kg^{-1} p.o. when administered twice at a 2 h interval in fasted rats, whereas PBZ (100 mg $kg^{-1} \times 2$) and indomethacin (10 mg $kg^{-1} \times 2$) produced serious gastric ulcers in all animals.

5. Conclusions

The pharmacological data obtained for compounds **2d**–**p** were compared with those previously reported for the unsubstituted analogues of type **2** [5]. It seems that 6-Cl, 7-Cl and 8-CH_3 substitution does not generally show any advantage for the analgesic activity, whereas it allows obtaining in some cases higher inhibition values in the carrageenin paw oedema test and the acetic acid peritonitis test. The above substitutions influenced the studied activities following different trends. Finally, compounds **2d**,**g** could be selected for their appreciable antiinflammatory activity and, at the same time, very low ulcerogenic index.

Table I. Pharmacological data.

Compound	Acute toxicity		Analgesic activity	Antiinflammatory activity		Ulcerogenic index
	Approximate LD_{50} (mg kg^{-1})		Phenylquinone writhing-test [a] (% protection)	Carrageenin paw rat oedema [a] (% inhibition)	Acetic acid peritonitis [a] (% inhibition)	
	p.o.	i.p.	10 mg kg^{-1}	100 mg kg^{-1}	10 mg kg^{-1}	2×300 mg kg^{-1}
Ia	>1000	~750	0	28*	22* [b]	–
Ib	>1000	~750	48*	33*	24* [b]	–
Ic	>1000	~750	44*	18	22* [b]	–
Id	>1000	~500	65*	19	18 [b]	–
2d	~800	~400	29*	45*	32*	80
2e	>1000	~800	37*	34*	25*	–
2f	>1000	~800	42*	10	16*	–
2g	~800	~400	18*	40*	43*	60
2h	>1000	~800	10	23*	8	–
2i	>1000	~800	21*	22*	19*	–
2l	>1000	~800	34*	34*	35*	–
2m	>1000	~800	58*	37*	25*	50
2n	>1000	~800	13	35*	24*	–
2o	>1000	~800	26*	33*	9	–
2p	>1000	~800	41*	33*	42*	40
PBZ	~700	~300	26*	56*	11	275 [c]
INDO	~25	~15	79* [d]	63* [e]	66* [f]	300 [c]

Oral administration for all tests. INDO = indomethacin, PBZ = phenylbutazone.* $P < 0.05$ Student's t-test versus controls.
[a] Values are percent of controls.
[b] 100 mg kg^{-1}.
[c] PBZ: 100 mg $kg^{-1} \times 2$, INDO: 10 mg $kg^{-1} \times 2$.
[d] INDO: 1 mg kg^{-1}.
[e] INDO: 10 mg kg^{-1}.
[f] INDO: 1 mg kg^{-1}.

Table II. Physical data of ethyl 1-methyl-5-(substituted 3,4-dihydro-4-oxoquinazolin-3-yl)-1*H*-pyrazole-4-acetates (**2d**–**p**).

Compound	M.p. (°C)	Formula	Analyses	IR (cm^{-1})	Yields (%)
2d	149	$C_{16}H_{15}N_4O_3Cl$	C, H, N	1735, 1680	51
2e	134	$C_{17}H_{17}N_4O_3Cl$	C, H, N	1735, 1695	51
2f	108–110	$C_{18}H_{19}N_4O_3Cl$	C, H, N	1740, 1690	51
2g	118–120	$C_{22}H_{19}N_4O_3Cl$	C, H, N	1735, 1685	46
2h	199	$C_{16}H_{15}N_4O_3Cl$	C, H, N	1750, 1675	55
2i	110–111	$C_{17}H_{17}N_4O_3Cl$	C, H, N	1737, 1695	54
2l	112	$C_{18}H_{19}N_4O_3Cl$	C, H, N	1735, 1680	52
2m	120–122	$C_{22}H_{19}N_4O_3Cl$	C, H, N	1730, 1680	50
2n	126	$C_{17}H_{18}N_4O_3$	C, H, N	1740, 1675	56
2o	123–125	$C_{18}H_{20}N_4O_3$	C, H, N	1735, 1670	56
2p	116	$C_{19}H_{22}N_4O_3$	C, H, N	1735, 1675	60

^{1}H-NMR (δ): **2d** 1.00 (3H, unresolved signal, CH_3); 3.48 (2H, s, CH_2); 3.90 (5H, superimposed singlets, CH_3+CH_2); 7.80–8.30 (5H, a set of signals; C_6H_3, pyrazole H-3, quinazolinone H-2). **2e** 1.02 (3H, t, CH_3, $J=7.00$ Hz); 2.25 (3H, s, CH_3); 3.31 (1H, d, H methylene, $J=16.50$ Hz); 3.5 (1H, d, H methylene, $J=16.50$ Hz); 3.91–3.94 (5H, superimposed signals, CH_3+CH_2); 7.70–8.07 (4H, a set of signals; C_6H_3+H-3 pyrazole). **2f** 1.01 (3H, t, CH_3, $J=6.70$ Hz); 1.17 (3H, t, CH_3, $J=7.66$ Hz); 2.38–2.54 (2H, 2 multiplets, C–*CH_2*–CH_3); 3.26 (1H, d, CH–CO, $J=16.65$ Hz); 3.45 (1H, d, CH–CO, $J=16.65$ Hz); 3.90–3.95 (5H, superimposed singlets, N–CH_3 + O–CH_2); 7.71–8.05 (4H, a set of signals; C_6H_3 and pyrazole H-3). **2g** 1.07 (3H, t, CH_3, $J=7.10$ Hz); 3.33 (dd superimposed with H_2O signal, CH_2); 3.70 (3H, s, CH_3); 3.94 (2H, q, CH_2, $J=7.10$ Hz); 7.32–8.16 (9H, a set of signals; C_6H_3, C_6H_5 and pyrazole H-3). **2h** 1.00 (3H, t, $J=6.85$ Hz); 3.48 (2H, s, CH_2); 3.69–3.93 (5H, superimposed signals, CH_3+CH_2); 7.63–8.30 (5H, a set of signals; C_6H_3, quinazolinone H-2 and pyrazole H-3). **2i** 1.02 (3H, t, CH_3, $J=6.43$ Hz); 2.25 (3H, s, CH_3); 3.31 (1H, d, methylene H, $J=16.75$ Hz); 3.51 (1H, d, methylene H, $J=16.75$ Hz); 3.92 (5H, superimposed singlets, CH_3+CH_2); 7.55–8.14 (4H, a set of signals; C_6H_3 and pyrazole H-3). **2l** 0.99 (3H, t, CH_3, $J=7.08$ Hz); 1.15 (3H, t, CH_3, $J=7.50$ Hz); 2.27–2.59 (2H, multiplets and DMSO signals, C–*CH_2*–CH_3); 3.25 (1H, d, methylene H, $J=16.62$ Hz); 3.45 (1H, d, methylene H, $J=16.62$ Hz); 3.86–3.94 (5H, superimposed signals, CH_3+CH_2); 7.54–8.12 (4H, a set of signals; C_6H_3+pyrazole H-3). **2m** 1.03 (3H, t, CH_3, $J=7.07$ Hz); 3.17–3.43 (dd and H_2O signal, C–CH_2–CO); 3.67 (3H, s, CH_3); 3.92 (2H, q, CH_2); 7.26–8.20 (9H, a set of signals; C_6H_5, C_6H_3 and pyrazole H-3). **2n** 1.05 (3H, t, $J=6.96$ Hz, CH_3); 2.65 (3H, s, CH_3); 3.54 (2H, s, CH_2); 3.94–4.02 (5H, s+q, CH_2+CH_3); 7.56–8.34 (4H, C_6H_3, quinazolinone H-2 and pyrazole H). **2o** 1.05 (3H, t, $J=7.5$ Hz, CH_3); 2.31 (3H, s, CH_3); 2.63 (3H, s, CH_3); 3.32 (1H, d, $J=16.6$ Hz, methylene CH); 3.54 (1H, d, $J=16.6$ Hz, methylene CH); 3.93–4.03 (5H, s+q, CH_2+CH_3); 7.48–7.93 (3H, C_6H_3 and pyrazole H). **2p** 1.06 (3H, t, $J=6.96$ Hz, CH_3); 1.27 (3H, t, $J=6.96$ Hz; CH_3); 2.36–2.72 (5H, s+m, CH_2+CH_3); 3.30 (1H, d, $J=16.58$ Hz, methylene CH); 3.5 (1H, d, $J=16.58$ Hz, methylene H); 3.92–4.03 (5H, s+q, CH_3+CH_2); 7.48–8.03 (3H, C_6H_3 and pyrazole H-3).

6. Experimental protocols

6.1. Chemistry

All melting points were taken in a Büchi-530 capillary apparatus and are uncorrected. IR spectra were recorded in a JASCO IR-810 spectrometer as Nujol mulls. ^{1}H-NMR spectra were obtained in DMSO-d_6 or $CDCl_3$ solutions in a Brüker AC-E 250 MHz spectrometer using tetramethylsilane as internal reference. Microanalyses were performed in the laboratories of the Dipartimento di Scienze Farmaceutiche, University of Catania, Italy, and were within ±0.4% of theoretical values.

*6.1.1. Ethyl 1-methyl-5-(substituted 3,4-dihydro-4-oxoquinazolin-3-yl]-1H-pyrazole-4-acetates (**2d**–**p**)*

The amino derivatives **1a**–**c** (2 g) [15] and the appropriate ethyl orthoester (3.5 mL) were refluxed for 5 h. After cooling at room temperature, the crystalline white solid that separated out was filtered off and recrystallized from 95%(v/v) ethanol. Yields 46–60%.

The data of compounds **2d**–**p** are reported in *table II*.

6.2. Biology

6.2.1. Materials and methods

Swiss male mice (20–23 g) and Sprague–Dawley rats (130–160 g) were used. The animals were starved for about 12 h before administration and maintained at a temperature of 22±2 °C. The tested compounds, indomethacin and phenylbutazone (PBZ), were suspended in 0.5% aqueous methylcellulose solution and administered orally or intraperitoneally. Control animals received the same amounts of the vehicle.

6.2.2. Behavioural effects and acute toxicity in mice [16]

Irwin's multidimensional screening–evaluative procedure was used on groups of five animals. The com-

pounds were administered at three doses orally (500, 700, 1000 mg kg^{-1}) or intraperitoneally (250, 500, 800 mg kg^{-1}). The animals were kept under observation for 6 h and the symptomatology was checked again 24 h later. The approximate LD_{50} was obtained from mortality 7 days later.

6.2.3. Analgesic activity: phenylbenzoquinone writhing test [17]

Analgesia was assessed by means of phenylbenzoquinone (PBQ)-induced writhing on a group of five male mice. Each mouse was given i.p. 0.25 mL of 0.02% PBQ in 5% ethanol and the number of writhes was counted for 5 min, beginning 5 min after the injection. The tested compounds and PBZ (10 mg kg^{-1}), as well as indomethacin (1 mg kg^{-1}), were orally administered 60 min before PBQ. The analgesic effect was expressed as percentage of protection in comparison with controls.

6.2.4. Antiinflammatory activity

6.2.4.1. Carrageenin-induced oedema [18]

Groups of four rats were used. The tested compounds and PBZ were given orally at 100 mg kg^{-1}, while indomethacin was given at 10 mg kg^{-1}. Sixty minutes later, 0.1 mL of 1% carrageenin solution was injected into the sub-plantar tissue of the right hind paw. The volume was measured by a mercury plethysmometer prior to the injection of carrageenin and 3 h later. The increase in volume of the paw 3 h after the injection of carrageenin was adopted as a measure of oedema. Swelling reduction in treated animals was calculated as the percentage of inhibition in comparison with controls.

6.2.4.2. Acetic acid peritonitis [19]

Groups of four rats were tested. Peritonitis was produced by an i.p. injection of acetic acid (10 mL kg^{-1} of 0.5% solution). Thirty minutes later, the rats were killed by ether and the peritoneal exudate was collected and measured. The tested compounds and PBZ were given orally at the dose of 10 mg kg^{-1}, 60 min before the injection of acetic acid. Indomethacin was administered in the same way at 1 mg kg^{-1}. The antiexudative response was expressed as the percentage of the exudate volume reduction compared with controls.

6.2.5. Ulcerogenic activity [20]

Groups of four rats were used. The compounds were given orally (300 mg kg^{-1}) to animals fasted for 24 h and after 2 h the treatment was repeated again. Indomethacin and phenylbutazone were administered twice at 10 and 100 mg kg^{-1}, respectively. Six hours after the first dose each rat was sacrificed by ether inhalation, the stomach removed, opened along the greater curvature and examined with a dissecting microscope for the presence of gastric ulcers. The severity of mucosal damage (Ulcerogenic index) was graded by means of scores from 0 (no lesion) to 4 (exceptional severe lesions). In order to take into account the percentage of rats having ulcers, an index of ulceration was calculated on the basis of the following formula:

$$\frac{\text{mean degree of ulcers} \times \text{number of animals with ulcers}}{\text{number of animals}} \times 100$$

6.2.6. Statistical analysis

The results are expressed as means; one-way analysis with Dunnett's comparison to controls and unpaired Student's *t*-test were used to determine statistical significance.

Acknowledgements

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