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4-Amino-3(2*H*)-pyridazinones bearing arylpiperazinylalkyl groups and related compounds: synthesis and antinociceptive activity

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Abstract

A series of 4-amino-3(2*H*)-pyridazinones substituted at position 2 with arylpiperazinylalkyl groups and analogues were synthesized and their antinociceptive effect was evaluated in the mouse abdominal constriction model. Preliminary SARs studies were performed. Several of the novel compounds dosed at 100 mg/kg s.c. significantly reduced the number of writhes induced by the noxious stimulus. Compound **12e** showed 100% inhibition of writhes and was able to protect all the treated animals from the effect of the chemical stimulus. Subsequent dose–response studies revealed **12e** to be almost 40-fold more potent than the structurally related Emorfazone.

Keywords: Arylpiperazinylalkylpyridazinones; Synthesis; Antinociceptive activity; Emorfazone; SARs studies

1. Introduction

Pain relief represents a major unmet medical need and millions of people worldwide have to use drugs for treatment of different levels of pain. Although non steroidal antiinflammatory agents (NSAIDs) and opioids have been the cornerstone of pain therapy for a long time, both classes are limited by severe side effects. NSAIDs, which are mainly used to treat mild to moderate inflammatory pain, induce gastric irritation and nephrotoxicity [1]. Opioids display an array of adverse reactions, such as respiratory depression, sedation, constipation [2]. Moreover, repeated administrations of these drugs induce tolerance to the analgesic effects and physical dependence. Neuropathic pain, which is a particular form of pain, can be controlled only with antidepressant and anticonvulsant drugs [3]. For all these reasons, the search for more safe novel antinociceptive agents remains an important challenge for medicinal chemists.

Among the pyridazine derivatives endowed with antinociceptive effects, Ag 246 **1** [4] and Emorfazone **2** [5] have emerged (Chart 1); the latter, which was launched in Japan, displays an interesting profile because its activity is not mediated by interaction with the prostaglandins system or by affinity for opioids receptors [6,7]. The literature reports many examples of antinociceptive agents bearing an arylpiperazinylalkyl moiety linked both to the 2-nitrogen of a pyridazine ring (compounds **3** and **4**) [8,9] and to a different lactamic system (compound **5**) [10].

Our studies in this field have allowed the identification of compound **6** ($ED_{50} = 14.9$ mg/kg, s.c.) as a potent antinociceptive agent in writhing test [9,11]. On this basis, we decided to combine the arylpiperazinyl moiety and the amino and vinyl functions (which are essential structural requirements for the activity of compound **6**) in the same phenylpyridazinone backbone, in order to verify if the contemporary presence of these structural elements is associated with better activity.

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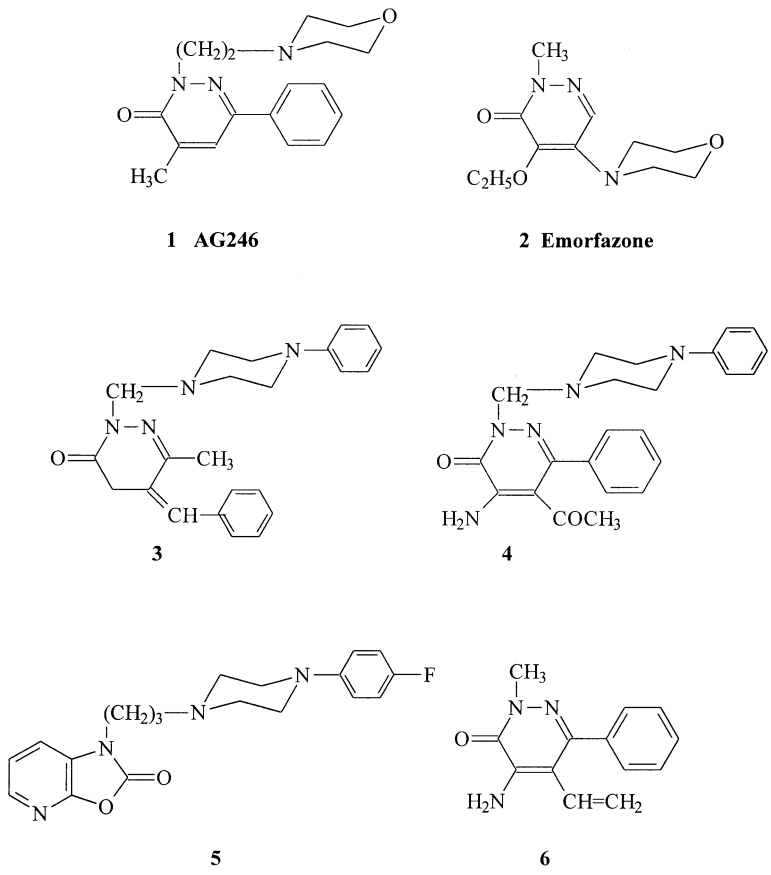


1 AG246 2 Emorfazone

3 4

5 6

Chart 1.

2. Chemistry

The synthetic pathway affording the final 5-vinylpyridazinones **12a**–**i** is depicted in Scheme 1. Compounds **8a**,**b** were obtained by treatment of the previously described isoxazolo[3,4-d]pyridazinones **7a**,**b** [12,13] with dibromoethane in standard conditions. The alkylation takes place regioselectivity at pyridazine-2-nitrogen in agreement with a variety of literature reports [14,15]. Displacement of the bromine in **8a**,**b** with the appropriate cycloalkylamine afforded **9a**–**i**, which, in turn, were transformed into the corresponding 5-acetyl-4-amino derivatives **10a**–**i** by reductive cleavage with hydrazine hydrate and 10% Pd/C. The final compounds **12a**–**i** were obtained by treatment of **10a**–**i** with sodium borohydride, followed by dehydratation of the secondary alcohols **11a**–**i** with poliphosphoric acid (PPA).

Scheme 2 shows the synthesis of the analogues **16a**–**c** bearing a three methylenic spacer. The introduction of the appropriate 1-aryl-4-(3-bromopropyl)piperazine moiety was carried out in standard conditions on compound **13** [12]; the intermediates **14a**–**c** were transformed into **16a**–**c** following the two steps procedure above. Again any trace of oxygen-alkylated derivative was detected in the reaction mixture.

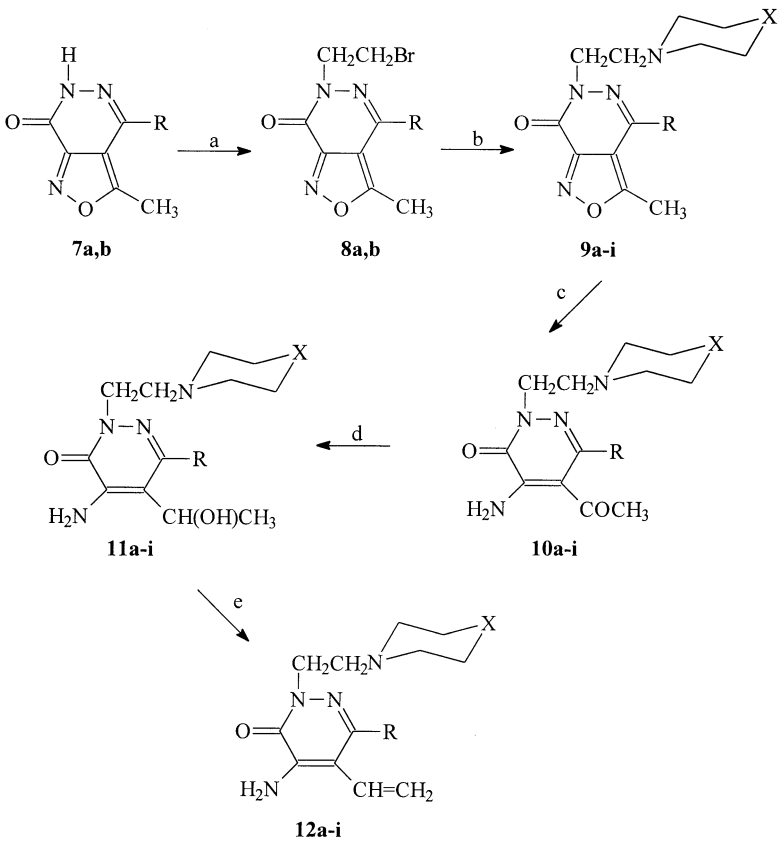
Mannich condensation between 2-methoxyphenylpiperazine and compound **18** [16], which in turn was obtained from **13** through the usual reduction [17] and dehydratation sequence, afforded the final **19** (Scheme 3).

The chemical and physical data of all compounds are reported in Tables 1–4.

3. Experimental procedures

3.1. Chemistry

All melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were recorded with Varian Gemini 200 instruments. Chemical shifts are reported in ppm, using the solvent as internal standard. Extracts were dried over Na_2SO_4 and the solvents were removed under reduced pressure. E. Merck F-254 commercial plates were used for analytical TLC to follow the course of reaction. Silica gel 60 (Merck 70–230 mesh) was used for column chromatography.

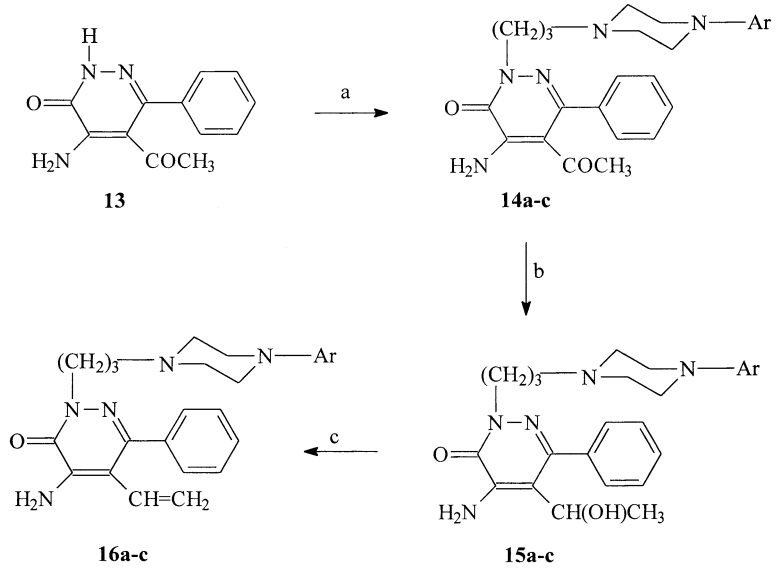


Scheme 1. Conditions: (a) $Br(CH_2)_2Br$, K_2CO_3, DMF; (b) cycloalkylamine, EtOH; (c) hydrazine hydrate, EtOH; (d) $NaBH_4$, MeOH; (e) PPA.

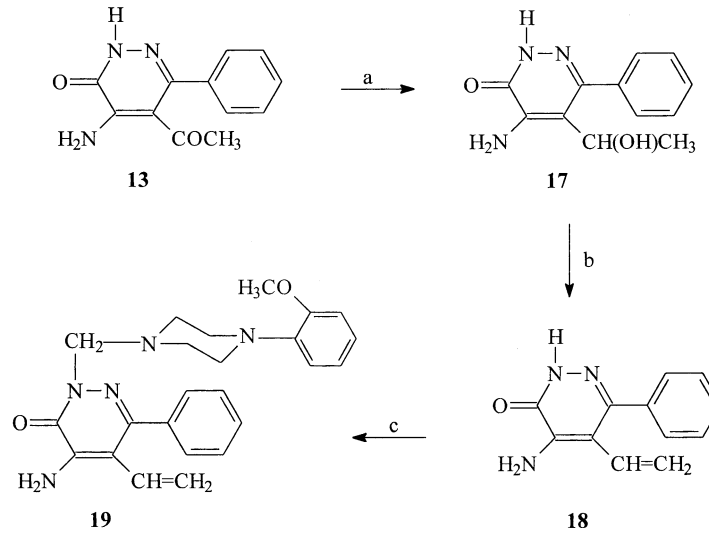
*3.1.1. General procedure for compounds **8a,b***

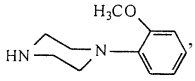
A mixture of the appropriate isoxazolopyridazinone **7a**,**b** (0.6 mmol), dibromoethane (1.2 mmol) and potassium carbonate (4 mmol) in anhydrous DMF (3 ml) was heated at 60 °C under stirring for 1–3 h. After the mixture cooled, water (12 ml) was added and the final products were recovered by suction.

*3.1.1.1. 6-(2-Bromoethyl)-3,4-dimethylisoxazolo[3,4-d]pyridazin-7(6H)-one (**8b**).* ^{1}H NMR ($CDCl_3$): δ 2.50



Scheme 2. Conditions: (a) Br-$(CH_2)_2$- –N N, K_2CO_3, DMF; (b) $NaBH_4$, MeOH; (c) PPA.



Scheme 3. Conditions: (a) $NaBH_4$, MeOH; (b) PPA; (c) HCHO,  , EtOH.

(s, 3H, 4-CH_3), 2.90 (s, 3H, 3-CH_3); 3.70 (t, $J = 8.1$ Hz, 2H, CH_2Br); 4.50 (t, $J = 8.1$ Hz, 2H, NCH_2).

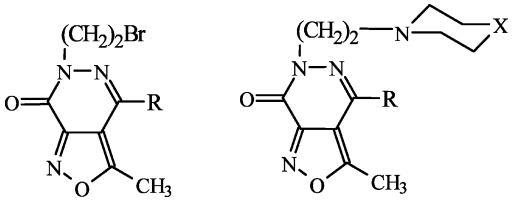
3.1.2. General procedure for compounds *9a–i*

A mixture of the appropriate 2-bromoethyl derivative **8a**,**b** (0.3 mmol), the appropriate cycloalkylamine (1 mmol), potassium carbonate (0.8 mmol) in anhydrous DMF (2 ml) was heated at 50–110 °C for 1–5 h. After the mixture cooled and cold water (10 ml) was added; the crude precipitate was recovered by suction with the exception of compounds **9b** and **9h** for which it was necessary extraction with $CHCl_3$ (3×15 ml) and evaporation of the solvent.

3.1.2.1. 3-Methyl-4-phenyl-6-[(4-phenylpiperazin-1-yl)ethyl]-isoxazolo[3,4-d]pyridazin-7(6H)-one (*9a*).

^{1}H NMR ($CDCl_3$): δ 2.50 (s, 3H, CH_3), 2.85–3.50 (m, 8H, piperazine); 3.40 (d, $J = 7.9$ Hz, 2H, NCH_2CH_2–piperazine); 4.45 (d, $J = 8.0$ Hz, 2H, NCH_2CH_2–piperazine); 6.80–7.00 (m, 5H, Ar); 7.54 (s, 5H, Ar).

Table 1



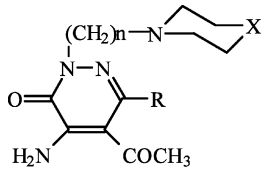
Physical and chemical data of compounds **8a**,**b**, **9a**–**i**, **21** and **22**

Comp. [a]	X	R	Yield (%)	M.p. (°C)	Formula [b]
8a		Ph	82	157–159	$C_{14}H_{12}BrN_3O_2$
8b		Me	60	127–130	$C_9H_{10}BrN_3O_2$
9a	NC_6H_5	Ph	72	158–160	$C_{24}H_{25}N_5O_2$
9b	$NC_6H_4OC_2H_5(o)$	Ph	93	Oil	$C_{26}H_{29}N_5O_3$
9c	$NC_6H_4CH_3(p)$	Ph	62	76–78	$C_{25}H_{27}N_5O_2$
9d	$NC_6H_4Cl(o)$	Ph	97	145–147	$C_{24}H_{24}N_5O_2Cl$
9e	$NC_6H_4CH_3(p)$	Me	69	117–119	$C_{20}H_{25}N_5O_2$
9f	CH_2	Ph	64	102–104	$C_{19}H_{22}N_4O_2$
9g	$CHCH_3$	Ph	62	90–92	$C_{20}H_{24}N_4O_2$
9h	$CHCH_2C_6H_5$	Ph	78	Oil	$C_{26}H_{28}N_4O_2$
9i	O	Ph	69	157–158	$C_{18}H_{20}N_4O_3$

[a] All compounds were recrystallized from EtOH with the exception of compounds **9b** and **9h** which were purified by column chromatography using as eluent cyclohexane/ethyl acetate 1:2 and $CHCl_3$/MeOH 9:1, respectively.

[b] The elemental analyses are within $\pm 0.4\%$ of the theoretical values for C, H, N.

Table 2



10a-i, 14a-c

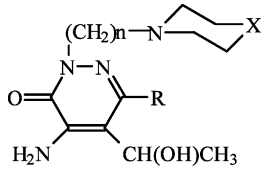
Physical and chemical data of compounds **10a**–**i** and **14a**–**c**

Comp. [a]	*N*	X	R	Yield (%)	M.p. (°C)	Formula [b]
10a	2	NC_6H_5	Ph	72	154–156	$C_{24}H_{27}N_5O_2$
10b	2	$NC_6H_4OC_2H_5(o)$	Ph	98	105–108	$C_{26}H_{31}N_5O_3$
10c	2	$NC_6H_4CH_3(p)$	Ph	86	149–151	$C_{25}H_{29}N_5O_2$
10d	2	$NC_6H_4Cl(o)$	Ph	62	149–151	$C_{24}H_{26}N_5O_2Cl$
10e	2	$NC_6H_4CH_3(p)$	Me	80	124–126	$C_{20}H_{27}N_5O_2$
10f	2	CH_2	Ph	72	132–133	$C_{19}H_{24}N_4O_2$
10g	2	$CHCH_3$	Ph	83	97–100 dec.	$C_{20}H_{26}N_4O_2$
10h	2	$CHCH_2C_6H_5$	Ph	95	Oil	$C_{26}H_{30}N_4O_2$
10i	2	O	Ph	90	152–155	$C_{18}H_{22}N_4O_3$
14a	3	NC_6H_5	Ph	98	145–147	$C_{25}H_{29}N_5O_2$
14b	3	$NC_6H_4CH_3$(p)	Ph	98	139–141	$C_{26}H_{31}N_5O_2$
14c	3	$NC_6H_4OC_2H_5$(o)	Ph	97	85–87 dec.	$C_{27}H_{33}N_5O_3$

[a] Compounds were recrystallized from EtOH with the exception of **10b** which was recrystallized from cyclohexane and **10h** which was purified by column chromatography using as eluent $CHCl_3$/MeOH 9:1.

[b] The elemental analyses are within ±0.4% of the theoretical values for C, H, N.

Table 3



11a-i, 15a-c

Physical and chemical data of compounds **11a**–**i** and **15a**–**c**

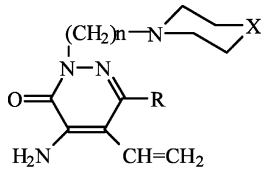
Comp.	*N*	X	R	Yield (%)	M.p. (°C)	Recryst. solvent	Formula [c]
11a	2	NC_6H_5	Ph	64	80–82	EtOH	$C_{24}H_{29}N_5O_2$
11b	2	$NC_6H_4OC_2H_5(o)$	Ph	85	70–75	Cyclohexane	$C_{26}H_{33}N_5O_3$
11c	2	$NC_6H_4CH_3(p)$	Ph	71	86–90	EtOH	$C_{25}H_{31}N_5O_2$
11d	2	$NC_6H_4Cl(o)$	Ph	91	82–84	EtOH	$C_{24}H_{28}N_5O_2Cl$
11e	2	$NC_6H_5CH_3(p)$	Me	71	166–168	Acetone	$C_{20}H_{29}N_5O_2$
11f	2	CH_2	Ph	72	Oil [a]		$C_{19}H_{26}N_4O_2$
11g	2	$CHCH_3$	Ph	80	73–76	A [b]	$C_{20}H_{28}N_4O_2$
11h	2	$CHCH_2C_6H_5$	Ph	68	65–70	EtOH	$C_{26}H_{32}N_4O_2$
11i	2	O	Ph	87	Oil [a]		$C_{18}H_{24}N_4O_3$
15a	3	NC_6H_5	Ph	50	70–72	Cyclohexane	$C_{25}H_{31}N_5O_2$
15b	3	$NC_6H_4CH_3(p)$	Ph	75	152–154	EtOH	$C_{26}H_{33}N_5O_3$
15c	3	$NC_6H_4OC_2H_5(o)$	Ph	50	84–87	Cyclohexane	$C_{27}H_{35}N_5O_3$

[a] Purified by column chromatography using as $CHCl_3$/MeOH 7:3 for **11f** and $CHCl_3$/MeOH 9:1 for **11i**.

[b] Cyclohexane/ethyl acetate 1:1.

[c] The elemental analyses are within ±0.4% of the theoretical values for C, H, N.

Table 4



12a-i, 16a-c, 19

Physical and chemical data of compounds **12a**–**i**, **16a**–**c** and **19**

Comp. [a]	*N*	X	R	Yield (%)	M.p. (°C)	Formula [b]
12a	2	NC_6H_5	Ph	56	120–125	$C_{24}H_{27}N_5O$
12b	2	$NC_6H_4OC_2H_5(o)$	Ph	40	59–61	$C_{25}H_{29}N_5O$
12c	2	$NC_6H_4CH_3(p)$	Ph	84	129–131	$C_{26}H_{31}N_5O_2$
12d	2	$NC_6H_4Cl(o)$	Ph	35	138–140	$C_{24}H_{26}N_5OCl$
12e	2	$NC_6H_4CH_3(p)$	Me	50	100–101	$C_{20}H_{27}N_5O$
12f	2	CH_2	Ph	57	Oil	$C_{19}H_{24}N_4O$
12g	2	$CHCH_3$	Ph	66	118–121	$C_{20}H_{26}N_4O$
12h	2	$CHCH_2C_6H_5$	Ph	57	118–120	$C_{26}H_{30}N_4O$
12i	2	O	Ph	42	Oil	$C_{18}H_{22}N_4O_2$
16a	3	NC_6H_5	Ph	76	115–118	$C_{25}H_{29}N_5O$
16b	3	$NC_6H_4CH_3(p)$	Ph	31	130–133	$C_{26}H_{31}N_5O$
16c	3	$NC_6H_4OC_2H_5(p)$	Ph	17	102–104	$C_{27}H_{33}N_5O_2$
19	1	$NC_6H_4OCH_3$(o)	Ph	19	159–161	$C_{24}H_{27}N_5O_2$

[a] All compounds were recrystallized from EtOH, with exception of **12f** and **12i** which were purified by column chromatography using as $CHCl_3$/MeOH 7:3 and $CHCl_3$/MeOH 9:1, respectively.

[b] The elemental analyses are within ±0.4% of the theoretical values for C, H, N.

*3.1.3. General procedure for compounds **10a**–**i***

The appropriate 2-substituted isoxazolo[3,4-d]pyridazinones (0.5 mmol) **9a**–**i** were suspended in EtOH (10 ml). Hydrazine hydrate (1.5 mmol) and 10% Pd/C (40 mg) were added and the mixture was refluxed for 1 h. Then, after cooling the catalyst was filtered off and the solvent was evaporated in vacuo affording **10a**–**i** as residue.

*3.1.3.1. 5-Acetyl-4-amino-6-phenyl-2-{[4(4-methyl)phenylpiperazin-1-yl]ethyl}-pyridazin-3(2H)-one (**10c**).* ^{1}H NMR ($CDCl_3$): δ 1.80 (s, 3H, $COCH_3$), 2.30 (s, 3H, C–CH_3); 2.80–3.15 (m, 8H, piperazine), 3.25 (t, $J = 7.8$ Hz, 2H, CH_2CH_2–piperazine); 4.45 (t, $J = 7.8$ Hz, 2H, CH_2CH_2–piperazine); 6.85 (m, 2H, Ar); 7.05 (m, 2H, Ar); 7.45 (s, 5H, Ar).

*3.1.4. General procedure for compounds **11a**–**i** and **15a**–**c***

Sodium borohydride (2.8 mmol) was added portionwise to a stirred solution of the appropriate 4-amino-5-acetyl derivatives **10a**–**i** or **14a**–**c** in MeOH (5–16 ml). The reaction mixture was stirred for 20–120 min at rt and, after concentration in vacuo, it was diluted with cold water. Compounds were recovered by suction, with the exception of **11b**, **11f**,**g** and **11i** which were obtained after extraction with CH_2Cl_2 (3 × 20 ml) and evaporation in vacuo.

*3.1.4.1. 4-Amino-5-hydroxyethyl-6-phenyl-2-[(4-phenylpiperazin-1-yl)propyl]-pyridazin-3(2H)-one (**15a**).* ^{1}H NMR ($CDCl_3$): δ 1.45 (d, $J = 7.8$ Hz, 3H, CH_3); 2.25–2.40 (m, 2H, $CH_2CH_2CH_2$); 2.90–3.35 (m, 10H, 8H piperazine and $CH_2CH_2CH_2$–piperazine); 4.20–4.40 (m, 2H, $NCH_2CH_2CH_2$–piperazine); 4.70–4.85 (m, 1H, CHOH); 5.95 (exch. br s, 1H, OH); 6.85–6.95 (m, 3H, Ar); 7.20–7.50 (m, 7H, Ar).

*3.1.5. General procedure for compounds **12a**–**i** and **16a**–**c***

The appropriate 5-hydroxyethylpyridazinones **11a**–**i**, **15a**–**c** (0.4 mmol) were reacted with PPA (40 mmol) for 2–24 h. For compounds **12e** and **16a**–**c** the reaction was carried out at 50 °C, for all others at rt. After treatment with ice water the mixture was neutralized with 1 N NaOH and compounds were recovered by suction (**12a**, **12c**–**e** and **12g**,**h**) or after extraction with CH_2Cl_2 (3 × 20 mmol) (**12b**, **12f**, **12i** and **16a**–**c**) and evaporation of the solvent.

*3.1.5.1. 4-Amino-6-phenyl-2[(4-phenylpiperazin-1-yl)propyl]5-vinylpyridazin-3(2H)-one (**16a**).* ^{1}H NMR ($CDCl_3$): δ 2.40–2.60 (m, 2H, $CH_2CH_2CH_2$); 2.95–3.20 (m, 6H, $CH_2CH_2CH_2$–piperazine and 4H piperazine); 3.50–3.65 (m, 4H, piperazine); 4.35 (t, $J = 7.8$ Hz, 2H, $CONCH_2$); 5.50 (d, 1H, $J = 18.3$ Hz, CH=CH_2); 5.60 (d, 1H, $J = 12.4$ Hz, CH=CH_2); 6.25 (dd, 1H, $J = 18.3$ Hz, $J = 12.4$ Hz, CH=CH_2); 6.85–7.00 (m, 3H, Ar); 7.25–7.35 (m, 2H, Ar); 7.45 (s, 5H, Ar).

*3.1.6. General procedure for compounds **14a–c***

A mixture of the appropriate 1-aryl-4-(3-bromopropyl)piperazine (0.75 mmol), 5-acetyl-4-aminopyridazinone (**13**) (0.5 mmol) and potassium carbonate (4.0 mmol) in acetone (DMF for **14b**) was heated at 70 °C for 1–3 h under stirring. After cooling, cold water was added and products were recovered by suction.

*3.1.6.1. 5-Acetyl-4-amino-6-phenyl-2-[(4-phenylpiperazin-1-yl)propyl]-pyridazin-3(2H)-one (**14a**).* ^{1}H NMR ($CDCl_3$): δ 1.80 (s, 3H, CH_3), 2.10–2-30 (m, 2H, $CH_2CH_2CH_2$), 2.60–2.80 (m, 6H, $CH_2CH_2CH_2$–piperazine and 4H piperazine); 3.20–3.35 (m, 4H, piperazine); 4.30 (t, $J = 7.9$ Hz, 2H, $CONCH_2$); 6.85–6.95 (m, 3H, Ar); 7.20–7.35 (m, 2H, Ar); 7.45 (s, 5H, Ar).

*3.1.7. Synthesis of 4-amino-6-phenyl-2-{[4-(2-methoxy)phenylpiperazin-1yl]methyl}-5-vinylpyridazin-3(2H)-one (**19**)*

To a suspension of compound **18** (0.05 mmol) in EtOH (3.5 ml), 2-methoxyphenylpiperazine (0.1 mmol) and 37% CH_2O (0.1 mmol) were added. The mixture was refluxed for 2 h under stirring, the solvent was evaporated in vacuo and the residue crystallized from EtOH.

^{1}H NMR ($CDCl_3$): δ 3.00–3.15 (m, 8H, piperazine); 3.85 (s, 3H, OCH_3); 3.90 (s, 2H, NCH_2N); 5.50–5.65 (m, 4H, $CH{=}CH_2$ and NH_2); 6.30 (dd, $J = 15.0$, 9.0 Hz, 1H, $CH{=}CH_2$); 6.80–7.05 (m, 4H, Ar); 7.35–7.50 (m, 5H, Ar).

3.2. Pharmacology

The antinociceptive effect of the compounds reported in Table 5 was evaluated by the *p*-phenylquinone-induced abdominal constriction test according the procedure described by Siegmund et al. [18] and modified by Milne [19]. Male CD-1 mice (21–35) maintained at 23 ± 1 °C, were injected intraperitoneally with *p*-phenylquinone (PPQ) (2 mg/kg using a 0.02% solution in 5% ethanol/95% distilled water, 20 min after subcutaneous administration of the test compound). Groups of 5 mice were used for each dose tested. The number of characteristic abdominal constrictions for each mouse was counted for a period of 8 min after the PPQ injection. The mean number of abdominal constrictions was compared for each treatment group with the mean number in the vehicle-treated control group. The percentage inhibition was then calculated for each compound-treated group. All compounds were dissolved in DMSO and administered subcutaneous in a volume of 5 ml/kg. The same volume of DMSO was administered to controls. The ED_{50} and related 95% confidence intervals were determined by the nonlinear fitting analysis using the software GraFit (Eriacus Software Limited, Horley, UK). The antinociceptive activity of the tested compounds was also evaluated as quantal protection, using the formula: $\%\ \text{quantal protection} = \frac{\text{protected}}{\text{treated}} \times 100$, where 'protected' means the number of animals completely protected from the effects of PPQ and 'treated' means the number of animals treated in each group.

4. Results and discussion

All the final compounds were evaluated as antinociceptive agents at the dose of 100 mg/kg s.c. in writhing test induced by PPQ and mouse abdominal constrictions were calculated. The obtained results are reported in Table 6.

In order to define some preliminary structure–activity relationships, the synthesized compounds can be examined considering three different chemical series:

a) 4-amino-5-vinylpyridazinones with piperidinyl(morpholinyl) ethyl chains (compounds **12f–i**)
b) 4-amino-5-vinylpyridazinones with arylpiperazinylalkyl chains (compounds **12a–e**, **16a–c** and **19**)
c) 4-amino-5-acetylpyridazinones **10c**, **10f** and **10g**.

In the first series, compound **12f**, bearing an unsubstituted piperidine was found completely devoid of activity; isosteric replacement of the CH_2 with oxygen (**12i**) resulted in a slight improvement of activity, whereas homologation of **12f** led to a strong increase of activity (compound **12g**, 63% inhibition); this compound, which was also able to protect 20% of the treated animal from the noxious stimulus, is one of the most active among the compounds described in this study. On the contrary, the phenylpiperidinyl analogue **12h** proved to be less active. These data seem to indicate that small lipophilic groups are the best arranged at position 4 of the cycloalkylamine.

Among the compounds belonging to the second series, the 4-phenyl derivative **12a** ($n = 2$) proved to be weak active (12% inhibition). Introduction of a chlorine (**12d**) or an ethoxy group (**12b**) in the ortho position left almost unchanged the activity, whereas introduction of a CH_3 in the para position (**12c**) was associated with a good level of activity (53% inhibition and 20% of quantal protection). When this same fragment was introduced in the substrate bearing a methyl group at position 6 of the pyridazine (**12e**) a dramatic increase of activity was observed (100% inhibition and 100% quantal protection). Dose–response studies revealed very high potency ($ED_{50} = 2.5$ mg/kg) (Fig. 1).

Elongation of the methylenic spacer to $n = 3$ gave interesting results, the 4-unsubstituted phenyl derivative **16a** displaying a significant level of activity (51%

Table 5
Analgesic activity of pyridazinone derivatives determined by *p*-phenylquinone-induced (PPQ) mouse abdominal constriction test

Comp. [a]	No. of abdominal constrictions	Inhibition (%)	Quantal protection (%)
12f	18.20±1.50	7	0
12g	7.40 *±3.26	63	20
12h	12.40 *±0.87	38	0
12i	14.40±2.87	27	0
12a	16.60±4.30	12	0
12b	12.40±4.76	34	20
12c	8.80±3.57	53	20
12d	13.20±3.46	30	0
12e	0.00 *±0.00	100	100
16a	9.60 *±2.68	51	0
16b	12.00±2.90	36	0
16c	4.60±1.89	76	40
19	7.80 *±3.25	60	20
10c	14.00±5.26	30	20
10f	19.40±1.72	1	0
10g	10.00±4.46	50	40
Saline	18.40±1.86		
Morphine [b]	0.80 *±0.80	96	80
Emorfazone [b]	7.00 *±2.02	64	20

[a] Compounds were dissolved in DMSO 100% and were administered (100 mg/kg, s.c.) 20 min before PPQ (2 mg/kg, i.p.).
[b] Compounds were dissolved in saline; morphine was administered at 1 mg/kg s.c. and Emorfazone at 100 mg/kg s.c..
* $P < 0.05$ vs. appropriate vehicle treated group ($n = 5$).

Table 6
Elemental analyses for the final compounds **12a–i**, **16a–c** and **19**

Comp.	Calculated (%)			Found (%)		
	C	H	N	C	H	N
12a	71.78	6.79	17.44	72.03	6.80	17.51
12b	72.25	7.05	16.86	72.50	7.03	16.92
12c	70.07	7.03	15.72	70.35	7.00	15.78
12d	66.11	6.02	16.07	65.90	6.00	16.01
12e	67.95	7.71	19.82	68.15	7.74	19.75
12f	70.33	7.47	17.27	70.50	7.44	17.34
12g	70.96	7.76	16.56	71.02	7.74	16.50
12h	75.32	7.31	13.52	75.05	7.44	13.50
12i	66.22	6.81	17.17	65.96	6.83	17.10
16a	72.25	7.05	16.86	72.39	7.02	16.80
16b	72.68	7.29	16.31	73.00	7.31	16.37
16c	70.55	7.25	15.24	70.81	7.22	15.30
19	69.03	6.53	16.78	68.85	6.50	16.84

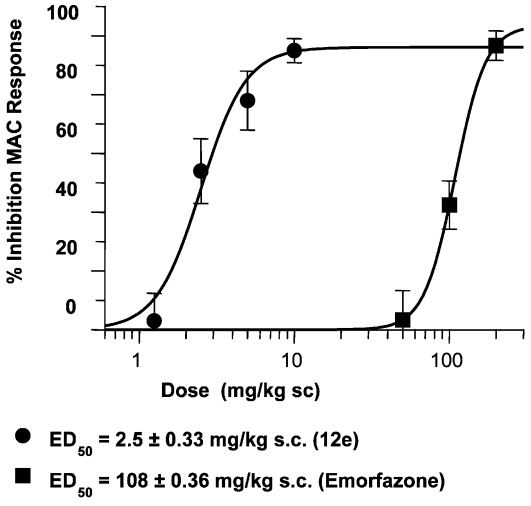


Fig. 1.

inhibition) and the para ethoxy phenyl analogue **16c** being even more active (76% inhibition, 40% quantal protection). Finally compound **19**, which is the only characterized by a mono methylenic spacer, was proved endowed with a good level of activity (60% inhibition, 20% quantal protection).

In the series of 4-amino-5-acetylpyridazinones, the structural requirements of the cycloalkylamino moiety at the end of the carbon chain demonstrated to be very similar to that of the 4-amino-5-vinyl analogues: in fact the best arranged system was the 4-methylpiperidine (compound **10g**, 50% inhibition and 40% quantal protection); the unsubstituted piperidine derivative **10f** was completely inactive, the *p*-tolyl derivative **10c** showed an intermediate value of inhibition (30%).

Taken together these results suggest that in the compounds examined in this study the presence of small lipophilic groups at position 4 of the cycloalkylamine and the nature of the substituent at position 6 of the pyridazine are important requirements for antinociceptive activity in writhing test. On the contrary, the nature of the function at position 5 and the length of the methylenic spacer seem to play only a marginal role.

This study allowed to identify compound **12e** as a very promising lead, useful for further chemical development. This novel agent, compared with the structurally related

Emorfazone **2** was over 40-fold more potent in the mouse abdominal constriction test (Fig. 1). A significant improvement in comparison with the previous lead **6** ($ED_{50} = 14.9$ mg/kg) was also observed. This finding seems to suggest that the molecular hybridization of structure **6** with that of the prototypes **1**, **3** and **5** may represent a rational approach to identify more potent antinociceptive agents belonging to the pyridazine chemical class. Studies in this direction are now in progress.

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