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Short communication

4,5-Functionalized 6-phenyl-3(2*H*)-pyridazinones: synthesis and evaluation of antinociceptive activity

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Summary — A series of 2-substituted 4,5-functionalized 6-phenyl-3(2*H*)-pyridazinones were synthesized and their antinociceptive activities were evaluated in the mouse abdominal constriction model. Single dose studies showed that compounds **11**, **18a** and **23** were more active than the reference drug, Emorfazone, in inhibiting the effects of the noxious chemical stimulus, *p*-phenylquinone. Subsequent dose–response studies revealed **18a** to be almost seven-fold more potent than Emorfazone.

Emorfazone / pyridazinone /antinociceptive activity

Introduction

The search for new analgesic agents devoid of the side-effects typical of morphine-like opioid agonists (such as respiratory depression, constipation and physical dependence), as well as of the gastro-intestinal problems associated with nonsteroidal antiinflammatory drugs (NSAIDs), has attracted considerable attention in recent years. In this regard a considerable number of 3(2*H*)-pyridazinone derivatives endowed with analgesic properties have been reported recently [1]. Among these compounds, Emorfazone **1** [2, 3] (which was launched as an analgesic in Japan at the beginning of the last decade), the 4,5-dihalo derivatives **2** [4], the 5-arylidene **3** [5] and the 4-carbamoylpyridazinones **4** [6] have emerged as being of particular interest. In addition, Flouzat *et al* [7] have recently reported new potent analgesics corresponding to the general structure **5** (chart 1). These compounds, which contain a five-membered *N*-substituted lactamic system, are characterized by the absence of either antiinflammatory activity or affinity for opioid receptors.

Stimulated by these findings, our attention has been focused on a group of 2-substituted 4,5-functionalized 6-phenyl-3(2*H*)-pyridazinones, arising from

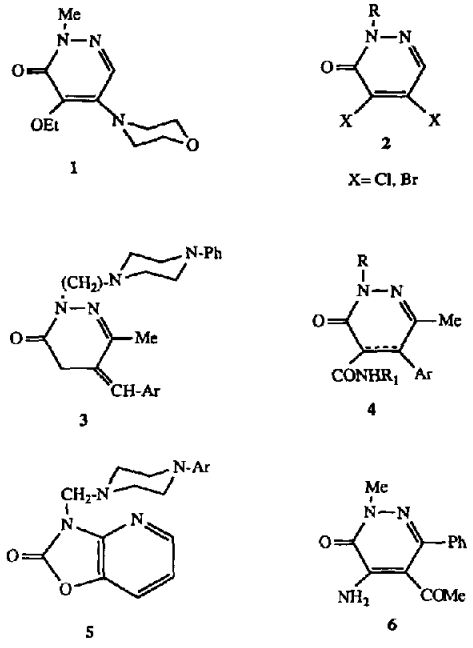


Chart 1.

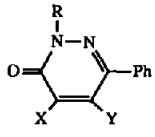
the chemical manipulation of compound **6**, previously described by us [8], which displays analgesic activity in the Randall–Selitto test. For some of these new compounds (table I), a pattern of substitutions at positions 2, 4 and 5 similar to that of compound **1** and the presence of a phenyl group featuring compounds of general formula **4**, can be recognized. The parent compound **6** has been further modified by introducing arylpiperazinylalkyl groups (compounds **11** and **23**), whose presence seems to play a fundamental role in determining the activity of the prototypes **3** and **5**, as well as of some recently developed analgesics containing a pyridazine moiety [9].

Chemistry

The synthetic strategies employed in this work are depicted in schemes 1–4. The isoxazolo[3,4-*d*]pyridazinone **7** was transformed into the 5-acetyl-4-aminopyridazinone **8** by hydrogenolytic opening of the isoxazole ring [10]. Alkylation of **8** with allyl bromide afforded the 2-allyl derivative **9** in good yields. Compound **11** was obtained starting from **7** by a Mannich condensation affording the intermediate 2-(4-phenylpiperazinomethyl)isoxazolo[3,4-*d*]pyridazinone **10** (71% yield) followed by catalytic hydrogenation (47% yield) (scheme 1).

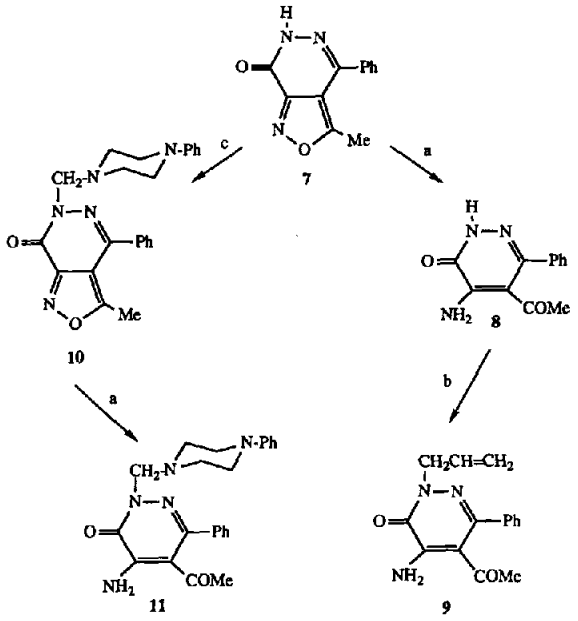
According to scheme 2, the methyl ester **13** (65% yield) and the nitrile **15** (54% yield) were synthesized starting from the common intermediate **12** (V Dal Piaz, unpublished results) by standard procedures. Reduction of **16** (V Dal Piaz *et al*, submitted for publication) with sodium borohydride afforded the new compound **17b** (72% yield) (scheme 3). From the known compound **17a** [11], **19** was synthesized in satisfactory yield. Treatment of **17a,b** with concentrated sulfuric acid led to the 5-vinylpyridazinones **18a,b** in moderate yield. Catalytic hydrogenation of **18a** afforded the corresponding 5-ethyl derivative **20**.

Table I. Chemical and physical data of 4,5-functionalized pyridazinones.

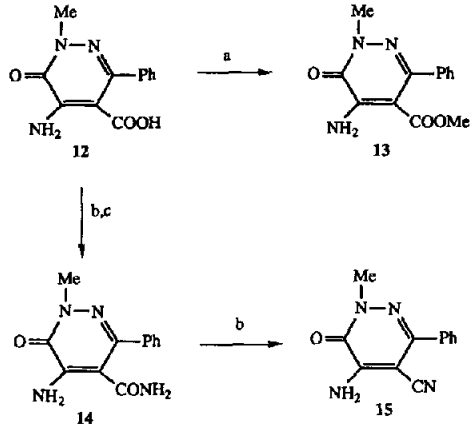


Compound	*R*	*X*	*Y*	*Yield (%)*	*Mp (°C) (solvent)*	*Crystallization solvent*	*Formula*[a]
9	$CH_2CH{=}CH_2$	NH_2	$COCH_3$	75	93–95	CHX[c]	$C_{15}H_{15}N_3O_2$
11	CH_2A[b]	NH_2	$COCH_3$	47	177–179	EtOH	$C_{23}H_{25}N_5O_2$
13	CH_3	NH_2	$COOCH_3$	65	184–186	$EtOH/H_2O$	$C_{13}H_{13}N_3O_3$
14	CH_3	NH_2	$CONH_2$	61	234–237	EtOH	$C_{12}H_{12}N_4O_2$
15	CH_3	NH_2	CN	54	246–248	MeOH	$C_{12}H_{10}N_4O$
17b	CH_3	OCH_3	$CH(OH)CH_3$	72	112–115	EtOH	$C_{14}H_{16}N_2O_3$
18a	CH_3	NH_2	$CH{=}CH_2$	48	122–123	$EtOH/H_2O$	$C_{13}H_{13}N_3O$
18b	CH_3	OCH_3	$CH{=}CH_2$	54	78–80	$EtOH/H_2O$	$C_{14}H_{14}N_2O_2$
19	CH_3	NH_2	$CH(OC_2H_5)CH_3$	60	187–188	EtOH	$C_{15}H_{19}N_3O_2$
20	CH_3	NH_2	CH_2CH_3	66	164–165	CHX	$C_{13}H_{15}N_3O$
23	CH_3	$NH(CH_2)_2A$[b]	$COCH_3$	63	163–165	EtOH	$C_{25}H_{29}N_5O_2$

[a]The elemental analyses were within ±0.4% of the theoretical values for C, H, N. [b]A= -N⟨ ⟩N-Ph. [c] CHX=cyclohexane.

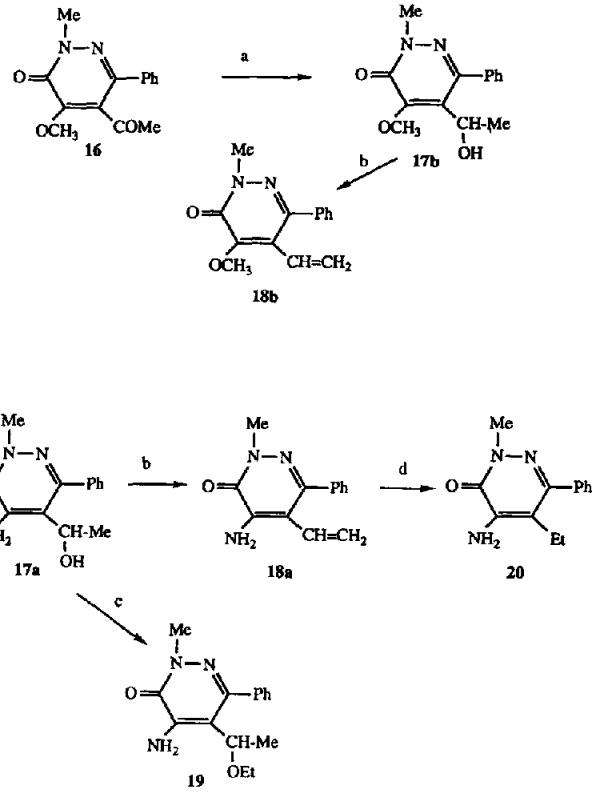


Scheme 1. Conditions: a) H_2, Pd/C, EtOH; b) CH_2 = $CHCH_2Br$, K_2CO_3, DMF; c) phenylpiperazine, CH_2O, EtOH.

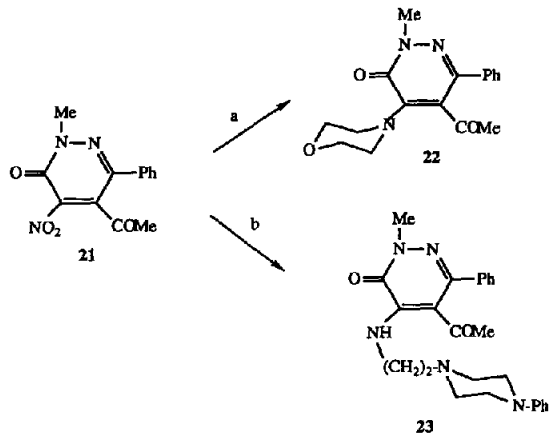


Scheme 2. Conditions: a) MeI, K_2CO_3, DMF; b) $SOCl_2$, 60°C; c) 30% aq NH_3.

Nucleophilic deplacement of the nitro group in compound **21** [12] afforded the known compound **22** [8] and the new phenylpiperazinoalkylamino derivative **23** in satisfactory yield (scheme 4). Chemical and physical data of the novel compounds are listed in table I.



Scheme 3. Conditions: a) $NaBH_4$, MeOH; b) conc H_2SO_4; c) EtOH, PPA, 100°C; d) H_2, Pd/C, EtOH.



Scheme 4. Conditions: a) morpholine, EtOH; b) $NH_2(CH_2)_2N$⟨⟩NPh, EtOH.

Results and discussion

With the exception of compounds **11**, **18a** and **23**, the novel compounds tested (including **6**, which is active in the Randall–Selitto test [8]) were inactive or only very weakly active in the mouse abdominal constriction test at a dose of 100 mg/kg sc (table II). Compounds **11** and **23** caused a reduction in the number of abdominal constrictions of 60 and 79%, respectively, and thus both compounds appeared to be more active than Emorfazone at this dose level using this method of assessing antinociceptive protection. Moreover, **11** and **23** induced complete protection in 40 and 60% of the treated animals, respectively, whereas at the same dose Emorfazone failed to completely protect any animal from the effects of *p*-phenylquinone. Compound **18a**, however, emerged as a compound of particular interest since at 100 mg/kg sc all of the treated animals were completely protected from the effects of the noxious chemical stimulus. Subsequent dose–response studies revealed an ED_{50} of 14.9 mg/kg sc, using a reduction in number of abdominal constrictions as the biological endpoint. Compared to the structurally related Emorfazone and paracetamol, compound **18a** was seven-fold more potent than Emorfazone and over 20-fold more potent than paracetamol in the mouse abdominal constriction test.

Table II. Analgesic activity of pyridazinone derivatives determined by *p*-phenylbenzoquinone-induced (PPQ) mouse abdominal constriction test.

Compound[a]	*Number of constrictions*	*Percentage inhibition*	*Quantal protection (%)*	*ED_{50}*[b] *(95% CI)*[c] *(mg/kg sc)*
Control	20.5 ± 1.4	0	0	
6	15.7 ± 2.1	23.7 ns[d]	0	
9	15.8 ± 2.3	23.1 ns	0	
11	8.1 ± 3.0	60.0*	40	
13	24.5 ± 1.6	−11.9 ns	0	
15	22.8 ± 2.5	−11.1 ns	0	
18a	0.0 ± 0.0	100	100	14.9 (7.9–27.3)
18b	18.4 ± 1.5	10.3 ns	0	
19	17.8 ± 6.3	8.2 ns	0	
20	18.8 ± 3.2	8.3 ns	0	
22	16.0 ± 2.8	22.0 ns	0	
23	4.2 ± 1.7	79.5***	60	
Emorfazone	11.2 ± 1.3	45.4**	0	103.8 (78.3–139.0)
Paracetamol				346.4 (208.4–576.0)
ASA				105.7 (49.3–168.4)

[a]All compounds were given at 100 mg/kg sc. [b]Calculated from dose–response curves. [c]Confidence intervals. [d]Not significant. ($n = 5$) *$P < 0.05$ Student's *t*-test; **$P < 0.01$ Student's *t*-test; ***$P < 0.001$ Student's *t*-test.

The structural requirements for antinociceptive activity in **18a**-like compounds appeared to be critical. Thus, the replacement of the amino group at position 4 with a different type of electron-donating group, such as methoxy (compound **18b**), resulted in a complete loss of activity. Similarly, replacement of the $CH{=}CH_2$ group at position 5 with a variety of different functionalities, such as $COCH_3$ (**6**), $COOCH_3$ (**13**), $CH(OC_2H_5)CH_3$ (**19**) and CN (**15**), produced inactive compounds. Saturation of the double bond of **18a** (compound **20**) also caused a dramatic reduction of activity, suggesting that electronic and/or conformational factors, connected with the presence of the vinyl group at position 5 of the pyridazine system, play a pivotal role for antinociceptive activity. The introduction of an allyl group at position 2, whose presence in Emorfazone-like compounds produces a significant increase of antinociceptive activity [2], appears to be detrimental in our series (compound **9**). Finally, the presence of a phenylpiperazinoalkyl group linked to the 2- or 4-position (compounds **11** and **23**) appears to produce a significant increase of activity with respect to the parent compound **6**, confirming the importance of this structural feature in this series of antinociceptive agents. The preliminary data obtained for **11** and **23** seem to suggest that the increase of antinociceptive activity does not depend on the position in which this substituent is introduced.

In conclusion, a new compound (**18a**) has been synthesized which is characterized by a level of antinociceptive activity in the mouse abdominal constriction test (ED_{50} = 14.9 mg/kg sc) greater than that seen with either Emorfazone or paracetamol. The structure of **18a** is characterized by a very low degree of acceptance as regards the substitution at positions 4 and 5 of the pyridazine system.

Further studies are in progress to define better SARs and the mechanism of action of the new promising agents described herein.

Experimental protocols

Chemistry

All melting points were determined on a Büchi apparatus and are uncorrected. IR spectra were measured as Nujol mulls with a Perkin-Elmer 681 spectrometer. ^{1}H-NMR spectra were recorded with Varian Gemini 200 instrument. 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. Silica-gel plates (Merck F_{254}) were used for analytical TLC. Recrystallization solvents are indicated in table I.

*5-Acetyl-2-allyl-6-phenyl-3(2H)-pyridazinone **9***
A mixture of 5-acetyl-4-aminopyridazinone **8** (0.1 g, 0.44 mmol), allyl bromide (0.13 g, 1.03 mmol) and anhydrous potassium carbonate (0.5 g) in anhydrous DMF (2 ml) was stirred at room temperature for 30 min. Cold water (20 ml) was then added and compound **9**, thus precipitated, was collected by suction. IR (cm^{-1}): 3400 and 3290 (NH_2), 1650 (2 x CO). ^{1}H-NMR δ: 1.80 (s, 3H, CH_3CO), 4.80 (d, J = 6.9 Hz, 2H, CH_2N), 5.30 (m, 2H, CH_2 = CH), 6.05 (m, 1H, $CH{=}CH_2$), 7.45 (s, 5H, C_6H_5).

*3-Methyl-4-phenyl-6-[(4-phenylpiperazin-1-yl)methyl]isoxazolo[3,4-d]pyridazin-7(6H)-one **10***
A solution of **7** (0.4 g, 1.7 mmol), 37% w/v aqueous formaldehyde (2 ml, 26 mmol) and 1-phenylpiperazine (0.42 g, 2.6 mmol) in 95% ethanol (15 ml) was refluxed under stirring for 1.5 h. After further stirring at room temperature for 1.5 h, the precipitate **10** was collected by suction (0.50 g, 71% yield); mp 146–148°C (crystallization solvent EtOH); anal $C_{23}H_{23}N_5O_2$ (C, H, N). IR (cm^{-1}): 1690 (CO). ^{1}H-NMR δ: 2.55 (s, 3H, CH_3), 3.00 (m, 4H, piperazine), 3.20 (m, 4H, piperazine), 5.25 (s, 2H, CH_2), 7.10 (m, 5H, C_6H_5N), 7.55 (s, 5H, C_6H_5).

*5-Acetyl-4-amino-6-phenyl-2-[(4-phenylpiperazin-1-yl)methyl]-pyridazin-3(2H)-one **11***
A mixture of **10** (0.85 g, 2.12 mmol) and 95% ethanol (35 ml) was hydrogenated in Parr apparatus with palladium on charcoal (10% Pd/C, 0.7 g) for 30 min. After filtration, compound **13** was obtained by evaporation of the solvent. IR (cm^{-1}): 3440 and 3310 (NH_2), 1670 (amidic CO), 1630 (ketonic CO). ^{1}H-NMR δ: 1.80 (s, 3H, CH_3CO), 3.00 (m, 4H, piperazine), 3.20 (m, 4H, piperazine), 5.25 (s, 2H, NCH_2N), 7.10 (m, 5H, C_6H_5N), 7.50 (s, 5H, C_6H_5).

*Methyl-4-amino-2-methyl-6-phenyl-2,3-dihydro-3-oxopyridazin-4-carboxylate **13***
A mixture of **12** (0.2 g, 0.82 mmol), iodomethane (0.68 g, 4.8 mmol) and anhydrous potassium carbonate (0.12 g) in anhydrous DMF (3 ml) was stirred at room temperature for 45 min. Dilution with cold water (25 ml) afforded the pure **13**. IR (cm^{-1}): 3430 and 3320 (NH_2), 1720 ($COOCH_3$), 1660 (amidic CO). ^{1}H-NMR δ: 3.45 (s, 3H, OCH_3), 3.80 (s, 3H, NCH_3), 7.10 (s, 2H, NH_2), 7.35 (m, 5H, C_6H_5).

*4-Amino-5-carboxyamido-2-methyl-6-phenyl-3(2H)-pyridazinone **14***
A suspension of **12** (0.15 g, 0.61 mmol) and $SOCl_2$ (3 ml) was stirred at 60°C for 30 min. After concentration *in vacuo*, the residue was carefully treated under cooling with 30% w/v aqueous ammonia (8 ml) and then stirred at 5°C for 1 h. The suspension was diluted with water (25 ml) and extracted with CH_2Cl_2 (3 x 25 ml). Evaporation of the solvent afforded the crude **14**. IR (cm^{-1}): 3400 and 3330 (NH_2), 1650 (lactamic CO), 1590 (amidic CO). ^{1}H-NMR δ: 3.80 (s, 3H, $COCH_3$), 5.00 (s, exch br, 2H, NH_2), 5.40 (s, exch br, 2H, NH_2), 7.50 (m 5H, C_6H_5).

*4-Amino-5-cyano-2-methyl-6-phenyl-3(2H)-pyridazinone **15***
A mixture of **14** (0.2 g, 0.82 mmol) and $SOCl_2$ (5 ml) was stirred at 60°C for 4 h. After evaporation *in vacuo*, the residue was carefully treated with cold water (10 ml) and the precipitate **15** collected by suction. IR (cm^{-1}): 3400 and 3340 (NH_2), 2220 (CN), 1660 (CO). ^{1}H-NMR δ: 3.85 (s, 3H, CH_3), 6.10 (s, exch br, 2H, NH_2), 7.50 (m, 3H, H_{arom}), 7.70 (m, 2H, H_{arom}).

*5-(1-Hydroxyethyl)-2-methyl-4-methoxy-6-phenyl-3(2H)-pyridazinone **17b***
To a solution of **16** (0.1 g, 0.39 mmol) in MeOH (6 ml), sodium borohydride (0.11 g, 2.9 mmol) was added portionwise over 15 min. The suspension was stirred at room temperature for 1 h. Cold water (25 ml) was added and extracted with

CH_2Cl_2 (3 x 25 ml). Evaporation of the solvent afforded the crude **17b**. IR (cm^{-1}): 3340 (OH), 1635 (CO). ^{1}H-NMR δ: 1.45 (d, J = 7.8 Hz, 3H, CH_3CH), 3.20 (d, J = 4.1 Hz, 1H, OH), 3.80 (s, 3H, CH_3N), 4.30 (s, 3H, CH_3O), 4.55 (m, 1H, CHOH), 7.35 (m, 5H, C_6H_5).

4-Amino-2-methyl-6-phenyl-5-vinyl-3(2H)-pyridazinone ***18a***
Compound **17a** [11] (0.1 g, 0.41 mmol) was treated with sulfuric acid (96% w/v, 2 ml), and the solution was stirred at room temperature for 2.5 h. After treatment with ice-cold water (20 ml), the precipitated **18a** was collected by suction. IR (cm^{-1}): 3480 and 3330 (NH_2), 1640 (CO). ^{1}H-NMR δ: 3.80 (s, 3H, CH_3), 5.45 (exch br, 2H, NH_2), 5.55 (d, 1H, J = 16 Hz, CH=CH_2), 5.60 (d, 1H, J = 10 Hz, CH=CH_2), 6.30 (dd, 1H, J = 16 Hz, 10 Hz, CH=CH_2), 7.40 (s, 5H, C_6H_5).

2-Methyl-4-methoxy-6-phenyl-5-vinyl-3(2H)-pyridazinone ***18b***
Starting from **17b** (0.11 g, 0.42 mmol) and following the same procedure described above, compound **18b** was isolated. IR (cm^{-1}): 1650 (CO). ^{1}H-NMR δ: 3.82 (s, 3H, NCH_3), 4.20 (s, 3H, OCH_3), 5.55 (d, J = 15 Hz, 1H, CH=CH_2), 5.85 (d, J = 9.0 Hz, 1H, CH=CH_2), 6.25 (m, 1H, CH=CH_2), 7.40 (m, 5H, C_6H_5).

4-Amino-5-[(1-ethoxy)ethyl)]-2-methyl-6-phenyl-3(2H)-pyridazinone ***19***
A mixture of **17a** (0.1 g, 0.37 mmol) anhydrous ethanol (10 ml) and PPA (1.5 g) was stirred at 100°C for 15 min. Dilution with cold water (20 ml) afforded **19** as a precipitate which was filtered off. IR (cm^{-1}): 3425 and 3330 (NH_2), 1640 (CO). ^{1}H-NMR δ: 1.15 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.40 (d, 3H, CH_3CH), 3.35 (m, 2H, OCH_2CH_3), 3.80 (s, 3H, CH_3N), 4.25 (q, J = 6.8 Hz, 1H, OC$H$$CH_3$), 5.90 (s, exch br, 2H, NH_2), 7.30 (m, 3H, H_{arom}), 7.70 (m, 2H, H_{arom}).

4-Amino-5-ethyl-2-methyl-6-phenyl-3(2H)-pyridazinone ***20***
A mixture of **18a** (0.1 g, 0.42 mmol) and 95% ethanol (20 ml) was hydrogenated in Parr apparatus with palladium on charcoal (10% Pd/C, 0.02 g) for 45 min. The title compound was isolated after filtration and evaporation of the solvent. IR (cm^{-1}): 3415 and 3315 (NH_2), 1660 (CO). ^{1}H-NMR δ: 1.00 (t, J = 6.9 Hz, 3H, CH_3CH_2), 2.30 (q, J = 6.9 Hz, 2H, CH_2CH_3), 3.80 (s, 3H, CH_3N), 4.95 (s, exch br, 2H, NH_2), 7.40 (m, 5H, C_6H_5).

5-Acetyl-4[(4-phenylpiperazin-1-yl)ethyl]-amino-2-methyl-6-phenylpiperazin-3(2H)-one ***23***
A mixture of **21** (0.2 g, 0.7 mmol) and 4-phenylpiperazin-1-yl-ethylamine [13] (0.25 g, 1.2 mmol) in 95% ethanol (7 ml) was stirred at room temperature for 1 h. After evaporation *in vacuo*, the residue was treated with ethanol and the precipitate **23** was collected by suction. IR (cm^{-1}): 3350 (NH), 1690 (ketonic CO), 1640 (amidic CO). ^{1}H-NMR δ: 1.85 (s, 3H $COCH_3$), 2.70 (m, 6H, $NHCH_2CH_2$ and 4H piperazine), 3.45 (m, 2H, $NHCH_2CH_2$), 3.25 (m, 4H, piperazine), 3.80 (s, 3H, NCH_3), 7.10 (m, 5H, C_6H_5N), 7.40 (s, 5H, C_6H_5), 8.00 (s, exch br, 1H, NH).

Pharmacology

The compounds in table II were evaluated by using the *p*-phenylquinone-induced abdominal constriction model of antinociception. The procedure of Siegmund [14], modified by Milne [15], was used. Male CD-1 mice (21–35 g), 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 sc administration of the test compound. Groups of 5 mice were used for each dose tested and a group of 10 animals was used as the control. The number of characteristic abdominal constrictions for each mouse was counted for a period of 8 min after the PPQ administration. 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 sc in a volume of 5 ml/kg. The same volume of DMSO was administered to controls. ED_{50} and the related confidence intervals were determined using the method of Finney [16]; a probability value of $p < 0.05$ was considered significant. 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.

Acknowledgments

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