Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 4, pp 655–659, 1997

PII: S0968-0896(97)00006-0
0968-0896/97 $17.00 + 0.00

Synthesis and Analgesic Effects of 3-Substituted 4,6-Diarylpyridazine Derivatives of the Arylpiperazine Class

Florence Rohet,[a] Catherine Rubat,[b] Pascal Coudert[a] and Jacques Couquelet[a],*

Groupe de Recherche en Pharmacochimie, [a]Laboratoire de Chimie Thérapeutique and [b]Laboratoire de Pharmacologie, Faculté de Pharmacie, Université d'Auvergne, 28, Place Henri Dunant F63001, Clermont-Ferrand, Cédex, France

Abstract—A new series of 4,6-diaryl pyridazines substituted in the 3-position by arylpiperazinyl moieties was synthesized and evaluated for analgesic activity. Five out of the nine tested compounds possessed significant antinociceptive effects in the phenylbenzoquinone-induced writhing test (PBQ test) with ED_{50} values ranging from 26.0 to 37.7 mg/kg ip. The most active derivatives **2a**, **2d** and **2h** had a low toxicity (LD_{50} > 800 mg/kg ip) but showed some sedative and neurotoxic effects from the dose of 50 mg/kg ip. The three selected pyridazines were devoid of activity in the hot-plate test. However, analgesic activity of **2d** and **2h** was significantly reversed by naloxone in the PBQ test. Administered at the low dose of 5 mg/kg ip, **2h** greatly potentiated the antinociceptive response induced by morphine (0.15 mg/kg sc). In addition, analgesic effects of **2h** (2.5 mg/kg ip) were also potentiated by 5-hydroxytryptophan combined with carbidopa. These results suggest that pyridazine **2h** induces analgesia, which is mediated via both opioid and serotonergic mechanisms. © 1997 Elsevier Science Ltd.

Introduction

Despite an ever growing body of knowledge of endogenous nociceptive and antinociceptive systems, many pain syndromes like rheumatoid arthritis and certain advanced cancers are still unsatisfactorily treated. A large variety of compounds related to natural opiates or with completely novel chemical structures have been investigated in order to develop new potent analgesic agents with the efficacy of morphine as pain killers but without the undesired and use-limiting side-effects (such as respiratory depression, dependence-inducing liability, nausea and inhibition of gastrointestinal motility).

Recently, several new arylpiperazine derivatives have been reported to exert potent and efficacious analgesic activity without displaying the behavioural properties associated with morphine and its congeners.[1–6] Among compounds of this type, we have found substantial analgesic activity in 5-benzyl and 4,6-diarylpyridazine derivatives.[7–10] Following our search for new antinociceptive agents containing an arylpiperazinyl moiety, we synthesized 4,6-diarylpyridazines substituted in the 3-position as outlined in Scheme 1. Pyridazines **2a–i** were evaluated for their analgesic properties in the phenylbenzoquinone-induced writhing test (PBQ-test) and compared to classical analgesic drugs as also to the chemically related antidepressant trazodone (Fig. 1), which was known to possess potent antinociceptive effects.[11,12] Then, the most promising compounds were scrutinized in order to assess their toxicity profile and their possible interactions with opioidergic and serotonergic systems.

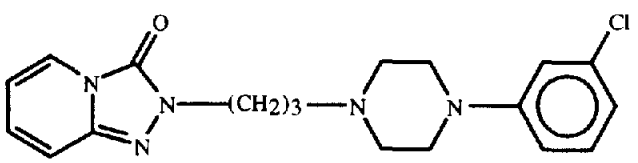


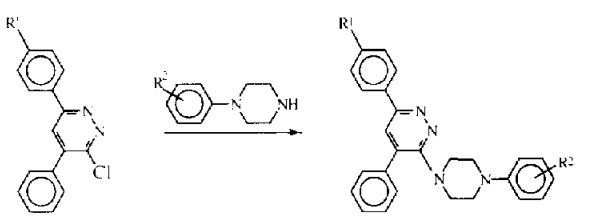
Figure 1. Trazodone.

Chemistry

The desired compounds were synthesized according to Scheme 1. One general procedure was used in preparing 3-substituted pyridazines **2a–i**. Starting 3-chloro-4,6-diarylpyridazines **1** were prepared by the reported method.[13] Heating of **1** with an excess of appropriate arylpiperazine provided the expected pyridazines **2a–i**. Physical constants and spectral data of **2** are reported in Table 1 and 2, respectively.

Results and Discussion

The analgesic activity of the pyridazine derivatives was evaluated with the mouse abdominal constriction test



1 2 a-i

Scheme 1.

Table 1. Physical constants of pyridazines **2a–i**

Compd	R^1	R^2	mp (°C)	Yield (%)	Formula
2a	H	H	185	45	$C_{26}H_{24}N_4$
2b	H	3-Cl	177	51	$C_{26}H_{23}N_4Cl$
2c	H	3-CF_3	181	25	$C_{27}H_{23}N_4F_3$
2d	F	H	220	30	$C_{26}H_{23}N_4F$
2e	F	3-Cl	160	45	$C_{26}H_{22}N_4FCl$
2f	F	3-CF_3	180	36	$C_{27}H_{22}N_4F_4$
2g	Cl	H	250	30	$C_{26}H_{23}N_4Cl$
2h	Cl	3-Cl	206	66	$C_{26}H_{22}N_4Cl_2$
2i	Cl	3-CF_3	214	65	$C_{27}H_{22}N_4ClF_3$

and data are reported in Table 3. Five compounds (**2a**, **2b**, **2d**, **2e**, **2h**) exhibited important antinociceptive effects with ED_{50} values ranging from 26.0 to 37.7 mg/kg ip. They possessed a much more potent activity than acetaminophen and noramidopyrine. However, they were three- to 50-fold less active than aspirin, trazodone, and morphine. In regard to the substitution on the phenyl nucleus in the 6-position of the pyridazine ring, 4-chloro derivatives (**2g** and **2h**) were less effective than the corresponding unsubstituted (**2a** and **2b**) and 4-fluoro (**2d** and **2e**) analogues. Substitution on the phenyl ring of the phenylpiperazine moiety by an *m*-trifluoromethyl group (**2c** and **2f**) markedly decreased activity versus non-substituted (**2a** and **2d**) and 3-chloro compounds (**2b** and **2e**). On the other hand, no substituent at the R^2 position (**2a** and **2d**) gave rise to the best activity.

On the basis of both the prescreening results and the nature of the substituents on the phenyl nuclei, pyridazines **2a**, **2d** and **2h** were selected for further pharmacological evaluation. To detect false positives in the PBQ-test in mice, according to Pearl et al.,[14] general behaviour was carefully observed in the acute toxicity assay, and the effects of the compounds were assessed on locomotor activity and in the rotarod test. Moreover, antinociceptive activity was also evaluated in the hot-plate test. The low toxicity of pyridazines was reflected by their high LD_{50} values up to 800 mg/kg ip, contrary to the arylpiperazine derivative used as reference drug, trazodone, with LD_{50} value of 223.4 mg/kg ip (Table 4).

With **2a**, **2d** and **2h** no deaths were seen over a period of 8 days following doses up to 800 mg/kg ip (Table 4). It should be noted that at the dose of 50 mg/kg ip **2a** showed deep sedation while **2d** and **2h** did not present a significant activity. Derivatives **2d** and **2h** prevented phenylbenzoquinone-induced reaction at doses that did not produce behavioural effects and that did not significantly affect rotarod performance. Only **2a** presented weak sedative and neurotoxic effects at 25–50 mg/kg ip. Any significant neurotoxicity evident in the rotarod test at 50–100 mg/kg ip disappeared by the second hour following administration of compounds. In the hot-plate test, no derivative was effective except the reference drug morphine, suggesting an absence of central effect.

Table 2. Spectral data of pyridazines **2a–i**

Compd	IR (KBr) ν (cm^{-1}) C=C C=N	^{1}H NMR (in $CDCl_3$) δ (ppm)
2a	1600, 1490	2.70–3.30 (m, 8H, $4CH_2$), 6.40–8.60 (m, 16H, $3C_6H_5$ + CH=)
2b	1600, 1490	3.00–3.60 (m, 8H, $4CH_2$), 6.60–8.30 (m, 15H, $2C_6H_5$ + C_6H_4 + CH=)
2c	1590, 1500	3.10–3.60 (m, 8H,$4CH_2$), 7.00–8.30 (m, 15H, $2C_6H_5$ + C_6H_4 + CH=)
2d	1600, 1490	3.00–3.60 (m, 8H, $4CH_2$), 6.70–8.20 (m, 15H, $2C_6H_5$ + C_6H_4 + CH=)
2e	1590, 1480	3.00–3.60 (m, 8H, $4CH_2$), 6.50–8.20 (m, 14H, C_6H_5 + $2C_6H_4$ + CH=)
2f	1600, 1490	3.00–3.60 (m, 8H, $4CH_2$), 6.90–8.20 (m, 14H, C_6H_5 + $2C_6H_4$ + CH=)
2g	1590, 1480	3.00–3.60 (m, 8H, $4CH_2$), 6.80–8.20 (m, 15H, $2C_6H_5$ + C_6H_4 + CH=)
2h	1600, 1490	3.00–3.60 (m, 8H, $4CH_2$), 6.80–8.20 (m, 14H, C_6H_5 + $2C_6H_4$ + CH=)
2i	1610, 1490	2.60–3.20 (m, 8H, $4CH_2$), 6.50–7.80 (m, 14H, C_6H_5 + $2C_6H_4$ + CH=)

With the aim of investigating possible opioid and serotonergic pathways involvement in the biological response, the selected compounds **2a**, **2d** and **2h** were administered successively with morphine, naloxone, and 5-hydroxytryptophan (5-HTP). Administered simultaneously with morphine 30 min before testing, **2h** induced a potent analgesia significantly greater than the sum of the individual effects induced by the drugs as illustrated in Figure 2. At the opposite, acute administration of subanalgesic dose of **2d** concomitant to morphine only indicated an additive action while **2a** failed to increase the sensitivity to morphine. Furthermore, to determine if test compounds elicited their analgesic activity through μ-opioid receptors, we examined the ability of naloxone to inhibit their activity in the PBQ-test. Thus, the opiate antagonist prevented the antinociceptive activity of morphine and **2h** in a similar manner (Fig. 3). When animals were given an

Table 3. Analgesic activity determined by phenylbenzoquinone-induced writhing test (PBQ)

Compd	ED_{50} mg/kg ip[a]
2a	26.0 (12.753.4)
2b	35.7 (22.456.9)
2c	>50
2d	33.9 (18.263.2)
2e	34.4 (16.472.1)
2f	>50
2g	>50
2h	37.7 (20.469.8)
2i	>50
Aspirin	7.8 (2.920.8)
Acetaminophen	231.3 (147.3363.2)
Noramidopyrine	68.5 (22.8205.3)
Morphine	0.57 (0.301.06)[b]
Trazodone	10.2 (7.114.6)

[a]Ninety-five per cent confidence intervals are given in parentheses.
[b]sc route.

Table 4. Hot-plate test, locomotor activity, rotarod test, and acute toxicity in mice

Compound	Dose (ip) mg/kg	Hot-plate test % of analgesia	Effect on locomotor activity (%) (+) increase; (−) decrease	Rotarod % of falls after treatment at 45 min	2 h	24 h	Acute toxicity LD_{50} mg/kg ip
	25		7.9 ± 15.0 (NS)[a]	40[b]	20 (NS)	0	
2a	50	Inactive	44.8 ± 9.9[b]	40[b]	20 (NS)	0	>800
	100	3.0 ± 2.9 (NS)	−53.8 ± 16.5[b]	40[b]	20 (NS)	20 (NS)	
	25	–	−24.3 ± 21.9 (NS)	0	0	0	
2d	50	Inactive	−20.3 ± 6.8 (NS)	60[b]	20	0	>800
	100	Inactive	−40.7 ± 11.8[b]	60[b]	0	0	
	25		−28.1 ± 14.7 (NS)	0	0	0	
2h	50	4.5 ± 1.2 (NS)	−29.6 ± 14.4 (NS)	20 (NS)	20 (NS)	0	>800
	100	6.8 ± 3.1 (NS)	−37.2 ± 14.5[b]	80[b]	20 (NS)	0	
Aspirin	25	Inactive	+3.1 ± 6.3 (NS)	10 (NS)	20 (NS)	10 (NS)	–
	100	1.0 ± 1.0 (NS)	+5.8 ± 11.6 (NS)	30[b]	40[b]	10 (NS)	–
Noramidopyrine	100	10.0 ± 6.0 (NS)	+60.7 ± 4.7[b]	40[b]	0	0	–
Morphine	10[c]	27.5 ± 2.9[b]	+58.8 ± 9.2[b]	20 (NS)	10 (NS)	10 (NS)	–
Trazodone	5	5.0 ± 3.0 (NS)	+29.0 ± 4.0[b]	33[b]	0	0	223.4 (215.0232.1)[d]
	10	28.0 ± 8.0 (NS)	+34.0 ± 11.0[b]	33[b]	11 (NS)	0	

[a]NS: not significant.
[b]$p < 0.05$.
[c]sc route.
[d]Ninety-five per cent confidence interval.

injection of **2d** + naloxone, the effect of the pyridazine was also reduced about 50%.

On the other hand, naloxone did not significantly affect the response of **2a** to the writhing test.

When administered together with 50 mg/kg ip of 5-HTP combined with the peripheral decarboxylase inhibitor carbidopa (25 mg/kg ip), **2a** and **2d** only gave rise to a synergy between the individual effects of the drugs (Fig. 4). Injection of **2h** with the same border-line doses of 5-HTP + carbidopa for the antinociceptive effect in this test, exhibited a significant potentiation of activity.

Conclusion

From all these data, it can be concluded that analgesic activity of pyridazine derivatives, especially that of **2h**, involved both opiate and serotonergic pathways. Although pharmacological studies are necessary in order to furnish additional evidence concerning the mechanism of action of **2h**, this compound appears to possess a large therapeutic index in view of both its LD_{50} superior to 800 mg/kg ip and its important analgesic properties at doses devoid of significant sedative and neurotoxic effects.

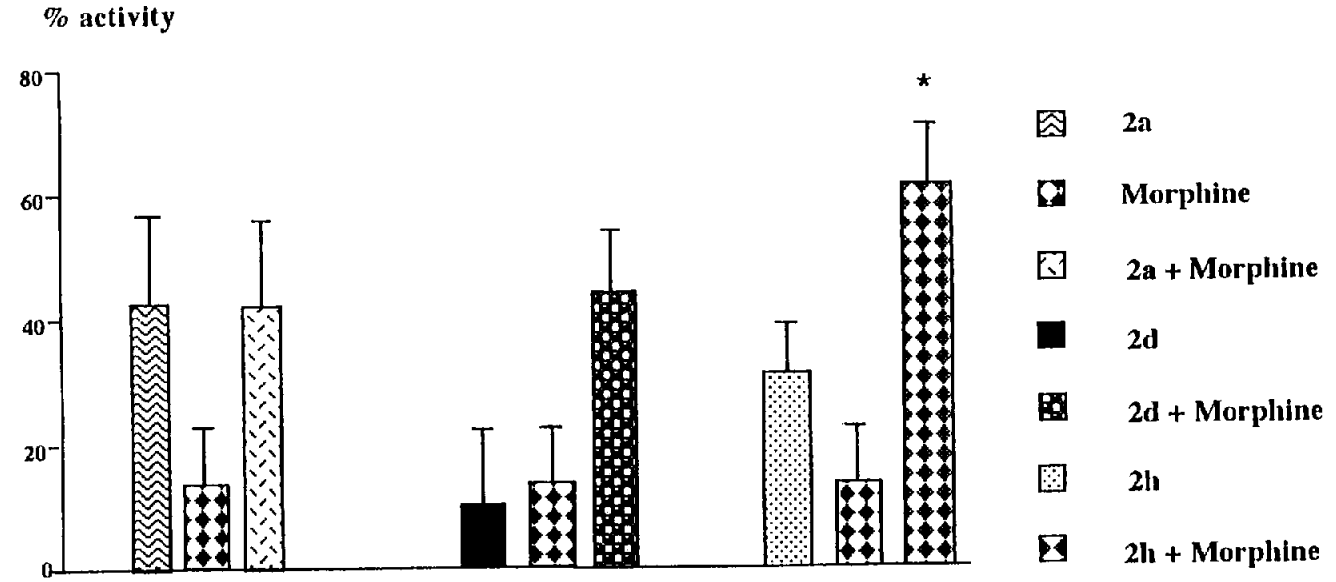


Figure 2. Potentiation of morphine (0.15 mg/kg sc)-induced analgesia by pyridazine **2a**, **2d**, and **2h** (5 mg/kg ip) in the PBQ-test. *$p < 0.05$ compared with morphine.

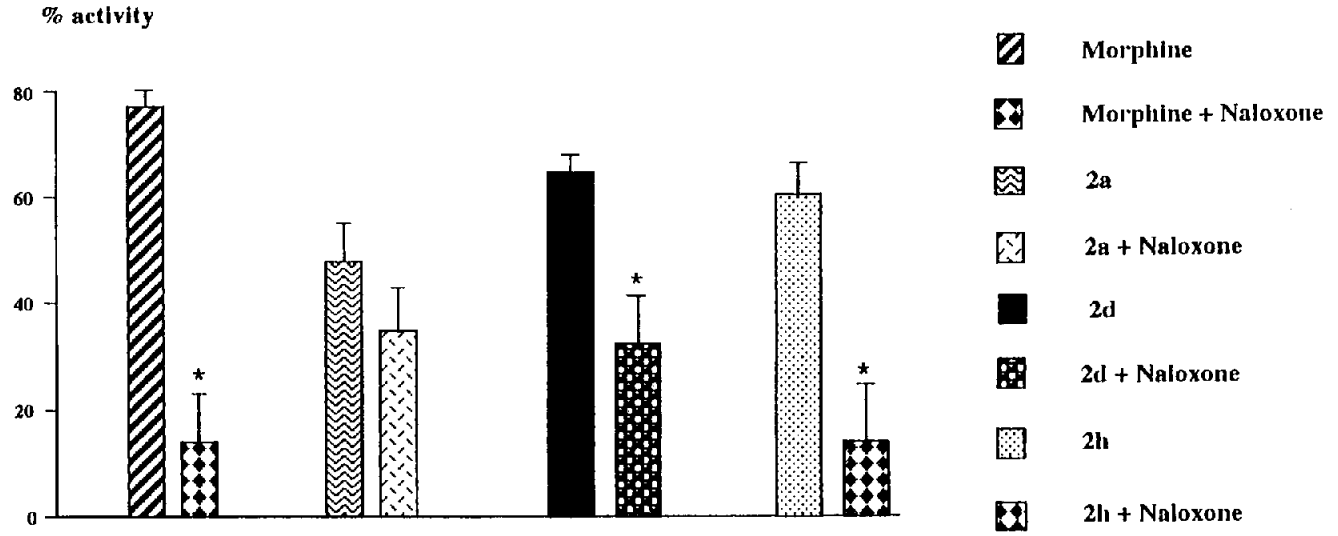


Figure 3. Effect of naloxone (1 mg/kg sc) on analgesic activity of morphine (1.5 mg/kg sc) and pyridazines **2a**, **2d**, and **2h** (50 mg/kg ip) in the PBQ-test. $^{*}p < 0.05$ compared with morphine, **2a**, **2d** and **2h**.

Experimental

Chemistry

Melting points were determined on a Kofler apparatus and were uncorrected. Infrared (IR) spectra were run as potassium bromide disks on a Beckman 4240 spectrophotometer. The proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained with a Bruker AC 400 (400 MHz). Chemical shifts were reported in ppm related to internal standard, tetramethylsilane. TLC was carried out on Merck silica gel 60 F 254 plates, and the purified compounds each showed a single spot. Elemental analyses were performed at the Service Central d'Analyses, Centre National de la Recherche Scientifique, 69390 Vernaison, France. Analytical results obtained for all compounds were within +0.4% of the theoretical value. Spectral (IR, NMR) data were compatible with the assigned structures in all cases.

A representative method used to prepare the target 3-arylpiperazinyl-4,6-diarylpyridazines **2a–i** is described in the following example.

3-[4-Phenylpiperazin-1-yl]-4,6-diphenyl pyridazine (2a). A suspension of 3-chloro-4,6-diphenyl pyridazine (2.66 g, 0.01 mol) in 30 mL of phenylpiperazine (0.197 mol) was refluxed for 4 h. After cooling, the resulting solid was collected and purified by chromatography on a silica gel column (35–70 μ, petroleum ether:ethyl acetate, 7:3 as eluent) to obtain pure **2a** as white crystals.

Pharmacology

In the studies described below, all compounds were administered intraperitoneally in saline (0.9% NaCl). Swiss male mice purchased from Depre (Saint-Doulchard, France) weighing 18–22 g were used in all experiments. Mice were kept in groups of 10 in a

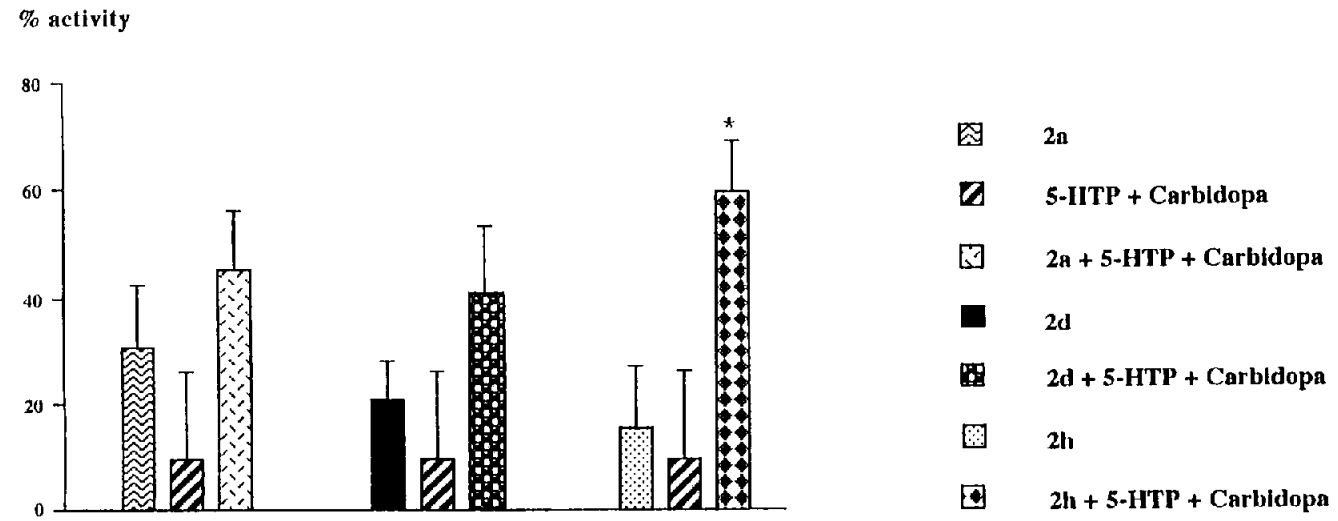


Figure 4. Analgesic effect of **2a**, **2d**, and **2h** (2.5 mg/kg ip) after intraperitoneal administration of 5-HTP (50 mg/kg ip) associated to carbidopa (25 mg/kg ip) in the PBQ-test. $^{*}p < 0.05$ compared with **2a**, **2d**, and **2h**.

temperature-controlled room with a 12 h light/dark cycle. Food and water were available ad libitum until the time of experiment. The allocation of animals in different groups was randomized and experiments were carried out in blind conditions.

Phenylbenzoquinone-induced writhing test.[15,16] A 0.02% solution (ethanol:water, 5:95) of PBQ maintained at 37 °C, was administered by intraperitoneal injection to mice, 30 min after intraperitoneal administration of drugs. The number of abdominal constrictions of each animal was counted between the 5th and the 15th min after the injection of the irritant.

Acute toxicity in mice. The compounds were administered intraperitoneally at doses of 100, 200, 400, 600 and 800 mg/kg. The animals were observed for 8 days in order to detect any sign of toxicity.

Locomotor activity.[17] The number of photocell beams crossed was recorded 30 min after drug administration (ip) in mice individually placed for 10 min in a photocell actimeter (Apelex, Massy, France).

Neurotoxicity. The rotarod test[18] was used to evaluate central nervous system toxicity. Neurologic toxicity was defined as the failure of the dosed animal to remain on a 3-cm diameter wood rod rotating at 6 rpm for 3 min. Experiments were carried out 45 min, 2 h and 24 h after drug administration.

Hot-plate test.[19] Animals were placed on a copper plate (Apelex, Massy, France) maintained at a constant temperature of 56 °C. The time necessary to induce the licking reflex of the forepaws was then recorded. Measurements were carried out 30 min after drug administration. A cut-off withdrawal latency of 40 s was used to prevent tissue damage.

Potentiation of the morphine analgesia.[20] Protocol used was the same as that in the PBQ-test. Morphine (0.15 mg/kg, sc) was injected at the same time as drugs, 30 min before the test.

Antagonism of drug antinociception by naloxone.[21] Protocol used for the evaluation of the effect of naloxone on drug-induced analgesia was similar to that described in the PBQ-test. Naloxone (1 mg/kg, sc) was injected 5 min before intraperitoneal administration of PBQ-solution.

Potentiation of drug antinociception by 5-hydroxytryptophan (5-HTP). Protocol used was adapted from the technique of Vonvoigtlander et al.[22] Experiments were carried out in the similar manner as in the PBQ-test. Carbidopa (25 mg/kg ip) was administered, followed 30 min later by 5-HTP (50 mg/kg, ip) and then after 15 min more by drugs. Twenty min later, the analgesic test was performed with administration of the PBQ-solution.

Data analysis. Statistical analysis of the results was performed using the method of Schwartz.[23] The ED_{50} values were determined by the method of Litchfield and Wilcoxon.[24] The significance of pharmacological data expressed as mean + S.E. was analysed by Student's *t*-test. Other results were analysed by means of the chi-square test with Yates' correction.

References

1. Sladowska, H. *Il Farm.* **1993**, *48*, 77.
2. Sladowska, H. *Il Farm.* **1993**, *48*, 85.
3. Erdogan, H.; Debaert, M.; Cazin, J. C. *Arzneim. Forsch./Drug Res.* **1991**, *41*, 73.
4. Palaska, E.; Unlu, S.; Erdogan, H.; Safak, C.; Gumusel, B.; Sunal, R. *Eur. J. Med. Chem.* **1993**, *28*, 963.
5. Flouzat, C.; Bresson, Y.; Mattio, A.; Bonnet, J.; Guilllaumet, G. *J. Med. Chem.* **1993**, *36*, 497.
6. Viaud, M. C.; Jamoneau, P.; Flouzat, C.; Bizot-Espiard, J. G.; Pfeiffer, B.; Renard, P.; Caignard, D. H.; Adam, G.; Guillaumet, G. *J. Med. Chem.* **1995**, *38*, 1278.
7. Rubat, C.; Coudert, P.; Tronche, P.; Bastide, J.; Bastide, P.; Privat, A. M. *Chem. Pharm. Bull.* **1989**, *37*, 2832.
8. Rubat, C.; Coudert, P.; Albuisson, E.; Bastide, J.; Couquelet, J.; Tronche, P. *J. Pharm. Sci.* **1992**, *81*, 1084.
9. Moreau, S.; Coudert, P.; Rubat, C.; Albuisson, E.; Couquelet, J. *Arzneim. Forsch./Drug Res.* **1996**, *46*, 800.
10. Rohet, F.; Rubat, C.; Coudert, P.; Albuisson, E.; Couquelet, J. *Chem. Pharm. Bull.* **1996**, *44*, 980.
11. Riblet, L. A.; Taylor, D. P. *J. Clin. Psychopharmacol.* **1981**, *1*, 175.
12. Valéri, P.; Pimpinella, G.; Morrone, L. A.; Romanelli, L. *Gen. Pharmac.* **1991**, *22*, 127.
13. Coudert, P.; Couquelet, J.; Tronche, P. *J. Heterocyclic Chem.* **1988**, 25, 799.
14. Pearl, J.; Stander, H.; Mc Kean, D. B. *J. Pharmacol. Exp. Ther.* **1968**, *167*, 9.
15. Siegmund, E.; Cadmus, R.; Lu, G. *Proc. Soc. Exp. Biol. Med.* **1957**, *95*, 729.
16. Lince, P. *J. Pharmacol.* **1972**, *3*, 513.
17. Boissier, J. R.; Simon, P. *Arch. Int. Pharmacodyn.* **1965**, *158*, 212.
18. Kinnard, W. J.; Carr, C. J. *J. Pharmacol. Exp. Ther.* **1957**, *121*, 354.
19. Woolfe, G.; McDonald, A. D. *J. Pharmacol. Exp. Ther.* **1944**, *80*, 300.
20. Fialip, J.; Marty, H.; Makambila, M. C.; Civiale, M. A.; Eschalier, A. *J. Pharmacol. Exp. Ther.* **1989**, *248*, 747.
21. Nevinson, M. J.; Gray, A. M.; Sewell, R. D. E. *British J. Pharmacol.—Proceedings Suppl.* **1991**, *104*, 267.
22. Vonvoigtlander, P. F.; Lewis, R. A.; Neff, G. L. *J. Pharmacol. Exp. Ther.* **1984**, *231*, 270.
23. Schwartz, D. Méthodes statistiques à l'usage des médecins et des biologistes; Editions Médicales Flammarion: Paris, 1984; p 263.
24. Litchfield, J. T.; Wilcoxon, E. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.

(*Received in U.S.A.* 14 *October* 1996; *accepted* 25 *November* 1996)