Synthesis and Trazodone-like Analgesic Activity of 4-Phenyl-6-aryl-2-[3-(4-arylpiperazin-1-yl)propyl]pyridazin-3-ones

Florence ROHET,[a] Catherine RUBAT,[b] Pascal COUDERT,[a] Eliane ALBUISSON,[c] and Jacques COUQUELET*,[a]

Groupe de Recherche en Pharmacochimie, Laboratoire de Chimie Thérapeutique,[a] Laboratoire de Pharmacologie,[b] and Laboratoire de Biomathématiques et Informatique Médicale,[c] Facultés de Médecine et Pharmacie, Université d'Auvergne, 28, Place Henri Dunant, 63001 Clermont-Ferrand Cedex, France.
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A series of 4,6-diaryl pyridazinones, chemically related to trazodone, was synthesized and evaluated for analgesic activity. With ED_{50} values ranging from 8.4 to 46.7 mg kg^{-1} i.p. in the phenylbenzoquinone-induced writhing test (PBQ test), most compounds were several times more potent than acetaminophen (ED_{50} = 231.3 mg kg^{-1} i.p.) and noramidopyrine (ED_{50} = 68.5 mg kg^{-1} i.p.). A multiple linear regression analysis demonstrated a correlation between antinociceptive activity and lipophilicity, as well as electronic and steric factors. The most active pyridazinones 2c and 2j exhibited minimal sedative and neurotoxic effects at the dose of 25 mg kg^{-1} i.p. They were devoid of activity in the hot plate test and their analgesic activity was not significantly reversed by naloxone in the PBQ test. The antinociceptive response induced by morphine (0.15 mg kg^{-1} s.c.) in the PBQ test was greatly potentiated by 2c and 2j administered at the low doses of 1 and 2.5 mg kg^{-1} i.p., respectively. On the other hand, their analgesic effects were enhanced synergistically by 5-hydroxytryptophan combined with carbidopa. All these data imply that a significant part of the antinociceptive effect induced by 2c and 2j may involve both opioid and serotonergic pathways. In addition, these two pyridazinones did not exhibit any antidepressant properties in the forced swimming test, nor did they potentiate yohimbine-induced toxicity.

Key words pyridazine derivative; 4,6-diaryl pyridazinone; analgesic activity; trazodone-related compound; structure–activity relationship

Various drugs acting mainly on the central nervous system possess arylpiperazine structures.[1] Thus, introduction of these moieties onto different heterocycles such as tetrahydropyrimidines,[1b] pyridine carboxylic acids,[2] benzoxazolinones[3] and oxazolopyridinones[4] produces analgesic compounds. The nontricyclic antidepressant trazodone and its related triazolo analogue nefazodone (Chart 1) also possess potent antinociceptive activity.[5,6] Complex interactions of these drugs with 5-HT_1, 5-HT_2 and/or opioidergic systems seem to play an important role in their analgesic effect.[5-7]

In a previous paper we have reported analgesic properties of 4,6-diaryl pyridazin-3-ones substituted at the 2-position with arylpiperazinylmethyl chains.[8] So, as part of a program to discover new analgesic compounds chemically related to trazodone and nefazodone, we have synthesized 4-phenyl-6-aryl-2-[3-(4-arylpiperazin-1-yl)propyl]pyridazin-3-ones. Preliminary pharmacological screening of all prepared compounds for analgesic activity was done with the phenylbenzoquinone-induced writhing test (PBQ test), and the influence of substituents on the two phenyl nucleic attached to the pyridazine and the piperazine rings was examined. The antinociceptive effects of the most active compounds were further scrutinized and their possible antidepressant activities were investigated.

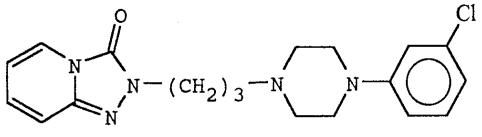
Chemistry

The *N*-substituted 4,6-diaryl pyridazinones **2** were synthesized from the previously described pyridazinones **1**[9] as shown in Chart 2. Compounds **1** were refluxed in a sodium ethylate solution, and then alkylated at the nucleophilic 2-N position of the pyridazine ring with the appropriate 1-chloropropyl 4-arylpiperazines.[10] The structures of derivatives **2a—o** were supported by elemental analysis (Table 1) and spectral data (Table 2).

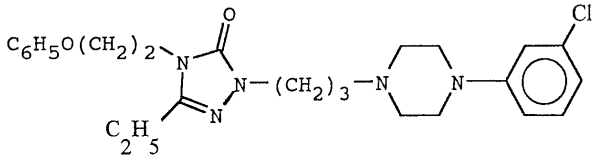
Pharmacological Results and Discussion

Behavioral effects and intraperitoneal acute toxicity were first investigated in mice. From 200 mg kg^{-1} i.p., test compounds produced sedation, which disappeared after 24 h. No other significant behavioral effects were observed even at doses up to 800 mg kg^{-1} i.p. and all animals were still alive after an observation period of one week.

All the pyridazine derivatives showed analgesic properties in the PBQ test in mice (Table 3). The writhing



Trazodone



Nefazodone

Chart 1

* To whom correspondence should be addressed.

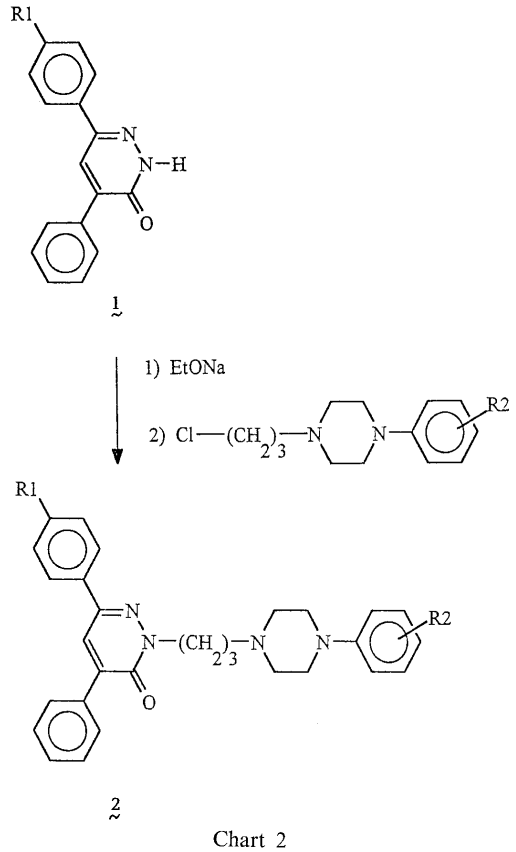


Chart 2

test is sensitive and, as a first approximation, predictive of activity for a variety of peripherally acting analgesics.[11] Examination of these data indicated that several of the synthesized compounds had activity of the same order as that of trazodone. Except for derivative **2h**, compounds unsubstituted on the phenyl nucleus at the 6-position of the pyridazine ring (**2a—e**) or possessing a fluorine atom at the *para*-position of the same aromatic nucleus (**2f, g, i, j**) exhibited the most potent analgesic activity, with ED_{50} values ranging from 8.4 to 29.2 mg kg^{-1} i.p. Introduction of a chloro substituent at the *para*-position (**2k, m—o**) decreased the activity with the exception of **2l**, bearing a 2-methoxy phenylpiperazinyl moiety on the side chain. Thus, it appeared that pyridazinone **2j** (ED_{50} = 84 mg kg^{-1}) possessed analgesic activity comparable to that of trazodone (ED_{50} = 10.2 mg kg^{-1} i.p.), and much more potent than that of classical analgesic drugs such as acetaminophen (ED_{50} = 231.3 mg kg^{-1} i.p.) and noramidopyrine (ED_{50} = 68.5 mg kg^{-1} i.p.). It is noteworthy that the activity in this series is observed over a large interval (*ca.* 4 to 7) of log k_w units, suggesting that the analgesic properties of the compounds under study are influenced not only by lipophilicity, but also by electronic and steric interactions.

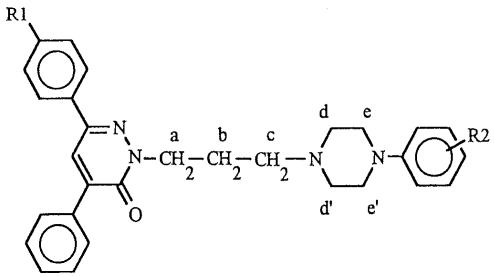
To corroborate this hypothesis, a Hansch analysis using log k_w as lipophilic index, Hammett's constants (σ_1 for R^1, σ_2 for R^2) as electronic parameters and Taft's constants

Table 1. Physical Data for Pyridazinones **2**

Compd.	R^1	R^2	Yield (%) Method	mp (°C) Solvent	Formula	Analysis (%) Calcd (Found)				
						C	H	Cl	F	N
2a	H	H	60	123	$C_{29}H_{30}N_4O$	77.33	6.67			12.44
			Y	A		(77.15	6.73			12.41)
2b	H	2-OCH_3	69	116	$C_{30}H_{32}N_4O_2$	75.00	6.67			11.67
			Y	D		(75.12	6.59			11.51)
2c	H	3-Cl	55	123	$C_{29}H_{29}ClN_4O$	71.83	5.98	7.33		11.56
			Y	B		(71.77	5.93	7.40		11.68)
2d	H	3-CF_3	52	95	$C_{30}H_{29}F_3N_4O$	69.50	5.60		11.00	10.81
			Z	E		(69.58	5.63		11.09	10.71)
2e	H	4-F	16	113	$C_{29}H_{29}FN_4O$	74.36	6.20		4.06	11.97
			Y	C		(74.12	6.31		4.15	11.81)
2f	F	H	26	123	$C_{29}H_{29}FN_4O$	74.36	6.20		4.06	11.97
			Y	A		(74.45	6.25		3.92	11.83)
2g	F	2-OCH_3	70	136	$C_{30}H_{31}FN_4O_2$	72.29	6.22		3.81	11.24
			Y	A		(72.18	6.31		3.65	11.29)
2h	F	3-Cl	31	97	$C_{29}H_{28}ClFN_4O$	69.25	5.57	7.06	3.78	11.14
			Z	F		(69.13	5.70	6.91	3.94	11.06)
2i	F	3-CF_3	60	85	$C_{30}H_{28}F_4N_4O$	67.16	5.22		14.18	10.45
			Z	E		(67.16	5.33		14.02	10.40)
2j	F	4-F	41	124	$C_{29}H_{28}F_2N_4O$	71.60	5.76		7.82	11.52
			Y	A		(71.49	5.65		7.78	11.73)
2k	Cl	H	64	84	$C_{29}H_{29}ClN_4O$	71.83	5.99	7.33		11.56
			Z	E		(71.72	5.93	7.25		11.49)
2l	Cl	2-OCH_3	46	120	$C_{30}H_{31}ClN_4O_2$	69.97	6.02	6.90		10.88
			Z	F		(70.09	5.94	6.79		10.93)
2m	Cl	3-Cl	47	50	$C_{29}H_{28}Cl_2N_4O$	67.05	5.39	13.68		10.79
			Z	G		(67.19	5.46	13.53		10.66)
2n	Cl	3-CF_3	58	52	$C_{30}H_{28}ClF_3N_4O$	65.16	5.07	6.42	10.32	10.14
			Z	H		(65.29	5.03	6.38	10.46	10.12)
2o	Cl	4-F	62	51	$C_{29}H_{28}ClFN_4O$	69.25	5.57	7.06	3.78	11.14
			Z	I		(69.31	5.53	7.01	3.64	11.19)

Y, Purified by recrystallization; Z, purified by column chromatography. A, ethanol; B, ethanol–water (95:5); C, ethanol–water (80:20); D, ethyl acetate; E, ethyl acetate–hexane (60:40); F, ethyl acetate–hexane (50:50); G, ethyl acetate–hexane (80:20); H, ethyl acetate–hexane (70:30); I, ethyl acetate–hexane (65:35).

Table 2. Spectral Data for Pyridazinones **2**



Compd.	IR (KBr) C=O	ν (cm^{-1}) C=N, C=C	^{1}H-NMR (in $CDCl_3$) δ ppm
2a	1640	1595 1490 1450	2.20 (2H, m, b), 2.60 (6H, m, c+d+d'), 3.15 (4H, m, e+e'), 4.40 (2H, t, a), 6.70—8.00 (16H, m, $3C_6H_5$+CH=)
2b	1640	1590 1490 1440	2.25 (2H, m, b), 2.70 (6H, m, c+d+d'), 3.15 (4H, m, e+e'), 3.90 (3H, s, OCH_3), 4.45 (2H, t, a), 6.80—8.10 (15H, m, $2C_6H_5+C_6H_4$+CH=)
2c	1640	1600 1490 1450	2.20 (2H, m, b), 2.60 (6H, m, c+d+d'), 3.20 (4H, m, e+e'), 4.45 (2H, t, a), 6.70—8.20 (15H, m, $2C_6H_5+C_6H_4$+CH=)
2d	1630	1590 1480 1440	2.20 (2H, m, b), 2.60 (6H, m, c+d+d'), 3.20 (4H, m, e+e'), 4.45 (2H, t, a), 6.90—8.20 (15H, m, $2C_6H_5+C_6H_4$+CH=)
2e	1640	1600 1510 1450	2.20 (2H, m, b), 2.60 (6H, m, c+d+d'), 3.10 (4H, m, e+e'), 4.45 (2H, t, a), 6.70—8.10 (15H, m, $2C_6H_5+C_6H_4$+CH=)
2f	1640	1590 1500 1445	2.20 (2H, m, b), 2.65 (6H, m, c+d+d'), 3.20 (4H, m, e+e'), 4.50 (2H, t, a), 6.70—8.10 (15H, m, $2C_6H_5+C_6H_4$+CH=)
2g	1640	1590 1500 1445	2.20 (2H, m, b), 2.70 (6H, m, c+d+d'), 3.15 (4H, m, e+e'), 3.90 (3H, s, OCH_3), 4.45 (2H, t, a), 6.85—8.10 (14H, m, $C_6H_5+2C_6H_4$+CH=)
2h	1635	1580 1500 1440	2.20 (2H, m, b), 2.60 (6H, m, c+d+d'), 3.20 (4H, m, e+e'), 4.45 (2H, t, a), 6.75—8.10 (14H, m, $C_6H_5+2C_6H_4$+CH=)
2i	1640	1600 1500 1440	2.20 (2H, m, b), 2.65 (6H, m, c+d+d'), 3.20 (4H, m, e+e'), 4.45 (2H, t, a), 6.80—8.20 (14H, m, $C_6H_5+2C_6H_4$+CH=)
2j	1640	1600 1500 1440	2.15 (2H, m, b), 2.60 (6H, m, c+d+d'), 3.10 (4H, m, e+e'), 4.40 (2H, t, a), 6.75—8.10 (14H, m, $C_6H_5+2C_6H_4$+CH=)
2k	1635	1600 1490 1445	2.20 (2H, m, b), 2.60 (6H, m, c+d+d'), 3.20 (4H, m, e+e'), 4.45 (2H, t, a), 6.80—8.00 (15H, m, $2C_6H_5+C_6H_4$+CH=)
2l	1640	1590 1490 1440	2.20 (2H, m, b), 2.70 (6H, m, c+d+d'), 3.10 (4H, m, e+e'), 3.85 (3H, s, OCH_3), 4.40 (2H, t, a), 6.85—8.10 (14H, m, $C_6H_5+2C_6H_4$+CH=)
2m	1640	1590 1490 1450	2.20 (2H, m, b), 2.55 (6H, m, c+d+d'), 3.15 (4H, m, e+e'), 4.45 (2H, t, a), 6.60—8.10 (14H, m, $C_6H_5+2C_6H_4$+CH=)
2n	1640	1590 1490 1450	2.20 (2H, m, b), 2.60 (6H, m, c+d+d'), 3.20 (4H, m, e+e'), 4.45 (2H, t, a), 6.90—8.00 (14H, m, $C_6H_5+2C_6H_4$+CH=)
2o	1630	1590 1500 1450	2.20 (2H, m, b), 2.60 (6H, m, c+d+d'), 3.10 (4H, m, e+e'), 4.45 (2H, t, a), 6.70—8.10 (14H, m, $C_6H_5+2C_6H_4$+CH=)

(Es_1 for R^1, Es_2 for R^2) as steric factors was performed. The values have been taken from the compilation by Hansch and Leo.[12] Equation 1 was derived from the 15 tested pyridazinones:

$$\log(1/ED_{50}) = -0.08(\pm 0.04)\log k_w + 0.30(\pm 0.12)Es_1 - 0.64(\pm 0.25) \quad (1)$$

$$n=15,\ r=0.714,\ r^2=0.510,\ s=0.183,\ F=6.247,\ p=0.013$$

where r^2 represents the squared correlation coefficient, s the standard deviation and F (as also p) the statistical significance of fit; the 95% confidence intervals are indicated in parentheses.

The data used in the analysis and $\log(1/ED_{50})$ values recalculated from this equation are given in Table 3. The less lipophilic compounds with less bulky R^1 substituents in their structure seemed to be associated with the most potent analgesic activity. Although it was significant ($p<0.05$), Eq. 1 gave a squared correlation coefficient of only 0.51. This weak correlation could be due to the deviant behavior of pyridazine **2h**. So a new Eq. 2 excluding derivative **2h** was calculated to clarify the relative contributions of the above-mentioned physicochemical parameters:

$$\log(1/ED_{50}) = -0.11(\pm 0.04)\log k_w - 1.26(\pm 0.37)\sigma_1 - 0.06(\pm 0.06)Es_2 - 0.50(\pm 0.22) \quad (2)$$

$$n=14,\ r=0.854,\ r^2=0.730,\ s=0.133,\ F=9.110,\ p=0.003$$

The recalculated $\log(1/ED_{50})$ values from this new equation are reported in Table 3. Figure 1 shows the plot of experimental *versus* predicted $\log(1/ED_{50})$ of the pyridazine derivatives. Clearly, Eq. 2 is a better model of the relationship ($r^2=0.73$). The negative coefficient for $\log k_w$ suggests that activity improves with weak or moderate lipophilicity, while the negative coefficient associated with σ_1 indicates that the presence of a weak electron-withdrawing R^1 substituent is also favorable. In addition, the potency increases as the bulkiness of the R^2 substituent increases.

In view of its potent analgesic effect and its chemical

Table 3. PBQ Test, Hansch Analysis and Capacity Factors ($\log k_w$) of Pyridazinones **2**

Compound	PBQ test				$\log k_w$
	ED_{50} (mg kg^{-1} i.p.)[a]	$\log(1/ED_{50})$ (obs.)	$\log(1/ED_{50})$ (calc.)[b]	$\log(1/ED_{50})$ (calc.)[c]	
2a	11.4 (6.1—21.1)	−1.06	−1.00	−0.99	4.387
2b	14.6 (7.2—29.6)	−1.16	−1.22	−1.26	7.174
2c	12.2 (7.7—19.4)	−1.09	−1.09	−1.06	5.576
2d	12.2 (5.4—27.5)	−1.09	−1.20	−1.12	6.918
2e	17.7 (8.0—39.3)	−1.25	−1.07	−1.05	5.244
2f	10.3 (5.6—18.8)	−1.01	−1.20	−1.15	5.143
2g	29.2 (17.9—47.6)	−1.47	−1.36	−1.34	7.212
2h	46.7 (22.3—97.6)	−1.67	−1.27	—	6.086
2i	16.0 (10.3—25.0)	−1.20	−1.35	−1.21	7.045
2j	8.4 (4.1—17.1)	−0.92	−1.20	−1.12	5.186
2k	26.3 (13.4—51.8)	−1.42	−1.32	−1.33	4.824
2l	15.5 (9.0—26.8)	−1.19	−1.37	−1.35	5.358
2m	38.4 (20.3—72.6)	−1.58	−1.58	−1.62	8.047
2n	44.1 (27.1—71.9)	−1.64	−1.62	−1.59	8.507
2o	30.5 (13.5—68.9)	−1.48	−1.38	−1.38	5.555
Acetaminophen	231.3 (147.3—363.2)	ND	ND	ND	ND
Noramidopyrine	68.5 (22.8—205.3)	ND	ND	ND	ND
Trazodone	10.2 (7.1—14.6)	ND	ND	ND	ND

a) The 95% confidence intervals are given in parentheses; ten animals were used for each dose in all experiments. *b*) Calculated from Eq. 1. *c*) Calculated from Eq. 2. ND, not determined.

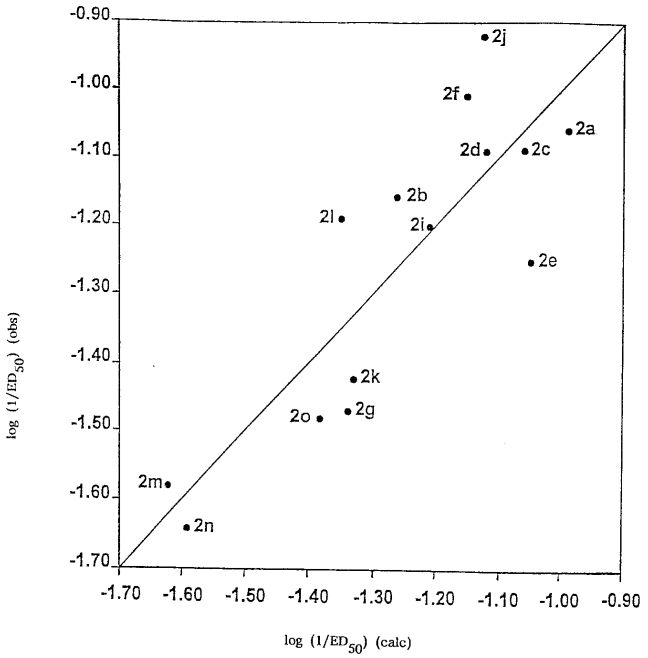


Fig. 1. Plot of Observed *versus* Calculated Analgesic Activity (from Eq. 2) of Pyridazine Derivatives

structure related to trazodone, the pyridazinone **2c**, as well as the most active derivative **2j**, was further pharmacologically investigated. Spontaneous motor activity was measured as a parameter of the sedative action of these compounds in the central nervous system (Table 4). In addition, determination of neurotoxicity in mice was performed at 25 and 100 mg kg^{-1} i.p. in the rotarod test. At 25 mg kg^{-1} i.p., pyridazinones **2c**, **2j** produced a weak but not significant decrease of about 15—25% in motor activity. This sedative effect was particularly intense at 100 mg kg^{-1} i.p. The reference drug trazodone also exhibited sedative properties from the low doses of 5 and 10 mg kg^{-1} i.p. In the rotarod test, compounds **2c**, **2j** did not display any significant neurotoxic effects even at the high dose of 100 mg kg^{-1} i.p., while trazodone gave rise to 33% falls in mice after injection of 5—10 mg kg^{-1} i.p.

The lack of activity in the hot plate test (Table 4) for the two derivatives associated with potent antinociceptive properties in the PBQ test implied that they might be peripherally acting agents without central analgesic effects. However, we can not reliably infer from these observations that the analgesic effect of pyridazinones has only a peripheral site of action. Mutual interactions between serotonin or catecholamine transmitters and opiate neurons have been reported.[13] It is therefore feasible that the antinociceptive properties of pyridazine derivatives could be mediated indirectly by central opioid mechanisms. When administered simultaneously with morphine 30 min before testing, **2c** and **2j** produced a potent analgesia significantly greater than the sum of the individual effects induced by the drugs, as illustrated in Fig. 2. Potentiation of morphine-induced analgesia by trazodone was much less intense than with **2c** or **2j**, while the activity of the specific serotonin reuptake inhibitor fluoxetine concurrently administered with morphine only appeared as a synergy between the individual effects of the drugs (Fig. 3). All these observations seemed to indicate possible interference between pyridazine derivatives and opiate systems, as has already been reported with many antinociceptive drugs.[14] However, analgesic activity of **2c** and **2j** was not significantly reversed by the opioid antagonist naloxone (Table 4). So the mechanism of the potentiation of morphine-induced analgesia by **2c** and **2j** might be interpreted in terms of an increase of opiate release.

A large body of evidence also suggests that serotonin neurons are implicated in mediating analgesia.[15] Therefore, in order to investigate possible interactions of **2c**, **2j** with 5-HT receptors, we attempted to potentiate their analgesic effects in the PBQ test with the serotonergic

Table 4. Locomotor Activity, Rotarod Test, Hot Plate Test and Effect of Naloxone on **2c**, **2j**, Trazodone and Morphine-Induced Analgesia

Compd.	Dose ($mg\,kg^{-1}$ i.p.)[a]	Decrease of motor activity (%)	Rotarod (% of falls after treatment)			Hot plate test analgesia (%)	PBQ test	Analgesia (%)
			45 min	2 h	24 h		Compd. or reference alone	Compd. +naloxone[b]
2c	25	25±17 (NS)	22 (NS)	22 (NS)	0	10±5 (NS)	65±10*	48±10 (NS)
	100	82± 4*	17 (NS)	17 (NS)	0	NT	NT	NT
2j	25	15± 9 (NS)	0	0	0	0±2 (NS)	58± 9*	56± 8 (NS)
	100	77± 6*	17 (NS)	33*	0	NT	NT	NT
Trazodone	5	29± 4*	33*	0	0	5±3 (NS)	41± 6*	58± 9 (NS)
	10	34±11*	33*	11 (NS)	0	28±8 (NS)	38± 4*	66± 8*
Morphine	1.5[c]	NT	NT	NT	NT	NT	78± 6*	39±10*
	5[c]	NT	NT	NT	NT	12±6 (NS)	NT	NT
	10[c]	NT	NT	NT	NT	28±3*	NT	NT

NT, not tested. * $p<0.05$. NS: not significant. *a*) Ten animals were used for each dose in all experiments. *b*) Administered at $1\,mg\,kg^{-1}$ s.c. *c*) The s.c. route.

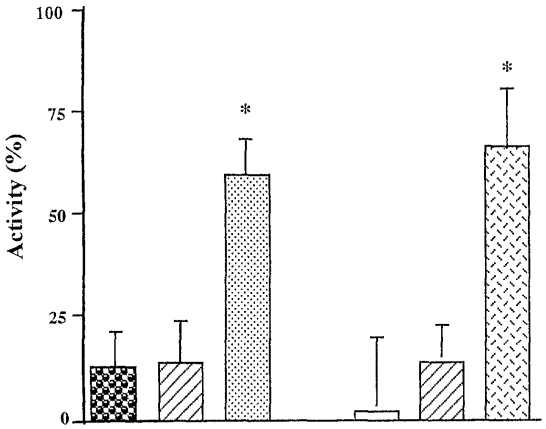


Fig. 2. Potentiation of Morphine-Induced Analgesia by Pyridazinones **2c** and **2j**

Ten animals were used for each dose in all experiments. * $p<0.05$. ▩, **2c** 1 mg/kg i.p.; ▨, morphine 0.15 mg/kg s.c.; ▦, **2c**+morphine; □, **2j** 2.5 mg/kg i.p.; ▣, **2j**+morphine.

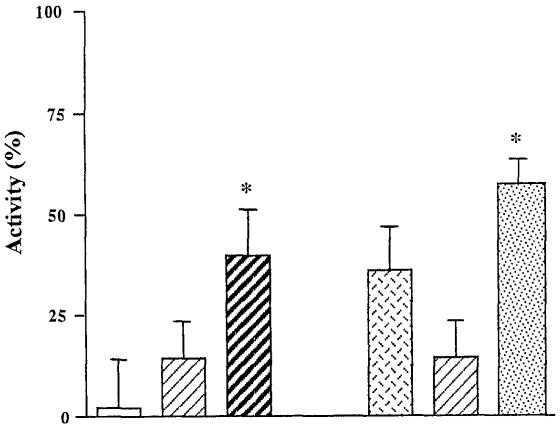


Fig. 3. Potentiation of Morphine-Induced Analgesia by Trazodone and Fluoxetine

Ten animals were used for each dose in all experiments. * $p<0.05$. □, trazodone 2 mg/kg i.p.; ▣, fluoxetine 3 mg/kg i.p.; ▨, morphine 0.15 mg/kg i.p.; ▩, trazodone+morphine; ▦, fluoxetine+morphine.

agonist 5-hydroxytryptophan (5-HTP) combined with the peripheral decarboxylase inhibitor carbidopa (Table 5). The data showed a marked synergic interaction of **2c**, **2j** and fluoxetine with 5-HTP combined with carbidopa. Overall, it can be concluded that the analgesic activity of **2c**, **2j** involved both opiate and serotonergic pathways.

Table 5. Effect of 5-Hydroxytryptophan on **2c**, **2j**, Trazodone and Fluoxetine-Induced Analgesia in the PBQ Test

Compd.	Dose ($mg\,kg^{-1}$ i.p.)[a]	Analgesia (%)	
		Compd. or reference alone	Compd. +5-HTP
2c	10	10± 6 (NS)	54± 5*
2j	2.5	8± 5 (NS)	53±11*
Trazodone	2.0	10± 6 (NS)	47± 9 (NS)
Fluoxetine	3.0	17±16 (NS)	52± 8*
Carbidopa +5-HTP	25 50	42±10*	—

* $p<0.05$. NS, not significant. *a*) Ten animals were used for each dose in all experiments.

Table 6. Antidepressant Activity of **2c**, **2j**, Trazodone and Clomipramine

Compd.	Forced swimming test[a] (% activity at $20\,mg\,kg^{-1}$, i.p.)	Yohimbine-induced toxicity[a] (ED_{50}, $mg\,kg^{-1}$, i.p.)
2c	17.5± 9.4 (NS)	Inactive at $200\,mg\,kg^{-1}$, i.p.
2j	15.0± 6.3 (NS)	Inactive at $200\,mg\,kg^{-1}$, i.p.
Trazodone	25.0±17.9 (NS)	183.1 (172.8—194.0)[b]
Clomipramine	11.9 (8.1—17.5)[b]	112.5 (91.9—137.8)[b]

a) Ten animals were used for each dose in all experiments. *b*) ED_{50} values with their 95% confidence intervals.

In addition to a marked antinociceptive action, the pharmacology of trazodone includes antianxiety and antidepressant components widely used in therapy.[5] Therefore it seemed interesting to evaluate the antidepressant properties of **2c** and **2j**, considering their structural similarity with the reference drug. In the force swimming test (Table 6), **2c** and **2j** did not significantly reduce immobility of mice, while clomipramine was very active with an ED_{50} value of $11.9\,mg\,kg^{-1}$ i.p. In the case of trazodone, the sedative effects observed from 5—$10\,mg\,kg^{-1}$ i.p. appeared to mask the reduced immobility observed at $20\,mg\,kg^{-1}$ i.p.

Unlike trazodone and clomipramine, **2c** and **2j** did not affect yohimbine-induced toxicity, even at a dose of $200\,mg\,kg^{-1}$ i.p. (Table 6).

So, in contrast to the chemically related drug trazodone,

2c and **2j** appear to be specific antinociceptive compounds on account of their lack of activity in the two last tests, which are predictive of antidepressant properties. To conclude, there is evidence that **2c** and **2j** may be useful to increase morphine's therapeutic index, as they potentiate analgesia when administered in conjunction with the opioid drug. In addition, their minimal sedative and neurotoxic effects up to the dose of 25 mg kg^{-1} i.p. make these pyridazine derivatives worthy of further investigations aimed at therapeutic use.

Experimental

All melting points were determined on a Kofler apparatus and are uncorrected. The IR spectra were obtained with a Beckman 4240 spectrophotometer. ^{1}H-NMR spectra were recorded on a Varian EM 360. Tetramethylsilane was used as an internal standard, and the abbreviations of signal patterns are as follows: s, singlet; t, triplet; m, multiplet. Elemental analyses (C, H, N, F and Cl within $\pm$0.4% of theoretical values) were performed at the Service Central d'Analyses, Centre National de la Recherche Scientifique, 69390 Vernaison, France.

General Procedure for the Preparation of 4-Phenyl-6-aryl-2-[3-(4-arylpiperazin-1-yl)propyl]pyridazin-3-ones 2 The appropriate pyridazinone **1** (4.8 mmol) was added to an ethanolic solution (100 ml) containing 0.11 g of sodium. The solution was stirred at room temperature for 1 h and then evaporated to dryness. The resulting pyridazinone sodium salt was dissolved in dimethylformamide (80 ml) containing a suitable 1-chloropropyl-4-arylpiperazine[10] (4.8 mmol). The solution was refluxed for 3 h with stirring and then evaporated to dryness. The oily residue was triturated with water until a precipitate appeared and the crude compound **2** was purified by recrystallization or by column chromatography on silica gel (Kieselgel 60, 230—400 mesh, Merck, Darmstadt, Germany).

Pharmacological Protocols In the studies described below, all compounds were administered intraperitoneally in saline (0.9% NaCl). Male C D mice purchased from Charles River S.A. France (Cleon, France), weighing 20—25 g, were used in the forced swimming test. Swiss male mice purchased from Depre (Saint–Doulchard, France) weighing 18—22 g were used in all other experiments. Mice were kept in groups of ten in a temperature-controlled room with 12 h light/dark cycle. Food and water were available *ad libitum* until the time of the experiment. The allocation of animals to different groups was randomized and the experiments were carried out in blind conditions.

Acute Toxicity in Mice The compounds were administered intraperitoneally at doses of 200, 400, 600 and 800 mg kg^{-1}. The animals were observed for 8 d in order to detect any sign of toxicity.

Phenylbenzoquinone-Induced Writhing Test A 0.02% solution (ethanol : water = 5 : 95) of PBQ (Eastman Kodak, Rochester, U.S.A.), 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 5 and 15 min after the injection of the irritant.[16]

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

Neurotoxicity The rotarod test[18] was used to evaluate central nervous system toxicity. Neurologic toxicity was defined as 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 and 24 h after drug administration.

Hot Plate Test 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 later.[19]

Potentiation of Morphine Analgesia The protocol used was the same as that in the PBQ test. Morphine (0.15 mg kg^{-1} s.c.) (Coopération Pharmaceutique Française, Melun, France) was injected at the same time as drugs, 30 min before the test.[20]

Antagonism of Drug Antinociception by Naloxone The protocol used for the evaluation of the effect of naloxone (Narcan,® Du Pont de Nemours, Paris, France) on drug-induced analgesia was similar to that described in the PBQ test. Naloxone (1 mg kg^{-1} s.c.) was injected 5 min before intraperitoneal administration of PBQ solution.[21]

Potentiation of Drug Antinociception by 5-Hydroxytryptophan (5-HTP) The protocol used was adapted from the technique of Vonvoigtlander *et al.*[22] Experiments were carried out in the same manner as in the PBQ test. Carbidopa (25 mg kg^{-1} i.p.) (ICN Biomedicals, Orsay, France) was administered, followed 30 min later by 5-HTP (50 mg kg^{-1} i.p.) (ICN Biomedicals, Orsay, France) and then after 15 more min, by drugs. Twenty minutes later, the analgesic test was performed with administration of the PBQ solution.

Forced Swimming Test Measurement of immobility in mice was carried out according to a modification of the method of Porsolt *et al.*[23] Acquired immobility of mice was evaluated 32—36 min after intraperitoneal drug or placebo treatment by recording the time for which the animals remained immobile after being placed (at 30 min) in vertical glass cylinders (height 25 cm, diameter 10 cm) containing 8 cm of water at 21—23 °C.

Yohimbine-Induced Toxicity Drugs were injected (i.p.) 30 min before yohimbine administration (30 mg kg^{-1} s.c.) and mortality was assessed 24 h later.[24]

Data Analysis Statistical analysis of the results was performed using the method of Schwartz.[25] The ED_{50} values were determined by the method of Litchfield and Wilcoxon.[26] The significance of pharmacological data expressed as mean $\pm$ S.E. was analyzed by using Student's t test. Other pharmacological data were analyzed by means of the chi-square test with Yates' correction.

Lipophilicity Measurements Lipophilicity was determined by reversed-phase, high-performance liquid chromatography.[27] A Varian 5000 liquid chromatography (Varian, Les Ulis, France) equipped with a detector operating at 254 nm was used. A Varian CDS 111 L integrator was used for peak registration and calculation of retention times. A column (15 × 6 mm i.d.) prepacked with octadecyl copolymer gel (particle size, 5 μm) was used as the nonpolar stationary phase. Mobile phases were prepared volumetrically from 50/50 to 90/10 combinations of methanol and aqueous 3-morpholinopropanesulfonic acid buffer (0.02 M, pH 7.4). The flow rate was 1 ml/min. Isocratic capacity factors (k_i) were defined as $k_i = (t_r - t_o)/t_o$, where t_r is the retention time of the solute and t_o is the column dead time determined with methanol as the nonretained compound. Log k_w was used as the lipophilic index obtained by linear extrapolation of log k_i to 100% water.

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