Synthesis and Pharmacological Evaluation in Mice of New Non-classical Antinociceptive Agents, 5-(4-Arylpiperazin-1-yl)-4-benzyl-1,2-oxazin-6-ones

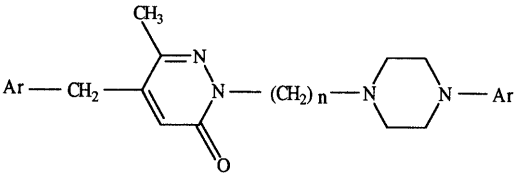
Monique BEBOT,[a] Pascal COUDERT,[a] Catherine RUBAT,[b] Danielle VALLEE-GOYET,[c] Daniel GARDETTE,[c] Sylvie MAVEL,[d] Eliane ALBUISSON,[e] and Jacques COUQUELET*,[a]

Groupe de Recherche en Pharmacochimie, Laboratoire de Chimie Thérapeutique,[a] Laboratoire de Pharmacologie,[b] Faculté de Pharmacie, 28, Place Henri Dunant, 63001 Clermont-Ferrand, Cedex, France, Laboratoire de Chimie des Substances Naturelles,URA 485 du CNRS,[c] Université Blaise Pascal, 63177 Aubière, Cedex, France, Laboratoire de Chimie Thérapeutique,[d] Faculté des Sciences Pharmaceutiques,Université François Rabelais, Avenue Monge, 37200 Tours, France, Laboratoire de Biomathématiques et Informatique Médicale,[e] Faculté de Médecine, 28, Place Henri Dunant, 63001 Clermont-Ferrand, Cedex, France. Received July 1, 1996; accepted November 15, 1996

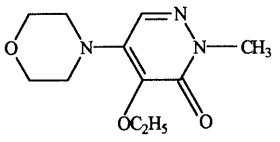
Several 5-(4-arylpiperazin-1-yl)-4-benzyl-1,2-oxazin-6-ones have been synthesized and tested for analgesic activity in a visceral pain model (phenylbenzoquinone-induced writhing test = PBQ test). A good correlation has been found between the antinociceptive effects of drugs and both their lipophilic and steric properties. The most active derivatives 5c and 5f, with intraperitoneal ED_{50} values of 10.5 and 10.3 mg kg^{-1} respectively, were more extensively investigated by evaluating their analgesic activity in a somatosensory pain model (hot plate test), as well as their sedative properties. Furthermore, naloxone suppressed the effect of 5c and 5f in the PBQ test, though these derivatives were ineffective to potentiate morphine analgesia. Pretreatment with yohimbine did not significantly attenuate the analgesic effects of 5c and 5f. In addition, pretreatment with 5-hydroxytryptophan associated with carbidopa also failed to potentiate the antinociceptive effects of 5c and 5f. So, a part of the analgesic activity of 5c and 5f seems to be related to an opioidergic mechanism, especially at the μ receptor level. Molecular modeling studies performed on the opiate drug morphine and on the most stable conformer of 5f showed structural similarities between these two molecules.

Key words 1,2-oxazin-6-one; arylpiperazine derivative; analgesic activity; structure-activity relationship

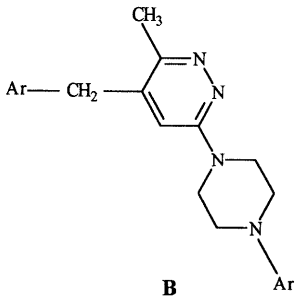
Clinical treatment of pain primarily depends on two classes of analgesics: non-steroidal anti-inflammatory drugs (NSAIDs, *e.g.*, aspirin, ibuprofen) and opiates. Non-narcotic analgesics act mainly peripherally by inhibiting prostaglandin synthesis[1,2] and are preferentially used in the management of mild to moderate nociceptive pain. On the other hand, the opiates act on specific central nervous system receptors, with clinical indications for moderate to severe pain.[3] Though nociceptive pain is generally well controlled by the currently available drugs, neurogenic and psychogenic pain disorders are not really assuaged by the various classical analgesic compounds, serotonergic tricyclic antidepressant drugs such as clomipramine excepted.[4] Moreover, NSAIDs induce irritant side effects on gastric mucosa,[5] while opiates



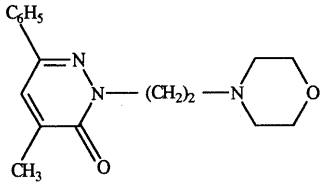
A



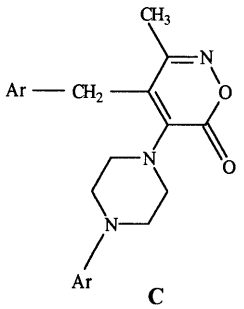
Emorfazone



B



Ag 246



C

* To whom correspondence should be addressed.

cause profound respiratory depression, postoperative nausea, constipation and physical dependence.[6] Therefore, there is a considerable therapeutic interest in novel antinociceptive agents without such side effects, perhaps with different modes of action.

Pyridazinones such as emorfazone and 2-morpholinoethyl-4-methyl-6-phenyl-3-pyridazone (Ag 246) have been shown to be analgesic agents without the above side effects[7,8] and we have prepared different series of active compounds in this area.[9-12] Pyridazine derivatives having an arylpiperazinyl moiety appeared to be the most active ones (structures A and B), according to many reports[13] on the antinociceptive properties of compounds containing an arylpiperazinyl unit in their structure.

As part of our efforts to discover new therapeutically advantageous analgesics, we adopted a strategy commonly used in medicinal chemistry, *i.e.*, the replacement of a nitrogen atom by oxygen in a drug template. Therefore, an oxazinone ring was substituted for the pyridazinone ring and arylpiperazinyl moieties were introduced at the 5-position of the heterocycle (structure C).

Here we describe the synthesis of 5-(4-arylpiperazin-1-yl)-4-benzyl-3-methyl-1,2-oxazin-6-ones. Further, the analgesic activity in the phenylbenzoquinone-induced writhing test (PBQ test) of the prepared compounds was examined, both as a preliminary pharmacological screening and to assess structure–activity relationships. Antinociceptive effects of the most active compounds were then investigated in more detail in order to determine possible interactions with opioidergic and serotonergic systems.

Chemistry

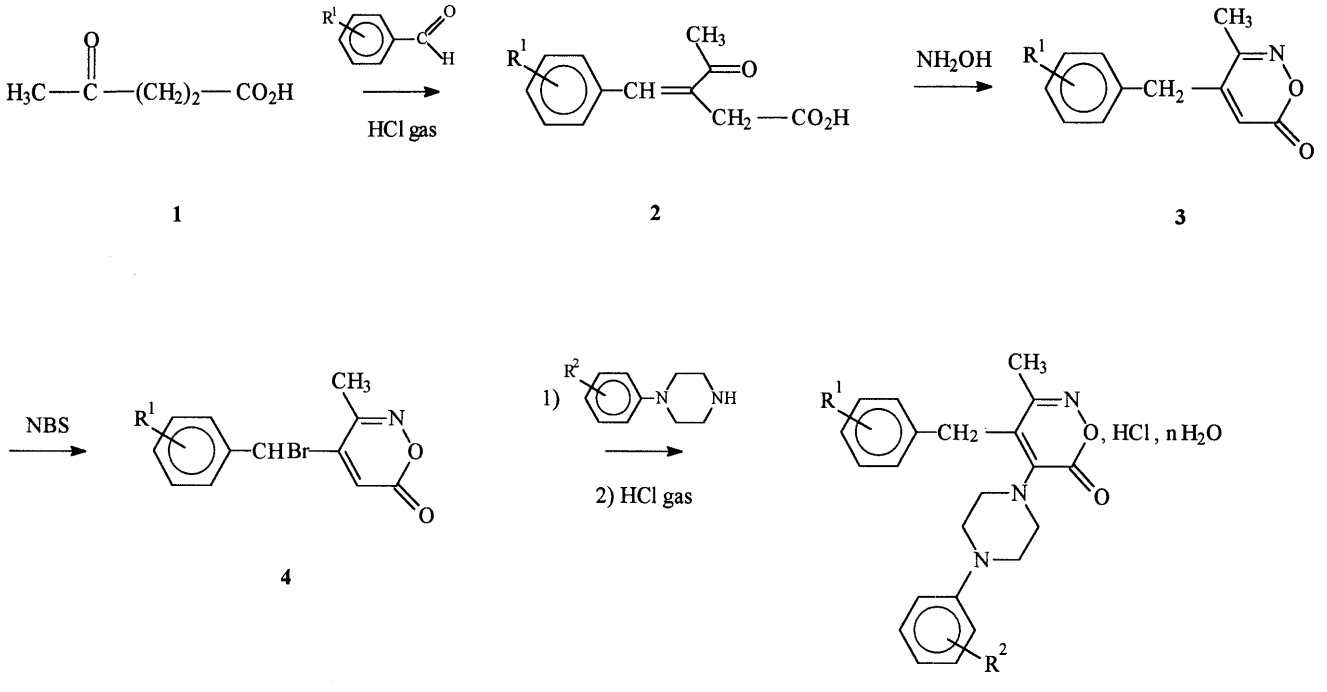
A new series of oxazinones **5** was prepared by means of a four-step reaction, as shown in Chart 1. Condensation of levulinic acid **1** with aldehydes in the presence of hydrogen chloride provided 3-acetyl-4-aryl-but-3-enoic acids **2** according to a previously described procedure.[14] The addition of hydroxylamine to keto-acids **2** afforded 4-benzyl-3-methyl-1,2-oxazin-6-ones **3** Tables 1 and 2), the structures of which were established by NMR NOE experiments. Irradiation of the methylene protons affected aromatic hydrogens, thus proving these groups to be in close proximity and consequently supporting the presence of an endocyclic double bond.

Radical bromination of **3** with *N*-bromosuccinimide (NBS) was catalyzed by 2,2′-azobisisobutyronitrile (AIBN) and resulted in monobromination at the benzylic position *via* a Wohl-Ziegler reaction.[15] The structure of **4** (Tables 3 and 4) was deduced from the correlation spectroscopy *via* long-range ^{1}H–^{13}C coupling (COLOC) data. In the case of **4a** ($R^1 = H$), the aromatic hydrogens at δ 7.30—7.40 ppm showed long-range correlations with the brominated carbon at δ 47.7 ppm, and the ethylenic proton at 6.82 ppm with the carbonyl group at δ 163.8 ppm.

According to an S_N2' mechanism,[16] reaction of **4** with arylpiperazines in methanol would furnish compounds **5** in basic form. The site of substitution was confirmed by long-range correlations between the methylenic signal of the benzyl group at δ 4.06 ppm (compound **5a**, $R^1 = R^2 = H$) and the aromatic carbons. Oxazinone salts **5a—m** (Tables 5 and 6) were obtained by treatment of the corresponding bases with hydrogen chloride in dry acetone.

Results and Discussion

Behavioral effects and intraperitoneal acute toxicity were first investigated in mice. At 400 mg kg^{-1} i.p., test compounds produced a more or less intense sedation in animals which disappeared after 24 h. No other significant



1 2 3 4 5

Chart 1

Table 1. Physical Data for Derivatives **3**

Compd.	R^1	Yield (%)	mp (°C)	Formula	Analysis Calcd (Found)				
					C	H	Cl	F	N
3a	H	54	93	$C_{12}H_{11}NO_2$	71.63	5.51			6.96
					(70.93	5.47			6.90)
3b	2-Cl	48	109	$C_{12}H_{10}ClNO_2$	61.16	4.28	15.04		5.94
					(60.91	4.21	15.28		6.07)
3c	4-F	51	133	$C_{12}H_{10}FNO_2$	65.75	4.60		8.67	6.39
					(65.97	4.41		8.71	6.50)
3d	4-CH_3	50	68	$C_{13}H_{13}NO_2$	72.54	6.09			6.51
					(72.19	6.21			6.67)

Table 2. Spectral Data for Derivatives **3**

Compd.	IR (KBr) ν (cm^{-1})			^{1}H-NMR (in $CDCl_3$) δ ppm
	C=O ester	C=O amide	C=C	
3a	1725	1630	1490	2.3 (s, 3H, CH_3), 3.7 (s, 2H, CH_2), 6.2 (s, 1H, CH=), 7.1—7.4 (m, 5H, C_6H_5)
3b	1725	1635	1475	2.4 (s, 3H, CH_3), 3.9 (s, 2H, CH_2), 6.0 (s, 1H, CH=), 7.2—7.5 (m, 4H, C_6H_4)
3c	1710	1625	1495	2.3 (s, 3H, CH_3), 3.8 (s, 2H, CH_2), 6.3 (s, 1H, CH=), 7.1—7.3 (m, 4H, C_6H_4)
3d	1730	1630	1510	2.3 (s, 3H, CH_3), 2.4 (s, 3H, C_6H_4–C$\underline{H}_3$), 3.8 (s, 2H, CH_2), 6.3 (s, 1H, CH=), 7.2—7.6 (m, 4H, C_6H_4)

Table 3. Physical Data for Derivatives **4**

Compd.	R^1	Yield (%)	mp (°C)	Formula	Analysis Calcd (Found)					
					C	H	Br	Cl	F	N
4a	H	52	Yellow oil	$C_{12}H_{10}BrNO_2$	51.45	3.60	28.52			5.00
					(51.16	3.46	28.63			5.28)
4b	2-Cl	20	Yellow oil	$C_{12}H_9BrClNO_2$	45.82	2.88	25.40	11.27		4.45
					(46.07	2.90	25.08	11.42		4.36)
4c	4-F	90	Yellow oil	$C_{12}H_9BrFNO_2$	48.35	3.04	26.80		6.37	4.70
					(47.98	3.08	27.02		6.15	4.94)
4d	4-CH_3	17	Yellow oil	$C_{13}H_{12}BrNO_2$	53.08	4.11	27.16			4.76
					(53.41	4.03	26.84			4.95)

Table 4. Spectral Data for Derivatives **4**

Compd.	IR (NaCl) ν (cm^{-1})			^{1}H-NMR (in $CDCl_3$) δ ppm
	C=O ester	C=O amide	C=C	
4a	1730	1620	1480	2.3 (s, 3H, CH_3), 6.0 (s, 1H, CHBr), 6.8 (s, 1H, CH=), 7.3—7.5 (m, 5H, C_6H_5)
4b	1710	1625	1460	2.4 (s, 3H, CH_3), 6.3 (s, 1H, CH), 6.7 (s, 1H, CH=), 7.3—7.6 (m, 4H, C_6H_4)
4c	1740	1630	1510	2.4 (s, 3H, CH_3), 5.9 (s, 1H, CH), 6.9 (s, 1H, CH=), 7.2—7.6 (m, 4H, C_6H_4)
4d	1720	1625	1500	2.3 (s, 3H, CH_3), 2.4 (s, 3H, C_6H_4–C$\underline{H}_3$), 5.9 (s, 1H, CH), 6.9 (s, 1H, CH=), 7.2—7.5 (m, 4H, C_6H_4)

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.

The oxazinone derivatives **5a—m** were evaluated for analgesic activity in the phenylbenzoquinone induced writhing test (PBQ test) in mice and compared with acetaminophen, noramidopyrine and trazodone. Although trazodone is currently in therapeutic use as an antidepressant agent, it possesses potent antinociceptive properties, partly due to the presence of a 3-chlorophenylpiperazinyl moiety in its structure.[17,18] This fact prompted us to use this drug as reference compound in the analgesic tests.

Compounds having a 3-chlorophenylpiperazinyl group (**5c**, **5f**, **5i**), as well as the two 4-fluorobenzyl derivatives (**5h**, **5k**), were the most effective ones with ED_{50} values ranging from 10 and 30 mg kg^{-1} i.p. (Table 7). On the other hand, introduction of a trifluoromethyl substituent at the meta position of the phenyl linked to the piperazine ring markedly decreased the activity with ED_{50} values up

Table 5. Physical Data for Oxazinones **5**

Compd.	R^1	R^2	Yield (%)	mp (°C)	Formula	Analysis Calcd (Found)				
						C	H	Cl	F	N
5a	H	H	60	138	$C_{22}H_{23}N_3O_2 \cdot HCl \cdot 0.5H_2O$	64.94	6.15	8.73		10.33
						(65.25	6.07	9.06		10.50)
5b	H	2-OCH_3	36	125	$C_{23}H_{25}N_3O_3 \cdot HCl$	64.56	6.12	8.29		9.82
						(64.93	6.21	8.11		9.88)
5c	H	3-Cl	38	120	$C_{22}H_{22}ClN_3O_2 \cdot HCl$	61.11	5.36	16.40		9.72
						(61.42	5.41	15.79		9.91)
5d	H	3-CF_3	62	102	$C_{23}H_{22}F_3N_3O_2 \cdot HCl \cdot 0.5H_2O$	58.17	5.09	7.47	12.00	8.85
						(58.40	5.09	7.27	12.18	9.07)
5e	H	4-F	62	127	$C_{22}H_{22}FN_3O_2 \cdot HCl$	63.54	5.57	8.52	4.57	10.10
						(63.27	5.55	8.42	4.58	9.89)
5f	2-Cl	3-Cl	26	109	$C_{22}H_{21}Cl_2N_3O_2 \cdot HCl \cdot 0.5H_2O$	55.54	4.87	22.35		8.83
						(55.25	4.87	22.43		8.71)
5g	2-Cl	4-F	46	86	$C_{22}H_{21}ClFN_3O_2 \cdot HCl \cdot 0.5H_2O$	57.52	5.05	15.44	4.14	9.15
						(57.37	5.04	15.55	4.23	9.29)
5h	4-F	H	57	110	$C_{22}H_{22}FN_3O_2 \cdot HCl$	63.54	5.57	8.52	4.57	10.10
						(63.33	5.52	8.48	4.65	9.99)
5i	4-F	3-Cl	56	108	$C_{22}H_{21}ClFN_3O_2 \cdot HCl \cdot 0.5H_2O$	57.52	5.05	15.44	4.14	9.15
						(57.66	5.01	15.62	4.15	9.28)
5j	4-F	3-CF_3	27	98	$C_{23}H_{21}F_4N_3O_2 \cdot HCl \cdot 0.5H_2O$	56.05	4.70	7.19	15.42	8.53
						(56.27	4.70	6.75	15.51	8.76)
5k	4-F	4-F	68	126	$C_{22}H_{21}F_2N_3O_2 \cdot HCl \cdot 0.5H_2O$	59.66	5.23	8.00	8.58	9.49
						(59.88	5.16	7.91	8.68	9.50)
5l	4-CH_3	H	33	114	$C_{23}H_{25}N_3O_2 \cdot HCl$	67.07	6.36	8.61		10.20
						(66.81	6.47	8.96		10.08)
5m	4-CH_3	3-Cl	37	107	$C_{23}H_{24}ClN_3O_2 \cdot HCl$	61.89	5.65	15.88		9.41
						(62.14	5.85	15.69		9.61)

Table 6. Spectral Data for Oxazinones **5**

Compd.	OH	IR (KBr) ν (cm^{-1})				^{1}H-NMR (in $CDCl_3$) δ ppm
		NH^+	C=O ester	C=O amide	C=C	
5a	3540—3300	2360—2100	1710	1620	1485	2.2 (s, 3H, CH_3), 3.2—3.5 (m, 9H, $4CH_2+0.5H_2O$), 4.1 (s, 2H, C_6H_5–C<u>H</u>$_2$), 6.9—7.4 (m, 10H, $2C_6H_5$), 7.8 (s, 1H, NH^+)
5b		2380—2100	1700	1600	1480	2.1 (s, 3H, CH_3), 3.3—4.5 (m, 8H, $4CH_2$), 3.9 (s, 2H, C_6H_5–C<u>H</u>$_2$), 4.0 (s, 3H, O–CH_3), 7.0—7.4 (m, 9H, $C_6H_5+C_6H_4$), 8.4 (s, 1H, NH^+)
5c		2400—2000	1705	1610	1495	2.1 (s, 3H, CH_3), 3.4—4.1 (m, 10H, $5CH_2$), 7.1—7.5 (m, 9H, $C_6H_5+C_6H_4$), 7.7 (s, 1H, NH^+)
5d	3540—3300	2400—2060	1690	1620	1490	2.1 (s, 3H, CH_3), 3.4—4.2 (m, 8H, $4CH_2$), 4.0 (s, 2H, C_6H_5–C<u>H</u>$_2$), 7.0—7.8 (m, 9H, $C_6H_5+C_6H_4$), 8.1 (s, 1H, NH^+)
5e		2360—2100	1705	1615	1515	2.1 (s, 3H, CH_3), 3.4—4.0 (m, 10H, $5CH_2$), 7.0—7.4 (m, 9H, $C_6H_5+C_6H_4$), 7.8 (s, 1H, NH^+)
5f	3620—3300	2200—1900	1720	1620	1470	2.1 (s, 3H, CH_3), 3.5—4.2 (m, 9H, $4CH_2+0.5H_2O$), 4.0 (s, 2H, C_6H_5–C<u>H</u>$_2$), 6.9—7.6 (m, 8H, $2C_6H_4$), 7.9 (s, 1H, NH^+)
5g	3670—3300	2460—2100	1720	1605	1510	2.1 (s, 3H, CH_3), 3.4—4.1 (m, 9H, $4CH_2+0.5H_2O$), 4.0 (s, 2H, C_6H_5–C<u>H</u>$_2$), 6.9—7.7 (m, 8H, $2C_6H_4$), 7.9 (s, 1H, NH^+)
5h		2400—2160	1720	1630	1510	2.1 (s, 3H, CH_3), 3.4—4.1 (m, 10H, $5CH_2$), 6.9—7.7 (m, 9H, $C_6H_4+C_6H_5$), 7.9 (s, 1H, NH^+)
5i	3680—3400	2420—2160	1715	1595	1510	2.1 (s, 3H, CH_3), 3.5—4.2 (m, 9H, $4CH_2+0.5H_2O$), 4.0 (s, 2H, C_6H_5–C<u>H</u>$_2$), 7.0—7.6 (m, 8H, $2C_6H_4$), 7.9 (s, 1H, NH^+)
5j	3600—3360	2360—2100	1715	1610	1510	2.1 (s, 3H, CH_3), 3.5—4.2 (m, 9H, $4CH_2+0.5H_2O$), 4.0 (s, 2H, C_6H_5–C<u>H</u>$_2$), 7.0—7.8 (m, 8H, $2C_6H_4$), 8.1 (s, 1H, NH^+)
5k	3620—3260	2380—2120	1700	1610	1510	2.1 (s, 3H, CH_3), 3.5—4.1 (m, 9H, $4CH_2+0.5H_2O$), 3.9 (s, 2H, C_6H_5–C<u>H</u>$_2$), 7.0—7.4 (m, 8H, $2C_6H_4$), 7.9 (s, 1H, NH^+)
5l		1440—1990	1700	1600	1490	2.1 (s, 3H, CH_3), 2.3 (s, 3H, C_6H_4–C<u>H</u>$_3$), 3.5—4.2 (m, 8H, $4CH_2$), 4.0 (s, 2H, C_6H_5–C<u>H</u>$_2$), 7.0—7.7 (m, 9H, $C_6H_4+C_6H_5$), 8.0 (s, 1H, NH^+)
5m		2440—2100	1700	1595	1490	2.1 (s, 3H, CH_3), 2.3 (s, 3H, C_6H_4–C<u>H</u>$_3$), 3.5—4.1 (m, 8H, $4CH_2$), 3.9 (s, 2H, C_6H_5–C<u>H</u>$_2$), 6.9—7.5 (m, 8H, $2C_6H_4$), 7.8 (s, 1H, NH^+)

to 75 mg kg^{-1} i.p. Thus, it appeared that **5c** (ED_{50} = 10.5 mg kg^{-1}) and **5f** (ED_{50} = 10.3 mg kg^{-1}) are equipotent to trazodone (ED_{50} = 10.2 mg kg^{-1}), while they are several times more active than the classical analgesic drugs acetaminophen (ED_{50} = 231.3 mg kg^{-1}) and noramidopyrine (ED_{50} = 68.5 mg kg^{-1}).The influence of hydrophobic effects on the activity was not clear from the log k_w values listed in Table 2. So, in order to clarify the structure–activity relationships in this series, a Hansch analysis using log k_w as a lipophilic index, Hammett's constants (σ_1 for R^1, σ_2 for R^2) as electronic parameters and Taft's constants (Es_1 for R^1, Es_2 for R^2) as steric factors, was performed. The constants have been taken from the compilation by Hansch and Leo.[19]

Because of the weak activity of four of our derivatives (**5d, 5g, 5j, 5m**), Eq. 1 was developed from a limited series of 9 oxazinones (**5a—c, 5e, 5f, 5h, 5i, 5k** and **5l**):

$$\log(1/ED_{50}) = 0.32(\pm 0.12)\log k_w - 2.97(\pm 0.60) \quad (1)$$
$$n=9,\ r^2=0.494,\ s=0.235,\ F=6.830\ (p=0.033)$$

In this equation, the numbers in parentheses are the 95% confidence intervals associated with the coefficients, n is the number of compounds employed, r^2 is the squared correlation coefficient corresponding to the fraction of observed variance accounted for, s is the standard deviation and F (as also p) the statistical significance of fit.

The log(1/ED_{50}) values recalculated from this equation are listed in Table 2.

The lipophilic descriptor log k_w explained only 49% of the variance in pharmacological data, although Eq. 1 was significant ($p<0.05$). So, a new Eq. 2 was recalculated excluding the most deviant oxazinone **5h**:

$$\log(1/ED_{50}) = 0.27(\pm 0.11)\log k_w - 0.28(\pm 0.19)Es_2 - 2.93(\pm 0.49)$$
$$n=8,\ r^2=0.776,\ s=0.177,\ F=8.186\ (p=0.027) \quad (2)$$

Equation 2 showed an important enhancement in the correlation (r^2 = 0.776) for the 8 remaining compounds and a further slight improvement in statistical significance: F = 8.186 (p = 0.027). The positive coefficient of log k_w suggests that the activity increases with increasing lipophilicity, while the negative coefficient associated with Es_2 indicates that the presence of a bulky substituent on the phenylpiperazinyl moiety is also beneficial. The different values of log(1/ED_{50}) recalculated from Eq. 2 are listed in Table 7 and shown in Fig. 1.

On the basis of their analgesic potency in the PBQ test, selected compounds **5c** and **5f** were further investigated. The effects of **5c**, **5f** on spontaneous and forced motor activity were evaluated in mice as parameters of their sedative properties and neurologic toxicity (Table 8). Derivatives **5c** and **5f** were equivalent in analgesic potency but had markedly different sedative activity. At 25 mg kg^{-1} i.p. **5c** caused a 59.6% decrease in spontaneous motor activity of animals, whereas **5f** did not produce a significant effect. At 50 mg kg^{-1} i.p. sedation appeared particularly intense only with **5c**. In the rotarod test, **5c** (50 mg kg^{-1} i.p.) displayed neurotoxic effects which disappeared after 2 h. Derivative **5f** did not show significant activity in this test. The reference drug trazodone exhibited sedative and neurotoxic effects from the low doses of 5 and 10 mg kg^{-1} i.p.

The potent antinociceptive effects of **5c**, **5f** exhibited

Table 7. PBQ Test, Hansch Analysis and Capacity Factors (log k_w) of Oxazinones **5a—m**

Compd.	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]	
5a	49.5 (26.4—92.5)	−1.70	−1.54	−1.72	4.546
5b	45.5 (26.2—79.2)	−1.66	−1.78	−1.77	3.778
5c	10.5 (5.1—21.4)	−1.02	−1.25	−1.20	5.469
5d	>75	—	—	—	5.720
5e	73.0 (38.2—139.4)	−1.86	−1.51	−1.57	4.637
5f	10.3 (5.2—20.3)	−1.01	−0.99	−0.99	6.287
5g	>75	—	—	—	5.230
5h	13.7 (7.1—26.2)	−1.14	−1.52	—	4.628
5i	27.9 (14.2—55.0)	−1.45	−1.39	−1.33	5.011
5j	>75	—	—	—	6.119
5k	24.7 (12.6—48.4)	−1.39	−1.40	−1.47	4.999
5l	38.7 (11.9—125.2)	−1.59	−1.43	−1.63	4.901
5m	>75	—	—	—	6.190
Acetaminophen	231.3 (147.3—363.2)	—	—	—	ND
Noramidopyrine	68.5 (22.8—205.3)	—	—	—	ND
Trazodone	10.2 (7.1—14.6)	—	—	—	ND

a) 95% confidence intervals in parentheses. *b*) Calculated from Eq. 1. *c*) Calculated from Eq. 2. ND: not determined.

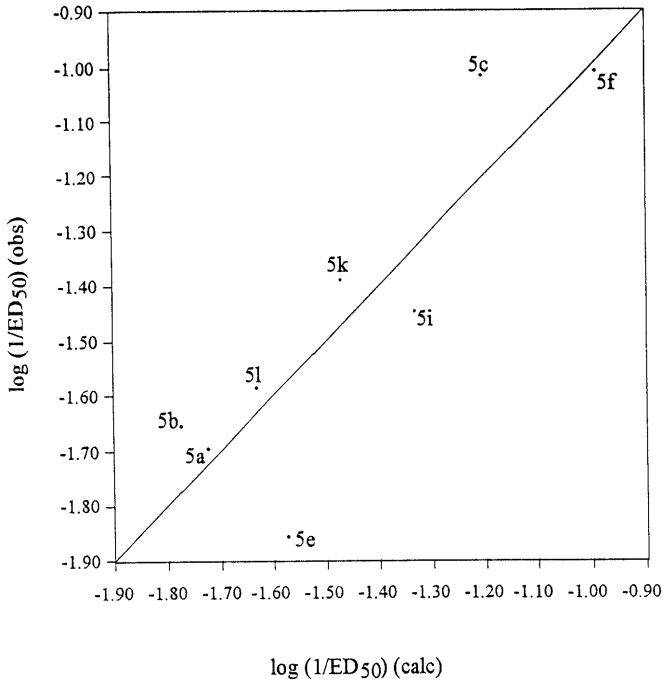


Fig. 1. Plot of Observed *versus* Calculated Analgesic Activity (from Eq. 2) of Oxazinones

in the PBQ test were not found in the hot plate test (Table 9). On account of their sedative effects, **5c** and **5f** were not administered at doses up to 25 mg kg^{-1} i.p. The lack of activity even at low doses suggests that the analgesic activity of oxazinones could be related either to a peripheral site of action or to a central non-opiate mechanism.[20] Most of the available evidence seems to support the role of monoamine transmitters (noradrenaline and serotonin) and of opiate neurons in the mechanism of pain control on the one hand and the mutual interactions of these systems on the other hand.[21]

Table 8. Sedative Activity and Neurotoxicity of Oxazinones **5c** and **5f**

Compd.	Dose (mg kg^{-1} i.p.)	Decrease of motor activity (%)	Rotarod test % of falls after treatment at		
			45 min	2 h	24 h
5c	25	59.6± 8.7*	0	0	0
	50	90.3± 2.0*	30*	0	0
5f	25	11.4±14.0 (NS)	10 (NS)	10 (NS)	0
	50	30.5± 8.1*	20 (NS)	10 (NS)	0
Trazodone	5	29± 4*	30*	0	0
	10	34±11*	30*	10 (NS)	0

* $p<0.05$. NS: not significant.

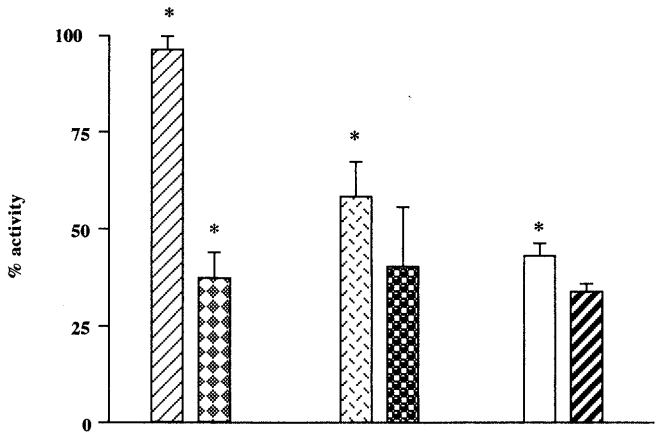


Fig. 2. Effect of Yohimbine (1 mg kg^{-1} *p.o.*) on Analgesic Activity of Clonidine, **5c** and **5f** in the PBQ Test

▨, clonidine (0.05 mg kg^{-1} s.c.); ▩, clonidine + yohimbine; ▨, **5c** (25 mg kg^{-1} i.p.); ▩, **5c** + yohimbine; □, **5f** (25 mg kg^{-1} i.p.); ▨, **5f** + yohimbine. * $p<0.05$.

Considering that activation of α_2-adrenoreceptors in the central nervous system produces analgesia in a number of animal models[22] and also that in the periphery, adrenergic mechanisms appear to be largely pronociceptive, we decided to investigate possible involvement of the α_2-adrenergic pathway in analgesic effects of oxazinones. Contrary to the observation with the α-sympathomimetic agent clonidine, the activity of **5c** or **5f** was not significantly attenuated by pretreatment with the α_2-adrenergic antagonist yohimbine (Fig. 2), suggesting that antinociceptive response was not mainly mediated by an α_2-adrenergic mechanism. In addition, no potentiation of **5c** or **5f** antinociception by 5-hydroxytryptophan (5-HTP) was observed, indicating that the analgesic properties of these compounds do not involve a serotonergic pathway, or that they involve a non-selective serotonergic mechanism (Table 9). Moreover, **5c** and **5f** were unable to potentiate morphine-induced analgesia, unlike trazodone. However, when animals were given an injection of **5c** (or **5f**) + naloxone, the effect of the former was significantly reduced in the same manner as was observed with morphine + naloxone (Fig. 3). On the other hand, naloxone not only failed to suppress analgesic activity of trazodone (10 mg kg^{-1} i.p.), but actually potentiated its effect. Similarly, Valeri *et al.*[18] and Messing *et al.*[23] respectively observed no blockade of the analgesic effect of trazodone (4 mg kg^{-1} i.p.) and fluoxetine, a specific inhibitor of serotonin uptake, by naloxone. Considering these last results, the antinociceptive properties of **5c** and **5f** might be due to modulation or activation of one or several endorphin relays, although these derivatives were ineffective as opiate adjuncts in both the PBQ test and hot plate test.

Taking into account the weak sedative activity of **5f** at the high dose of 50 mg kg^{-1} i.p., this derivative appears to have a superior analgesic profile to **5c**. Thus, it appeared interesting to compare **5f** and morphine by use of the molecular modeling software SYBYL 6.03[24] in order to seek structural analogies between the two molecules. Although all opiates share the feature of a protonated amine, it is hypothesized that this proton immediately transfers, without a barrier, to a nearby anionic site, resulting in neutralization. Therefore, as was previously demonstrated,[25] the neutral form is the most realistic

Table 9. Potentiation of Morphine-induced Analgesia by **5c**, **5f** and Effect of 5-Hydroxytryptophan on **5c**, **5f**-induced Analgesia

Compd.	Dose (mg kg^{-1} i.p.)	PBQ test (% analgesia)		Hot plate test (% analgesia)		Effect of 5-HTP	
		compd or reference alone	compd + morphine	compd or reference alone	compd + morphine	compd or reference alone	comp + 5-HTP
5c	3	28.5±15.6 (NS)	14.4±13.2 (NS)	NT	NT	14.2± 9.7 (NS)	41.4±11.6 (NS)
	25	NT	NT	5.3±2.2 (NS)	12.9± 4.2 (NS)	NT	NT
5f	3	17.3± 8.8 (NS)	18.5± 9.3 (NS)	NT	NT	13.7±10.6 (NS)	37.0±12.3 (NS)
	25	NT	NT	3.0±2.1 (NS)	5.0± 2.2 (NS)	NT	NT
Trazodone	2	32.8± 3.8 (NS)	43.0±12.1*	NT	NT	10.3± 6.7 (NS)	47.2± 9.1 (NS)
	10	NT	NT	28.6±8.1*	73.3±12.1*	NT	NT
Morphine	0.15a	8.6± 5.8 (NS)	—	NT	—	NT	NT
	5a	NT	—	10.9±1.7*	—	NT	NT
Carbidopa +5-HTP	25	—	—	—	—	42.3±10.3*	—
	50	—	—	—			

a) s.c. route. * $p<0.05$. NS: not significant. NT: not tested.

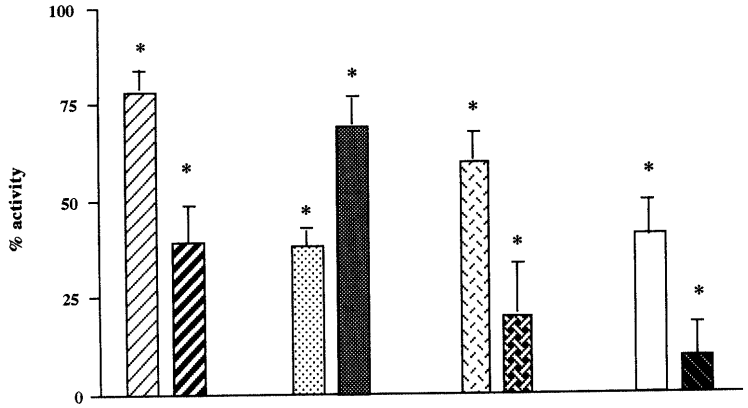


Fig. 3. Effect of Naloxone (1 mg kg^{-1} s.c.) on Analgesic Activity of Morphine, Trazodone, **5c** and **5f** in the PBQ Test

▨, morphine (1.5 mg kg^{-1} s.c.); ▨, morphine + naloxone; ▨, trazodone (10 mg kg^{-1} i.p.); ■, trazodone + naloxone; ▨, **5c** (25 mg kg^{-1} i.p.); ▨, **5c** + naloxone; □, **5f** (25 mg kg^{-1} i.p.); ■, **5f** + naloxone. $*p<0.05$.

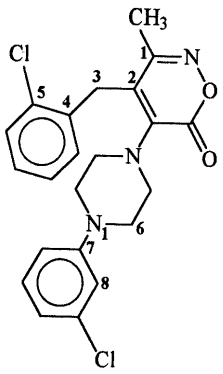


Fig. 4. Dihedral Angles Used in the Initial Conformational Analysis of **5f** by the Multitorsion Grid Search Method of SYBYL

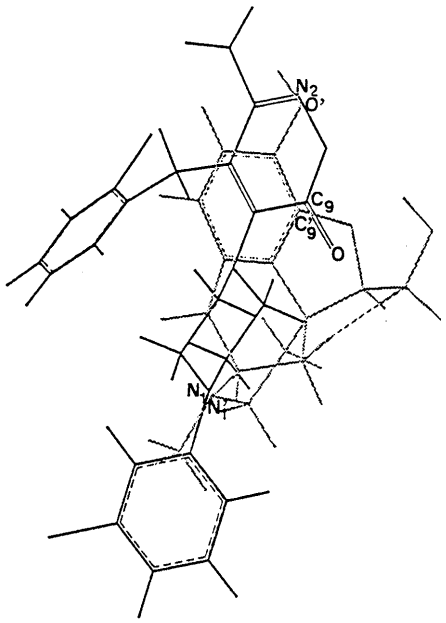


Fig. 5. Superimposition of Compound **5f** (Bold Solid) and Morphine (Solid)

simulation of the ligand in the environment of an anionic receptor site. Thus, the input geometries were generated and initially minimized using the Tripos force field[24] for the neutral form of **5f** and morphine. A conformational analysis was carried out for **5f**. Rotation around the C_2–C_3 ($\theta 1$), C_3–C_4 ($\theta 2$) and N_1–C_7 ($\theta 3$) bonds (Fig. 4) from 0 to 360° gave a four-dimensional (E, $\theta 1$, $\theta 2$, $\theta 3$) graph. Its two-dimensional projection on the E, $\theta 1$ plane gave a curve with two minima of closely related energies having an energy barrier of 20.9 kJ mol^{-1}. Further refinement using AM1 calculation shows that the conformer having the benzyl group far from the methyl group, and the phenyl nucleus of the phenylpiperazinyl moiety and the oxazine ring in the same plane, is more stable by more than 11.7 kJ mol^{-1}.

Superimposition of this conformer and morphine, also optimized by the AM1 method, was performed with the Fit procedure within SYBYL. The structural features taken into account in the matching processes were: (i) a cationic head (N_1 for **5f** and N'_1 for morphine), (ii) an electronegative atom (N_2 for **5f** and O′ for morphine); (iii) in addition, a third atom was chosen to determine a plane having an electron-rich zone (C_9 for **5f** and C'_9 for morphine). The fitting experiments showed a good superimposition (Fig. 5; RMS = 0.252).

Thus, **5f** appeared to possess the stereochemical requirements of classical opiates for binding to μ receptor,[25,26] namely an aromatic system (oxazine ring) placed at an appropriate distance from a tertiary ionizable aminic function (N_1) and electronegative atoms (N_2, O), which could be involved in the formation of hydrogen bonds.

Electrostatic isopotential maps of both molecules were generated by SYBYL from MOPAC charges and showed great similarities (Figs. 6 and 7). In particular, they exhibited the same contour maps of negative electrostatic potential adjacent to the oxazine ring for **5f** and to the dihydrobenzofuran moiety for morphine.

In conclusion, a structurally unique class of antinociceptive agents was investigated. Structure-activity relationships were studied with emphasis on the quantitative effects of substituents present on the phenyl moiety of compounds **5**. The oxazinone **5f** was found to possess a superior analgesic profile with low neurosedative effects. Antagonism of **5f**-induced analgesia by naloxone suggested possible interactions of this derivative with μ receptors. This idea was supported by molecular modeling studies which revealed evident structural similarities between **5f** and morphine. Although the routine use of opiates is rarely

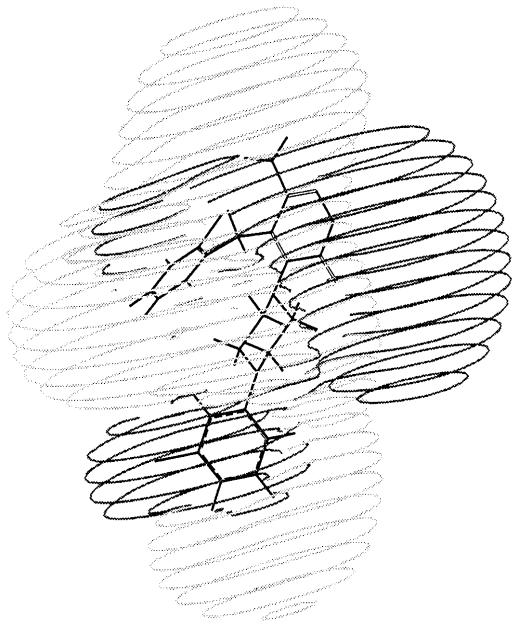


Fig. 6. Stereoview of Electrostatic Isopotential Surfaces (E) of Compound **5f**

$E \leq -1$ kcal/mol; bold solid. $E \geq 1$ kcal/mol; solid.

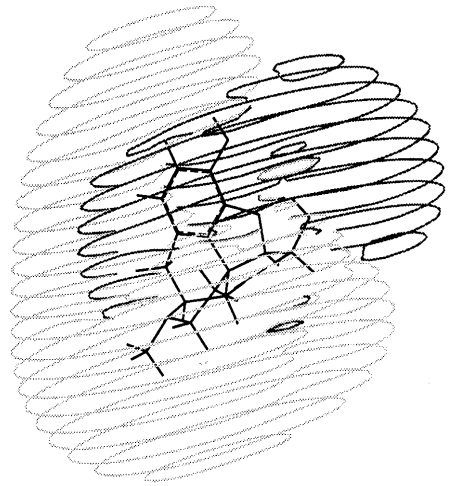


Fig. 7. Stereoview of Electrostatic Isopotential Surfaces (E) of Morphine

$E \leq -1$ kcal/mol; bold solid. $E \geq 1$ kcal/mol; solid.

associated with clinically significant respiratory depression in the absence of pulmonary disease, opiate overdose can be a problem, particularly in the immediate postoperative period. Therefore, compound **5f**, a non-classical anti-nociceptive drug could be a promising candidate for the treatment of pain.

Experimental

All chemicals were obtained from Janssen Chimica, Noisy le Grand, France. Melting points were determined on a Reichert apparatus without correction. IR spectra were recorded on a Beckman 4240 spectrophotometer. NMR spectra were obtained on a Bruker DPX 200 MHz. The chemical shifts were reported in parts per million (δ, ppm) downfield from tetramethylsilane, which was used as an internal standard for $CDCl_3$ solution. The abbreviations of signal patterns are as follows: s, singlet; t, triplet; m, multiplet. Reaction progress and purity of products were checked by analytical TLC using silica gel plates (60 F_{254}, E. Merck, Darmstadt, Germany). Spots were visualized with UV_{254} light or iodine. Column chromatography was performed with Kieselgel 60 (230—400 mesh) silica gel (Merck). Microanalyses were performed by the Service Central d'Analyses du C.N.R.S., Vernaison, France, and the analytical results obtained were within $\pm 0.4\%$ of the theoretical values.

4-Benzyl-3-methyl-1,2-oxazin-6-ones (3) The keto-acid **2** (15 mmol) was dissolved in pyridine (30 ml), then 1.04 g (15 mmol) of hydroxylamine hydrochloride and 1.5 ml of water were added to the solution. The mixture was refluxed for 10 h, cooled and then poured into 20% iced hydrochloric acid (100 ml). The resulting solution was extracted with chloroform (2 × 25 ml), dried over sodium sulfate and evaporated to afford crude products, which were recrystallized from a mixture of ethanol–water, 40 : 60.

4-(α-Bromobenzyl)-3-methyl-1,2-oxazin-6-ones (4) An oxazinone **3** (5 mmol) was dissolved in carbon tetrachloride (30 ml), then *N*-bromosuccinimide (5 mmol) and a catalytic amount (5 mg) of AIBN were added to the solution. The mixture was refluxed for 24 h. It was allowed to cool, then filtered and evaporated to dryness. The resulting residue was purified by column chromatography (eluent; ethyl acetate–hexane, 30 : 70).

5-(4-Arylpiperazin-1-yl)-4-benzyl-3-methyl-1,2-oxazin-6-one Hydrochlorides (5) The appropriate arylpiperazine (10 mmol) was added to a solution of α-bromobenzyl oxazinone **4** (5 mmol) in methanol (30 ml). The mixture was stirred at room temperature for 24 h. After evaporation the residue was triturated in water (100 ml) and extracted with 30 ml of methylene chloride. The organic layer was dried over sodium sulfate and, after evaporation, the residue was purified by column chromatography (eluent; ethyl acetate–cyclohexane, 40 : 60). The oxazinone **5** was dissolved in dry acetone. The salt was prepared by bubbling gaseous hydrochloric acid into a cooled solution of the base. The resulting precipitate was washed with diethyl ether, collected by filtration and dried.

Pharmacological Methods In the studies described below, all compounds were administered intraperitoneally in saline (0.9% NaCl). Swiss male mice weighing 18—22 g, purchased from Depre (Saint-Doulchard, France), were used in all experiments. Mice were kept in groups of ten in a temperature-controlled room with a 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 under blind conditions.

Acute Toxicity in Mice The compounds were administered intraperitoneally at doses of 100, 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[27] 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 the 5th and the 15th min after the injection of the irritant.

Locomotor Activity[28] 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).

Neurotoxicity The rotarod test[29] 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 h and 24 h after drug administration.

Hot Plate Test[30] 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. A cut-off withdrawal latency of 40 s was used to prevent tissue damage.

Potentiation of Morphine Analgesia[31] The protocol used was the same as that of 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.

Antagonism of Drug Antinociception by Naloxone[32] 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 for the PBQ test. Naloxone (1 mg kg^{-1}, s.c.) was injected 5 min before intraperitoneal administration of PBQ solution.

Antagonism of Drug Antinociception by Yohimbine[33] Thirty minutes after simultaneous administration of drugs (i.p.) and yohimbine (1 mg kg^{-1}, p.o.), (Sigma, Montluçon, France), PBQ solution was given i.p. Beginning 5 min after injection of PBQ, writhing behavior of mice was scored for a period of 10 min.

Potentiation of Drug Antinociception by 5-HTP The protocol used was adapted from the technique of Vonvoigtlander *et al.*[34] Experiments were carried out in a similar manner to 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 a further 15 min by drugs. Twenty min later, the analgesic test was performed with administration of PBQ solution.

Data Analysis Statistical analysis of the results was performed using the method of Schwartz.[35] The ED_{50} values were determined by the method of Litchfield and Wilcoxon.[36] The significance of pharmacological data expressed as mean ± S.E. was evaluated by using Student's t test. Other results were analyzed by means of the chi-square test with Yates' correction.

Lipophilicity Measurements Lipophilicity was determined by reversed-phase high-performance liquid chromatography.[37] A Varian 5000 liquid chromatograph (Varian, Les Ulis, France) equipped with a detector operating at 254 nm was used. A Varian CDS 111L integrator was employed 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.

Molecular Modeling Studies The topographical and electrostatic characterization of the studied molecules was performed using the SYBYL 6.03 software package on a Silicon Graphics Personal IRIS 4D35TG workstation. Structures were built within SYBYL and minimized by MAXIMIN 2 with the Tripos force field, under *in vacuo* conditions, to provide reasonable standard geometries. Molecules were deemed to be minimized when there was a minimum energy change of less than 0.021 kJ mol^{-1} for one iteration.

The conjugate gradient method was used for minimization. All AM1 calculations involved the singlet state. Molecules were deemed to be minimized when the gradient fell to less than 0.021 kJ mol^{-1}. The conformational space of **5f** was explored using the SYBYL search facility. Torsion angles were defined around the single bonds C_2–C_3, C_3–C_4 and N_1–C_7, and a grid search was performed, allowing these bonds to rotate with a 360° revolution by 20° increments. The lowest-energy conformers thus obtained were submitted to AM1 calculations (MOPAC version 5.0)[38] to optimize their geometry and to determine atomic charge distributions.

A structural fitting between morphine and **5f** was done by using the FIT option of SYBYL. The molecular electrostatic potential surfaces were calculated in SYBYL and partitioned into two intervals as follows: -1 kcal mol^{-1} and down, and 1 kcal mol^{-1} and up.

Acknowledgment The authors wish to thank Professor J.C. Gramain of the Université Blaise Pascal de Clermont-Ferrand for helpful discussions during this work and Mrs. S. Nahmias and Mr. F. Leal for their technical assistance.

References

1) Amadio P., *Am. J. Med.*, **77** (Suppl. 3A), 17—25 (1984).
2) Bannwarth B., Demotes-Mainard F., Schaeverbeke T., Labat L., Dehais J., *Fundam. Clin. Pharmacol.*, **9**, 1—7 (1995).
3) Martin W. R., *Pharmacol. Rev.*, **35**, 283—323 (1983).
4) Budd K., *Anaesthesia*, **33**, 531—534 (1978).
5) Clinch D., Banerjee A. K., Ostick G., Levy D. W., *J. R. Coll. Physicians London*, **17**, 228—230 (1983).
6) Jaffe J. H., Martin W. R., "The Pharmacological Basis of Therapeutics," ed. by Goodman L. S., Gilman A., Rall T. W., Nics A. S., Taylor P., Pergamon Press, New York, 1990, pp. 485—521.
7) Sato M., Yamaguchi A., *Arzneim.-Forsch.*, **32**, 379—382 (1982).
8) Wermuth C.G., Leclerc G., Melounou P., *Chim. Ther.*, **5**, 243—246 (1970).
9) Rubat C., Coudert P., Couquelet J., Bastide P., Bastide J., *Chem. Pharm. Bull.*, **36**, 1558—1561 (1988).
10) Rubat C., Coudert P., Tronche P., Bastide J., Bastide P., Privat A. M., *Chem. Pharm. Bull.*, **32**, 2832—2835 (1989).
11) Rubat C., Coudert P., Albuisson E., Bastide J., Couquelet J., Tronche P., *J. Pharm. Sci.*, **81**, 1084—1087 (1992).
12) Moreau S., Coudert P., Rubat C., Albuisson E., Couquelet J., *Arzneim. Forsch./Drug Res.*, **46**, 800—805 (1996).
13) *a*) Sladowska H., *Il Farm.*, **48**, 77—84 (1993); *b*) *Idem, ibid.*, **48**, 85—94 (1993); *c*) Erdogan H., Debaert M., Cazin J. C., *Arzneim.-Forsch.*, **41**, 73—76 (1991); *d*) Palaska E., Unlu S., Erdogan H., Safak C., Gumusel B., Sunal R., *Eur. J. Med. Chem.*, **28**, 963—967 (1993); *e*) Flouzat C., Bresson Y., Mattio A., Bonnet J., Guillaumet G., *J. Med. Chem.*, **36**, 497—503 (1993); *f*) 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.*, **38**, 1278—1286 (1995).
14) Taoufik J., Couquelet J., *Synthesis*, **8**, 660—661 (1992).
15) Ziegler K., Spath A., Schaaf E., Schumann W., Winkelmann E., *Ann. Chem.*, **551**, 80—119 (1942).
16) Magid R. M., *Tetrahedron*, **36**, 1901—1930 (1901).
17) Riblet L. A, Taylor D. P., *J. Clin. Psychopharmacol.*, **1** (Suppl. 6), 175—225 (1981).
18) Valeri P., Pimpinella G., Morrone L. A., Romanelli L., *Gen. Pharmac.*, **22**, 127—131 (1991).
19) Hansch C., Leo A., "Substituent Constants for Correlation Analysis in Chemistry and Biology," Wiley Interscience, New York, 1979, pp. 69—161.
20) Collier H. O. J., Dinneen L. C., Johnson C. A., Schneider C., *Br. J. Pharmacol. Chemother.*, **32**, 295—310 (1968).
21) *a*) Groppetti A., Braga P.C., Biella G., Parenti M., Rusconi L., Mantegazza P., *Neuropharmacology*, **28**, 499—505 (1988); *b*) Messing R. B., Lytle L. D., *Pain*, **4**, 1—21 (1977); *c*) Taiwo Y. O., Levine J. D., *J. Neurosci.*, **8**, 1346—1349 (1988); *d*) Schmitt H., Le Douarec J. C., Petillot N., *Neuropharmacology*, **13**, 289—294 (1974); *e*) Kuraishi Y., Harada Y., Aratani S., Satoh M., Takagi H., *Brain Res.*, **273**, 245—252 (1983).
22) *a*) Petrovaara A., *Prog. Neurobiol.*, **40**, 691—709 (1993); *b*) Hong Y., Abbott F. V., *Eur. J. Pharmacol.*, **301**, 41—48 (1996).
23) Messing R. B., Phebus L., Fisher L., Lytle L. D., *Psychopharmacol. Commun.*, **1**, 511—521 (1975).
24) Tripos Associates, Inc., 1699 S. Harley Rd., Suite 303, St Louis, MO 63144.
25) Cometta-Morini C., Maguire P. A., Loew G. H., *Mol. Pharmacol.*, **41**, 185—196 (1992).
26) *a*) Michel A., Villeneuve G., Di Maio J., *J. Comput.-Aided Mol. Design*, **5**, 553—569 (1991); *b*) Barlocco D., Cignarella G., Greco G., Novellino E., *J. Comput.-Aided Mol. Design*, **7**, 557—571 (1993); *c*) Froimowitz M., *NIDA Res. Monogr.*, **134**, 178—194 (1993).
27) *a*) Siegmund E., Cadmus R., Lu G., *Proc. Soc. Exp. Biol. Med.*, **95**, 729—731 (1957); *b*) Linee P., *J. Pharmacol.* (Paris), **3**, 513—515 (1972).
28) Boissier J. R., Simon P., *Arch. Int. Pharmacodyn. Ther.*, **158**, 212—221 (1965).
29) Kinnard W. J., Carr C. J., *J. Pharmacol. Exp. Ther.*, **121**, 354—361 (1957).
30) Woolfe G., McDonald A. D., *J. Pharmacol. Exp. Ther.*, **80**, 300—307 (1944).
31) Fialip J., Marty H., Makambila M.C., Civiale M.A., Eschalier A., *J. Pharmacol. Exp. Ther.*, **248**, 747—751 (1989).
32) Nevinson M. J., Gray A. M., Sewell R. D. E., *British J. Pharmacol.-Proceedings Suppl.*, **104**, 267P (1991).
33) Luttinger D., Ferrari R., Perrone M. H., Haubrich D. R., *J. Pharmacol. Exp. Ther.*, **232**, 883—889 (1985).
34) Vonvoigtlander P. F., Lewis R. A., Neff G. L., *J. Pharmacol. Exp. Ther.*, **231**, 270—274 (1984).
35) Schwartz D., "Méthodes Statistiques à l'Usage des Médecins et des Biologistes," ed. Médicales Flammarion, Paris, 1984, pp. 263—307.
36) Litchfield J. T., Wilcoxon E., *J. Pharmacol. Exp. Ther.*, **96**, 99—113 (1949).
37) *a*) El Tayar N., Van de Waterbeemd H., Testa B., *J. Chromatogr.*, **320**, 293—304 (1985); *b*) Bechalany A., Röthlisberger T., El Tayar N., Testa B., *ibid.*, **473**, 115—124 (1989).
38) Merz K. M., Besler B. H., MOPAC version 5.0 ESC, QCPE n° 589, 1990, with AM1 force field parameters.