J. Pharm. Pharmacol. 1998, 50: 575–582
Received October 30, 1997
Accepted February 18, 1998

Synthesis of Thiazolotriazine Derivatives and Their Antinociceptive Effects in Mice

VERONIQUE ISSARTEL, PASCAL COUDERT, CATHERINE RUBAT*, SOLANGE NHAMIAS AND JACQUES COUQUELET

Groupe de Recherche en Pharmacochimie and Laboratoire de Chimie Thérapeutique and Laboratoire de Pharmacologie, Faculté de Pharmacie, 28 Place Henri Dunant, 63000 Clermont-Ferrand, France*

Abstract

A series of 2-(4-arylpiperazin-1-yl-methyl)-4-methyl-1-oxo-5,6,8,8a-tetrahydro-thiazolo[3,4-*d*][1,2,4]triazines was prepared and tested for antinociceptive activity.

The compounds were prepared by the Mannich reaction from the corresponding 2-unsubstituted thiazolotriazines. When administered intraperitoneally most were found to have potent analgesic activity in the mouse during tests of phenylbenzoquinone-induced abdominal constriction; ED50 values (doses resulting in half the maximum effect) ranged from 10 to 87 mg kg^{-1}. Derivatives with a 3-chloro- or 4-fluorophenylpiperazinylmethyl side-chain in the 2-position of the bicyclic system were, when administered intraperitoneally at doses greater than 25 mg kg^{-1}, also effective in the hot-plate test without associated sedative effects. The compounds have a large therapeutic index; intraperitoneal LD50 values (doses which result in the death of half the animals) were > 700 mg kg^{-1}. Naloxone attenuated the analgesic activity of the 3-chloro derivative, suggesting the participation of μ-receptors in the antinociceptive effects of this drug. In addition, a non-opioid mechanism, probably related to enhancement of the release of 5-hydroxytryptamine and noradrenaline, or inhibition of the neuronal re-uptake of these compounds, has been evinced to explain the analgesic properties of the 3-chloro or 4-fluoro derivatives.

These results provide evidence for the involvement of noradrenergic and 5-hydroxytryptaminergic pathways in the analgesic activity of **3** and **4**. Because of their potential effectiveness, the 3-chloro- or 4-fluorophenylpiperazinylmethyl derivatives might be suitable for treatment of a wide variety of painful conditions and could be attractive reserve agents for patients dissatisfied with opioids.

Although clinical treatment of pain is dominated by two classes of analgesic, the cyclooxygenase inhibitors (aspirin and other non-steroidal anti-inflammatory drugs) and the opiates (morphine and related compounds), α-sympathomimetic agents and monoamine-uptake inhibitors are also important in the management of pain (Schmitt et al 1974; Valeri et al 1991; Ardid et al 1992; Pick et al 1992). Thus, clonidine, an α_2-adrenoceptor agonist, is a useful adjunct to opioid agents and has analgesic action when administered epidurally (Patel et al 1996). Similarly 5-hydroxytryptamine-uptake inhibitors such as clomipramine are efficacious in the treatment of pain related to some advanced cancers (Eschalier 1990). In addition a growing knowledge of endogenous nociceptive and antinociceptive systems has led to the discovery of non-traditional centrally-acting analgesic drugs such as tramadol. This cyclohexanol derivative combines inhibition of neuronal monoamine re-uptake with weak affinity for opioid receptors (Raffa et al 1995). This original mechanism of action results in a drug which does not induce the well known gastrointestinal lesions generated by non-steroidal anti-inflammatory drugs and which has minimal morphine-like side-effects (tolerance, physical dependence, respiratory depression). Development of compounds which activate descending pain inhibitory systems is, therefore, particularly appropriate for the treatment of persistent and chronic pain.

Research on arylpiperazinyl derivatives has led to the discovery of many analgesic structures, for example benzoxazolinones (Erdogan et al 1991;

Correspondence: J. Couquelet, Laboratoire de Chimie Thérapeutique, Faculté de Pharmacie, 28 Place Henri Dunant, 63000 Clermont-Ferrand, France.

Gökhan et al 1996), triazoles and benzotriazoles (Pick et al 1992; Caliendo et al 1995), oxazolopyridines (Flouzat et al 1993), pyridopyridazines (Sladowska et al 1995) acting on opioid and 5-HT systems. In an effort to develop novel analgesic compounds containing arylpiperazinyl groups we have previously synthesized several pyridazine and pyrazolotriazine derivatives which had potent antinociceptive properties in animal models (Mavel et al 1993; Rubat et al 1995; Moreau et al 1996; Rohet et al 1996). Continuing this work on heteropolycyclic compounds with analgesic activity, a new series of thiazolotriazines with an arylpiperazinomethyl chain has been prepared and tested in mice. We wanted to evaluate the effect of changing the pyrazole ring of previously described pyrazolotriazines (Mavel et al 1993) to a thiazole ring, because this substitution seemed compatible with retention of biological activity, considering that different thiazole derivatives had antinociceptive activity (Bordi et al 1992; Palagiano et al 1995). These studies and the secondary pharmacological results of selected compounds will be discussed herein.

Materials and Methods

Materials

Chemicals used for synthesis were reagent-grade from Aldrich (St Quentin Fallavier, France). Carbidopa (ICN Biomedicals, Orsay, France), clonidine (Catapressan, Boehringer Ingelheim, Paris, France), fluoxetine (Prozac, Lilly, St Cloud, France), L-5-hydroxytryptophan (Sigma, Montluçon, France), morphine hydrochloride (Coopération Pharmaceutique Française, Melun), naloxone (Narcan, Du Pont de Nemours, Paris, France) and trazodone (Pragmarel, UPSA, Rueil Malmaison, France) were dissolved in saline. Yohimbine (Sigma) was dissolved in deionized water.

Phenylbenzoquinone (Eastman Kodak, Rochester, USA) was used as a 0·02% solution in 5% ethanol.

Synthesis

Melting points were determined on a Reichert apparatus and are uncorrected. IR spectra were recorded with a Beckman 4240 spectrophotometer. NMR spectra were obtained with a Brüker 400 MSL. Chemical shifts are reported in δ (ppm) downfield from tetramethylsilane, used as an internal standard in $CDCl_3$ solution. Abbreviations used for signal patterns are: s, singlet; d, doublet; t, triplet; m, multiplet. Reaction progress and purity of products were checked by analytical TLC on silica gel 60 F_{254} plates (E. Merck, Darmstadt, Germany). Spots were revealed by illumination with UV_{254} light. Microanalyses were performed by the Service Central d'Analyses du CNRS (Vernaison, France).

Synthesis of 2-(4-aryl-piperazin-1-yl-methyl)-4-methyl-1-oxo-5,6,8,8a-tetrahydro-thiazolo[3,4-d][1,2,4]triazines **2–8**

Thiazolotriazinone **1** (1·71 g, 0·01 mol) was added to an aqueous solution of formaldehyde (35%; 1 mL, 0·012 mol) and the appropriate arylpiperazine (0·01 mol) in ethanol (75 mL). The mixture was heated under reflux for 10 h. After partial evaporation, the solution was cooled and the precipitate formed was collected by filtration and recrystallized from ethanol (Tables 1 and 2).

Animals and experimental procedure

Swiss male mice, 18–22 g, 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 freely available until the time of the experiment. The allocation of animals to different groups was randomized and the experiments were performed under blind conditions. Except otherwise indicated all compounds were administered intraperitoneally in saline (0·9% NaCl).

Acute toxicity in mice

The compounds were administered at doses of 100, 200, 400, 600 and 800 mg kg^{-1}. The animals were observed for 8 days to detect any sign of toxicity.

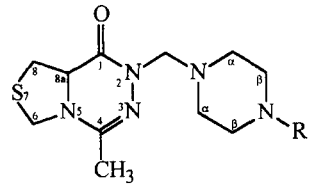
Phenylbenzoquinone-induced abdominal constriction (Siegmund et al 1957; Linee 1972)

A solution (ethanol/water, 5 : 95) of phenylbenzoquinone (0·02%) maintained at 37°C, was administered to mice by intraperitoneal injection 30 min after intraperitoneal administration of the drugs. The number of abdominal constrictions of each animal was counted between the 5 and 15 min after injection of the irritant.

Table 1. Physical constants of compounds **2–8**.

Compound	R	Formula	MW	Yield (%)	Mp (°C)
2	C_6H_5	$C_{17}H_{23}ON_5S$	345·5	35	79
3	3-ClC_6H_4	$C_{17}H_{22}ON_5SCl$	380·0	53	90
4	4-FC_6H_4	$C_{17}H_{22}ON_5SF$	363·5	36	131
5	2-$OCH_3C_6H_4$	$C_{18}H_{25}O_2N_5S$	375·5	54	125
6	3-$CF_3C_6H_4$	$C_{18}H_{22}ON_5SF_3$	413·5	70	82
7	2-Pyridyl	$C_{16}H_{22}ON_6S$	346·5	71	149
8	2-Pyrimidinyl	$C_{15}H_{21}ON_7S$	347·5	38	153

Table 2. ^{1}H NMR characteristics (δ (ppm) $CDCl_3$) of compounds **2–8**.



Compound	CH_3	CH2α CH2β	H8	H8a	H6	CH_2	R
2	2·00 (s)	2·90 (m) 3·10 (m)	3·00 (t, J = 10 Hz) 3·40 (dd, J = 10, 6 Hz)	4·00 (dd, J = 10, 6 Hz)	4·30 (d, J = 9·2 Hz) 4·75 (d, J = 9·2 Hz)	4·70 (s)	6·80–7·30 (5H, m)
3	2·00 (s)	2·80 (m) 3·10 (m)	3·00 (t, J = 10 Hz) 3·40 (dd, J = 10, 6 Hz)	3·90 (dd, J = 10, 6 Hz)	4·20 (d, J = 9·2 Hz) 4·70 (d, J = 9·2 Hz)	4·60 (s)	6·70–7·10 (4H, m)
4	2·00 (s)	2·90 (m) 3·10 (m)	3·00 (t, J = 10 Hz) 3·40 (dd, J = 10, 6 Hz)	4·00 (dd, J = 10, 6 Hz)	4·30 (d, J = 9·2 Hz) 4·70 (d, J = 9·2 Hz)	4·60 (s)	6·80–7·00 (4H, m)
5	2·00 (s)	2·90 (m) 3·10 (m)	3·00 (m) 3·40 (dd, J = 10, 6 Hz)	3·95 (dd, J = 10, 6 Hz)	4·25 (d, J = 9·2 Hz) 4·70 (d, J = 9·2 Hz)	4·65 (s)	3·85 (3H, s) 6·80–7·00 (4H, m)
6	2·00 (s)	2·80 (m) 3·20 (m)	3·00 (m) 3·40 (m)	3·90 (m)	4·25 (d, J = 10·3 Hz) 4·70 (d, J = 10·3 Hz)	4·60 (s)	7·00–7·30 (4H, m)
7	2·00 (s)	2·80 (m) 3·50 (m)	3·00 (t, J = 10 Hz) 3·40 (dd, J = 10, 6 Hz)	3·90 (dd, J = 10, 6 Hz)	4·30 (d, J = 9·2 Hz) 4·70 (d, J = 9·2 Hz)	4·65 (s)	6·60 (2H, m) 7·50 (1H, m) 8·20 (1H, m)
8	1·95 (s)	2·70 (m) 3·75 (m)	2·90 (t, J = 10 Hz) 3·30 (dd, J = 10, 6 Hz)	3·85 (dd, J = 10, 6 Hz)	4·20 (d, J = 9·2 Hz) 4·60 (d, J = 9·2 Hz)	4·70 (s)	6·40 (1H, m) 8·20 (2H, m)

Locomotor activity (Boissier & Simon 1965)
Thirty minutes after intraperitoneal administration of the drug mice were placed individually in a photocell actimeter (Apelex, Massy, France) and the number of photocell beams crossed in 10 min was recorded.

Hot-plate test (Woolfe & McDonald 1944)
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 made 30 min after drug administration. A cut-off withdrawal latency of 40 s was used to prevent tissue damage.

Potentiation of morphine analgesia (Fialip et al 1989)
The protocol used was the same as in the phenylbenzoquinone test. Morphine (0·15 mg kg^{-1}) was injected subcutaneously at the same time as the drugs, 30 min before the test.

Antagonism of drug antinociception by naloxone (Nevinson et al 1991)
The protocol used for the evaluation of the effect of naloxone on drug-induced analgesia was similar to that described for the phenylbenzoquinone test. Naloxone (1 mg kg^{-1}) was injected subcutaneously 5 min before intraperitoneal administration of phenylbenzoquinone solution.

Antagonism of drug antinociception by yohimbine (Luttinger et al 1985)
Thirty minutes after simultaneous intraperitoneal administration of the drug and oral administration of yohimbine (1 mg kg^{-1}), phenylbenzoquinone solution was given intraperitoneally. Beginning 5 min after injection of phenylbenzoquinone the number of abdominal constrictions of each animal was counted for a period of 10 min.

Potentiation of drug antinociception by 5-hydroxytryptophan
The protocol used was adapted from the technique of Vonvoigtlander et al (1984). Experiments were performed in a similar manner to the phenylbenzoquinone test. Carbidopa (25 mg kg^{-1}) and, 30 min later, 5-hydroxytrytophan (50 mg kg^{-1}) were administered intraperitoneally and the drug was administered after a further 15 min. The analgesic test was performed 20 min later by administration of phenylbenzoquinone solution.

Data analysis
The LD50 (doses which result in the death of half the animals) and ED50 (doses resulting in half the maximum effect) values, and their 95% confidence intervals were determined by the method of Litchfield & Wilcoxon (1949). In some experiments results obtained after administration of different doses of the test drugs were compared with

those from controls or from other treatment groups by use of Student's t-test. $P < 0.05$ was accepted as the level of statistical significance (Schwartz 1984).

In the hot-plate test, the results are expressed as the percentage of analgesia for the different groups (drugs + saline; morphine + saline).

Results and Discussion

Chemistry

The starting thiazolotriazine **1** was prepared by a procedure described elsewhere (Issartel et al 1997). The new series of 2-substituted thiazolotriazines **2–8** were obtained by the Mannich reaction with formaldehyde and *N*-arylpiperazine (Figure 1). Physical constants and spectral data of **2–8** were reported in Tables 1 and 2, respectively. All ^{13}C NMR spectra of the final compounds showed signals at approximately 68 and 162 ppm, attributable to the N–CH_2–N and C=O groups, respectively.

Pharmacological studies

The compounds were first evaluated for their analgesic activity in the phenylbenzoquinone test and for their acute toxicity in mice. The results, shown in Table 3, include those obtained for morphine, aspirin, paracetamol and noramidopyrine. Trazodone (Figure 2) was also chosen as a reference drug in the assays because its chemical

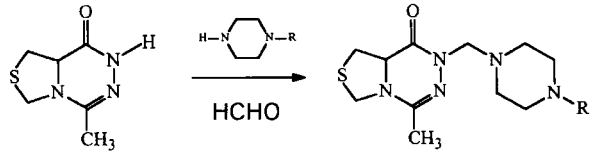


Figure 1. Synthesis of 2-substituted thiazolotriazinones **2–8**.

Table 3. Phenylbenzoquinone test ED50 values and the acute toxicities (LD50) of compounds **2–8**.

Compound	ED50 (mg kg^{-1})	LD50 (mg kg^{-1})
2	86·9 (34·9–216·4)	> 800
3	22·0 (13·9–34·8)	750·0 (535·7–1050·0)
4	10·0 (4·0–25·0)	> 800
5	55·7 (31·6–98·0)	NT
6	48·7 (39·9–59·4)	710·0 (591·7–852·0)
7	> 100	NT
8	87·0 (42·0–180·1)	NT
Morphine	0·6 (0·3–1·1)	NT
Aspirin	7·8 (2·9–20·8)	NT
Paracetamol	228·6 (167·9–311·7)	NT
Noramidopyrine	68·3 (26·9–173·7)	NT
Trazodone	10·2 (8·1–12·9)	223·4 (215·0–232·1)

Values in parentheses are 95% confidence limits. NT = not tested. Compounds were administered intraperitoneally except for morphine (subcutaneously).

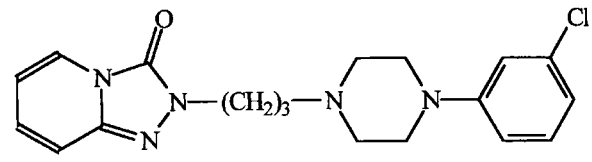


Figure 2. The structure of trazodone.

structure is closely related to those of the thiazolotriazines and because of its potent analgesic properties (Riblet & Taylor 1981; Valeri et al 1991).

A statistically significant antinociceptive effect was measured for six of the seven thiazolotriazines; after intraperitoneal administration ED50 values ranged from 10 to 87 mg kg^{-1}. Substitution on the phenyl ring of the phenylpiperazine group (**3–6**) markedly increased the activity in comparison with that of the unsubstituted derivative **2** and those of the bioisosteric analogues **7** and **8**. The best analgesic effect was obtained for compounds with an electron—withdrawing substituent on the phenyl nucleus-a chlorine atom at the 3- position (compound **3**) or a fluorine atom at the 4- position (compound **4**). These two last derivatives were characterized by ED50 values much lower than those of currently used drugs (22 and 10 mg kg^{-1}, respectively, for **3** and **4** compared with 228·6 and 68·3 mg kg^{-1}, respectively, for paracetamol and noramidopyrine). The toxic profile of thiazolotriazines was assessed by determination of acute toxicity in mice for some representative derivatives of this series (Table 3). The intraperitoneal LD50 values were found to be in excess of 700 mg kg^{-1}. Because, after intraperitoneal injection, the pharmacologically active analgesic doses are 10 to 87 mg kg^{-1}, it can be concluded that these compounds have large therapeutic indices, especially in comparison with the structurally related compound trazodone (ED50 10·2 mg kg^{-1} and LD50 223·4 mg kg^{-1}, both after intraperitoneal injection). After an intraperitoneal dose of 100 mg kg^{-1} all the substances investigated except compound **6** significantly reduced the spontaneous locomotor activity of mice (Table 4). At therapeutic doses equivalent to 1/30 LD50, the most active derivatives (**3** and **4**) in the phenylbenzoquinone test did not influence motor activity.

When the hot-plate test was used to verify the analgesic properties of thiazolotriazines **3**, **4** and **6**, for which ED50 values after intraperitoneal administration were lower than 50 mg kg^{-1} in the abdominal constriction test, antinociceptive activity was found for compounds **3** and **4** only (Table 4). The discrepancy between the results from the two

Table 4. Results from locomotor activity and hot-plate tests.

Compound	Dose (mg kg^{-1})	Decrease of motor activity (%)	Hot-plate test reaction time (s)
2	100	77·7 ± 4·1**	NT
3	25	1·0 ± 7·2	11·6 ± 0·9*
	100	44·8 ± 6·3*	16·5 ± 2·8**
4	25	3·3 ± 13·6	13·7 ± 1·7*
	100	71·6 ± 5·1**	14·5 ± 1·2**
5	100	77·1 ± 1·7*	NT
6	100	24·3 ± 17·3	10·4 ± 1·0
7	100	73·3 ± 6·5*	NT
8	100	62·8 ± 8·4*	NT
Morphine	7·5	NT	12·0 ± 0·5*
Trazodone	5	29·2 ± 11·2*	10·0 ± 1·0
	10	34·6 ± 11·6*	17·4 ± 2·6*

The antinociceptive effects of drugs is expressed as the time necessary to induce the forepaw-licking reflex in mice. The reaction time for saline (control) was 8·4 ± 0·5 s. NT = not tested. Compounds were administered intraperitoneally except for morphine (subcutaneously). *$P < 0.05$, **$P < 0.01$, significantly different from control result.

tests could be attributed to their different sensitivity—it is known that ED50 values obtained from the abdominal constriction test are usually markedly lower than those obtained in the hot-plate test (Ardid et al 1992; Sladowska et al 1995; Viaud et al 1995). Results from the thermal test in mice showed a significant increase in foot-licking latency after an intraperitoneal dose of 25 mg kg^{-1} of compounds **3** and **4**. Because of their analgesic potency and their low toxicity, **3** and **4** were selected for further pharmacological evaluation with particular attention to interactions with opioidergic and monoaminergic systems.

When administered simultaneously with morphine 30 min before testing, **4** and trazodone potentiated the analgesia induced by the opioid agonist, as illustrated in Table 5. The interaction of **3** and the specific 5-hydroxytryptamine re-uptake inhibitor fluoxetine with morphine corresponded to simple addition of the effects of the individual drugs. Moreover a 1 mg kg^{-1} dose of naloxone dramatically reduced the analgesic activity of morphine and **3** (Table 6). In contrast, naloxone did not antagonize the effect of both **4** and 5-hydroxytryptamine re-uptake inhibitors as previously described by Valeri et al (1991). From these observations it can be postulated that thiazolotriazines interfere with opiate systems, as has already been reported for many analgesic drugs (Biegon & Samuel 1980; Somoza et al 1981; Isenberg & Cicero 1984; Granados-Soto et al 1995). It can also be concluded that activation of μ-receptors by direct or indirect mechanisms contribute to the analgesic effect of **3** because the non-specific opioid antagonist naloxone has greater affinity for μ-receptors. Potentiation of morphine-induced analgesia by **4** could be interpreted in terms of increased opiate release. In addition, because there is mutual interaction between 5-hydroxytryptamine or catecholamine transmitters and opiate neurons (Groppetti et al 1988; Taiwo & Levine 1988; Pini et al 1997), involvement of monoaminergic systems in the antinociceptive properties of **3** and **4** was investigated. Thus, treatment with the precursor of 5-hydroxytryptamine, 5-hydroxytryptophan, combined with the peripheral decarboxylase inhibitor carbidopa enhanced the analgesic effect of thiazolotriazines **3** and **4** in mice (Table 7). This activity seemed to be potentiation of the individual effects of the drugs. Similar behaviour was observed for the 5-hydroxytryptamine re-uptake inhibitor trazodone, which also potentiated analgesia in conjunction with 5-hydroxytryptophan. Trazodone might form 3-chlorophenylpiperazine during its biotransformation in-vivo (Caccia & Garattini 1990) and 5-hydroxytryptaminergic activity of arylpiperazinyl groups has been established (Fuller et al 1978; Maj et al 1979; Kahn & Wetzler 1991; Fontaine 1993; Takeshita & Yamaguchi 1995). These results provide support for the hypothesis that the antinociceptive action of **3** and **4** is partly a central event mediated through 5-hydroxytryptamine.

Table 5. Analgesic effect of subcutaneous morphine (0·15 mg kg^{-1}) in the phenylbenzoquinone test after acute intraperitoneal administration of **3**, **4**, trazodone and fluoxetine.

Compound	Dose (mg kg^{-1})	Analgesia (%)		
		Morphine + saline	Drug + saline	Drug + morphine
		7·5 ± 7·0		
3	5		28·7 ± 7·3	36·6 ± 10·1*
4	5		35·9 ± 7·6	55·3 ± 5·8*
Trazodone	2		10·3 ± 6·4	43·0 ± 12·2*
Fluoxetine	3		20·1 ± 2·9	30·0 ± 3·9*

Results are means ± s.e.m. *$P < 0.05$, significantly different from result for morphine + saline.

Table 6. Effect of subcutaneous naloxone (1 mg kg^{-1}) on analgesia induced by **3**, **4**, morphine, trazodone and fluoxetine in the phenylbenzoquinone test.

Compound	Dose (mg kg^{-1})	Analgesia (%)	
		Drug + saline	Drug + naloxone
3	15	64·0 ± 10·9	19·5 ± 16·3*
4	15	59·5 ± 12·3	59·9 ± 8·9
Morphine	1·5	77·2 ± 3·9	14·1 ± 9·2*
Trazodone	5	41·8 ± 6·0	58·3 ± 9·4
Fluoxetine	30	68·6 ± 7·2	68·0 ± 4·1

Compounds were administered intraperitoneally except for morphine (subcutaneously). Results are means ± s.e.m. *$P < 0.05$, significantly different from result for drug + saline.

Because activation of the α_2-adrenoreceptors in the central nervous system results in antinociception (Luttinger et al 1985; Suh et al 1995), we examined the capacity of the α_2-adrenergic antagonist yohimbine to prevent the analgesic effect elicited by intraperitoneal administration of test drugs in the phenylbenzoquinone test. It seemed that yohimbine clearly attenuated the antinociceptive effects of **3** and **4** and that of the sympathomimetic agent clonidine (Table 8). Accordingly, the noradrenergic system also participates in inhibition of chemical nociception by **3** and **4**.

In conclusion, these results provide evidence for the involvement of noradrenergic and 5-hydroxytryptaminergic pathways in the analgesic activity of **3** and **4**. This non-opioid mechanism is probably related to enhancement of the release of 5-hydroxytryptamine and noradrenaline or to inhibition of the neuronal re-uptake of the compounds. In addition, an opioid component of the antinociceptive effect of **3** was evinced by administration of naloxone. Because of their potential effectiveness, thiazolotriazine derivatives **3** and **4** might be suitable for a wide variety of painful conditions and could represent attractive reserve agents for patients dissatisfied with opioids.

References

Ardid, D., Marty, H., Fialip, J., Privat, A. M., Eschalier, A., Lavarenne, J. (1992) Comparative effects of different uptake inhibitor antidepressants in two pain tests in mice. Fundam. Clin. Pharmacol. 6: 75–82

Biegon, A., Samuel, D. (1980) Interaction of tricyclic antidepressants with opiate receptors. Biochem. Pharmacol. 29: 460–461

Boissier, J. R., Simon, P. (1965) Action de la caféine sur la motilité spontanée de la souris. Arch. Int. Pharmacodyn. Ther. 158: 212–221

Bordi, F., Mor, M., Plazzi, P. V., Silva, C., Morini, G., Impicciatore, M., Barocelli, E., Chiavarini, M. (1992) 4-(1,2-Benzisothiazol-3-yl)alkanoic and phenylalkanoic acids: synthesis and anti-inflammatory, analgesic and antipyretic activities. Il Farmaco 47: 551–565

Table 7. Potentiation by intraperitoneal 5-hydroxytryptophan (50 mg kg^{-1}) + carbidopa (25 mg kg^{-1}) of analgesia induced by intraperitoneal administration of **3**, **4**, trazodone and fluoxetine in the phenylbenzoquinone test.

Compound	Dose (mg kg^{-1})	Analgesia (%)		
		Drug + saline	5-Hydroxytryptophan +carbidopa	Drug+ 5-hydroxytryptophan carbidopa
			29·5 ± 16·0	
3	2	3·3 ± 2·1		51·7 ± 13·5*
4	2	4·2 ± 2·9		64·8 ± 12·3*
Trazodone	2	10·1 ± 5·9		47·2 ± 8·9
Fluoxetine	3	19·8 ± 2·8		51·3 ± 5·4*

Results are means ± s.e.m. *$P < 0.05$, significantly different from result for drug + saline.

Table 8. Effect of oral yohimbine (1 mg kg^{-1}) on analgesia induced by intraperitoneal administration of **3**, **4** and clonidine in the phenylbenzoquinone test.

Compound	Dose (mg kg^{-1})	Analgesia (%)	
		Drug + saline	Drug + clonidine
3	10	66·2 ± 4·9	35·6 ± 11·2*
4	15	70·6 ± 7·1	49·7 ± 3·6*
Clonidine	0·05	95·2 ± 4·6	37·5 ± 7·4*

Results are means ± s.e.m. *$P < 0.05$, significantly different from result for drug + saline.

Caccia, S., Garattini, S. (1990) 1-Arylpiperazines as active metabolites of (4-substituted aryl-1-piperazinyl) alkyl heterocyclic drugs. Acta Pharm. Jugosl. 40: 441–460

Caliendo, G., Di Carlo, R., Greco, G., Meli, R., Novellino, E., Perissuti, E., Santagada, V. (1995) Synthesis and biological activity of benzotriazole derivatives structurally related to trazodone. Eur. J. Med. Chem. 30: 77–84

Erdogan, H., Debaert, M., Cazin, J. C. (1991) Synthesis of some 2-benzoxazolinone derivatives and their analgesic properties. Arzneim. Forsch. 41: 73–76

Eschalier, A. (1990) Antidepressants and pain management. In: Besson, J. M. (ed.) Serotonin and Pain. Excerpta Medica, ICS 879, 305–325

Fialip, J., Marty, H., Makambila, M. C., Civiale, M. A., Eschalier, A. (1989) Pharmacokinetic patterns of repeated administration of antidepressants in animals. Their relevance in a study of the influence of clomipramine on morphine analgesia in mice. J. Pharmacol. Exp. Ther. 248: 747–751

Flouzat, C., Bresson, Y., Mattio, A., Bonnet, J., Guillaumet, G. (1993) Novel nonopioid non-antiinflammatory analgesics: 3-(aminoalkyl)- and 3-[(4-aryl-1-piperazinyl)alkyl]oxazolo[4,5-*b*]pyridin-2(3*H*)-ones. J. Med. Chem. 36: 497–503

Fontaine, R. (1993) Novel serotonergic mechanisms and clinical experience with nefazodone. Clin. Neuropharmacol. 16 (Suppl. 3): S45–S50

Fuller, R. W., Snoddy, H. D., Mason, N. R., Molloy, B. B. (1978) Effect of 1-(*m*-trifluoromethylphenyl)-piperazine on ^{3}H-serotonin binding to membranes from rat brain in vitro and on serotonin turnover in rat brain in vivo. Eur. J. Pharmacol. 52: 11–16

Gökhan, N., Erdogan, H., Tel, B., Demirdamar, R. (1996) Analgesic and antiinflamatory activity screening of 6-acyl-3-piperazinomethyl-2-benzoxazoline derivatives. Eur. J. Med. Chem. 31: 625–628

Granados-Soto, V., Flores-Murrieta, F. J., Castaneda-Hernandez, G., Hong, E., Lopez-Munoz, F. J. (1995) Evidence against the participation of μ- and κ-opioid receptors in the analgesic activity of ketorolac in rats. J. Pharm. Pharmacol. 47: 514–517

Groppetti, A., Braga, P. C., Biella, G., Parenti, M., Rusconi, L., Montegazza, P. (1988) Effect of aspirin on serotonin and met-enkephalin in brain: correlation with the antinociceptive activity of the drug. Neuropharmacology 28: 499–505

Isenberg, K. E., Cicero, T. J. (1984) Possible involvement of opiate receptors in the pharmacological profile of antidepressant compounds. Eur. J. Pharmacol. 103: 57–63

Issartel, V., Spenher, V., Coudert, P., Seilles, E., Couquelet, J. (1998) Synthesis of new pyrrolo[1,2-*d*][1,2,4]triazines and thiazolo[3,4-*d*][1,2,4]triazines as immunostimulating agents. Bioorg. Med. Chem. 6: 349–354

Kahn, R. S., Wetzler, S. (1991) *m*-Chlorophenylpiperazine as a probe of serotonin function. Biol. Psychiatry 30: 1139–1166

Linee, P. (1972) Test des crampes abdominales provoquées chez la souris par la phénylbenzoquinone. J. Pharmacol. (Paris) 3: 513–515

Litchfield, J. T., Wilcoxon, E. (1949) A simplified method of evaluating dose-effects experiments. J. Pharmacol. Exp. Ther. 96: 99–113

Luttinger, D., Ferrari, R., Perrone, M. H., Haubrich, D. R. (1985) Pharmacological analysis of α_2-adrenergic mechanisms in nociception and ataxia. J. Pharmacol. Exp. Ther. 232: 883–889

Maj, J., Palider, W., Rawlow, A. (1979) Trazodone a central serotonin antagonist and agonist. J. Neural Transm. 44: 237–248

Mavel, S., Rubat, C., Coudert, P., Privat, A. M., Couquelet, J., Tronche, P., Bastide, P. (1993) Synthesis of new 5-substituted pyrazolo [1,5-*d*][1,2,4]triazine derivatives and their analgesic, anti-inflammatory and antipyretic properties. Arzneim. Forsch. 43: 464–468

Moreau, S., Coudert, P., Rubat, C., Albuisson, E., Couquelet, J. (1996) Synthesis of peripherally acting analgesic 3-aryl piperazinyl-5-benzyl-pyridazines. Arzneim. Forsch. 46: 800–805

Nevinson, M. J., Gray, A. M., Sewell, R. D. E. (1991) Antagonism of nefopam anti-nociception by naloxone and RX821002. Br. J. Pharmacol. 104 (Proc. Suppl.): 267P

Palagiano, F., Arenare, L., Luraschi, E., De Caprarus, P., Abignente, E., D'Amico, M., Filipelli, W., Rossi, F. (1995) Research on heterocyclic compounds. XXXIV. Synthesis and SAR study of some imidazo[2,1-*b*]thiazole carboxylic and acetic acids with antiinflamatory and analgesic activities. Eur. J. Med. Chem. 30: 901–909

Patel, S. S., Dunn, C. J., Bryson, H. M. (1996) Epidural clonidine: a review of its pharmacology and efficacy in the management of pain during labour and postoperative and intractable pain. CNS Drugs 6: 474–497

Pick, C. G., Paul, D., Eison, M. S., Pasternak, G. W. (1992) Potentiation of opioid analgesia by the antidepressant nefazodone. Eur. J. Pharmacol. 211: 375–381

Pini, L. A., Vitale, G., Ottani, A., Sandrini, M. (1997) Naloxone-reversible anti-nociception by paracetamol in the rat. J. Pharmacol. Exp. Ther. 280: 934–940

Raffa, R. B., Nayak, R. K., Liao, S., Minn, F. L. (1995) The mechanism(s) of action and pharmacokinetics of tramadol hydrochloride. Rev. Contemp. Pharmacother. 6: 485–497

Riblet, L. A., Taylor, D. P. (1981) Pharmacology and neurochemistry of trazodone. J. Clin. Psychopharmacol. 1 (Suppl. 6): 17S–22S

Rohet, F., Rubat, C., Coudert, P., Albuisson, E., Couquelet, J. (1996) Synthesis and trazodone-like analgesic activity of 4-phenyl-6-aryl-2-[3-(4-arylpiperazin-1-yl)propyl]pyridazin-3-ones. Chem. Pharm. Bull. 44: 980–986

Rubat, C., Coudert, P., Bastide, P., Tronche, P. (1995) Behavioural profile of two potential antidepressant pyridazine derivatives including arylpiperazinyl moieties in their structure, in mice. J. Pharm. Pharmacol. 47: 162–170

Schmitt, H., Le Douarec, J. C., Petillot, N. (1974) Antinociceptive effects of some α-sympathomimetic agents. Neuropharmacology 13: 289–294

Schwartz, D. (1984) Méthodes statistiques l'usage des médecins et des biologistes. Editions Médicales Flammarion, Paris, pp 263–307

Siegmund, E., Cadmus, R., Lu, G. (1957) A method for evaluating both non-narcotic and narcotic analgesic. Proc. Soc. Exp. Biol. Med. 95: 729–731

Sladowska, H., Potoczek, J., Sieklucka-Dzuiba, M., Rajtar, G., Mlynarczyk, M., Kleinrok, Z. (1995) Investigations on the synthesis and properties of some *N*-arylpiperazinylalkyl derivatives of 1,4-dioxo (1,4,5-trioxo)-1,2,3,4-tetra(and 1,2,3,4,5,6,hexa)hydro-pyrido[3,4-d]pyridazines. Il Farmaco 50: 37–46

Somoza, E., Galindo, A., Bazan, E., Guillamon, A., Valencia, A., Fuentes, J. A. (1981) Antidepressants inhibit enkephalin binding to synaptosome-enriched fractions of rat brain. Neuropsychobiology 7: 297–301

Suh, H. W., Song, D. K., Choi, Y. S., Lee, B. Y., Kim, Y. H. (1995) Spinal serotoninergic and noradrenergic receptors are partially involved in immobilization stress-induced anti-nociception in the mouse. Pharmacol. Commun. 7: 71–77

Taiwo, Y., Levine, J. D. (1988) Prostaglandins inhibit endogenous pain control mechanisms by blocking at spinal noradrenergic synapses. J. Neurosci. 8: 1346–1349

Takeshita, N., Yamaguchi, I. (1995) *Meta*-chlorophenylpiperazine attenuates formalin-induced nociceptive responses through 5-$HT_{1/2}$ receptors in both normal and diabetic mice. Br. J. Pharmacol. 116: 3133–3138

Valeri, P., Pimpinella, G., Morrone, L. A., Romanelli, L. (1991) Anti-nociceptive effects of trazodone and *m*-chlorophenylpiperazine (mCPP) in mice: interaction with morphine. Gen. Pharmacol. 22: 127–131

Viaud, M. C., Jamoneau, P., Flouzat, C., Bizot-Espiard, J. G., Pfeiffer, B., Renard, P., Caignard, D. H., Adam, G., Guillaumet, G. (1995) *N*-Substituted oxazolo-[5,4-b]pyridin-2(1*H*)-ones: a new class of non-opiate anti-nociceptive agents. J. Med. Chem. 38: 1278–1286

Vonvoigtlander, P. F., Lewis, R. A., Neff, G. L. (1984) Kappa opioid analgesia is dependant on serotoninergic mechanisms. J. Pharmacol. Exp. Ther. 231: 270–274

Woolfe, G., McDonald, A. D. (1944) Evaluation of the analgesic action of pethidine-hydrochloride. J. Pharmacol. Exp. Ther. 80: 300–307