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Pharmacological properties and SAR of new 1,4-disubstituted piperazine derivatives with hypnotic–sedative activity

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Abstract

Preparation, pharmacological properties and structure–activity relationships of new pyrimidylpiperazine derivatives, exhibiting sedative and hypnotic activity in mice, are reported. The hypnotic activity of the compounds was comparable with that of zopiclone (the known hypnotic–sedative agent), their interaction with ethanol, however, being much lower. The obtained results suggested that zopiclone and pyrimidylpiperazines **2**, **4** and **5** exerted their pharmacological activity through a different mechanism – zopiclone through the interaction with benzodiazepine receptors and compounds **2**, **4** and **5** through an unidentified molecular target. The pharmacological properties of compound **3** could be the result of a mixed mechanism of action, combining the properties of zopiclone and those of compounds **2**, **4** and **5**. A common feature of zopiclone and compounds **2** and **3** was that, after their systemic administration, independently of mechanism of action, together with the hypnotic effect a reduction of the 5-HT turnover in the mouse brain was observed. Minimum structural requirements for the hypnotic activity were formulated. Structural considerations have shown that removing the α-carbonyl group did not influence the drug's ability to inhibit the locomotor activity. However, it did influence its ability to disturb motor coordination or abolish the righting reflex within non-lethal doses.

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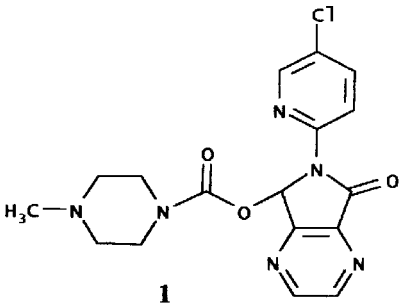
Introduction

Compounds possessing hypnotic and sedative activity in general form a common functional group exhibiting specific action on the CNS. They produce sedative effects, in higher doses causing hypnosis and even general anaesthesia. The compounds belong to diverse chemical classes and their mechanism of action is somewhat unclear although it can be said that they (particularly barbiturates) produce a stabilising effect on neuronal membranes. It is now apparent that a variety of hypnotic–sedatives and general anaesthetics have potent actions on certain ion channels or neurotransmitter receptors. One action common to a variety of general anaesthetics is the potentiation of the inhibitory synaptic transmission mediated by gamma aminobutyric acid A ($GABA_A$) receptor complex (Lovinger et al 1993). The two main classes of hypnotic–sedative agents are benzodiazepines and barbiturates. The benzodiazepines, which are used as hypnotics, sedatives and anxiolytics bind to the benzodiazepine site of the $GABA_A$ receptor complex and



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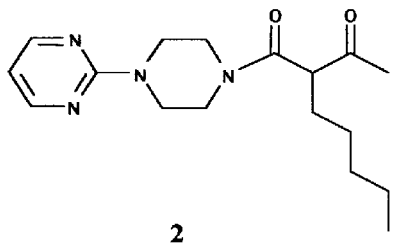

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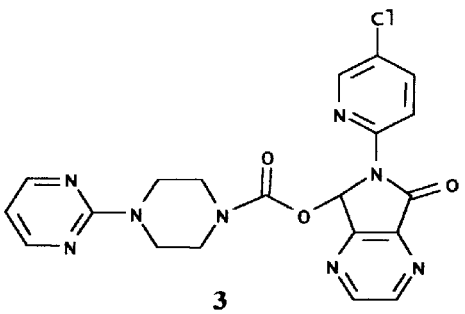

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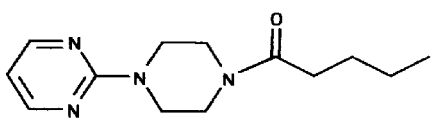

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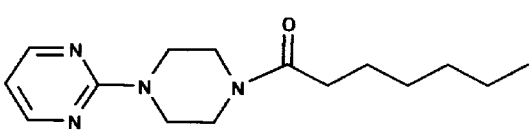

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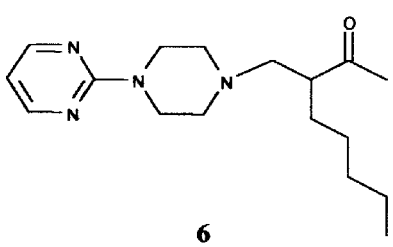

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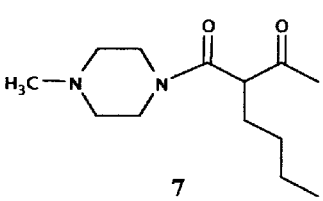

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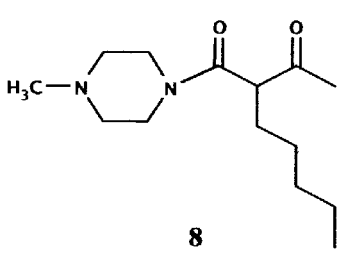

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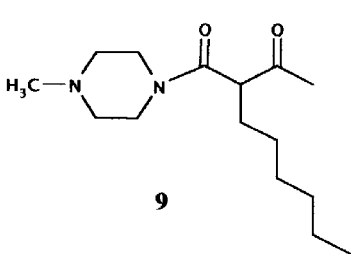

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Figure 1 Structure of compounds **1**–**9**.

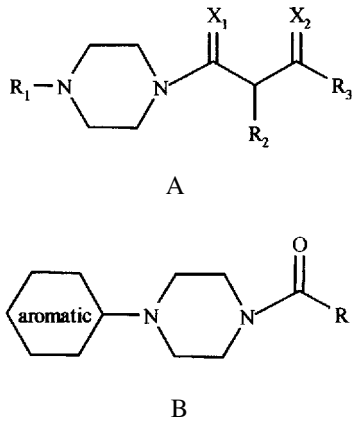

Figure 2 Considered pharmacophoric elements (A) and minimum structural requirements for hypnotic activity (B) of pyrimidylpiperazine derivatives.

enhance the binding of its neurotransmitter, gamma-aminobutyric acid (GABA) (Haefely 1989; Hobbs et al 1996). Barbiturates also modulate the GABA neurotransmission (Olsen 1987). The antagonistic effects of some non-volatile anaesthetics on excitatory ionotropic *N*-methyl-D-aspartate (NMDA) glutamate receptors are supposed to be involved in their behavioural actions (Lodge & Johnson 1990; Irifune et al 1992; Mihic & Harris 1997 and references therein). There is also evidence of involvement of some biogenic amines in the state of sleep and arousal although their role is not yet completely understood.

It is well recognised that central histamine controls some behavioural effects and many antihistamines produce drowsiness and impaired performance. Of particular interest are classical antihistamines, antagonists of H_1-histamine receptors, which readily cross the blood–brain barrier. Their central effects, particularly sedation, have been recognised for some time (for a review see White & Rumbold 1988). Studies in man with the enantiomers of chlorphenamine (chlorpheniramine) and dimethindene strongly suggest that the sedation can arise from H_1-receptor antagonism alone (Nicholson et al 1991).

Serotonin (5-HT) seems to play an important role in sleep regulation. It is known that neuronal serotonin has been implicated in various physiological functions, including sleep, sexual and aggressive behaviour, thermoregulation, locomotion, feeding, pain modulation, learning and memory (Vogt 1982). Portas & McCarley (1994) found that in freely moving cats the serotonin level in the dorsal raphe nucleus was decreased by 50% during slow-wave sleep and to 38% during REM (rapid eye movement) sleep (as compared with waking). It was also found that a single injection of *p*-chlorophenylalanine (which inhibits one of the early steps of serotonin synthesis) produced total insomnia in the cat and that the administration of 5-hydroxytryptophan, a precursor of serotonin, reversed the insomnia (Jouvet 1969; Pujol et al 1971; Petitjean et al 1985), suggesting that serotonin is important for sleep. Imeri et al (1994) reported that the extracellular amount of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), measured in-vivo in freely moving rats, increased with wakefulness and decreased with sleep. Also, during pharmacologically induced sleep caused by a hypnotic dose of flunitrazepam, reduction of 5-HIAA levels in rat brain was observed (de St Hilare-Kafi & Gaillard 1988). It is assumed that if increased transmission by a particular monoamine is responsible for sleep after sleep deprivation (when the propensity for sleep is high), transmission by that amine should be elevated in the brain regions responsible for the sleep induction. On the other hand, if sleep is responsible for restoring a monoamine system, deprivation should reduce transmission by that amine (Bergmann et al 1994). Reported changes in levels of serotonin and its metabolite following sleep deprivation have been inconsistent. Thus, no change (Tsuchiya et al 1969; Stern et al 1971; Wasemann & Weiner 1982; Wasemann et al 1983), decrease (Weiss et al 1968; Tsuchiya et al 1969; Schildkraut & Hartmann 1972) and increase (Hernández-Peón et al 1969; Cramer et al 1973) in serotonin levels have all been observed in the brain of sleep deprived rats. For the serotonin metabolite, 5-HIAA, increase (Weiss et al 1968; Bliss et al 1972; Cramer et al 1973; Wojcik & Radulovacki 1981) or no change (Borbély et al 1980; Wasemannn & Weiner 1982; Wasemann et al 1983) in the levels was observed. Serotonin turnover was, however, found to increase in the brain of a sleep-deprived rat and hamster (Hery et al 1970; Toru et al 1984; Asikainen et al 1995, 1997).

In our previous papers (Chilmonczyk et al 1995, 1996) we described preparative, pharmacological and QSAR (quantitative structure–activity relationship) studies of several 1,4-disubstituted piperazine derivatives, a new class of hypnotic–sedatives. Here we report the comparison of pharmacological data between some new 1-(2-pyrimidyl)piperazine derivatives (**3**–**9**; Figure 1; obtained according to Mazgajska (2001)) and the known hypnotic–sedative agent zopiclone (Karen & Rennie 1986) (**1**) as well as the most active in the previously described series, compound **2** (patent PL 300318). Our previous results concerned compounds of general structure A (Figure 2) possessing aromatic (R_1 = 2-pyrimidyl) and piperazine rings, α,β-dicarbonyl

arrangement ($X_1 = X_2 = O$) and an alkyl or alkoxy substituent (or both) (R_2 = alkyl, R_3 = Me or OEt). In this study, the importance of the above substituents in compounds **3**–**9** for the hypnotic–sedative activity was evaluated in-vivo. The other part of the work concerns the determination of serotonin levels and those of its metabolite in the brain of sleeping mice after the administration of compounds **1**, **2** and **3**. To examine possible molecular targets of the compounds' hypnotic activity, binding studies to benzodiazepine, $GABA_A$, NMDA, $glycine_B$ and H_1-histamine receptors were performed. Additionally, the influence on the second messengers cyclic adenosine monophosphate (cyclic AMP) and inositol triphosphate (IP_3) production was determined.

Materials and Methods

Behavioural experiments

All the behavioural experiments with animals were performed in accordance with the Polish governmental regulations (decree on animals protection DZ.U. 97. 111.724, 1997).

The experiments were conducted on male Albino-Swiss mice (20–28 g). The mice were kept at room temperature of 20–21°C on a natural day–night cycle; they were housed in plastic boxes (55 × 35 × 20 cm) in groups of 20, with free access to food and water throughout the experiment. Experimental and control groups consisted of 10 mice each. The tested compounds were administered intraperitoneally as suspensions in a 1% Tween 80 (compounds **1**–**3** and **5**) or as solutions in physiological saline (compounds **4** and **6**–**9**) in a volume of 10 mL kg^{-1}. The control groups of mice received the same amounts of the solvent. ED50 (effective dose) or LD50 (lethal dose) values were calculated on the basis of the effect of at least 3 doses, according to the Litchfield & Wilcoxon method (1949).

Spontaneous locomotor activity
The spontaneous locomotor activity of single mice was measured for 30 min in photoresistor actometers at 1 h after administration of the tested compounds.

Rota-rod test
Preselected mice (those staying on a rotating rod for 2 min) were placed on a rotating rod and observed for 2 min. The number of mice falling from the rota-rod was recorded. The studied compounds were administered 1 h before the test.

Righting reflex
The loss of the righting reflex for at least 15 s was accepted as a criterion of a sedative or general anaesthetic action. Observations were conducted immediately after the administration of the tested compounds. In those mice in which the righting reflex was abolished after treatment with the tested compounds, spinal reflexes (a reaction to pinching a distal part of the tail) and the corneal reflex (abolition of the reflex occurs when the irritation of the cornea of both eye balls for 1 s does not produce eyelid closing) were checked.

Ethanol narcosis time
The ethanol narcosis time was regarded as the period during which the mice lost the righting reflex. The tested compounds were administered 1 h before the injection of ethanol (4 g kg^{-1}, i.p., as 40% solution).

Convulsions
Convulsions were induced by pentetrazole (80 mg kg^{-1}, i.p.) at 1 h after the administration of the tested compounds. The number of mice reacting with clonic convulsions was recorded.

Four-plate test
A four-plate test was carried out according to the method of Aron et al (1971). The number of punished crossings was calculated at 1 h after the administration of the tested compounds.

Labelled ligands

[^{3}H]Pyrilamine, [^{14}C]cyclic AMP, [^{3}H]5,7-DCKA and [^{3}H]CGP-39653 were obtained from DuPont-NEN. [^{3}H]Adenine, [^{3}H]flunitrazepam, [^{3}H]muscimol and the kit for selective IP_3 determination (TRK 1000 [^{3}H]IP_3-RRA) were purchased from Amersham.

Binding data

Male Wistar rats (180–230 g) were used in all binding ($GABA_A$, benzodiazepine, NMDA, $glycine_B$ and H_1 receptors) and cyclic AMP and phosphatidylinositol (PI) turnover experiments. Experiments were repeated three times for each compound.

Benzodiazepine receptors
The experiment was performed according to a method described previously by Sabato et al (1981) using [^{3}H] flunitrazepam. The radioactivity was assayed in a Beckman LS 6000TA counter.

$GABA_A$ receptors
The experiment was performed according to a method described previously by Olpe et al (1990) using [^{3}H] muscimol. The radioactivity was assayed in a Beckman LS 6000TA counter.

NMDA receptors
The binding assay was performed as described by Nowak et al (1996) using [^{3}H]CGP-39653 (48.9 Ci mmol^{-1}). The radioactivity was assayed in a Beckman LS 6000TA counter.

$Glycine_B$ receptors
The method was performed as described by Nowak et al (1996) using [^{3}H]5,7-DCKA (14 Ci mmol^{-1}). The radioactivity was assayed in a Beckman LS 6000TA counter.

Histamine H_1 receptors
The experiment was performed according to a method described previously by Agut et al (1997) using [^{3}H] pyrilamine (20 Ci mmol^{-1}). The radioactivity was measured in a Beckman LS 3801 counter.

Assay of cyclic AMP formation
Assay of cyclic AMP formation was carried out by the method described in detail by Nowak & Sek (1994) on rat striatum and cerebral cortex. Some experiments were carried out on guinea-pig cerebral cortex tissue. The synthesis of [^{3}H]cyclic AMP in brain slices prelabelled with [^{3}H]adenine was determined by the method of Shimizu et al (1969). The [^{3}H]cyclic AMP formed was isolated by a sequential co-chromatography (Dowex 50W$\times$4 and aluminium oxide columns) with a tracer [^{14}C]cyclic AMP (52.3 Ci mol^{-1}), used for the measurement of the recovery of each assay; the mean recovery was 48–50%) according to the method of Salomon et al (1974). The final eluate was tested for radioactivity in a liquid scintillation counter (^{3}H/^{14}C channel). The data presented were corrected for recovery and expressed as percent conversion ([^{3}H]cyclic AMP$\times$100/total ^{3}H). Basal activity – 0.28$\pm$0.07(6).

Stimulation of hydrolysis of membrane phosphatidylinositols
This method was used as described previously by Brown et al (1984) with a modification of Nalepa et al (1990) using [^{3}H] *myo*-inositol solution. Radioactivity was measured in a scintillation counter (Pharmacia, Wallac 1410). Carbachol and histamine were used as the reference compounds and stimulated the basic IP_3 production by 220–360% (depending on the experiment).

Determination of blood and brain [^{3}H][1-(4-pyrimidin-2-yl-piperazin-1-yl)-2-pentylbutan-1,3-dione] ([^{3}H]2) levels

The [^{3}H]**2** levels in the rat brain were determined according to a previously described general procedure (Brown et al 1986). Male Wistar rats (120–140 g) were used in the experiments. The rats were kept in standard environmental conditions (a natural day–night cycle, with free access to food and water). [H^3]**2** (8.5 mCi mmol^{-1}) was administered intraperitoneally as a suspension in 1% Tween 80 at a dose 154.8 mg kg^{-1} (containing 7.74 mg of [H^3]**3**). At 1, 3, 5, 10, 20, 30, 60, 120 and 180 min following injection, rats were killed by decapitation. The blood was collected, centrifuged and 0.5 mL of the plasma was placed into scintillation vials (in duplicate). The brains were immediately removed, and the hippocampus (left and right), striatum (left and right), hypothalamus and two fragments of cerebral cortex were dissected out. The tissue samples were washed in physiological saline (cleaned from peripheral vasculature if necessary), dried on a piece of paper, weighed and placed into scintillation vials. Each tissue sample (blood and brain) was suspended in 0.5 mL of tissue solubilizer (Soluene-350; Packard) and left overnight at room temperature. Then each sample was treated with 10 mL of scintillator fluid (Hionic – Fluor, Packard) and submitted to tritium measurement with a liquid scintillation counter (Wallac 1410, Pharmacia). The penetration of the drug into the brain was estimated as the ratio of radioactivity in the brain tissue (measured per 1 g of the tissue) to that in peripheral blood (measured per 1 mL of the plasma).

Determination of serotonin and 5-HIAA levels

Serotonin and 5-HIAA contents in the frozen tissue samples were measured by HPLC with electrochemical detection according to procedure described elsewhere (Gołembiowska 1990; Nowak et al 1992) on male Albino-Swiss mice (20–28 g). The mice were kept in standard environmental conditions (a natural day–night cycle, with free access to food and water). Compounds **1**–**3** were injected intraperitoneally in the form of 1% suspension in Tween 80 (1 mL/mouse). For biochemical assays the mice were killed by decapitation and serotonin and 5-HIAA levels were determined by HPLC with electrochemical detection. A Waters 616 liquid chromatograph with Waters 464 electrochemical detector was equipped with Waters Spherisorb 10$\times$4.6 mm guard cartridge and 5 μm Waters Spherisorb ODS2 250$\times$ 4.6 mm column. The mobile phase consisted of 31.2 g NaH_2PO_4, 0.32 g heptanesulfonic acid, 0.6 g ethylene

diamine tetra-acetic acid (EDTA), 130 mL acetonitrile, water up to 2 L adjusted to pH 2.88 with 3 M H_3PO_4. The flow rate was maintained at 1 mL min^{-1}. The applied voltage was set at 0.60 V vs Ag/AgCl electrode. The compounds were quantified by peak height comparison with standard run on the day of the analysis, with a sensitivity of 3–100 pg.

Statistics

The statistical significance of the results was calculated using *t*-test (*STATISTICA*, StatSoft).

Results and Discussion

The results of pharmacological tests obtained for compounds **1**–**9** are summarized in Tables 1 and 2. All the new pyrimidylpiperazines tested (**3**–**9**) showed a sedative effect on spontaneous locomotor activity of mice, and their ED50 values ranged from 50 (**3**) to 88 mg kg^{-1} (**8**). In that test, the potency of compound **1** (zopiclone), used as a reference drug, was the highest (ED50 12 mg kg^{-1}). Compounds **2**–**5** in doses 3–6 times (ED50 195 (**5**)–300 (**3**) mg kg^{-1}) those that reduced the locomotor activity disturbed the locomotor coordination of mice on the rota-rod; the rest of the compounds, in doses up to 200 or 300 mg kg^{-1}, did not change that behaviour of mice despite the fact that in a dose of 200 (**6**, **8**, **9**) or 300 mg kg^{-1} (**7**) they caused death of 20–40% of mice. Zopiclone (**1**) disturbed the motor coordination of mice with an ED50 value of 51 mg kg^{-1}, which was 4 times the dose at which the locomotor activity was reduced by 50%. Compounds **1**–**5**, but not **6**–**9**, given in high doses (ED50 175 (**2**)–200 mg kg^{-1}) induced the abolition of the righting reflex (soon after their administration) which lasted up to 30 min after the administration of the highest doses. At the same time compounds **1**–**5** did not affect the spinal reflexes.

In the potentiation of ethanol narcosis time, compounds **1**–**5** exhibited differentiated activity. The

Table 1 ED50 (effective dose) (mg kg^{-1}, i.p.) values for central depressant effects of compounds **1**–**9** in mice.

Compound	Inhibition of locomotor activity[a]	Disturbances of motor coordination[a] (rota-rod test)	Abolition of the righting reflex	Prolongation of ethanol narcosis time[a]
1	12 (8.3–17.4)	51 (40.2–64.8)	225 (155.2–362.3)	2.3 (1.7–3.2)
2	28 (18.1–43.4)	210 (156.7–281.4)	175 (126.8–241.5)	85 (63.0–114.8)
3	50 (29.4–85.0)	300 (258.6–348.0)	200 (173.9–264.5)	20 (15.0–25.6)
4	73 (8.0–111.0)	260 (244.4–276.6)	200 (181.8–222.0)	30 (23.1–39.0)
5	65 (45.8–92.3)	195 (169.1–224.8)	185 (156.8–218.3)	> 100
6	55 (36.7–82.5)	> 200[b]	> 200[b]	NT
7	60 (40.0–90.0)	> 300[b]	> 300[b]	NT
8	88 (65.2–118.8)	> 200[b]	> 200[b]	NT
9	75 (53.6–105.0)	> 200[b]	> 200[b]	NT

95% Confidence limits are given in parentheses. [a]The compounds were administered 1 h before the tests. [b]Lethal doses. NT, not tested.

Table 2 Acute toxicity (lethal dose (LD50), mg kg^{-1}, i.p.) of compounds **1**–**5** in mice after 24 h, 48 h and 7 days.

Compound	Time		
	24 h	48 h	7 days
1	750 (675.7–832.5)	640 (598.1–684.8)	655 (595.5–720.5)
2	550 (458.3–660.0)	400 (333.3–480.0)	300 (230.8–390.0)
3	910 (805.3–1028.3)	850 (720.3–1003.0)	850 (772.7–935)
4	465 (422.7–511.5)	415 (360.9–477.3)	390 (333.3–456.3)
5	280 (233.3–336.0)	270 (228.8–318.6)	135 (112.5–162.0)

95% Confidence limits are given in parentheses.

Table 3 Therapeutic indices (LD50[a]/ED50) for compounds **1**–**5** in mice.

Compound	1	2	3	4	5
Inhibition of locomotor activity	62.5	19.6	18.2	6.4	4.3
Disturbance of motor coordination	14.7	2.6	3	1.8	1.4
Abolition of the righting reflex	3.3	3.1	4.6	2.3	1.5
Prolongation of ethanol sleeping time	326.1	6.5	45.5	15.5	< 2.8
Anticonvulsant activity	131.6	—	11.4	—	—
Potential anxiolytic activity (four-plate test)	75[b]	—	—	—	—

[a] After 24 h. [b] LD50/MED (lethal dose/minimum effective dose) (10 mg kg^{-1}).

strongest interaction was shown by compound **1** which in low doses increased the ethanol narcosis time in mice (ED50 2.3 mg kg^{-1}). A weaker effect was induced by compounds **3** and **4** (ED50 20 mg kg^{-1} and 30 mg kg^{-1}, respectively) and the effect of compounds **2** and **5** was slight (ED50 85 mg kg^{-1} and >100 mg kg^{-1}, respectively). However, within the series of the tested pyrimidylpiperazines **2**–**5**, only compound **3** inhibited convulsions produced by pentetrazole (ED50 80 mg kg^{-1}). A more potent anticonvulsant activity was produced by zopiclone (**1**; ED50 5.7 mg kg^{-1}). Compounds **2**–**9** at doses up to 30 mg kg^{-1} did not reveal potential anxiolytic properties in the four-plate test (data not shown), while compound **1** (2.5–15 mg kg^{-1}) dose-dependently increased the number of punished crossings in that test ($F(4.42) = 7.39$, $P < 0.001$), its MED (minimum effective dose) being 10 mg kg^{-1}. Acute toxicity (LD50) was evaluated for compounds **1**–**5** (Table 2), but not for **6**–**9,** which caused death of mice after their administration in doses of 200–300 mg kg^{-1}. The lowest toxicity was shown by compounds **1** and **3**: their LD50 values (after 24 h) were 750 mg kg^{-1} and 910 mg kg^{-1}, respectively, and practically did not change throughout a 7-day observation period. The toxicity of compounds **2** and **4** was at a medium level (ED50 ~ 500 mg kg^{-1}) and increased at 48 h and 7 days after their administration. The highest toxicity was revealed by compound **5**: its LD50 value was 280 mg kg^{-1} after 24 h and increased to twice that after 7 days. The ED50 values for zopiclone (**1**), determined in the locomotor activity, rota-rod and pentetrazole-induced convulsions tests, as well as its MED for the anxiolytic-like activity (a four-plate test), were similar to those quoted by other authors (Julon et al 1985; Perrault et al 1990). On the other hand, the LD50 value for zopiclone, determined in our study, was significantly lower than that quoted by Julon et al (1985).

The pharmacological profile of the most interesting pyrimidylpiperazines (**2**–**5**) shows that the compounds, like zopiclone (**1**), display features characteristic for sedative (an inhibition of the spontaneous locomotor activity, disturbances of motor coordination and the prolongation of ethanol narcosis time) and hypnotic drugs (abolition of the righting reflex). However, their therapeutic indices (LD50/ED50) are differentiated (Table 3). Compounds **1**–**3** appear to have the most favourable indices: for the abolition of the righting reflex the therapeutic indices for the three compounds were similar (3.3, 3.1 and 4.6, respectively); for the disturbances in the locomotor coordination and for the inhibition of the locomotor activity compounds **3** (2.6 and 18.2, respectively) and **2** (3 and 19.6, respectively) had, however, considerably lower indices than zopiclone (**1**; 14.7 and 62.5, respectively). On the other hand, compounds **2** and **3** showed a much weaker interaction with ethanol, their therapeutic indices being, respectively, 50 and 7 times lower than that for zopiclone.

The sedative–hypnotic activity of compounds **2** and **5** seems to be unspecific. Unlike zopiclone (**1**; IC50 175 nM) and compound **3** (IC50 1260 nM), compounds **2** and **5** did not show an affinity for the benzodiazepine receptor (IC50 > 10000). Compound **2** also did not exhibit an affinity for the $GABA_A$, NMDA, $glycine_B$ and H_1-receptors (IC50 > 10000). It was, however, shown that compound **2** could cross the blood–brain barrier. The maximum concentration of compound [^{3}H]**2** in the rat brain was observed 10 min after intraperitoneal administration ($c_{brain}/c_{blood} = 1.77 \pm 0.35$ – hypothalamus, 1.55 ± 0.19 – hippocampus, 2.06 ± 0.31 – striatum, 1.51 ± 0.19 – cortex), reaching the basal level after ca 60 min.

To trace the activity of the most interesting compounds, **2** and **3**, at any receptors coupled to the most common second messenger systems, their influence on phosphatidylinositol (PI) turnover and cyclic AMP production was examined. Compounds **2** and **3** in concentrations up to 0.1 mM did not significantly

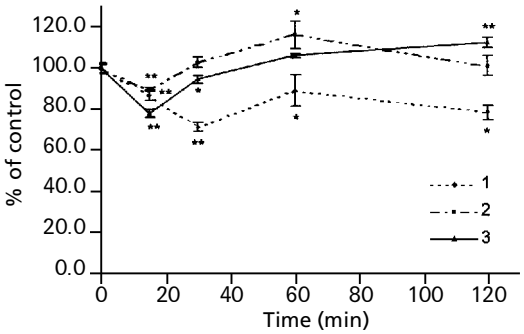

Figure 3 Serotonin turnover (5-HIAA/serotonin) after the systemic administration of compounds **1**, **2** and **3** (50 mg kg^{-1}) to mice. Control values (ng (g tissue)$^{-1}$) for compounds **1** and **2**: serotonin, 444.1±19.0; 5-HIAA, 164.2±5.5; for compound **3**: serotonin, 478.6±22.3; 5-HIAA, 150.5±6.1. *$P < 0.05$; **$P < 0.01$ vs control.

influence either basal or forskolin-stimulated cyclic AMP production. However, compound **2** in high concentration (1 mM) moderately increased (by 196.5%, $P < 0.001$) the basal cyclic AMP production, the effect probably not being related to the interaction with a receptor system. Compounds **2** and **3** in concentrations up to 3 mM did not significantly influence either basal or stimulated (histamine or carbachol) PI turnover (as controlled by IP_3 production). The obtained data could suggest that the compounds did not activate receptors coupled either to phospholipase C or to adenyl cyclase.

The determination of levels of serotonin and its metabolite in the brain of rats after the administration of compounds **1**, **2** and **3** has shown the decrease in serotonin turnover (as measured by 5-HIAA/serotonin level, Figure 3) with the minimum at 15 (for **2** and **3**) or 30 min (for **1**) after administration. The decrease in serotonin turnover produced by compound **1** remained during 2 h of measurements, whereas for 60 or 120 min after administration of compounds **2** or **3**, 5-HIAA/serotonin ratios were unchanged or increased. The results were consistent with those obtained for flunitrazepam (de St Hilaire-Kafi & Gaillard 1988).

Studied pyrimidylpiperazines exhibited an interesting profile of pharmacological activity. Although the reference drug zopiclone (**1**) displayed stronger sedative activity than compounds **2** and **3**, all the three compounds exhibited a similar hypnotic activity. At the same time, however, the interaction of zopiclone with ethanol was considerably stronger than those of compounds **2** and **3**, the smallest interaction being found for compound **2**. Since the toxicity of compound **2** rose after 7 days of administration, it seems reasonable to further optimise its structure to find a compound with a better pharmacological profile.

Conclusion

The obtained results suggested that zopiclone and pyrimidylpiperazines **2**, **4** and **5** exerted their pharmacological activity through a different mechanism – zopiclone through the interaction with benzodiazepine receptors and compounds **2**, **4** and **5** through an unidentified molecular target (compounds **2** and **5** did not bind to benzodiazepine or other receptors). Compound **3** exhibited a moderate affinity for benzodiazepine receptors, but its pharmacological properties could be the result of a mixed mechanism of action combining the properties of zopiclone and those of compounds **2**, **4** and **5**. A common feature of zopiclone and compounds **2** and **3** was that after their systemic administration, independently of the mechanism of action, together with the hypnotic effect, a reduction in the serotonin turnover in the mouse brain was observed.

Structural considerations have shown that removing the carbonyl group in β-position ($X_2 = H_2$, Figure 2; compounds **3**, **4** and **5**, Figure 1) had no major effect on the sedative–hypnotic potency of the compounds (as measured by the inhibition of the locomotor activity, disturbance of motor coordination or abolition of the righting reflex) as compared with compound **2** (Table 1). On the other hand, removing the carbonyl group in α-position ($X_1 = H_2$, compound **6**) or replacing the aromatic pyrimidine ring with a methyl group (R_1 = Me, compounds **7**–**9**) did not influence the drug's ability to inhibit locomotor activity. However, those compounds lost their ability to disturb motor coordination or abolish the righting reflex within non-lethal doses. Therefore a hypnotic activity could be attributed to compounds possessing a piperazine ring 1,4-disubstituted with aromatic and α-carbonyl moieties (Figure 2B).

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