ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 55 (2000) 6–12

IL FARMACO

Synthesis and pharmacological properties of 1-(4-substituted)butyl derivatives of amides of 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylic acid

Helena Śladowska [a,*], Maria Sieklucka-Dziuba [c], Robert Rejdak [b], Zdzisław Kleinrok [b]

[a] *Department of Chemistry of Drugs, Wrocław Medical University, Tamka 1, 50-137 Wrocław, Poland*
[b] *Department of Pharmacology, Medical School, Jaczewskiego 8, 20-090 Lublin, Poland*
[c] *Department of Hygiene, Medical School, Radziwiłłowska 11, 20-085 Lublin, Poland*

Received 22 February 1999; accepted 23 September 1999

Abstract

The synthesis of 1-(4-substituted)butyl derivatives of amides of 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylic acid and the results of the preliminary pharmacological screening are described in this paper. Some of them showed a weak analgesic action and caused suppression of the spontaneous locomotor activity of mice.

Keywords: Pyrido[2,3-*d*]pyrimidines; (4-Substituted)butyl derivatives; Pharmacological activity

1. Introduction

In a previous paper [1], the synthesis and properties were reported for 1-[4-(4-aryl)- and 1-[4-[4-(2-pyrimidinyl)]-1-piperazinyl]butyl derivatives of ethyl 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylate (**Ia**–**c**) (Fig. 1).

In the pharmacological screening compounds **Ia**,**b** depressed the spontaneous locomotor activity of mice and showed anxiolytic action in 'four plates' test. All caused hypothermia in normothermic mice. Contrary to compounds **Ia**,**b**, derivative **Ic** had high toxicity. In continuing research in this series of compounds, we stated recently [2] that the replacement of an ester group by an amide one and introduction of a 2-hydroxy-3(4-phenyl-1-piperazinyl)propyl substituent in position 1 of the appropriate 2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine gave non-toxic substances (**IIa**–**c**) characterized by a strong analgesic activity (Fig. 2).

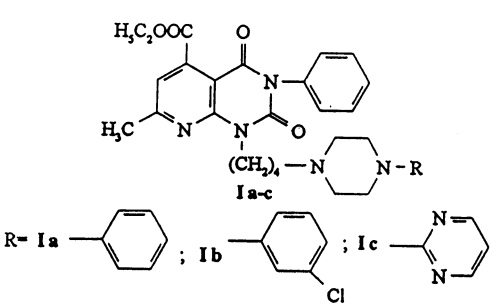

Fig. 1.

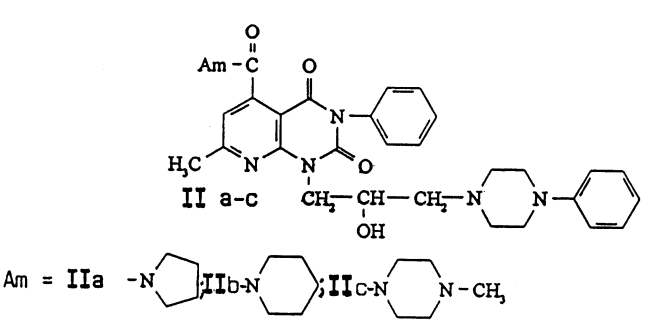

Fig. 2.

* Corresponding author.

0014-827X/00/$ - see front matter
PII: S0014-827X(99)00099-3

In correlation with these studies, we now synthesized 1-(4-substituted)butyl derivatives of amides of 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylic acid (**5**–**14**) combining certain structural elements of both **I** and **II**. We hoped that the obtained compounds would have a depressive influence on the CNS of animals. Our expectations followed (amongst others) from the presence in their structures of 4-aryl- or 4-(2-pyrimidinyl)-l-piperazinyl- and 2-(1,2,3,4-tetrahydroisoquinolinyl)butyl substituents, characteristic of the selective anxiolytics — Buspirone type [3–7] as well as from the presence of pyrrolidinyl — or a piperidinoamide moiety having influence on the analgesic action of the appropriate 2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidines [2,8].

2. Chemistry

The starting materials for the synthesis of compounds **3**–**14** were the appropriate amides of 7-methyl-3-phenyl-2,4 - dioxo - 1,2,3,4 - tetrahydropyrido[2,3 - *d*]pyrimidine-5-carboxylic acid (**1**, **2**) previously synthesized [2]. Their potassium salts were condensed with 1,4-dibromobutane with the aim of obtaining 4-bromobutyl derivatives (**3**, **4**). This last reaction was carried out in anhydrous DMF solution at room temperature. Then, 4-bromobutyl derivatives **3** and **4** were transformed into the compounds **5**–**14** by heating with the suitable amines (*N*-phenyl-, *N*-*o*-methoxyphenyl-, *N*-methyl-, *N*-(2-pyrimidinyl)-piperazines, 1,2,3,4-tetrahydroisoquinoline, 1-(2-aminoethyl) piperidine) in acetonitrile solution and in the presence of anhydrous potassium carbonate. The compounds described in this paper (Fig. 3) are crystalline substances. Their structures were confirmed by spectral (IR, ^{1}H NMR) and elemental analyses.

For pharmacological screening we chose four substances: **5**–**7** and **13**, Three of them (**5**–**7**) are pyrrolidinylamide derivatives. As follows from our earlier investigations [2], pyrrolidinylamide of 1-[2-hydroxy-3(4-phenyl-1-piperazinyl)]propyl-7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylic acid produced considerably stronger analgesic effect than the corresponding piperidinoamide. Compounds **5**–**7** differ from one another in the kind of the substituents at the N-4 of piperazine bounded with the butyl chain. These substituents (phenyl (**5**), *o*-methoxyphenyl (**6**), CH_3 (**7**)) have different electronic and lipophilic features. Introducing them we wanted to verify their possible influence on the CNS action. For testing we also selected compound **13**, being a piperidinoamide derivative containing in position 1 4-[2-(1,2,3,4-tetrahydroisoquinolinyl)]butyl substituent. According to Mokrosz et al. [7], the replacement of 4-substituted piperazine in the side-chain of Buspirone by the 1,2,3,4-tetrahydroisoquinolinyl group did not change its affinity to the $5HT_{1A}$ receptors and pharmacological profile. Based on this, we wanted to know whether this replacement would be possible in this group of compounds.

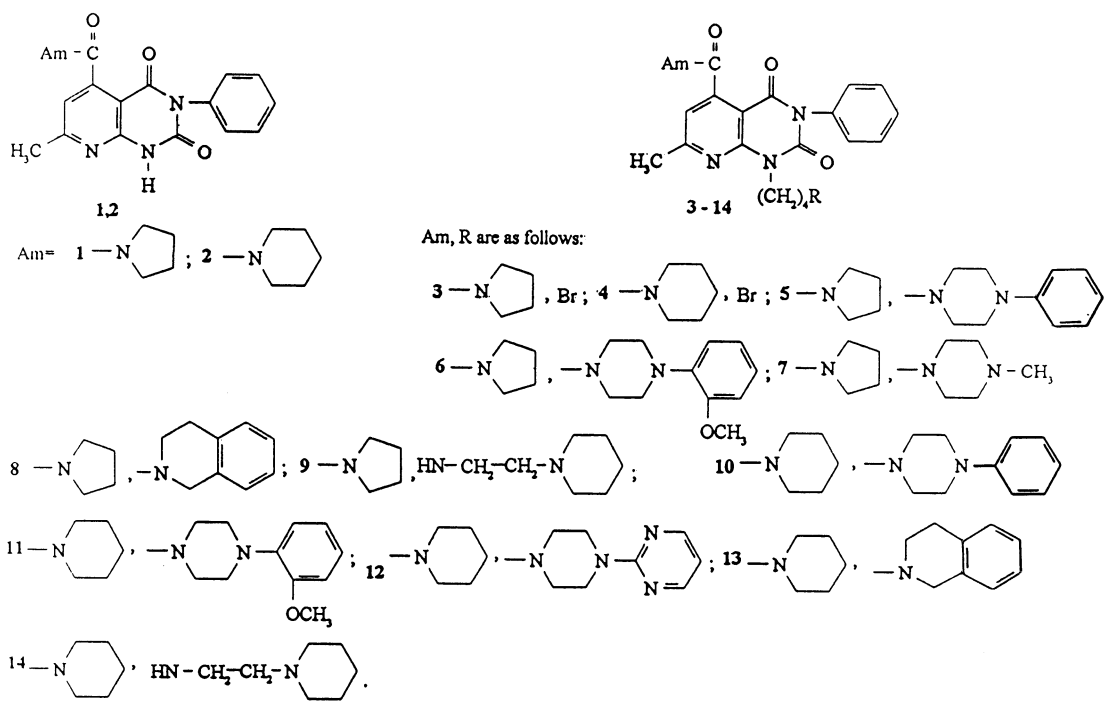

Fig. 3.

Table 1
Properties of the investigated compounds

Comp.	Formula (molecular wt.)	M.p. (°C) (solvent)	Yield (%)	IR absorptions in KBr (cm^{-1})		
				CO	Mono- and disubstituted benzene	CH_2
3	$C_{23}H_{25}N_4O_3Br$ (485.378)	182–184 (ethanol)	60	1650, 1670, 1720	700, 755	2880, 2930
4	$C_{24}H_{27}N_4O_3Br$ (499.404)	175–178 (ethanol)	70	1650, 1680, 1720	700, 760	2840, 2920
5	$C_{33}H_{38}N_6O_3$ (566.68)	196–198 (ethanol)	75	1650, 1680, 1720	700, 765	2800, 2860, 2905
6	$C_{34}H_{40}N_6O_4$ (596.71)	179–182 (ethanol)	60	1640, 1680, 1720	700, 760	2800, 2910
7	$C_{28}H_{36}N_6O_3$ (504.62)	150–152 (ether)	60–65	1645, 1670, 1720	700, 760	2800, 2940
8	$C_{32}H_{35}N_5O_3$ (537.64)	176–180 (ether)	40	1650, 1670, 1725	700, 750	2900, 2920
9	$C_{30}H_{40}N_6O_3$ (532.67)	126–130 (petroleum ether)	50	1650, 1680, 1720	700, 760	2800, 2920
10	$C_{34}H_{40}N_6O_3$ (580.71)	199–201 (ethanol)	70	1650, 1680, 1715	700, 760	2800, 2900
11	$C_{35}H_{42}N_6O_4$ (610.73)	120–123 (ether/petroleum ether)	50	1640, 1680, 1720	700, 750	2800, 2900
12	$C_{32}H_{38}N_8O_3$ (582.688)	187–191 (methanol/ether)	55	1650, 1680, 1720	700, 760	2840, 2900
13	$C_{33}H_{37}N_5O_3$ (551.67)	147–150 (ether)	55	1650, 1670, 1720	700, 755	2840, 2930
14	$C_{31}H_{42}N_6O_3$ (546.69)	120–124 (petroleum ether)	56–60	1650, 1680, 1720	700, 760	2840, 2910

3. Experimental

3.1. Chemistry

All the results of the C, H, N determinations (carried out by a Carlo Erba Elemental Analyzer model NA-1500) were within $\pm 0.4\%$ of the theoretical values. All melting points are uncorrected. The IR spectra, in KBr pellets, were measured with Zeiss Jena specord model IR 75 and ^1H NMR spectra were determined in $CDCl_3$ on a Tesla 587 A spectrometer (80 MHz) using TMS as internal standard.

3.1.1. General procedure for obtaining compounds *3* and *4*

0.01 mol of potassium was dissolved in 150 ml of anhydrous ethanol and to this solution 0.01 mol of the appropriate amide (**1** or **2**) was added. After dissolving the solid substance, ethanol was distilled off under diminished pressure and a dry crystalline residue was treated with 16 g of 1,4-dibromobutane in 60 ml of anhydrous DMF. The obtained suspension was stirred at room temperature until the alkaline reaction disappeared. Then 200 ml of ether were added to the reaction mixture and it was stirred again for 1 h. After filtration the solvents were evaporated completely under diminished pressure. The residue was treated with a small amount of distilled water and the crystalline substance was collected on a filter and purified.

The properties of compounds **3** and **4** are given in Table 1 and the assignments in their ^1H NMR spectra are presented below: ^1H NMR of **3**: $\delta = 1.77–2.14$, m-8H (H of pyrrolidine + Hβ,γ of butyl); 2.64, s-3H (CH_3 in 7); 3.04–3.20, t-2H (H of pyrrolidine); 3.41–3.68, m-4H (H of pyrrolidine + Hδ of butyl); 4.41, t(distorted)-2H (Hα of butyl); 6.94, s-1H (H in 6); 7.17–7.57, m-5H (H arom.).

^1H NMR of **4**: $\delta = 1.4–2.13$, m-10H (H of piperidine + Hβ,γ of butyl); 2.63, s-3H (CH_3 in 7); 3.13–3.20, m-2H (H of piperidine): 3.43–4.17, m-4H (H of piperidine + Hδ of butyl): 4.42, t-2H (Hα of butyl); 6.89, s-1H (H in 6); 7.16–7.51, m-5H (H arom.).

3.1.2. General procedure for obtaining compounds *5–14*

To 0.003 mol of compound **3** or **4** in 75 ml of anhydrous acetonitrile, 0.75 g of anhydrous potassium carbonate and 0.0045 (**5–7**, **10–12**), 0.005 (**8**, **13**), 0.012 (**9**, **14**) mol of the suitable amine (*N*-phenyl-, *N*-*o*-methoxyphenyl-, *N*-methyl-, *N*-(2-pyrimidynyl)-piperazines, 1,2,3,4-tetrahydroisoquinoline, 1-(2-aminoethyl) piperidine) were added. The mixture was refluxed for 12 h. Then, after filtration the solvent was evaporated under

reduced pressure and the residue was purified by crystallization from the solvent given in Table 1.

The properties of compounds **5**–**14** are presented in Table 1, and the assignments in the ^{1}H NMR spectra of some of them are presented below:

^{1}H NMR of **6**: $\delta = 1.55$–2.03, m-8H (H of pyrrolidine + Hβ,γ of butyl); 2.49–2.71, m-9H (CH_3 in 7 + H_2C–N(CH_2–)$_2$); 3.12–3.16. m-6H (H of pyrrolidine + H of piperazine); 3.6–3.67, t-2H (H of pyrrolidine); 3.86, s-3H (OCH_3); 4.42, t-2H (Hα of butyl); 6.93–7.50, m-10H (H arom.).

^{1}H NMR of **7**: $\delta = 1.41$–1.92, m-8H (H of pyrrolidine + Hβ,γ of butyl); 2.28–2.62, m-16H ($2 \times CH_3$ + H of piperazine + Hδ of butyl); 3.04–3.13, t-2H and 3.61–3.66, t(distorted)-2H (H of pyrrolidine); 4.30–4.40, t-2H (Hα of butyl); 6.92, s-1H (H in 6); 7.19–7.52, m-5H (H arom.).

^{1}H NMR of **9**: $\delta = 1.47$–1.94, m-14H (H of piperidine + H of pyrrolidine) + Hβ,γ of butyl); 2.35–2.76, m-13H ($3 \times CH_2$ + H of piperidine + CH_3 in 7); 3.01–3.14, t-2H and 3.60–3.66, t(distorted)-2H (H of pyrrolidine); 4.40–4.48, t-2H (Hα of butyl); 6.91, s-1H (H in 6); 7.19–7.49, m-5H (H arom.).

^{1}H NMR of **10**: $\delta = 1.30$–2.00, m-10H (H of piperidine + Hβ,γ of butyl); 2.40–2.72, m-9H (CH_3 in 7 + H_2C–N(CH_2–)$_2$); 2.92–3.20, m-6H (H of piperazine + H of piperidine); 3.88–4.13, m-2H (H of piperidine); 4.33–4.50, t-2H (Hα of butyl); 6.88–7.5, m-11H (H arom.).

^{1}H NMR of **11**: $\delta = 1.30$–2.00, m-10H (H of piperidine + Hβ,γ of butyl); 2.25–2.72, m-9H (CH_3 in 7 + H_2C–N(CH_2–)$_2$); 2.87–3.29, m-6H (H of piperazine + H of piperidine); 3.74–4.08, m-5H (OCH_3 + H of piperidine); 4.25–4.50, t-2H (Hα of butyl); 6.88–7.45, m-10H (H arom.).

^{1}H NMR of **12**: $\delta = 1.30$–2.00, m-10H (H of piperidine + Hβ,γ of butyl); 2.27–2.89, m-9H (CH_3 in 7 + H_2C–N(CH_2–)$_2$); 2.90–4.07, m-8H (H of piperidine + H of piperazine); 4.07–4.50, t(distorted)-2H (Hα of butyl); 6.40–6.51, t-1H; 8.25–8.31, d-2H (H of pyrimidine); 6.88, s-1H (H in 6); 7.29–7.42, m-5H (H arom.).

^{1}H NMR of **13**: $\delta = 1.51$–1.82, m-10H (H of piperidine + Hβ,γ of butyl); 2.61–3.05, m-9H (CH_3 in 7 + Hδ of butyl + H of tetrahydroisoquinoline); 3.05–3.25, m-2H; 3.75–4.2, t-2H (H of piperidine): 3.65, s-2H (H of tetrahydroisoquinoline); 4.27–4.65, t-2H (Hα of butyl); 6.87–7.44, m-10H (H arom.).

3.2. Pharmacology

Compounds **5**–**7** and **13** were investigated pharmacologically.

3.2.1. Material and methods

The experiments were carried out on male and female Albino–Swiss mice (body weight, 20–25 g) and male Wistar rats (200–250 g). Investigated compounds were administered intraperitoneally (i.p.) as suspensions in 3% Tween 80 in the constant volume of 10 ml/kg in mice and 5 ml/kg in rats. The compounds were administered in doses equivalent to 1/10, 1/20 and 1/40 of LD_{50}. Control animals received the equivalent volume of solvent. Each experimental group consisted of eight animals.

The following pharmacological tests were performed:

1. Acute toxicity in mice.
2. Motor coordination in the rota-rod test in mice.
3. Spontaneous locomotor activity in mice
4. Amphetamine-induced locomotor hyperactivity in mice.
5. Pain reactivity in the 'writhing syndrome' test in mice.
6. Pain reactivity in the 'hot plate' test in mice.
7. Anxiolytic properties in 'four plates' test in mice.
8. Pentetrazol-induced seizures in mice.
9. Maximal electric shock in mice
10. Head twitches induced by 5-hydroxytryptophane in mice.
11. Arterial blood pressure in rats.

Acute toxicity was assessed by the methods of Litchfield and Wilcoxon [9] and presented as LD_{50} calculated from the mortality of mice after 24 h.

Motor coordination was measured according to the method of Gross et al. [10]. The effects were evaluated 15, 30, 45, 60, 75, 90 and 105 min after the administration of the investigated compounds.

Spontaneous locomotor activity in mice was measured by the use of Digiscan Optical Animal Activity Monitoring System (Omnitech Electronics, Inc., Columbus, OH). Thirty minutes after injection of the investigated compounds, mice were placed separately in Plexiglas cages ($20 \times 20 \times 30$ cm) for 1 h. The apparatus monitors animal locomotor activity via a grid of invisible infrared light beams, which in an equal number traverse the animal cage from front to back and from left to right. Each crossing of the light beam was recorded automatically and subjected to rapid analysis by the Digiscan Analyzer using computer program OMNI-PRO, Version 2.40. Horizontal activity (the total number of beam interruptions that occurred in the horizontal sensor during observation time) was evaluated after 30 and 60 min.

Amphetamine hyperactivity in mice was induced by D,L-amphetamine 2.5 mg/kg s.c. The investigated com pounds were injected 30 min before amphetamine was administered. The locomotor hyperactivity was measured 30 and 60 min later in the Digiscan Optical Animal Activity Monitoring System.

Pain reactivity was measured by the 'writhing syndrome' test of Koster et al. [11]. The test was performed on mice by the i.p. injection of a 0.6% solution of acetic

Table 2
Acute toxicity of the investigated compounds ($n = 8$)

Comp.	LD_{50} (mg/kg i.p.)	Confidence limit
5	389.5	139.5–694.2
6	244.5	92.8–443.9
7	304.8	248.0–374.7
13	405.7	321.9–511.4

acid in a volume of 10 ml/kg, 60 min after the administration of investigated compounds. The number of writhing episodes was counted for 30 min after the injection of 0.6% acetic acid.

Pain reactivity was also measured in the 'hot plate' test according to the method of Eddy and Leimbach [12]. Animals were placed individually on the metal plate heated to 56°C. The time(s) for the appearance of the pain reaction (licking of the forepaws or jumping) was measured. The experiments were performed 60 min after the administration of the investigated compounds.

Anxiolytic properties were assessed by the 'four plates' test in mice, according to Aron et al. [13], 60 min after the administration of investigated compounds in doses that had no effect on the spontaneous locomotor activity. Mice were placed in the cages with four plates in the floors (11×7 cm) with a 4 mm gap between each. After 15 s of adaptation the number of crossings was counted for 1 min. Each crossing was punished with direct current (180 V, 0.5 A) but not more often than every 3 s.

Pentetrazol seizures in mice were induced by pentetrazol administration at a dose of 100 mg/kg s.c. 30 min after the investigated compounds. The animals were observed during 30 min and the number of mice developing clonic and tonic seizures as well as mortality was recorded in that period.

Maximal electric shock was induced by means of alternating current (50 Hz, 25 mA, 0.2 s) with the use of ear clip electrodes according to the method of Swinyard et al. [14]. The criterion for the convulsive response was the tonic extension of the hind limbs. The test was performed 60 min after administration of the investigated compounds.

Head twitch behavior was induced by the administration of 5-hydroxytryptophane (5-HTP) at a dose of 180 mg/kg i.p. 30 min after the investigated compounds were administered. Animals were observed 60 min after 5-HTP administration.

Arterial blood pressure was determined according to the method of Gerold and Tschirky [15] using the UGO-BASILE equipment (blood pressure recorder, Cat. No. 8006). Systolic blood pressure on the tail artery was measured 30 min after administration of the investigated compounds.

Table 3
Influence of the investigated compounds on the spontaneous locomotor activity in mice ($n = 8$)

Comp.	Dose (part of LD_{50})	Dose (mg/kg)	No. of impulses ± SEM after:	
			30 min	60 min
Control			3925.0 ± 522.7	6389.8 ± 854.6
5	1/10	38.95	2154.0 ± 349.5*	3328.7 ± 435.45**
	1/20	19.47	3445.0 ± 326.7	4963.2 ± 538.2
6	1/10	24.45	1125.8 ± 362.0***	2147.5 ± 431.4***
	1/20	12.22	1976.1 ± 650.4*	2789.1 ± 492.8**
	1/40	6.11	3261.0 ± 823.4	4829.0 ± 387.4
7	1/10	30.48	4120.5 ± 452.5	6566.6 ± 707.7
13	1/10	40.57	2224.2 ± 488.0*	3326.8 ± 757.9*
	1/20	20.28	3966.3 ± 842.7	6884.3 ± 634.2

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Table 4
Influence of the investigated compounds on the pain reactivity in 'writhing syndrome' test in mice ($n = 8$)

Comp.	Dose (part of LD_{50})	Dose (mg/kg)	Mean no. of writhings ± SEM
Control			7.5 ± 0.8
5	1/10	38.95	3.2 ± 0.5***
	1/20	19.47	4.5 ± 0.4**
	1/40	9.73	6.1 ± 1.8
6	1/10	24.45	6.4 ± 1.6
7	1/10	30.48	3.8 ± 1.2*
	1/20	15.24	7.5 ± 0.6
13	1/10	40.57	2.7 ± 0.8***
	1/20	20.28	4.8 ± 0.6*
	1/40	10.14	6.5 ± 1.1

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

3.2.2. Statistics

The results obtained were presented as means and evaluated statistically using Student's t-test or exact Fischer's test.

4. Results and discussion

The LD_{50} values for the investigated compounds **5**–**7** and **13** after their i.p. administration in mice are presented in Table 2. They indicate that the tested substances were quite toxic with LD_{50} values between 244.5 and 405.7 mg/kg. The most toxic derivative was **6** ($LD_{50} = 244.5$ mg/kg). None of the investigated com-

Table 5
Influence of the investigated compounds on the pain reactivity in 'hot plate' test in mice ($n = 8$)

Comp.	Dose (part of LD_{50})	Dose (mg/kg)	Time of reaction on pain stimulus ± SEM (s)
Control			6.2 ± 1.1
5	1/10	38.95	9.3 ± 0.7*
	1/20	19.47	7.6 ± 0.9
6	1/10	24.45	6.0 ± 2.0
7	1/10	30.48	11.1 ± 1.4**
	1/20	15.24	8.3 ± 1.6
13	1/10	40.57	9.2 ± 0.6*
	1/20	20.28	6.5 ± 0.8

* $P < 0.05$.
** $P < 0.01$.

pounds had neurotoxic properties at the dose of 38.95 (**5**), 24.45 (**6**), 30.48 (**7**), 40.57 (**13**) mg/kg as they did not affect the motor coordination in the rota-rod test. Compounds **5** and **13** suppressed spontaneous locomotor activity during a 1 h observation period at the highest dose used 38.95 (**5**), 40.57 (**13**) mg/kg. Compound **6** was active in this test up to the dose of 12.22 mg/kg. Derivative **7** did not affect spontaneous locomotor activity (Table 3). Furthermore compounds **5**, **7** and **13** possessed analgesic activity assayed in the 'writhing syndrome' test. Compounds **5** and **13** were active up to a dose of 19.47 (**5**), 20.28 (**13**) mg/kg, and **7** at a dose of 30.48 mg/kg (Table 4). Analgesic activity of compounds **5**, **7** and **13** was confirmed in a 'hot plate' test at doses of 38.95 (**5**), 30.48 (**7**), 40.57 (**13**) mg/kg. Derivative **6** did not show analgesic activity in both tests performed (Table 5). In the remaining tests all investigated compounds were inactive.

From the presented results it follows that **6**, being a (4-*o*-methoxyphenyl-1-piperazinyl)butyl derivative of the appropriate pyrrolidinylamide, proved to be, contrary to **5** (without the OCH_3 group), the least active and the most toxic compound. Piperidinoamide **13**, containing in position 1 4-[2-(1,2,3,4-tetrahydroisoquinolinyl)]butyl substituent, was the least toxic compound and similar to **5**, active in three tests.

In spite of the differences in the structures, compounds **5** and **13** have the same pharmacological profile. Their LD_{50} values are also similar. The small differences in the strength of action, e.g. in the 'writhing syndrome' test, may be explained by the fact that piperidinoamide produces a weaker analgesic effect than pyrrolidinylamide. This indicates that at least in two tests ('writhing syndrome' and 'hot plate'), the replacement of the *N*-substituted piperazinyl group by 1,2,3,4-tetrahydroisoquinolinyl one in this series of compounds is possible. Amide **7**, containing a small lipophilic methyl group at the nitrogen atom in position 4 of the piperazine, showed only analgesic activity in two tests at the highest dose used. But in the 'writhing syndrome' test it has a weak action.

The investigated compounds have the same activity profile as 1-[2-hydroxy-3(4-phenyl-1-piperazinyl)]propyl derivatives (**IIa–c**) but they are considerably more toxic in comparison with **IIa–c** (LD_{50} for **IIa–c** were > 2000 mg/kg [2]. The last compounds showed strong analgesic properties in the 'writhing syndrome' test up to a dose of 1.56 (**IIa**), 25 (**IIb**), or 6.25 mg/kg (**IIc**). This indicates that in this test, compounds **5**, **7** and **13** were less active than **IIa**,**c**. In comparison with **IIb**, derivatives **5** and **13** indeed an exerted analgesic effect in lower doses, but particularly in the case of **13** (at the dose of 20.28 mg/kg) it was a weak action. A weak activity was also displayed by **7**. In the 'hot plate' test compounds **IIa–c** were active as strong analgesic agents up to doses of 25 (**IIa**), 100 (**IIb**) and 50 mg/kg (**IIc**). The investigated compounds showed a weak activity in this test. This might be due (among others) to the fact that in comparison with **IIb** and **IIc** they could not be administered in higher doses because of their toxicity. Compounds **IIb**,**c** suppressed weakly the spontaneous locomotor activity of mice up to a dose of 50 mg/kg. The tested compounds (with the exception of **13**) gave stronger effect in this test in lower doses. Substances **5–7** and **13** were also more toxic in comparison with **Ia**,**b** (LD_{50} for **Ia**,**b** were as follows: 1600 (**Ia**), 1800 mg/kg (**Ib**) but less toxic than **Ic** (LD_{50} for **Ic** = 183.7 mg/kg) [1]). Contrary to **Ia**,**b** they were devoid of anxiolytic activity, but with the exception of **6** they showed weak analgesic properties. The comparison of the activity of compound **5** (amide) with the action of **Ia** (ester) indicates that in this case, the amide group causes the analgesic effect.

References

[1] H. Śladowska, M. Sieklucka-Dziuba, L. Staniak, Z. Kleinrok, Synthesis and properties of 1-aryl(pyrimidinyl)piperazinyl alkyl derivatives of ethyl 3-phenyl-7-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylate, Farmaco 49 (1994) 499–503.

[2] H. Śladowska, M. Sieklucka-Dziuba, G. Rajtar, M. Sadowski, Z. Kleinrok, Investigations on the synthesis and properties of amides of 7-methyl-3-phenyl-1-[2-hydroxy-3(4-phenyl-1-piperazinyl)propyl]-2,4-dioxo-1,2,3,4-tetrahydropyrido-[2,3-*d*]pyrimidine-5-carboxylic acid, Farmaco (1999) in press.
[3] Wu Yao-Hua, K.R. Smith, J.W. Rayburn, J.W. Kissel, Psychosedative agents. *N*-(4-phenyl-1-piperazinylalkyl)-substituted cyclic imides, J. Med. Chem. 12 (1969) 876–881.
[4] Wu Yao-Hua, J.W. Rayburn, L.E. Allen, H.C. Ferguson, J.W. Kissel, Psychosedative agents. 2. 8-(4-substituted-1-piperazinylalkyl)-8-azaspiro[4,5]decane-7,9-diones, J. Med. Chem. 15 (1972) 477–479.
[5] J.P. Yevich, D.L. Temple Jr., J.S. New, D.P. Taylor, L.A. Riblett, Buspirone analogues. 1. Structure–activity relationships in a series of *N*-aryl- and heteroarylpiperazine derivatives, J. Med. Chem. 26 (1983) 194–203.
[6] J.S. New, J.P. Yevich, M.S. Eison, D.P. Taylor, A.S. Eison, L.A. Riblett, C.P. VanderMaelen, D.L. Temple Jr., Buspirone analogues. 2. Structure–activity relationships of aromatic imide derivatives, J. Med. Chem. 29 (1986) 1476–1482.
[7] J.L. Mokrosz, A. Dereń-Wesołek, E. Tatarczyńska, B. Duszyńska, A.J. Bojarski, M.J. Mokrosz, E. Chojnacka-Wójcik, 8-{4-[2-(1,2,3,4-Tetrahydroisoquinolinyl)]butyl}-8-azaspiro[4,5]decane-7,9-dione. A new 5-HT_{1A} receptor ligand with the same activity profile as Buspirone, J. Med. Chem. 39 (1996) 1125–1129.
[8] H. Śladowska, T. Zawisza, Synthesis and properties of amides of 1-benzyl-3-methyl- and 1-butyl-3-phenyl-7-methyl-4-oxo-2-thioxo-(2,4-dioxo)-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-6-carboxylic acids, Farmaco 41 (1986) 952–963.
[9] I.T. Litchfield, F. Wilcoxon, A simplified method of evaluating dose-effect experiments, J. Pharmacol. Exp. Ther. 96 (1949) 99–113.
[10] F. Gross, J. Tripod, R. Meier, Zur pharmakologischen Charakterisierung des Schlafmittels Doriden, Med. Wschr. 85 (1955) 305–309.
[11] R. Koster, M. Anderson, E.J. de Bear, Acetic acid for analgesic screening, Fed. Proc. Fed. Am. Soc. Exp. Biol. 18 (1959) 412.
[12] N.B. Eddy, D. Leimbach, Synthetic analgesics. II Dithienyl–butenyl and dithienyl–butylamines, J. Pharmacol. Exp. Ther. 107 (1953) 385–389.
[13] C. Aron, D. Simon, C. Larousse, J.R. Boissier, Evaluation of rapid technique for detecting minor tranquilizers, Neuropharmacology 10 (1971) 459–469.
[14] E.A. Swinyard, W.C. Brown, L.S. Goodman, Comparative assays of antiepileptic drugs in mice and rats, J. Pharmacol. Exp. Ther. 106 (1952) 319–330.
[15] M. Gerold, H. Tschirky, Measurement of blood pressure in unanaesthetized rats, Artzneim. -Forsch. 18 (1968) 1285–1287.