ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 54 (1999) 773–779

IL FARMACO

Investigations on the synthesis and pharmacological 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

Helena Śladowska [a,*], Maria Sieklucka-Dziuba [c], Grażyna Rajtar [b], Mirosław Sadowski [c], 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

Synthesis of amides of 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylic acid (**6**–**10**) and their 1-[2-hydroxy-3(4-phenyl-1-piperazinyl)propyl] derivatives (**11**–**15**) are described. Some of them displayed strong analgesic activity.

Keywords: Pyrido[2,3-*d*]pyrimidines; *N*-2-Hydroxy-3(4-phenyl-1-piperazinyl)propyl derivatives; Amides; Synthesis; Pharmacological activity

1. Introduction

It was stated previously [1] that compounds **1** and **2** were characterized by low toxicity and displayed analgesic properties. Derivative **1** also weakly reduced hyperthermia induced by *m*-chlorophenylpiperazine (antidepressant action).

These findings as well as the analgesic, anxiolytic, sedative and anti-inflammatory activities of various derivatives of pyrido[2,3-*d*]pyrimidine, obtained in our department (e.g. Refs. [2–4]), encouraged us to continue the synthesis in this group of compounds. The starting point for further investigations was our accidental statement that the introduction of the 2-hydroxy-3-(4-phenyl-1-piperazinyl)propyl substituent at the nitrogen atom of some 3,4-pyridinedicarboximides gave non-toxic substances ($LD_{50} > 2000$ mg kg^{-1}), displaying very strong analgesic activity [4]. Because 3,4-pyridinedicarboximides and 2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidines show some structural similarity (imide moiety, pyridine ring), it seemed interesting to state whether substitution of the above-mentioned pharmacophoric group in position 1 of compound **1** would distinctly influence its pharmacological activity (Fig. 1). In order to do this from a chemical point of view, the subsitution of the ester group in compound **1** by an amide group proved to be

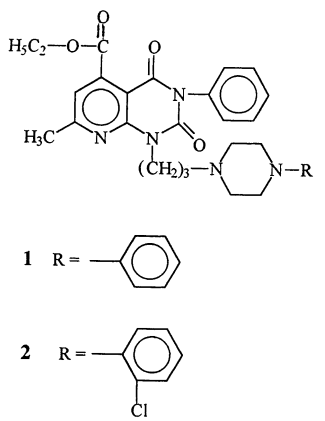

Fig. 1.

* Corresponding author.

0014-827X/99/$ - see front matter
PII: S0014-827X(99)00101-9

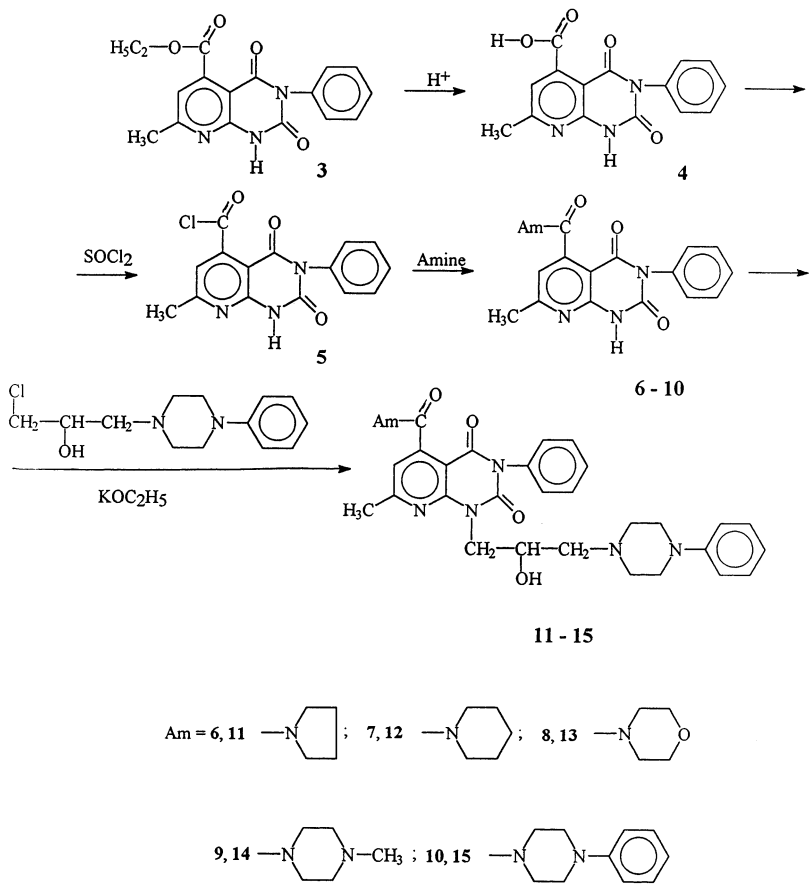

Fig. 2.

necessary. According to statements made above the aim of this paper was the synthesis of some amides 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 (**11**–**15**) and evaluation of their action on the CNS in preliminary pharmacological tests. We hoped that the compounds obtained would show (among others) stronger analgesic properties in comparison with those of compound **1**. Furthermore, the 2-hydroxy-3(4-phenyl-1-piperazinyl)-propyl substituent has similar structure to that of the side chains of β-blockers. Therefore, we also evaluated the influence of some amides on the arterial blood pressure in rats.

2. Chemistry

The starting material was ethyl 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]-pyrimidine-5-carboxylate (**3**) [6]. It was subjected to acid hydrolysis giving the corresponding pyrido-[2,3-*d*]pyrimidine-5-carboxylic acid (**4**) [2,5]. Acid **4** was transformed into the acid chloride **5** by heating with $SOCl_2$ in anhydrous benzene solution. The last gave crystalline substances with the following cyclic amines: pyrrolidine (**6**), piperidine (**7**), morpholine (**8**), *N*-methyl- and *N*-phenylpiperazines (**9**, **10**). The amides **6**–**10** underwent condensation with 2-hydroxy-3(4-phenyl-1-piperazinyl)-propyl chloride in anhydrous ethanol and in the presence of potassium ethoxide. As a result derivatives **11**–**15** were obtained (Fig. 2).

The structures of all compounds synthesized were confirmed by elemental and spectral analyses (IR, ^{1}H NMR).

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 a Zeiss Jena specord model IR 75 and ^{1}H NMR spectra were determined in $CDCl_3$ on a Tesla 587 A spectrometer (80 MHz) using TMS as internal standard.

*3.1.1. 7-Methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrido-[2,3-d]pyrimidine-5-carboxylic acid (**4**)*

Ethyl 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrido[2,3-*d*]pyrimidine-5-carboxylate (**3**) (8 g) was treated with a mixture of 480 ml of glacial acetic acid and 240 ml of concentrated hydrochloric acid and refluxed for 6 h. The acids were then distilled off under reduced pressure and to the dry residue 200 ml of distilled water were added. The separated product was collected on a filter, washed with water, dried and purified by crystallization from ethanol. After drying in vacuo at 130° it melted >300°C. It had identical physical and chemical properties to 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylic acid ($C_{15}H_{11}N_3O_4 = 297.26$) synthesized previously [3,6].

*3.1.2. Chloride of 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-5-carboxylic acid (**5**)*

7-Methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]-pyrimidine-5-carboxylic acid (4 g) (**4**) was treated with 60 ml of $SOCl_2$ and 80 ml of anhydrous benzene. The suspension was refluxed for 7 h. Benzene and an excess of $SOCl_2$ were distilled off under diminished pressure and 40 ml of anhydrous benzene were added to the residue. Benzene was again evaporated in vacuo. This procedure was repeated twice in order to remove all traces of $SOCl_2$. The crude product was used for the reaction without further purification.

*3.1.3. General procedure for obtaining amides of 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido-[2,3-d]pyrimidine-5-carboxylic acid (**6**–**10**)*

Chloride **5** (0.014 mol) obtained above was dissolved in 80 ml of anhydrous benzene. To this solution 0.035 mol of a suitable cyclic amine (pyrrolidine, piperidine, morpholine, *N*-methyl- and *N*-phenylpiperazines) was introduced. The mixture was then stirred at room temperature (r.t.) for 5 h. The separated product was collected on a filter and after drying it was washed with 100 ml of distilled water. Only in the case of amide **10** was the solid substance first washed with ethanol (120 ml) and then with distilled water.

All amides were purified by crystallization from ethanol.

The properties of compounds **6**–**10** are listed in Table 1 but the assignments of their ^{1}H NMR spectra are shown below.

^{1}H NMR of **6**: $\delta = 1.89$ (m-4H), 3.05–3.13 (m-2H), 3.61–3.66 (m-2H, H of pyrrolidine), 2.69 (s-3H, CH_3 in **7**), 6.96 (s-1H, H in **6**), 7.22–7.5 (m-5H, H arom), 10.5 (s-1H, NH).

^{1}H NMR of **7**: $\delta = 1.60$ (m-6H), 2.7–4.06 (m-4H, H of piperidine), 2.50, (s-3H, CH_3 in **7**), 6.86 (s-1H, H in **6**), 7.17–7.56 (m-5H, H arom), 11.5 (s, broad, 1H, NH).

^{1}H NMR of **8**: $\delta = 2.68$ (s-3H, CH_3 in **7**), 3.18 (t-2H), 3.59–3.92 (m-6H, H of morpholine), 6.88 (s-1H, H in **6**), 7.20–7.52 (m-5H, H arom), 11.54 (s-1H, NH).

^{1}H NMR of **9**: $\delta = 2.31$–2.62 (m-10H, $2 \times CH_3 + 4$H of piperazine), 3.17–3.32 (t-2H), 3.66 (m-2H, H of piperazine), 6.91 (s-1H, H in **6**), 7.26–7.46 (m-5H, H arom), 8.88 (s-1H, NH).

^{1}H NMR of **10**: $\delta = 2.62$ (s-3H, CH_3 in **7**), 2.77–3.72 (m-6H) and 3.72–4.28 (m-2H, H of piperazine), 6.90–7.42 (m-11H, H arom), 11.06 (s-1H, NH).

*3.1.4. General procedure for obtaining amides of 7-methyl-3-phenyl-1-[2-hydroxy-3(4-phenyl-1-piperazinyl)propyl]-2,4-dioxo-1,2,3,4-tetrahydro-pyrido[2,3-d]pyrimidine-5-carboxylic acid (**11**–**15**)*

Potassium (0.01 mol) was dissolved in anhydrous ethanol (150 ml) and to this solution 0.01 mol of a suitable amide of 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylic acid was introduced. After dissolving the solid substance,

Table 1
Properties of the investigated compounds

Comp.	Formula (molecular weight)	M.p. (°C) (solvent)	Yield (%)	IR absorptions in KBr (cm^{-1})		
				CO	OH or NH	Monsubstituted benzene
6	$C_{19}H_{18}N_4O_3$ (350.37)	314–316 (ethanol)	75	1640, 1665, 1720	3060, 3100–3130	700, 770
7	$C_{20}H_{20}N_4O_3$ (364.39)	333–335 (ethanol)	60	1645, 1670, 1720	3060, 3100	700, 760
8	$C_{19}H_{18}N_4O_4$ (366.37)	303–305 (ethanol)	55	1640, 1680, 1730	3060, 3100–3120	705, 770
9	$C_{20}H_{21}N_5O_3$ (379,41)	312–314 (ethanol)	65	1650, 1670, 1715	3120, 3200	700, 770
10	$C_{25}H_{23}N_5O_3$ (441,47)	283–286 (ethanol)	50	1660, 1680, 1730	3060, 3110	710, 770
11	$C_{32}H_{36}N_6O_4$ (568.66)	198–201 (ethanol)	44	1650, 1670, 1725	3370–3420	700, 760
12	$C_{33}H_{38}N_6O_4$ (582.68)	185–187 (ethanol)	45	1640, 1670, 1720	3360–3400	700, 760
13	$C_{32}H_{36}N_6O_5$ (584.66)	195–197 (methanol [a] or ether)	60	1640, 1670, 1720	3400–3420	700, 760
14	$C_{33}H_{39}N_7O_4$ (597.698)	209–211 (methanol)	55	1650, 1680, 1730	3340–3400	700, 770
15	$C_{38}H_{41}N_7O_4$ (659.764)	226–228 (ethanol)	45	1640, 1680, 1730	3360–3400	700, 770

[a] Compound **13** crystallizes with 1 mol of CH_3OH.

2-hydroxy-3(4-phenyl-1-piperazinyl)propyl chloride (0.012 mol) was added. The reaction mixture was refluxed until the alkaline reaction disappeared. After the filtration ethanol was distilled off to about 1/3 of its original volume and left to crystallize. The separated product (**11**–**14**) was collected on a filter. Only in the case of amide **15** was the solid organic substance precipitated during the heating of the reagents. It was collected on a filter, washed with distilled water and dried.

An additional amount of **15** was isolated from the filtrate (after evaporation of it to a small volume) and added to the main product. All amides (**11**–**15**) were purified by crystallization from the solvents given in Table 1.

The properties of compounds **11**–**15** are listed in Table 1 and the assignments of their ^{1}H NMR spectra are shown below.

^{1}H NMR of **11**: $\delta = 1.88$ (m-4H), 3.6–3.65 (m-2H, H of pyrrolidine), 3.14–3.20 (m-6H, 4H of piperazine + 2H of pyrrolidine), 2.56–2.73 m-9H CH_3 in 7 +-CH_2-N<(CH_2-)(CH_2-); 4.33–4.64 (m-4H, $H_{\alpha,\beta}$ of propyl + OH), 6.84–7.5 (m-11H, H arom).

^{1}H NMR of **12**: $\delta = 1.66$ (m-6H), 3.75–3.90 (m-2H, H of piperidine), 3.15–3.44 (m-6H, 4H of piperazine + 2H of piperidine), 2.56–2.73 (m-9H CH_3 in 7 +-CH_2-N<(CH_2-)(CH_2-); 4.28–4.65 (m-4H, $H_{\alpha,\beta}$ of propyl + OH), 6.85–7.50 (m-11H, H arom).

^{1}H NMR of **13**: $\delta = 2.59$–2.74, (m-9H CH_3 in 7 +-CH_2-N<(CH_2-)(CH_2-); 3.16–3.26 (m-6H, 4H of piperazine + 2H of morpholine); 3.62 (m-2H); 3.73–3.76, (m-4H, H of morpholine); 4.30–4.75, (m-4H, Hα, β of propyl + OH); 6.84–7.46, m-11H (H arom).

^{1}H NMR of **14**: $\delta = 2.29$–2.73 (m-16H, $2 \times CH_3 + 8$H of piperazine + H_α of propyl), 3.15–3.20 (m-6H), 3.65–3.95 (m-2H, H of piperazine), 4.15–4.77 (m-4H, $H_{\alpha,\beta}$ of propyl + OH), 6.84–7.49 (m-11H, H arom).

^{1}H NMR of **15**: $\delta = 2.58$–2.76 m-9H CH_3 in 7 + -CH_2-N<(CH_2-)(CH_2-); 3.16–3.35 (m-10H, H of piperazine), 3.77–4.85 (m-6H, 2H of piperazine + $H_{\alpha,\beta}$ of propyl + OH), 6.86–7.52 (m-16H, H arom).

3.2. Pharmacology

Compounds **11**, **12** and **14** were investigated pharmacologically.

3.2.1. Materials and methods

The experiments were carried out on male and female Albino-Swiss mice (body weight of 20–25 g) and male Wistar rats (200–250 g). The compounds investigated were administered intraperitoneally (i.p.) as suspensions in 3% Tween 80 at a constant volume of 10 ml kg^{-1} in rats. The compounds were administered in doses equivalent to 1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280 or 1/2560 of LD_{50}. For all compounds 2000 mg kg^{-1} was taken to be the initial dose. 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 [7] and presented as the LD_{50} calculated from the mortality of mice after 24 h.

Motor coordination was measured according to the method of Gross et al. [8]. The mice were placed for 2 min on the rod rotating with the speed of 4 rpm. 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 in circular photoresistor actometers (32 cm in diameter). After the injection of the investigated compounds the animals were placed in the actometers for 1 h. Each crossing of the light beam was recorded automatically. The number of impulses was noted after 30 and 60 min.

Amphetamine hyperactivity in mice was induced by D,L-amphetamine 2.5 mg kg^{-1} s.c. The investigated compounds were injected 30 min before the amphetamine was administered. The locomotor hyperactivity was measured 30 and 60 min later in the photoresistor actometers.

Pain reactivity was measured by the 'writhing syndrome' test of Koster et al. [9]. The test was performed in mice by the i.p. injection of a 0.6% solution of acetic acid in a volume of 10 ml kg^{-1} 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 [10]. Animals were placed individually on the metal plate heated to 56°C. The time (s) of 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.

Table 2
Influence of the investigated compounds on the spontaneous locomotor activity in mice ($n = 8$)

Comp.	Dose (part of LD_{50})	Dose (mg kg^{-1})	No. of impulses ± SEM after:	
			30 min	60 min
Control			364.8 ± 70.8	554.4 ± 98.9
11	1/10	200.0	248.3 ± 11.3	375.7 ± 48.4
12	1/10	200.0	165.0 ± 42.2 *	187.0 ± 16.5 **
	1/20	100.0	195.9 ± 22.0 *	257.8 ± 23.3 *
	1/40	50.0	238.0 ± 26.0	324.4 ± 15.1 *
	1/80	25.0	292.7 ± 40.7	472.0 ± 113.1
14	1/10	200.0	192.5 ± 33.3 *	262.1 ± 63.5 *
	1/20	100.0	156.7 ± 48.0 *	270.5 ± 80.9 *
	1/40	50.0	223.0 ± 23.5	322.1 ± 13.1 *
	1/80	25.0	276.4 ± 35.6	397.4 ± 98.0

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

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

Pentetrazol seizures in mice were induced by pentetrazol administration at a dose of 100 mg kg^{-1} s.c. 30 min after the injection of 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. [12]. 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 behaviour was induced by the administration of 5-hydroxytryptophane (5-HTP) at a dose of 180 mg kg^{-1} i.p. 30 min after the investigated compounds were administered. Animals were observed 60 min after 5-HTP administration [13].

Arterial blood pressure was determined according to the method of Gerold and Tschirky [14] 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.

3.2.2. Statistics

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

4. Results and discussion

The LD_{50} values (i.p. in mice) for the tested compounds **11**, **12** and **14** are > 2000 mg kg^{-1}. It indicated that the tested compounds were not toxic. Furthermore, none of the compounds in doses equivalent to 200 mg kg^{-1} showed neurotoxic properties as they did not affect the motor coordination in the rota-rod test. Compounds **12** and **14** suppressed the spontaneous locomotor activity of mice during the 1 h observation period up to the dose of 50 mg kg^{-1}. Compound **11** was inactive in this test (Table 2). All compounds, administered at a dose of 200 mg kg^{-1}, did not affect the excitatory action of amphetamine in mice, but they possessed strong analgesic activity, assayed in the 'writhing syndrome' test up to the dose of 1.56 mg kg^{-1} (**11**), 25 mg kg^{-1} (**12**) and 6.25 mg kg^{-1} (**14**) (Table 3).

Strong analgesic properties of the investigated derivatives were also confirmed in the 'hot plate' test. They were active up to doses of 25 mg kg^{-1} (**11**), 100 mg kg^{-1} (**12**) and 50 mg kg^{-1} (**14**) (Table 4). None of the investigated compounds, administered at a dose of 200 mg kg^{-1}, affected the pulse rate and arterial blood

Table 3
Influence of the investigated compounds on the pain reactivity in the 'writhing syndrome' test in mice ($n = 8$)

Comp.	Dose (part of LD_{50})	Dose (mg kg^{-1})	Mean no. of writhings ± SEM
Control			9.5 ± 1.27
11	1/10	200.0	0 ***
	1/20	100.0	0.5 ± 0.32 ***
	1/40	50.0	0.67 ± 0.38 ***
	1/80	25.0	0.74 ± 0.24 ***
	1/160	12.5	0.95 ± 0.32 ***
	1/320	6.25	1.25 ± 0.55 ***
	1/640	3.12	2.00 ± 0.41***
	1/1280	1.56	4.20 ± 0.62 **
	1/2560	0.78	6.75 ± 1.04
12	1/10	200.0	0 ***
	1/20	100.0	0.25 ± 0.16 ***
	1/40	50.0	0.38 ± 0.26 ***
	1/80	25.0	3.37 ± 0.67 ***
	1/160	12.5	4.75 ± 1.49
14	1/10	200.0	0 ***
	1/20	100.0	0 ***
	1/40	50.0	0 ***
	1/80	25.0	1.00 ± 0.56 ***
	1/160	12.5	3.00 ± 1.10**
	1/320	6.25	4.10 ± 0.93 **
	1/640	3.12	6.87 ± 0.59

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

Table 4
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^{-1})	Time of reaction on pain stimulus ± SEM (s)
Control			4.09 ± 0.30
11	1/10	200.0	15.70 ± 1.10 ***
	1/20	100.0	8.46 ± 1.33**
	1/40	50.0	6.37 ± 0.70*
	1/80	25.0	6.95 ± 0.77**
	1/160	12.5	5.02 ± 0.45
12	1/10	200.0	8.70 ± 0.74***
	1/20	100.0	6.81 ± 0.52***
	1/40	50.0	5.70 ± 0.81
14	1/10	200.0	13.00 ± 0.90***
	1/20	100.0	8.46 ± 1.46*
	1/40	50.0	6.67 ± 0.71**
	1/80	25.0	4.67 ± 0.33

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

pressure in rats. In the remaining tests all the examined amides were inactive.

From the data presented above it follows that among the three studied substances compound **11** (being the pyrrolidinylamide derivative) showed the strongest selective analgesic activity. Piperidinoamide **12** proved to be the least active analgesic agent. It indicates that the kind of amide group has influence on the strength of the analgesic action.

The lead compound **1** showed low toxicity (LD_{50} = 1561 mg kg^{-1}), but in the rota-rod test it exerted weak neurotoxic properties at the highest dose used (156.1 mg kg^{-1}), whereas amides **11**, **12** and **14** did not reveal any neurotoxic effects even at a dose of 200 mg kg^{-1}. Ester **1** displayed analgesic properties in the 'writhing syndrome' test up to a dose of 25 mg kg^{-1} similar to amide **12**, but in the case of **12** it was a very strong action. Similarly to **11**, **12**, and **14** compound **1** was inactive both as an anxiolytic agent, in the 'four plates' test, and also as anticonvulsant (pentetrazole and the maximal electric shock induced seizures in mice). Furthermore, **1** did not affect the excitatory action of amphetamine in mice at a dose of 156.1 mg kg^{-1}. The same effect was observed after administration of amides in doses of 200 mg kg^{-1}. Compounds **1**, **11**, **12** and **14** did not change the number of head twitches induced by 5-HTP in mice.

From the data presented above it can be seen that the described modifications of compound **1** caused reduction in the toxicity (LD_{50} for **11**, **12** and **14** > 2000 mg kg^{-1}), elimination of the neurotoxic properties in all cases and, in the case of compounds **11** and **14**, made the substance more active as an analgesic agent. The results obtained also indicate that the introduction of the 2-hydroxy-3(4-phenyl-1-piperazinyl)-propyl group on some 2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]-pyrimidine derivatives caused the same pharmacological effects as in the case of some 3,4-pyridinedicarboximides, mentioned in Section 1. In both chemical groups non-toxic substances were obtained, characterized by very strong analgesic activity.

Except for **11**, in both cases analgesic action was associated with the weak suppression of the spontaneous locomotor activity of mice, but only at the high doses (50–200 mg kg^{-1}).

References

[1] H. Śladowska, Investigations on the synthesis and properties of arylpiperazinylalkyl derivatives of some dihydro- and tetrahydropyrido[2,3-*d*]pyrimidines, Farmaco 48 (1993) 77–84.
[2] H. Śladowska, A. Bartoszko-Malik, T. Zawisza, Investigations on the reaction course of diethyl 2-chloro-6-methylpiridine-3,5-dicarboxylate with *N*-amino and *N*-hydroxyalkyl derivatives of thiourea, Farmaco 41 (1986) 899–912.
[3] H. Śladowska, A. Bartoszko-Malik, T. Zawisza, Synthesis and properties of the derivatives of 2-alkylthio-4-oxo-3,4 (and 1,4)-dihydropyrido[2,3-*d*]pyridmide-5- and -6-carboxylic acids, Farmaco 45 (1990) 111–121.
[4] H. Śladowska, M. Sieklucka-Dziuba, L. Staniak, Z. Kleinrok, Synthesis and properties of l-aryl(pyrimidinyl)piperazinylalkyl 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.
[5] H. Śladowska, J. Potoczek, M. Sieklucka-Dziuba, G. Rajtar, Z. Kleinrok, in press.
[6] H. Śladowska, A. Bartoszko-Malik, T. Zawisza, Synthesis and properties of new derivatives of ethyl 7-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylate, Farmaco 45 (1990) 101–110.
[7] I.T. Litchfield, F. Wilcoxon, A simplified method of evaluating dose-effect experiments, J. Pharmacol. Exp. Ther. 96 (1949) 99–113.
[8] F. Gross, J. Tripod, R. Meier, Zur pharmakologischen Charakterisierung des Schlafmittels Doriden, Med. Wschr. 85 (1995) 305–309.

[9] R. Koster, M. Anderson, J.E. de Bear, Acetic acid for analgesic screening, Fed. Proc. Fed. Am. Soc. Exp. Biol. 18 (1959) 412.
[10] N.B. Eddy, D. Leimbach, Synthetic analgesics, II. Dithienylbutenyl and dithienyl-butylamines, J. Pharmacol. Exp. Ther. 107 (1953) 385–389.
[11] C. Aron, D. Simon, C. Larousse, J.R. Bïssier, Evaluation of a rapid technique for detecting minor tranquilizers, Neuropharmacology 10 (1971) 459–469.
[12] 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.
[13] M. Nakamura, H. Fukushima, Effects of reserpine, parachlorophenylalanine, 5,6-dihydroxytryptamine and fludiazepam on the head twitches induced by 5-hydroxytryptamine or 5-methoxytryptamine in mice, J. Pharm. Pharmacol. 30 (1978) 254–256.
[14] M. Gerold, H. Tschirky, Measurement of blood pressure in unanaesthetized rats, Artzneim. Forsch. 18 (1968) 1285–1287.