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Synthesis and pharmacological properties of *N*,*N*-dialkyl(dialkenyl)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

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Abstract

Synthesis of *N*,*N*-dialkyl(dialkenyl)amides of 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylic acid (**5**–**9**) and their 1-[2-hydroxy-3-(4-phenyl-1-piperazinyl)propyl] derivatives (**10**–**14**) is described. Compounds **10**–**14** were tested for analgesic and sedative activities as well as for μ-opioid receptors binding affinities. All the amides, being the object of investigation, displayed an interesting analgesic action, which in case of the compounds **10**–**12** and **14** was superior to that of acetylsalicylic acid in two different tests. Furthermore all the amides (**10**–**14**) significantly suppressed the spontaneous locomotor activity, prolonged barbiturate sleep in mice and showed a weak affinity to μ-opioid receptors.

Keywords: Pyrido[2,3-*d*]pyrimidines; *N*-2-hydroxy-3-(4-phenyl-1-piperazinyl)propyl derivatives; Amides; Synthesis; Pharmacological activity; Affinity to μ-opioid receptors

1. Introduction

It was stated previously [1] that some of 1-[2-hydroxy-3-(4-phenyl-1-piperazinyl)propyl] derivatives of amides of 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylic acid (**1**–**3**) (Fig. 1) displayed an interesting analgesic action in the 'writhing syndrome' and 'hot plate' tests and were not toxic ($LD_{50} > 2000$ mg/kg). In the case of the compounds **2** and **3** the analgesic action was associated with the weak suppression of the spontaneous locomotor activity in mice but only at the high doses (50–200 mg/kg). Among the three investigated substances, pyrrolidinylamide derivative **1** exhibited the strongest analgesic effects. Piperidinoamide **2** was the least active compound. The results obtained indicate that the kind of amide group influences the strength of the analgesic action. Having regard to the above statement and continuing our studies we synthesized then *N*,*N*-dialkyl(dialkenyl)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 (**10**–**14**) (Fig. 2) in order to ascertain whether the changes in the structure of amide group would distinctly influence their toxicity, analgesic and sedative activities. Compounds **10**–**14** were also tested for μ-opioid receptors binding affinities.

Newly obtained amides similarly as the compounds **1**–**3** have an asymmetric carbon atom (bearing OH group). But in the pharmacological tests they were used in the form of racemates (±).

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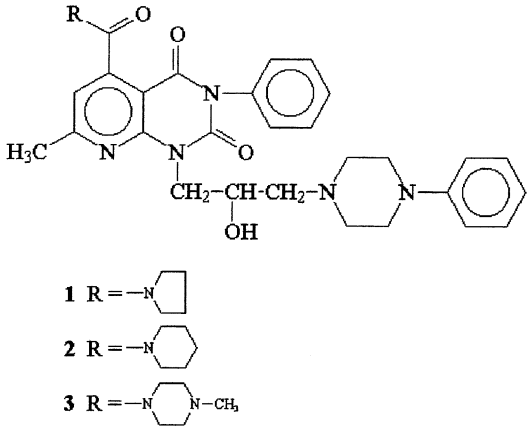


Fig. 1.

2. Chemistry

Starting material was chloride of 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylic acid (**4**) previously synthesized [1]. It was transformed into suitable amides **5**–**9** in the reaction with diethyl-, diallyl-, di-*n*-propyl-, diisopropyl- and diisobutylamines in toluene solution. The amides **5**–**9** underwent condensation with 2-hydroxy-3-(4-phenyl-1-piperazinyl)propyl chloride [2] in anhydrous ethanol and in the presence of potassium ethoxide giving derivatives **10**–**14**.

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

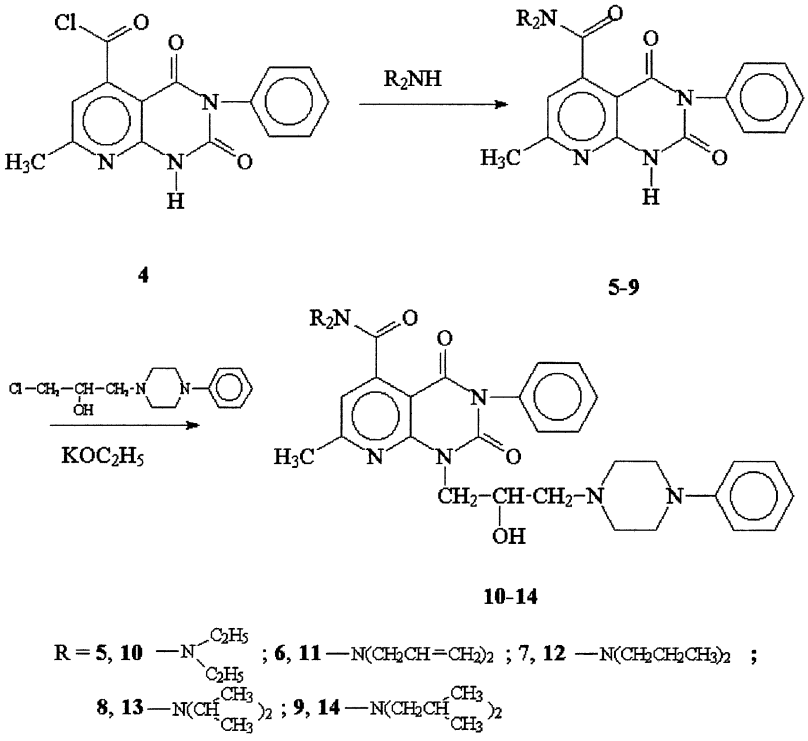
3. Experimental

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 ±0.4% of the theoretical values. All melting points (m.p.) were uncorrected. The IR spectra, in KBr pellets, were measured with a Zeiss Jena specord model IR 75 and specord M 80 (Jena). ^{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. General procedure for obtaining N,N-dialkyl(dialkenyl)amides of 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-5-carboxylic acid (5–9)

Chloride of 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylic acid (**4**) (0.014 mol) was dissolved in 80 ml of anhydrous toluene. To this solution 0.035 mol of a suitable amine (diethyl-, diallyl-, di-*n*-propyl-, diisopropyl- and diisobutylamines) 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



4 5-9 10-14

R = 5, 10 —N(C2H5)2 ; 6, 11 —N(CH2CH=CH2)2 ; 7, 12 —N(CH2CH2CH3)2 ;
8, 13 —N(CH(CH3)2)2 ; 9, 14 —N(CH2CH(CH3)2)2

Fig. 2.

Table 1
Properties of the investigated compounds

Comp.	Formula (molecular wt)	M.p. (°C) (solvent)	Yield (%)	IR absorptions in KBr (cm^{-1})		
				CO	NH or OH	Mono-substituted benzene
5	$C_{19}H_{20}N_4O_3$ (352.38)	271–274 (ethanol)	60	1635, 1675, 1720	3060, 3160–3200	700, 760
6	$C_{21}H_{20}N_4O_3$ (376.40)	267–269 (ethanol)	58	1640, 1655, 1725	3080, 3100–3200	700, 770
7	$C_{21}H_{24}N_4O_3$ (380.43)	229–231 (ethanol)	50	1620, 1680, 1730	3290–3380	700, 760
8	$C_{21}H_{24}N_4O_3$ (380.43)	287–289 (diluted ethanol)	49	1650, 1680, 1735	3080, 3140	700, 750
9	$C_{23}H_{28}N_4O_3$ (408.49)	228–230 (ethanol)	50	1620, 1670, 1720	3100–3140	700, 760
10	$C_{32}H_{38}N_6O_4$ (570.67)	139–141 (ethanol/ether)	55	1650, 1680, 1725	3360–3400	700, 750–760
11	$C_{34}H_{38}N_6O_4$ (594.69)	116–119 (ether/petroleum ether)	40	1660, 1680, 1730	3360	705, 760
12	$C_{34}H_{42}N_6O_4$ (598.72)	165–168 (ethanol)	45	1640, 1680, 1720	3360–3400	700, 760
13	$C_{34}H_{42}N_6O_4$ (598.72)	226–228 (ethanol)	45	1635, 1670, 1720	3400	700, 750
14	$C_{36}H_{46}N_6O_4$ (626.78)	143–145 (ethanol)	44	1650, 1680, 1730	3390	710, 780

was washed with 100 ml of distilled water. The obtained substance was purified by crystallization from the solvents given in Table 1.

The properties of amides (**5**–**9**) are listed in Table 1 but data concerning their ^{1}H NMR spectra are shown below:

^{1}H NMR of **5**: $\delta = 1.18$ (2 overlapping t-6H); 2.62 (s-3H); 3.09, 3.53 (2 q-4H); 6.85 (s-1H); 7.38 (m-5H); 11.42 (s-1H).

^{1}H NMR of **6**: $\delta = 2.65$ (s-3H); 3.66 (d-2H); 4.13 (d-2H) and 5.54 (m-6H); 6.85 (s-1 H); 7.35 (m-5H); 11.54 (s-1H).

^{1}H NMR of **7**: $\delta = 0.88$ (2 overlapping t-6H); 1.63 (m-4H); 2.70 (s-3H); 2.98 and 3.41 (2 distorted t-4H); 6.88 (s-1H); 7.34 (m-5H); 10.77 (s-1H).

^{1}H NMR of **8**: $\delta = 1.10$ (d-6H) and 1.54 (d-6H); 2.67 (s-3H); 3.50 (m-2H); 6.82 (s-1H); 7.34 (m-5H); 10.32 (s-1H).

^{1}H NMR of **9**: $\delta = 0.88$ (m-12H); 2.02 (m-2H); 3.20 (m-7H); 6.86 (s-1H); 7.34 (m-5H,); 10.81 (s-1H).

*3.1.2. General procedure for obtaining N,N-dialkyl(dialkenyl)amides of 7-methyl-3-phenyl-1-[2-hydroxy-3-(4-phenyl-1-piperazinyl)propyl]-2,4-dioxo-1,2,3,4-tetra-hydropyrido[2,3-d]pyrimidine-5-carboxylic acid (**10**–**14**)*

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 (**5**–**9**) was introduced. After dissolving the solid substance, 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 1/4 of its original volume and left to crystallize. The separated product (**12**–**14**) was collected on a filter and purified by crystallization from the solvents given in Table 1. In case of amides **10** and **11** ethanol was evaporated to dryness. A residue was crystallized from the suitable solvent (Table 1).

The properties of compounds **10**–**14** are listed in Table 1 and data concerning their ^{1}H NMR spectra are shown below:

^{1}H NMR of **10**: $\delta = 1.20$ (2 overlapping t-6H); 2.65 (m-9H); 3.14 (m-6H,); 3.49 (q-2H,); 4.34 (m-4H); 7.15 (m-11H).

^{1}H NMR of **11**: $\delta = 2.64$ (m-9H); 3.20 (m-4H); 3.70 (d-2H); 4.32 (m-6H); 5.47 (m-6H); 7.16 (m -11H).

^{1}H NMR of **12**: $\delta = 0.86$ (2 overlapping t-6H); 1.66 (m-4H); 3.08 (m-17H); 4.39 (m-4H); 7.16 (m-11H).

^{1}H NMR of **13**: $\delta = 1.10$ (d-6H) and 1.54 (d-6H); 2.63 (m-9H); 3.36 (m-6H); 4.39 (m-4H); 6.89 (m-11H).

^{1}H NMR of **14**: $\delta = 0.88$ (m-12H); 1.98 (m-2H); 3.01 (m-17H); 4.44 (m-4H); 7.18 (m-11H).

3.2. Pharmacology

3.2.1. Material and methods

3.2.1.1. Substances. Acetylsalicylic acid (Polopiryna, ZF Starogard Gdañski, PL), [^{3}H]dihydromorphine (Amersham), levallorphan (Sigma), morphine (Morphinum hydrochloricum, Polfa-Kutno, PL), phenylbenzoquinone (INC Pharmaceuticals, Inc, NY), thiopental sodium (Thiopentone sodium, HEFA-FRENON Arzneimittel, Germany).

3.2.1.2. Animals. The experiments were carried out on male Albino-Swiss mice (body weight 18–26 g) and male Wistar rats (body weight 120–200 g). Animals were housed in constant temperature facilities and exposed to a 12 h light:12 h dark cycle and maintained on a standard pellet diet and tap water was given *ad libitum*. Control and experimental groups consisted of 6–8 animals each. The compounds investigated were administered intraperitoneally (i.p.) as a suspension in 0.5% methylcellulose at a constant volume of 10 ml/kg.

3.2.1.3. Statistical analysis. The statistical significance was calculated using a Student's *t*-test. The ED_{50} values and their confidence limits were calculated according to the method of Litchfield and Wilcoxon [3].

3.2.1.4. Acute toxicity. Acute toxicity was assessed by the methods of Litchfield and Wilcoxon [3] and presented as LD_{50} calculated from the mortality of mice after 24 h.

3.2.1.5. Pain reactivity. Pain reactivity was measured in the 'hot plate' test according to the method of Eddy and Leimbach [4]. Animals were placed individually on the metal plate heated to 56 °C. The time (s) necessary to induce the licking reflex of the forepaws or jumping was recorded by a stop-watch. A cut off time of 60 s was used to present tissue damage. The experiment was performed 30 min after administration of the investigated compounds at graded doses of 25, 50, 100 and 200 mg/kg i.p.

3.2.1.6. 'Writhing syndrome'. 'Writhing syndrome' test was performed in mice according to Hendershot and Forsaith [5]. Different doses of the tested compounds ranging from 0.78 to 100 mg/kg were administered i.p. Twenty five minutes later, 0.02% solution (ethanol:-water, 5:95) of phenylbenzoquinone was injected i.p. in a constant volume of 0.25 ml. Five minutes after injection of the irritating agent, the number of 'writhing' episodes in the course of 10 min was counted. The analgesic effect of individual doses was expressed in per cent:

$$\%\ \text{analgesic effect} = 100 - \frac{\sum \text{of writhing incidents in experimental group}}{\sum \text{of writhing incidents in control group}} \times 100$$

The ED_{50} values and their confidence limits were estimated by the method of Litchfield and Wilcoxon [3].

3.2.1.7. Spontaneous locomotor activity. Spontaneous locomotor activity in mice was measured in circular photoresistor actometers (32 cm in diameter). The investigated compounds were injected i.p. at a dose-range of 1.56–50 mg/kg. Thirty minutes after the injection of the investigated compounds mice were placed for 30 min in the actometers. Each crossing of the light beam was recorded automatically. The amount of impulses was noted after 30 min.

3.2.1.8. Thiopental anesthesia. Thiopental sodium in a narcotic dose (55 mg/kg) was injected i.p. 30 min after administration of the tested compounds. Duration of thiopental-induced sleep was measured from disappearance to return of the righting reflex.

3.2.1.9. Radioligand binding assay [6]. Male Wistar rats were sacrificed by decapitation and the whole brains were removed rapidly and placed in ice-cold Tris–buffer. Brains were homogenized in 30 volumes of ice-

Table 2
Influence of the investigated compounds, acetylsalicylic acid and morphine on pain reactivity in the 'hot-plate' test in mice

Comp.	Dose (mg/kg)	Time of reaction on pain stimulus in seconds ±SEM	Prolongation of the reaction time (%)
Control		15.5±1.0	
10	200	47.3±10.9****	205.2
	100	25.9±4.0***	67.1
	50	27.0±6.5*	74.2
	25	12.7±2.7	
11	200	31.6±5.5***	103.9
	100	27.5±5.2***	77.4
	50	15.3±6.5	
12	200	27.8±5.6***	79.3
	100	24.0±7.1	54.8
13	200	17.3±3.8*	11.6
	100	16.0±3.4	3.2
Control		12.3±1.4	
14	200	28.8±6.9****	85.8
	100	21.8±4.9**	40.6
	50	13.8±3.0	12.2
Control		14.5±3.6	
Acetylsalicylic acid	400	31.3±4.2**	116.5
	200	19.6±4.1	35.1
	100	16.2±4.9	11.7
Control		18.4±1.0	
Morphine	6	30.6±3.9**	66.3
	3	29.6±6.0*	60.9
	1	19.4±2.1	5.4

Each group consisted of six to eight animals. *$p < 0.05$, **$p < 0.02$, ***$p < 0.01$, ****$p < 0.001$.

Table 3
Influence of the investigated compounds, acetylsalicylic acid and morphine on the pain reactivity in the 'writhing syndrome' test in mice

Comp.	Dose (mg/kg)	Mean number of writhings ± SEM	Analgesic effect (%)	ED_{50} (mg/kg)
Control	0	29.7 ± 3.5		
10	100	0.7 ± 0.3****	97.6	5.31 (2.56–11.0)
	50	1.8 ± 1.2****	93.9	
	25	4.8 ± 2.3****	83.8	
	12	7.3 ± 3.8***	75.4	
	6.25	10.5 ± 2.8***	64.6	
	3.125	20.8 ± 2.7	30.0	
	1.56	24.3 ± 2.3	18.2	
11	100	5.33 ± 2.7****	82.0	1.53 (0.21–10.9)
	50	7.33 ± 4.0***	75.3	
	25	8.2 ± 3.0***	72.4	
	12.5	9.2 ± 2.9***	69.0	
	6.25	11.6 ± 3.5***	60.9	
	3.125	12.6 ± 2.6**	57.6	
	1.56	14.8 ± 5.2*	50.2	
	0.78	17.0 ± 3.8	42.7	
12	50	4.0 ± 1.8****	86.5	2.53 (0.78–8.15)
	25	5.0 ± 1.8****	83.2	
	12.5	6.3 ± 2.9****	78.8	
	6.25	7.0 ± 2.3****	76.4	
	3.125	13.7 ± 2.8**	53.9	
	1.56	20.0 ± 3.4	32.6	
13	50	7.0 ± 3.6***	76.4	1.93 (0.36–10.25)
	25	7.6 ± 2.6***	74.4	
	12.5	8.4 ± 4.0***	71.7	
	6.25	11.0 ± 3.9***	63.0	
	3.125	12.0 ± 4.5***	59.6	
	1.56	15.5 ± 3.4*	47.8	
	0.78	19.0 ± 4.5	36.0	
14	50	6.8 ± 2.6***	77.1	4.8 (1.42–16.18)
	25	9.2 ± 2.7***	69.0	
	12.5	9.4 ± 2.4***	68.3	
	6.25	9.8 ± 2.4***	67.0	
	3.125	14.2 ± 2.7**	52.2	
	1.56	23.6 ± 2.3	20.5	
Control		19.2 ± 3.2		
Acetylsalicylic acid	100	3.2 ± 1.2****	83.3	39.15 (29.1–48.4)
	50	8.5 ± 1.3**	55.7	
	30	11.2 ± 2.1	41.7	
Morphine	10	1.2 ± 0.8****	93.7	2.44 (1.18–5.02)
	3	7.5 ± 2.9**	60.9	
	1	16.2 ± 3.51	15.6	

Each group consisted of six to eight animals. ****$p < 0.001$, ***$p < 0.01$, **$p < 0.02$, *$p < 0.05$.

cold 0.05 M Tris–buffer, pH 7.7. The homogenate was centrifuged at 48 000 × g for 15 min at 4 °C. The pellets were suspended in the same volume of buffer and incubated at 37 °C for 30 min, centrifuged at 4 °C for 10 min. The final pellet was resuspended in 50 volumes of 0.05 M fresh Tris–buffer, to pH 7.7, and used for binding studies. A 240 μl sample of the latter membrane suspension was incubated at 25 °C for 30 min with 30 μl of the buffer solution of the investigated compound (1 nM–100 μM) or levallorphan and 30 μl of a [^{3}H]DHM (0.5 nM). The incubation was followed by a rapid vaccum filtration through Whatman GF/C glass filters. The filters were washed twice with 100 μl of ice-cold Tris–buffer. The radioactivity was counted in a Wallac 1409 DSA Instrument (Finland).

Levallorphan is used for the determination of non-specific binding.

4. Results

4.1. Acute toxicity

All investigated compounds were not toxic (LD_{50} > 2000 mg/kg).

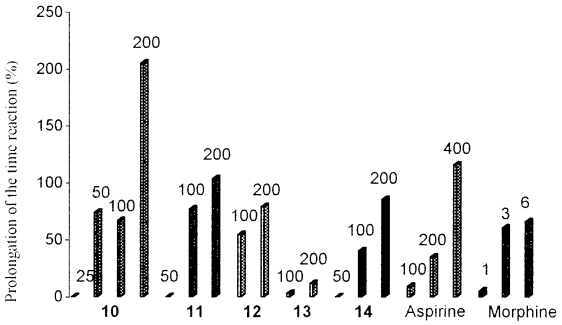


Fig. 3. Comparison of analgesic effects of tested compounds to those of acetylsalicylic acid and morphine in the 'hot plate' test in mice. The columns represent prolongation of the time reaction (%) for each tested dose of compound (mg/kg) in comparison to control.

4.2. Analgesic activity

Analgesic activities were evaluated in the 'hot plate' and in the phenylbenzoquinone-induced 'writhing' tests. All compounds produced significant analgesic activity in both tests (Tables 2 and 3).

In the 'hot plate' test, compound **10** exhibited a significant effect up to the dose of 50 mg/kg, whereas compounds **11** and **14** had analgesic action in doses up to of 100 mg/kg. Amides **12** and **13** decreased the pain sensitivity in mice in this test up to the dose of 200 mg/kg. It was interesting to observe that compounds **10**–**12** and **14** displayed activity superior to that of acetylsalicylic acid in the 'hot plate' method. The results are summarized in Table 2 and Fig. 3.

In the phenylbenzoquinone-induced 'writhing' test, compounds **11**, **13** and **12** showed analgesic effects which were superior or comparable to those of morphine, whereas analgetic action of amides **14** and **10** was weaker than that of morphine. It was interesting to observe that all compounds displayed activity superior to that of acetylsalicylic acid (Table 3).

4.3. Locomotor activity

All compounds tested produced a significant decrease in locomotor activity of mice during a 30 min observation period. Compounds **13** and **14**, given at doses of 12.5, 6.25 and 3.125 mg/kg, inhibited spontaneous locomotor activity in mice by 63–67, 59 and 49–39%, respectively. The other compounds significantly decreased locomotor activity in mice by 58.5–47% when administered at doses of 50 or 25 and 12.5 mg/kg (Table 4).

4.4. Thiopental anesthesia

All the compounds tested significantly prolonged barbiturate sleep in mice. The most potent effect was produced by compounds **12** and **13** which were active up to a dose of 12.5 mg/kg, whereas compounds **10** and **11** acted up to a dose of 25 mg/kg. Amide **14** produced a significant effect up to a dose of 50 mg/kg (Table 5, Fig. 4).

4.5. Radioligand binding assay

Table 6 illustrates the effect of the tested compounds on μ-opioid receptors affinity, as determined by standard ligand-binding techniques. All the tested com-

Table 4
Influence of the investigated compounds on the spontaneous locomotor activity in mice

Comp.	Dose (mg/kg)	Number of movements ±SEM during 30 min	% inhibition of locomotor activity	ED_{50} (mg/kg)
Control		484.0±58.1		
10	50	203.8±46.0***	57.8	31.4 (20.9–47.0)
	25	268.0±78.5*	44.6	
	12.5	301.8±80.3	37.6	
11	25	192.4±36.9***	60.2	14.6 (8.6–24.8)
	12.5	255.0±53.7*	47.3	
	6.25	311.8±50.5	35.6	
12	50	197.2±38.8***	59.3	27.5 (15.2–49.5)
	25	233.8±43.8**	51.7	
	12.5	319.8±101.4	33.9	
13	12.5	177.2±33.3***	63.4	4.5 (3.75–5.4)
	6.25	198.8±48.0**	58.9	
	3.125	248.2±66.2*	48.7	
	1.56	347.0±27.9	28.3	
14	25	137.5±10.7***	71.6	6.2 (3.3–11.8)
	12.5	161.5±8.6***	66.6	
	6.25	196.2±24.1***	59.5	
	3.125	294.4±52.2*	39.2	
	1.56	340.2±75.3	29.7	

Each group consisted of six to eight animals. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 5
Influence of the investigated compounds on the thiopental sleeping time

Comp.	Dose (mg/kg)	Duration of sleeping time ± SEM (min)	Prolongation (%)
Control		30.0 ± 5.7	
10	50	84.6 ± 19.6**	182.0
	25	65.4 ± 14.9*	118.0
	12.5	38.0 ± 5.6	26.7
11	50	76.2 ± 15.5**	154.0
	25	66.8 ± 15.2*	122.7
	12.5	45.6 ± 18.2	52.0
12	25	114.4 ± 27.6***	281.3
	12.5	88.0 ± 20.6**	193.3
	6.25	61.0 ± 17.2	103.3
13	25	90.6 ± 18.3***	202.0
	12.5	83.0 ± 22.8*	176.7
	6.25	54.2 ± 12.5	80.7
14	100	139.7 ± 32.5***	365.7
	50	115.0 ± 30.1**	283.3
	25	57.3 ± 15.2	91.0

Each group consisted of six to eight animals. ****$p < 0.001$, ***$p < 0.01$, **$p < 0.02$, *$p < 0.05$.

pounds displaced [^{3}H]dihydromorphine from binding sites in low micromolar concentration ($IC_{50} = 15.8–46.4$ μM). This effect was considerably lower than that of levallorphan and morphine [7], but comparable to tramadol, which possessed a modest affinity for μ-opioid receptors (K_i values in the micromolar range [8]).

5. Discussion

From the data presented above it follows that submitted to investigation compounds (**10**–**14**), independently of the kind of the substituents at amide nitrogen atom, showed activity in all tests performed and were non-toxic. In the 'writhing syndrome' test *N*,*N*-diethylamide derivative **10** proved to be the least active compound. Replacement of ethyl groups by allyl ones (compound **11**) caused considerable increase of analgesic action in this test. Similar effect was observed in case of di-isopropyl- and di-*n*-propylamides **13** and **12**. On the contrary, in the 'hot plate' test amide **10** showed the strongest analgesic activity. The other compounds displayed analgesic properties at the higher doses (100–200 mg/kg). In all cases analgesic action was associated with the suppression of the spontaneous locomotor activity in mice. In the last test the most active compounds appeared to be **13** and **14**, i.e. amides containing the branched alkyl groups at the amide nitrogen atom. Moreover all compounds prolonged the time of thiopental anesthesia. In this test the most active amides were compounds **12** and **13** with *N*,*N*-di-*n*-propyl- and diisopropyl substituents. Independently of the differences in the structure of amide groups all tested compounds showed a weak affinity to μ-opioid receptors.

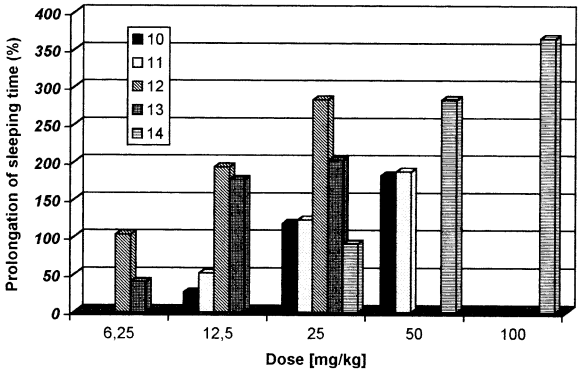


Fig. 4. Effect of tested compounds on the thiopental sleeping time.

Previously [1] synthesized amides **1**–**3** displayed analgesic properties in the 'writhing syndrome' test up to the dose of 1.56 mg/kg (**1**), 25 mg/kg (**2**) and 6.25 mg/kg (**3**). These data indicate that in this test, diallyl- and diisopropylamides (**11** and **13**) produced the analgesic effects in the same doses as pyrrolidinylamide **1** whereas diethylamide **10** was comparable on that score with the compound **3**. Di-*n*-propyl- and diisobutylamides (**12** and **14**) acted weaker than compound **1** but stronger than derivatives **2** and **3**.

Table 6
Competition of tested compounds for opioid receptors labelled with [^{3}H]dihydromorphine

Comp.	IC_{50} ± SEM (μM)
10	46.4 ± 2.5
11	29.7 ± 4.5
12	15.8 ± 1.5
13	32.0 ± 5.9
14	22.5 ± 3.1
Levallorphan	0.226 ± 0.09 nM

In the 'hot plate' test amides **1**–**3** displayed the analgesic activity up to the dose of 25 mg/kg (**1**), 100 mg/kg (**2**) and 50 mg/kg (**3**). All the newly synthesized compounds (**10**–**14**) were less active in this test than amide **1**. Diethylamide **10** produced the analgesic effects up to the dose of 50 mg/kg similarly to *N*-methylpiperazinylamide **3**. Amides **11** and **14** acted in this test like piperidinoamide **2** whereas compounds **12** and **13** were less active than the previously synthesized substances **1**–**3**. Amides **2** and **3** suppressed the spontaneous locomotor activity in mice up to the dose of 50 mg/kg. Compound **1** was inactive in this test. All the investigated substances (**10**–**14**) were more active in this test than derivatives **2** and **3**.

From the results presented it can be seen that the described chemical changes in the structure of amide groups in compounds **1**–**3** did not influence the toxicity of the obtained amides (LD_{50} for **1**–**3** and **10**–**14** > 2000 mg/kg). At the same time the performed modifications did not cause the increase of the analgesic activity in relation to the amide **1** ('lead' compound) in the 'writhing syndrome' test. All the studied compounds acted stronger than derivative **2**. Most of them were more active than amide **3** in this test. In the 'hot plate' test all the tested compounds showed weaker analgesic activity than that of the amide **1**. Depending upon the kind of substituents in amide group some of the substances **10**–**14** produced analgesic effects similar to those of compounds **2** and **3** or acted weaker than amides **2** and **3**. On the contrary, the newly synthesized amides suppressed the spontaneous locomotor activity in mice stronger than compounds **1**–**3**. Furthermore substances **10**–**14** significantly prolonged the time of thiopental induced sleep in mice. As amides **1**–**3** were not examined in this test it was not possible to compare their activity.

The above data reveal clearly that the tested compounds showed an interesting analgesic properties in the 'writhing syndrome' and 'hot plate' tests. Analgesic activity of compounds **10**–**12** and **14** was superior to that of acetylsalicylic acid in both tests. Usually the 'hot plate' test is used by many investigators for evaluation of analgesics, acting centrally, while the 'writhing syndrome' test is used for evaluation of peripheral analgesics.

Furthermore amides **10**–**14** inhibited the spontaneous locomotor activity and prolonged barbiturate sleep in mice. On the basis of the ligand binding data, we suggest that a weak affinity to μ-opioid receptors probably plays a role in the mechanism of action of these compounds. But the explanation of a precise mechanism of action will demand the further pharmacological investigations.

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