Acta Poloniae Pharmaceutica – Drug Research, Vol. 64 No. 4 pp. 369–376, 2007 ISSN 0001-6837
Polish Pharmaceutical Society

SYNTHESIS AND PHARMACOLOGICAL PROPERTIES OF 1-[2-HYDROXY-3-(4-*o*,*m*,*p*-HALOGENOPHENYL)- AND 3-(4-*m*-CHLOROPHENYL)-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 WITH ANALGESIC AND SEDATIVE ACTIVITIES

ALEKSANDRA SABINIARZ[a], HELENA ŚLADOWSKA[a], BARBARA FILIPEK[b], JACEK SAPA[b], MAGDALENA DUDEK[b] and KATARZYNA ŚLEPOKURA[c]

[a] Department of Chemistry of Drugs, Wrocław University of Medicine, Tamka 1, 50-137 Wrocław, Poland
[b] Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University, Medical College, Medyczna 9, 30-688 Kraków, Poland,
[c] Faculty of Chemistry, University of Wrocław, Joliot-Curie 14, 50-383 Wrocław, Poland

Abstract: Synthesis of 1-[2-hydroxy-3-(4-*o*,*m*,*p*-halogenophenyl)- and 3-(4-*m*-chlorophenyl)-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 (**18**, **20**-**23**, **25**, **27**-**30** and **19**, **24**, **26**) is described. All substances were active as analgesic agents in "writhing syndrome" test and except of **18** and **23** they acted stronger than acetylsalicylic acid. All final derivatives tested significantly suppressed the spontaneous locomotor activity of mice.

Keywords: pyrido[2,3-d]pyrimidines; 1-chloro-3-(4-aryl-1-piperazinyl)propan-2-ols; chlorides of 2-hydroxy-7-aryl-7-aza-4-azoniaspiro[3.5]nonane; amides; synthesis; analgesic and sedative activities

In our previous papers (1, 2) we described the preparation and pharmacological properties 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**-**8**) (Fig. 1).

All the obtained compounds displayed an interesting analgesic action in the "writhing syndrome" and "hot plate" tests and were not toxic (LD_{50} > 2000 mg/kg).

Among the three derivatives of amides, containing the moieties of cyclic amines (**1**-**3**), pyrrolidinylamide derivative **1** exhibited the strongest analgesic effects (1). Piperidinoamide **2** was the least active compound. These data indicated that in this group of compounds the strength of the analgesic action is depended on the kind of amide group. Based on this statement 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 (**4**-**8**) (Fig. 1) (2). All obtained compounds showed antinociceptive activity in both tests and were non-toxic. In "writhing syndrome" test diallyl and diisopropylamides (**5** and **7**) produced the analgesic effects in the same doses as compound **1**. In the "hot plate" test derivative **4** showed the strongest analgesic activity, whereas in the "writhing syndrome" test it proved to be the least active compound. Except of amide **1,** all the investigated substances (**2**-**8**) significantly inhibited the spontaneous locomotor activity. Furthermore, amides **4**-**8** were tested for affinity for μ-opioid receptors. Their action in this test was comparable to tramadol (2).

These findings encouraged us to continue the research in this group of compounds in order to obtain new information concerning the structure – activity relationship (SAR).

According to above, we carried out further modification of the structure of four active derivatives 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 (**1**-**3**, **5**). It consisted in introduction of chlorine and fluorine atoms into the benzene ring at the N-4 atom of piperazine. We wanted to know if the increase of lipophilicity of these compounds and

* Corresponding author: e-mail address: fastola@poczta.onet.pl

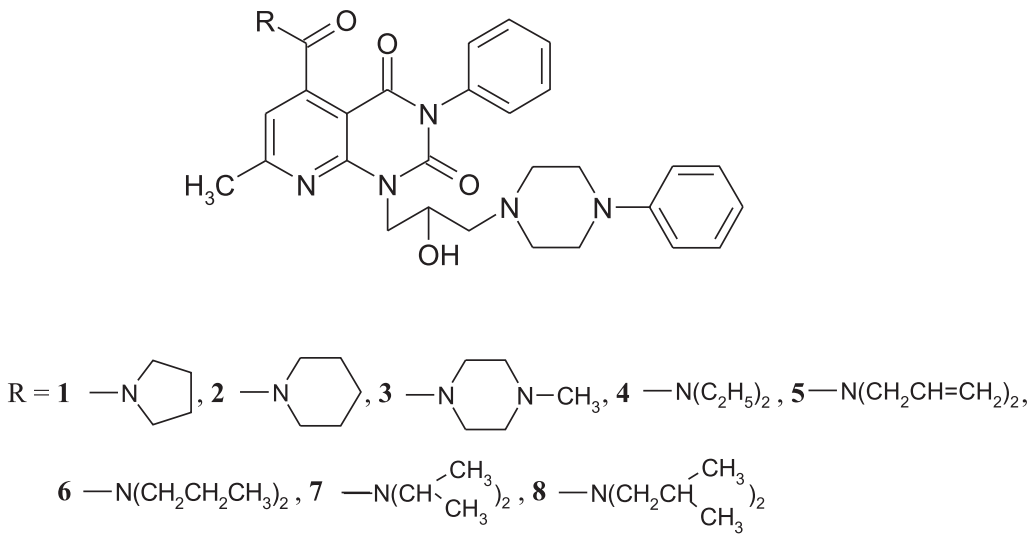

Figure 1.

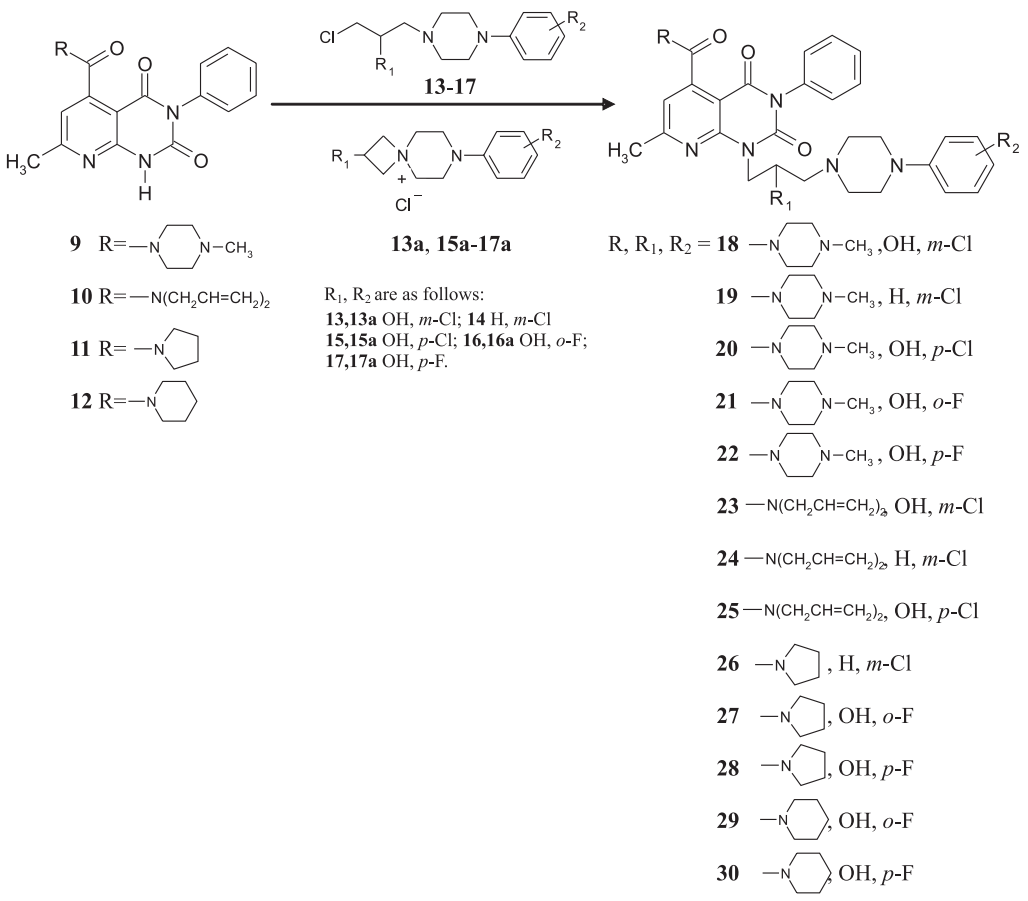

Figure 2.

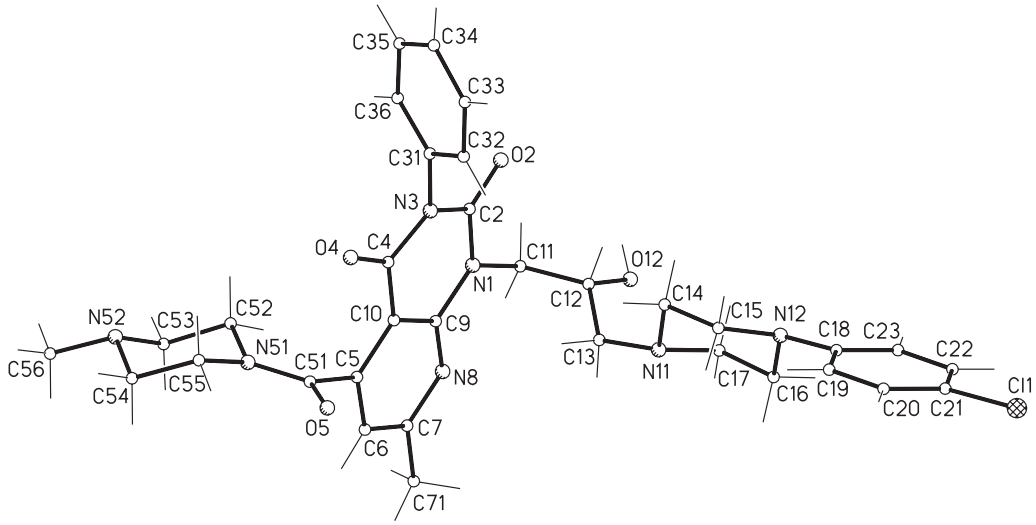

Figure 3. A view of the molecule of compound **20**

situation of halogen atoms in the phenyl substituent (*ortho*, *meta*, *para* positions) would influence their toxicity, analgesic and sedative activities. With this aim we carried out the synthesis of the appropriate amides (**18**-**30**), presented in Fig. 2. In the case of compounds **19**, **24** and **26** an elimination of the hydroxy group in the side-alkyl chain was additionally performed.

Chemistry

The starting materials for synthesis of compounds **18**-**30** were suitable amides of 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-5-carboxylic acid (**9**-**12**) previously synthesized (1, 2). They were condensed with the appropriate chlorides **13**-**17** or their cyclic isomers **13a**, **15a**-**17a** (3) in anhydrous ethanol and in the presence of potassium ethoxide giving derivatives **18**-**30**. The structure of the obtained amides was confirmed by elemental analysis, spectral methods: IR, ^{1}H-NMR and X-ray crystallography.

X-ray investigation was made for compound **20** (Fig. 3) (the strongest analgesic agent) in order to establish the structure of the alkylation product. From Fig. 3 it follows that the alkylation takes place at the nitrogen atom in position 1.

Full specification of molecular and crystalline structure will be published in crystallographic journal.

EXPERIMENTAL

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 the melting points are uncorrected. The IR spectra, in KBr pellets, were measured with a Zeiss Jena Specord M 80. ^{1}H-NMR spectra were determinated in $CDCl_3$ on a Tesla 587 A spectrometer (80 MHz) when not otherwise indicated, using TMS as an internal standard.

Chromatographic separations were performed on using silica gel 60 (70-230 mesh, Lancaster Synthesis).

GENERAL METHOD FOR SYNTHESIS OF 1-[2-HYDROXY-3-(4-ARYL-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 (**18**, **20**-**23**, **25**, **27**-**30**)

Potassium (0.01 mol) was dissolved in anhydrous ethanol (150 mL) and to this solution 0.01 mol of the appropriate amide of 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-5-carboxylic acid (**9**-**12**) was introduced. After dissolving the solid substance, to the clear solution 0.012 mol of the suitable chloride (**13**, **15**-**17** or **13a**, **15a**-**17a**) was added. The mixture was refluxed until the alkaline reaction disappeared. After the filtration, ethanol was evaporated to a small volume (in the case of compounds **18**, **20**, **22**, **25**, **28**-**30**) and left to crystallize. The separated product was collected on a filter and purified by crystallization from the solvents given below. In the case of amides **21**, **23** and **27** ethanol was evaporated completely and an oily residue was purified by column chromatography (eluents are given below).

Compound **18**: $C_{33}H_{38}ClN_7O_4$ (M. W. 632.16), m. p. 179-181°C (ethanol), yield 58 %, ^{1}H- NMR: δ (ppm) = 2.01-2.46 (m, 7H); 2.48-2.87 (m, 9H); 3.01-3.35 (m, 6H); 3.42-4.03 (m, 2H); 4.13-4.89 (m, 4H); 6.65-7.62 (m, 10H), IR (cm^{-1}): 1635, 1670, 1725 (CO), 3150-3220 (OH), 700, 750, 760 (benzene).

Compound **20**: $C_{33}H_{38}ClN_7O_4$ (M. W. 632.16), m. p. 233-236°C (ethanol), yield 56 %, ^{1}H- NMR: δ (ppm) = 2.05-2.43 (m, 7H); 2.45-2.79 (m, 9H); 2.94-3.24 (m, 6H); 3.28-3.85 (m, 2H); 3.93-4.65 (m, 4H); 6.72-7.57 (m, 10H), IR (cm^{-1}): 1655, 1675, 1725 (CO), 3120-3200 (OH), 700, 755, 825 (benzene).

Compound **21**: $C_{33}H_{38}FN_7O_4$ (M. W. 615.71), m. p. 205-207°C ($CHCl_3$: CH_3OH, 5:1 v/v, R_f = 0.64), yield 52 %, ^{1}H NMR: δ (ppm) = 1.99-2.46 (m, 7H); 2.48-2.87 (m, 9H); 2.92-3.30 (m, 6H); 3.55-3.95 (m, 2H); 4.11-4.90 (m, 4H); 6.83-7.50 (m, 10H), IR (cm^{-1}): 1635, 1670, 1725 (CO), 3120-3260 (OH), 695, 740, 750 (benzene).

Compound **22**: $C_{33}H_{38}FN_7O_4$ (M. W. 615.71), m. p. 198-200°C (ethanol), yield 57 %, ^{1}H- NMR: δ (ppm) = 2.12-2.44 (m, 7H); 2.51-2.82 (m, 9H); 2.94-3.28 (m, 6H); 3.39-3.96 (m, 2H); 4.01-4.75 (m, 4H); 6.81-7.57 (m, 10H), IR (cm^{-1}): 1635, 1670, 1720 (CO), 3120-3220 (OH), 695, 750, 820 (benzene).

Compound **23**: $C_{34}H_{37}ClN_6O_4$ (M. W. 629.16), m. p. 156-158°C ($CHCl_3$:CH_3OH, 20:1 v/v, R_f = 0.47), yield 56 %, ^{1}H-NMR: δ (ppm) = 2.45-2.84 (m, 9H); 3.04-3.34 (m, 4H); 3.59-3.79 (d, 2H); 4.06-4.73 (m, 6H); 4.96-6.10 (m, 6H); 6.66-7.71 (m, 10H), IR (cm^{-1}): 1635, 1675, 1720 (CO), 3040-3120 (OH), 695, 745, 770 (benzene).

Compound **25**: $C_{34}H_{37}ClN_6O_4$ (M. W. 629.16), m. p. 110-112°C (ethanol), yield 58 %, ^{1}H- NMR: δ (ppm) = 2.45-2.85 (m, 9H); 3.03-3.27 (m, 4H); 3.57-3.77 (d, 2H); 4.00-4.67 (m, 6H); 4.90-6.10 (m, 6H); 6.69-7.59 (m, 10H), IR (cm^{-1}): 1660, 1680, 1730 (CO), 3120-3200 (OH), 705, 765, 815 (benzene).

Compound **27**: $C_{32}H_{35}FN_6O_4$ (M. W. 586.66), m. p. 144-146°C (acetone:cyklohexane, 4:1 v/v, R_f = 0.58), yield 47 %, ^{1}H-NMR: δ (ppm) = 1.72-2.00 (m, 4H); 2.44-2.84 (m, 9H); 2.93-3.22 (m, 6H); 3.44-3.77 (m, 2H); 4.10-4.41 (m, 1H); 4.46-4.68 (m, 3H); 6.83-7.58 (m, 10H), IR (cm^{-1}): 1630, 1675, 1725 (CO), 3040-3120 (OH), 705, 745-770 (benzene).

Compound **28**: $C_{32}H_{35}FN_6O_4$ (M. W. 586.66), m. p. 212-214°C (ethanol), yield 54 %, ^{1}H- NMR (300 MHz): δ (ppm) = 1.82-2.00 (m, 4H); 2.54-2.82 (m, 9H); 3.06-3.18 (m, 6H); 3.52-3.78 (m, 2H); 4.22-4.35 (m, 1H); 4.45-4.60 (m, 3H); 6.82-7.55 (m, 10H), IR (cm^{-1}): 1665, 1695, 1735 (CO), 3080-3120 (OH), 710, 780, 840 (benzene).

Compound **29**: $C_{33}H_{37}FN_6O_4$ (M. W. 600.69), m. p. 150-152°C (ethanol), yield 54 %, ^{1}H-NMR: δ (ppm) = 1.34-1.78 (m, 6H); 2.63-2.85 (m, 9H); 2.96-3.24 (m, 6H); 3.33-4.13 (m, 2H); 4.15-4.68 (m, 4H); 6.82-7.63 (m, 10H), IR (cm^{-1}): 1640, 1675, 1720 (CO), 3040-3120 (OH), 695, 745, 760 (benzene).

Compound **30**: $C_{33}H_{37}FN_6O_4$ (M. W. 600.69), m. p. 200-202°C (ethanol), yield 59 %, ^{1}H NMR: δ (ppm) = 1.37-1.94 (m, 6H); 2.45-2.83 (m, 9H); 2.97-3.27 (m, 6H); 3.34-4.12 (m, 2H); 4.15-4.69 (m, 4H); 6.78-7.61 (m, 10H), IR (cm^{-1}): 1645, 1670, 1720 (CO), 3100-3180 (OH), 700, 755, 815 (benzene).

Table 1. Influence of the compounds investigated on the pain reaction in the „hot-plate" test in mice

Comp. No.	Dose (mg/kg)	Prolongation of the reaction time (%)
18	200 100	33.5 24.0
19	200 100	63.9* 14.5
20	200 100 50	91.2*** 55.5* 25.9
21	200 100	15.7 5.8
22	200 100	52.3* 30.2
23	200 100	0 0
24	200 100	41.6 27.0
25	200 100	24.1 19.2
26	200 100	89.4*** 14.0
27	200 100	39.4 38.7
28	200 100	17.0 16.8
29	200 100	40.7 16.9
30	200 100	44.5 15.3

Each group consisted of 6-8 animals. *** $p < 0.01$; * $p < 0.05$

GENERAL METHOD FOR SYNTHESIS OF 1-[3-(4-ARYL-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 (**19**, **24**, **26**).

0.022 mol of potassium was dissolved in 150 mL of anhydrous ethanol and to this solution 0.01 mol of the appropriate amide (**9**-**11**) was added. To the clear solution 0.012 mol of 1-(3-chlorophenyl)-4-(3-chloropropyl)piperazine monohydrochloride (**14** × HCl) (Aldrich) was introduced. The obtained

suspension was stirred at first at room temperature (about 1 h) then boiled. The course of the reaction was controlled by TLC (mobile phase $CHCl_3$:CH_3OH (10:1 v/v)) R_f = 0.47 (**19**), 0.87 (**24**), 0.71 (**26**). Then, after filtration, ethanol was evaporated to an oily residue (**19**) or to a small volume in case of **24** and **26** and left to crystallize. The solid substances (**24**, **26**) were collected on a filter and purified by crystallization from the solvents given below. The oily product **19** was purified by column chromatography.

Compound **19**: $C_{33}H_{38}ClN_7O_3$ (M. W. 616.16), m. p. 186-188°C ($CHCl_3$:CH_3OH, 10:1 v/v, R_f = 0.47), yield 62 %, ^{1}H-NMR: δ (ppm) = 1.71-2.38 (m, 9H); 2.45-2.77 (m, 9H); 3.01-3.31 (m, 6H,); 3.35-4.18 (m, 2H); 4.25-4.61 (t, 2H); 6.67-7.59 (m, 10H), IR (cm^{-1}): 1635, 1670, 1715 (CO), 690, 755, 765 (benzene).

Compound **24**: $C_{34}H_{37}ClN_6O_3$ (M. W. 613.16), m. p. 161-163°C (ethanol), yield 59 %, ^{1}H-NMR (300 MHz): δ (ppm) = 1.92-2.06 (m, 2H); 2.52-2.65 (m, 9H); 3.12-3.28 (m, 4H); 3.63-3.78 (distorted d, 2H); 4.05-4.25 (m, 2H); 4.38-4.60 (m, 2H); 5.03-5.28 (m, 4H); 5.58-5.95 (2 × m, 2 × H); 6.65-7.53 (m, 10H), IR (cm^{-1}): 1640, 1680, 1720 (CO), 705, 755, 770 (benzene).

Compound **26**: $C_{32}H_{35}ClN_6O_3$ (M. W. 587.12), m. p. 190-192°C (ethanol), yield 56 %, ^{1}H-NMR (300 MHz): δ (ppm) = 1.72-2.08 (m, 6H); 2.52-2.70 (m, 9H); 3.06-3.22 (m, 6H); 3.52-3.78 (m, 2H); 4.36-4.58 (m, 2H); 6.72-7.52 (m, 10H), IR (cm^{-1}): 1640, 1670, 1720 (CO), 700, 740-770 (benzene).

Pharmacology

MATERIALS AND METHODS

Substances

Acetylsalicylic acid (Polopiryna, ZF Starogard Gdański, PL), morphine (Morphinum hydrochloricum, Polfa-Kutno, PL), phenylbenzoquinone (INC Pharmaceuticals, Inc. N.Y.).

Animals

The experiments were carried out on male Albino-Swiss mice (body weight 18-26 g). Animals were housed in constant temperature facilities exposed to 12:12 h light-dark cycle and maintained on a standard pellet diet and tap water given *ad libitum*. All procedures conformed to the Animal Care and Use Committee Guidelines, and were approved by the Ethical Committee of Jagiellonian University, Kraków.

Control and experimental groups consisted of 6-8 animals each. The investigated compounds were administered intraperitoneally (i.p.) as the suspension in 0.5% methylcellulose in constant volume of 10 mL/kg.

Statistical analysis

The statistical significance was calculated using the Student's t-test. The ED_{50} values and their confidence limits were calculated according to the method of Litchfield and Wilcoxon (4).

Acute toxicity was assessed by the methods of Litchfield and Wilcoxon (4) and presented as LD_{50} calculated from the mortality of mice after 24 hours.

"Writhing syndrome" in mice according to Hendershot and Forsaith (5). Different doses of the tested compounds ranging from 3.125 mg/kg to 200 mg/kg were administered i.p. Twenty five minutes later, 0.02% solution (ethanol-water, 5:95 v/v) of phenylbenzoquinone was injected intraperitoneally 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 percent:

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

The ED_{50} values and their confidence limits were estimated by the method of Litchfield and Wilcoxon (4).

Pain reactivity was measured in the "hot plate" test according to the method of Eddy and Leimbach (6). Animals were placed individually on the metal plate heated to 56°C. The time (in seconds) of appearance of the pain reaction (licking of the forepaws or jumping) was recorded by a stop-watch. The experiments were performed 30 min after administration of the investigated compounds at graded doses of 100 and 200 mg/kg i.p.

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 1-200 mg/kg. Thirty minutes after the injection, mice were placed in the actometers for 30 min. Each crossing of the light beam was recorded automatically. The amount of impulses was noted after 30 min.

RESULTS AND DISCUSSION

Acute toxicity.

After intraperitoneal administration compounds **18**-**26**, **28** and **29** were not toxic (LD_{50} > 2000 mg/kg). Only the amides **27** and **30** had a little higher toxicity (LD_{50} = 1580 mg/kg (**27**), LD_{50} = 1800 mg/kg (**30**)). Toxic doses of all tested deriva-

tives caused sedation and a decrease of the locomotor activity. The examined substances showed lower toxicity than aspirin (ASA) and morphine after intraperitoneal administration (aspirin LD_{50} = 167 mg/kg (7), morphine LD_{50} = 140 mg/kg (8)).

Analgesic properties of the obtained compounds were assessed by the "hot plate" and "writhing syndrome" tests:

"Hot plate" test

In this test only derivative **20**, given i.p. at the doses of 200 and 100 mg/kg, showed strong analgesic activity, whereas compounds **19**, **22** and **26** produced significant analgesic effects at the dose of 200 mg/kg. The summarized data are shown in Table 1.

"Writhing syndrome" test in mice

All amides tested displayed analgesic activity in this test. The most potent effects were produced by **20** and **28** (ED_{50} = 6.4 mg/kg and ED_{50} = 6.9 mg/kg, respectively). Two derivatives **18** and **23** showed similar antinociceptive properties to ASA. Eleven substances investigated (**19**-**22** and **24**-**30**) in this test were more active than acetylsalicylic acid but these analgesic effects were weaker than those of morphine. The ED_{50} values and therapeutic indexes for the compounds studied are presented in Figure 4.

Locomotor activity

All derivatives tested significantly suppressed the spontaneous locomotor activity of mice during a 30 min observation period. The most potent effects were produced by derivative **28** (ED_{50} = 2.9 mg/kg). On the contrary, the weakest activity in this test was displayed by amide **22** (ED_{50} = 128 mg/kg). The ED_{50} values and therapeutic indexes for the compounds investigated are presented in Figure 5.

From the data presented above it follows that the most active substance in the "writhing syndrome" test was N-methylpiperazinylamide derivative **20** with *p*-chlorophenyl substituent at N-4 of piperazine. Displacement of chlorine atom in **20** from *para* to *meta* position (substance **18**) was unprofitable. *Para*-chloroisomer **20** acted in this test considerably stronger than *meta*-chloroisomer **18**. The replacement of chlorine atom in **20** by fluorine (compound **22**) weakened the antinociceptive effects. The situation of fluorine atom in *para*-position of the phenyl (**22**) was less advantageous for analgesic activity than that in *ortho* (**21**). Elimination of the hydroxy group in the side-alkyl chain in **18** (amide **19**) caused an increase of the analgesic action.

From among diallylamide derivatives (**23**–**25**) the most active substance in the "writhing syndrome" test was compound **25**, containing *p*-chlorophenyl substituent. Its *m*-chlorophenyl isomer (**23**) acted considerably weaker. The similar situation was observed in the case of above-mentioned N-methylpiperazinylderivatives **20** and **18**. Elimination of the hydroxy group in the propyl linker in **23** (amide **24**) caused an increase of antinociceptive properties. The same relationship was stated in case of compounds **18** and **19** being N-methylpiperazinylamide derivatives.

In the group of pyrrolidinylamide derivatives **26**-**28**, substance **28** with *p*-fluorophenyl substituent

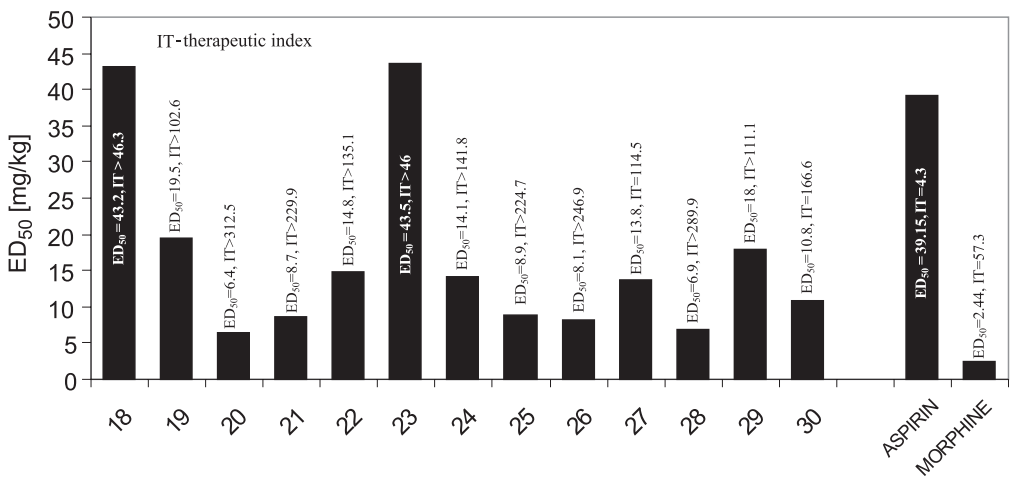

Figure 4. Influence of the compounds investigated on the pain in the "wrirng syndrome" test in mice.

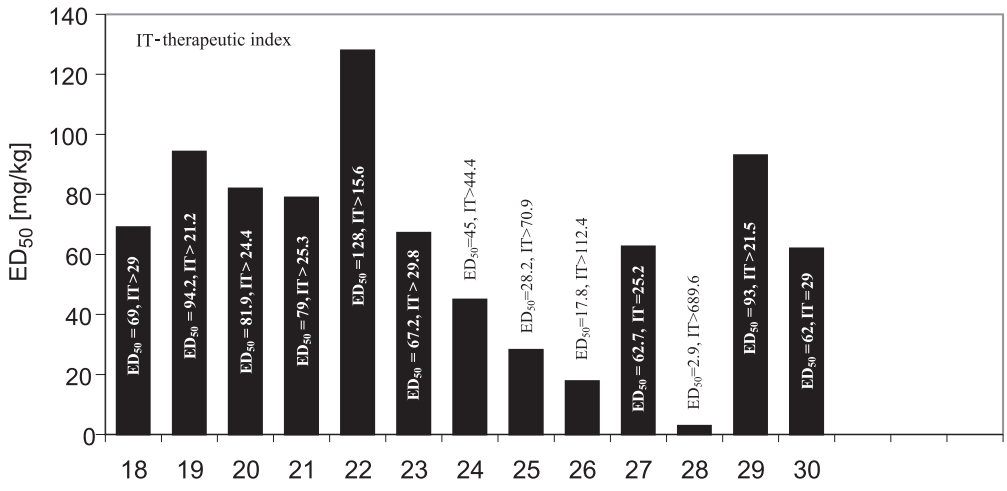

Figure 5. Influence of the compounds on the spontaneous locomotor activity in mice.

was characterized by the strongest analgesic activity. Its *ortho*-fluoroisomer **27** was less active as analgesic agent in "writhing syndrome" test. Derivative **26** containing *m*-chlorophenyl group and propyl chain displayed a little weaker antinociceptive properties than amide **28**.

From two compounds **29** i **30** being piperidineamide derivatives, substance **30**, with *p*-fluorophenyl substituent acted stronger than *ortho*-fluoroisomer **29**.

Amides **18** and **23** dispalyed in the "writhing" test almost the same analgesic properties despite of the differences in the structure of amide groups. From among three compounds **19**, **24** and **26** devoid of the hydroxy group in propyl chain the strongest "anti-writhing" agent was pyrrolidinylamide derivative **26**. The ED_{50} values indicate also that between two analogues **20** and **25** (with *p*-chlorophenyl group) the more active substance in this test was N-methylpiperazinylamide derivative **20**. The differences in analgesic activity displayed also analogues **21**, **27** and **29**, containing the same substituent with *o*-fluorophenyl group at N-1 of pyridopyrimidine system. In this case N-methylpiperazinylamide derivative **21** generated the strongest antinociceptive effects. On the contrary to this, in the group of compounds **22**, **28** and **30** with *p*-fluorophenyl substituent the strongest analgesic activity was observed in case of compound **28** (pyrrolidinylamide derivative). The above-mentioned data confirmed our earlier statement (1, 2) that in this series of compounds the strength of analgesic action depends among others on the structure of the amide group.

In the "hot plate" test only four compounds (**19**, **20**, **22** and **26**) produced analgesic effects. **19**, **20** and **22** belong to the group of N-methylpiperazinylamide, **26** is the pyrrolidinylamide derivative. Similarly as in the "writhing syndrome" test, the most active substance was compound **20** with *p*-chlorophenyl substituent. Elimination of the hydroxy group with simultaneous displacement of chlorine atom from *para* to *meta* position in **20** (amide **19**), as well as replacement of chlorine atom in **20** by fluorine (**22**) weakened the analgesic properties in this test. An exchange of N-methylpiperazinyl group for pyrrolidinyl one in **19** (substance **26**) increased antinociceptive effects.

In all the cases analgesic action was associated with the suppression of the spontaneous locomotor activity in mice. In this test the most active compound was **28** (pyrrolidinylamide with *p*-fluorophenyl group). The weakest inhibition of the locomotor activity was produced by N-methylpiperazinylamide analogue (**22**). *Meta*-chlorophenyl derivatives of N-methylpiperazinyl- (**18**) and diallylamide (**23**) caused similar suppression of the spontaneous locomotor activity in mice. Elimination of the hydroxy group in the propyl linker in **18** and **23** (amides **19** and **24**) weakened this effect in **19** but in case of **24** the increase of inhibition was observed. From two compounds **20** and **25** containig *p*-chlorophenyl substituent, diallylamide derivative **25** was more active in this test. The N-methylpiperazinylamide derivative **22** possessing fluorine atom in *para*-position generated weaker inhibition of the locomotor activity than *o*-fluoroisomer (**21**).

However, opposite effect was observed in case of derivatives of pyrrolidinyl- and piperidinoamides, *para*-fluoroisomers (**28** and **30**) acted in this test stronger than *ortho*-fluoroderivatives (**27** and **29**) and similar situation took place in "writhing syndrome" test.

Previously (1, 2) synthesized amides **1**-**3** and **5** displayed an interesting analgesic action in the "writhing syndrome" test (values of ED_{50} are as follows: 1.44 mg/kg (**1**), 13 mg/kg (**2**), 6.1 mg/kg (**3**), 1.53 mg/kg (**5**)). These data indicated that in this test a majority of the studied compounds showed weaker analgesic properties than the above mentioned compounds **1**-**3** and **5**. The most active substance **20** (ED_{50} = 6.4 mg/kg) displayed similar analgesic activity to that of the parent structure **3**. Antinociceptive effects of **30** (ED_{50} = 10.8 mg/kg) were stronger than these of **2,** indicating that introduction of fluorine atom in *para* position of phenyl in piperidinoamide **2** increased analgesic action.

In the "hot plate" test **1**-**3** and **5** displayed analgesic properties up to the dose of 25 mg/kg (**1**), 50 mg/kg (**3**), 100 mg/kg (**2** and **5**) (1, 2). A majority of the newly synthesized amides were inactive in this test. Three compounds (**19**, **22** and **26**) which were active at the dose of 200 mg/kg showed weaker analgesic activity in relation to the parent substances **3** and **1**. Derivative **20** acted up to the dose of 100 mg/kg, similarly to **2** and **5**, but its antinociceptive effects were less advantageous than those of **3**.

Amides **2**, **3** and **5** suppressed significantly the spontaneous locomotor activity in mice (their ED_{50} = 94.4 mg/kg (**2**), 121.2 mg/kg (**3**) and 14.6 mg/kg (**5**)) (1, 2). Compound **1** was inactive in this test. Introduction of fluorine atom in *para*-position of phenyl at N-4 piperazine in **1** (compound **28** with ED_{50} = 2.9 mg/kg) caused the appearance of the potent sedative action. Amide **28** proved to be the most active substance in this test from all described before (1, 2). Other modifications in pyrrolidinylamide **1** (**26**, **27**) were also profitable in this test. Except of amide **22,** all N-methylpiperazinylamide derivatives suppressed stronger spontaneous locomotor activity in mice than parent substance **3**. Fluoroderivatives of **2** (**29** and **30**) acted stronger (**30**) or similarly (**29**) in relation to **2** in this test. An introduction of halogens into the phenyl ring at N-4 of piperazine in **5** (amides **23**-**25**) caused a considerable decrease of the sedative action.

CONCLUSION

In continuation of our investigations we now report on the synthesis, analgesic activity and influence on the spontaneous locomotor activity in mice of a new analogues of the earlier obtained active compounds (1, 2). The analgesic properties of the amides synthesized in this study were measured using the "hot-plate" and phenylbenzoquinone-induced "writhing" assays.

In the "hot plate" test only compounds **20** produced significant antinociceptive effects at doses of 200 and 100 mg/kg, whereas compounds **19**, **22** and **26** were significantly active at a dose of 200 mg/kg. In the "writhing syndrome" test all derivatives studied displayed stronger than (**19**-**22**, **24**-**30**) or similar to (**18**, **23**) analgesic activity of ASA but weaker one than morphine.

Our investigations indicate that the potent antinociceptive properties in these tests possessed compound **20**, which was non-toxic.

Furthermore, all newly tested compounds significantly suppressed the spontaneous locomotor activity of mice.

The mode of action and activity of compound **20** was promising enough to continue further experiments.

REFERENCES

1. Śladowska H., Sieklucka-Dziuba M., Rajtar G., Sadowski M., Kleinrok Z.: Farmaco 54, 773 (1999).
2. Śladowska H., Sabiniarz A., Filipek B., Kardasz M., Maciąg D.: Farmaco 58, 25 (2003).
3. Śladowska H., Sabiniarz A., Szkatuła D., Filipek B., Sapa J.: Acta Pol. Pharm. – Drug Res. 63, 245 (2006).
4. Litchfield J. T., Wilcoxon F.: J. Pharmacol. Exp. Ther. 96, 99 (1949).
5. Hendershot L. C., Forsaith J.: J. Pharmacol. Exp. Ther. 125, 237 (1959).
6. Eddy N. B., Leimbach D.: J. Pharmacol. Exp. Ther. 107, 385 (1953).
7. http://chem.sis.nlm.nih.gov/chemidplus/ (aspirin).
8. http://chem.sis.nlm.nih.gov/chemidplus/(morphine).

Received: 24.01.2007