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SYNTHESIS AND PROPERTIES OF 4-ALKOXY-2-[2-HYDROXY-3-(4-*o*,*m*,*p*-HALOGENOARYL-1-PIPERAZINYL)PROPYL]-6-METHYL-1H-PYRROLO-[3,4-C]PYRIDINE-1,3(2*H*)-DIONES WITH ANALGESIC AND SEDATIVE ACTIVITIES

HELENA ŚLADOWSKA[a]*, ALEKSANDRA SABINIARZ[a], DOMINIKA SZKATUŁA[a], BARBARA FILIPEK[b] and JACEK SAPA[b]

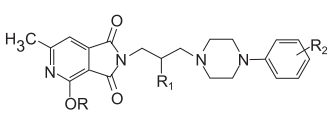
[a] Department of Chemistry of Drugs, Wrocław Uniwersity of Medicine, Tamka 1, 50-137 Wrocław, Poland
[b] Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University, Collegium Medicum, Medyczna 9, 30-688 Kraków, Poland

Abstract: Synthesis of N-substituted derivatives of 4-alkoxy-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-diones (**17-26**) is described. The chlorides, containing OH group, used in the above synthesis can exist in two isomeric forms: chain (**12, 14-16**) and cyclic (**12a, 14a-16a**). All final imides studied exhibited analgesic activity in the "writhing syndrome" test which was superior than that of acetylsalicylic acid. In the "hot plate" test only two compounds (**19, 20**) were active as antinociceptive agents. Furthermore, all compounds tested significantly suppressed the spontaneous locomotor activity of mice.

Keywords: 4-Alkoxy-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione derivatives; 1-chloro-3-(4-aryl-1-piperazinyl)propan-2-ols and their isomeric cyclic forms; synthesis; analgesic and sedative activities

It has been reported previously (1) that 4-alkoxy-6-methyl-2-[2-hydroxy-3-(4-phenyl-1-piperazinyl)propyl]-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-diones (3,4-pyridinedicarboximides) (**1, 2**) displayed potent analgesic activity and were not toxic (LD_{50} > 2000 mg/kg). Compound **1** with methoxy substituent in position 4 was more active than its ethoxy homologue **2**.

Introduction of trifluoromethyl or methoxy groups at the phenyl ring at the N-4 position of piperazine in the imide **1** (compounds **3** and **5**) caused a decrease of the analgesic action both in the "writhing" syndrome and "hot plate" tests. A similar relationship was noticed in the case of compounds **2** and **4** in the first test. In the "hot plate" test derivative **4** was more active than the parent imide **2**. An elimination of the hydroxy group in β-position of the propyl moiety in compounds **1** and **2** (imides **6** and **7**) weakened the analgesic properties while introduction of the methoxy group in *ortho*-position of phenyl ring in **6** and **7** (compounds **8** and **9**) caused an increase of the analgesic activity (2). At the same time these modifications resulted in the non-toxic substances (LD_{50} for **3–9** > 2000 mg/kg).



R, R_1, R_2 are as follows: **1** CH_3, OH, H; **2** C_2H5, OH, H; **3** CH_3, OH, *m*-CF_3; **4** C_2H_5, OH, *m*-CF_3; **5** CH_3, OH, *o*-OCH_3; **6** CH_3, H, H; **7** C_2H_5, H, H; **8** CH_3, H, *o*-OCH_3; **9** C_2H_5, H, *o*-OCH_3

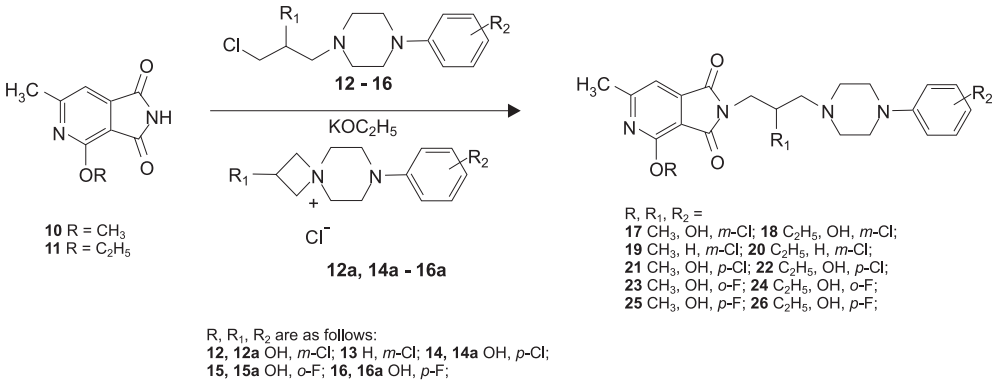
Figure 1. The structure of compounds **1** – **9**.

It was interesting to observe that in the "writhing" test all the above mentioned compounds displayed activity superior to that of acetylsalicylic acid (ASA). In the "hot plate" test imide **7** was inactive at a dose of 200 mg/kg similarly as aspirin whereas the other compounds acted stronger than ASA. Except for **1**, in all the cases analgesic activity was associated with the significant suppression of the spontaneous locomotor activity of mice (1, 2).

These findings encouraged us to continue the synthesis 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 1H-pyrrolo-[3,4-c]pyridine-1,3(2H)-diones **1**, **2**. It consisted in intro-

* Corresponding author: e-mail address – stanislawa@bf.uni.wroc.pl



Scheme 1.

duction of chlorine and fluorine atoms into the phenyl ring at the N-4 position of piperazine. We wanted to know if the increase of lipophilicity of these compounds and situation of halogens in phenyl substituent (*ortho*, *meta* and *para* positions) would influence their toxicity, analgesic and sedative activities. With this aim we carried out the synthesis of the appropriate 1H-pyrrolo[3,4-c}pyridine-1,3(2H)-diones (**17–26**), presented in Scheme 1.

In the case of the compounds **19** and **20** an elimination of the hydroxy group in the side-alkyl chain was additionally performed (analogues of **6** and **7**).

We expected that the compounds obtained would exhibit the analgesic and sedative properties.

Chemistry

The starting materials for the synthesis of the above mentioned compounds were 4-methoxy- and 4-ethoxy-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-diones (2-methoxy- and 2-ethoxy-6-methyl-3,4-pyridinedicarboximides (**10**, **11**)) (3). They were condensed with the appropriate chlorides **12–16** or their cyclic isomers (**12a**, **14a–16a**) in anhydrous ethanol in the presence of potassium ethoxide giving compounds **17–26**. The structures of the obtained compounds were confirmed by elemental and spectral (IR, ^{1}H NMR) analyses.

The above mentioned 2-hydroxy-3-(4-aryl-1-piperazinyl)propyl chlorides were synthesized in the reaction of epichlorohydrin with appropriate N-halogenoarylpiperazines in ethanol according to the literature data (7, 8). Carrying out this reaction we noticed that the structure of the obtained products was dependent on temperature. At room temperature water non-soluble substances were formed whereas the same reaction performed at 40°C afforded isomeric water-soluble compounds which gave positive reaction for chloride ions with silver nitrate. Similar observations were made by us earlier (1) in case of using to this reaction N-*o*-methoxy- and N-*m*-trifluoromethylphenyl piperazines. Based on the results of our previous research (confirmed by X-ray analysis) (1) as well as elemental and spectral analyses, we ascribed to the water-insoluble substances the structures of 2-hydroxy-3-(4-aryl-1-piperazinyl)propyl chlorides (**12**, **14–16**). The isomeric water-soluble compounds were appropriate derivatives of 7-aza-4-azoniaspiro[3,5]nonane chloride (**12a**, **14a–16a**) (Scheme 1). In the literature (7) there is a mention concerning 1-chloro-3-[4-(3 and 4-chlorophenyl)-1-piperazinyl]propan-2-ols (**12**, **14**) which without isolation (after reaction of epichlorohydrin with suitable piperazines) were used as intermediates in the synthesis of 5-(1-aryl-4-piperazinyl)methyl-2-amino-2-oxazolines with antidepressant activity.

EXPERIMENTAL

Chemistry

All the results of the C,H,N determinations (carried out using 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 Specord M 80 (C. Zeiss, Jena). ^{1}H NMR spectra were determined in $CDCL_3$ on a Tesla 587 A spectrometer (80 MHz), when not otherwise indicated, using TMS as an internal standard.

General method for synthesis of 2-[2-hydroxy-3-(4-aryl-1-piperazinyl)]propyl derivatives of 4-methoxy and 4-ethoxy-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-diones (**17**, **18**, **21–26**).

A 0.01 mol of potassium was dissolved in 120 mL of anhydrous ethanol and to this solution 0.01 mol of 4-methoxy- or 4-ethoxy-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3-(2H)-dione (**10**, **11**) was added. The reaction mixture was refluxed for 15 min in case

of the imide **10** and to the obtained suspension (imide **10**) or clear solution (imide **11**) 0.012 mol of the appropriate chloride (**12**, **14–16** or **12a, 14a–16a**) was added. The mixture was refluxed until the alkaline reaction was finished. After filtration the clear solution (**18**, **24**) was left to crystallize. Then the separated product (**18**, **24**) was collected on a filter and purified by the crystallization from the solvent, given in Table 1. In the case of compounds **17**, **21–23**, **25** and **26** the solid substances precipitated during the reaction. After cooling they were also collected on a filter and dried. The purity of imides **21**, **22**, **25** and **26** was sufficient for pharmacological tests. Because of their sparing solubility the small samples of these substances were purified for analysis whereas imides **17** and **23** were recrystallized. The appropriate solvents used for crystallization are presented in Table 1.

The properties of compounds **17**, **18**, **21–26** are listed in Table 1 but the assignments of their ^{1}H NMR spectra are shown below:

^{1}H NMR of **17**: δ (ppm) = 2.35–2.85 (m, 9H, C**H**$_3$ + **-H**$_2$C-N-(C**H**$_2$)$_2$-); 2.94–3.27 (m, 4H, -(C**H**$_2$)$_2$-N-Ph); 3.70–3.85 (distorted d, 3H, Hα of propyl + O**H**); 3.90–4.23 (m, 4H, Hβ of propyl + OC**H**$_3$); 6.58–7.24 (m, 5H, arom. H).

^{1}H NMR of **18**: δ (ppm) = 1.29–1.61 (t, 3H, C**H**$_3$-CH$_2$-); 2.32–2.79 (m, 9H, C**H**$_3$ + **-H**$_2$C-N-(C**H**$_2$)$_2$-); 3.03–3.32 (distorted t, 4H, -(**H**$_2$C)$_2$-N-Ph); 3.62–3.82 (distorted d, 3H, Hα of propyl + O**H**); 3.90–4.23 (m, 1H, -C**H**-); 4.39–4.78 (q, 2H, -C**H**$_2$-CH$_3$); 6.63–7.32 (m, 5H, arom. H).

^{1}H NMR of **21** in DMSO-d$_6$ (300 MHz): δ (ppm) = 2.35–2.53 (m, 9H, C**H**$_3$ + **-H**$_2$C-N-(C**H**$_2$)$_2$-); 2.85–3.00 (m, 4H, -(C**H**$_2$)$_2$-N-Ph); 3.50–3.59 (m, 3H, Hα of propyl + O**H**); 3.90–4.15 (m, 4H, OC**H**$_3$ + HB of propyl); 6.70–7.45 (m, 5H, arom. H).

^{1}H NMR of **22** in DMSO-d$_6$ (300 MHz): δ (ppm) = 1.29–1.34 (t, 3H, C**H**$_3$-CH$_2$-); 2.28–2.60 (m, 9H, C**H**$_3$ + **-H**$_2$C-N-(C**H**$_2$)$_2$-); 2.80–3.00 (m, 4H, -(**H**$_2$C)$_2$-N-Ph); 3.50–3.65 (m, 3H, Hα of propyl + O**H**); 3.80–4.00 (m, 1H, -C**H**-); 4.40–4.50 (q, 2H, -C**H**$_2$-CH$_3$); 6.83–7.30 (m, 5H, arom. H).

^{1}H NMR of **23**: δ (ppm) = 2.36–2.84 (m, 9H, C**H**$_3$ + **-H**$_2$C-N-(C**H**$_2$)$_2$-); 2.91–3.22 (m, 4H, -(C**H**$_2$)$_2$-N-Ph); 3.66–3.84 (m, 3H, Hα of propyl + O**H**); 3.88–4.23 (m, 4H, Hβ of propyl + OC**H**$_3$); 6.84–7.30 (m, 5H, arom. H).

^{1}H NMR of **24**: δ (ppm) = 1.26–1.64 (t, 3H, C**H**$_3$-CH$_2$-); 2.31–2.82 (m, 9H, C**H**$_3$ + **-H**$_2$C-N-(C**H**$_2$)$_2$-); 2.89–3.24 (distorted t, 4H, -(**H**$_2$C)$_2$-N-Ph); 3.58–3.84 (distorted d, 3H, Hα of propyl + O**H**); 3.90–4.19 (m, 1H, -C**H**-); 4.35–4.79 (q, 2H, -C**H**$_2$-CH$_3$); 6.59–7.33 (m, 5H, arom. H).

^{1}H NMR of **25** in DMSO-d$_6$ (300 MHz): δ (ppm) = 2.31–2.60 (m, 9H, C**H**$_3$ + **-H**$_2$C-N-(C**H**$_2$)$_2$-); 2.80–2.95 (m, 4H, -(C**H**$_2$)$_2$-N-Ph); 3.50–3.60 (m, 3H, Hα of propyl + O**H**); 3.90–4.05 (m, 4H, OC**H**$_3$ + Hβ of propyl); 6.82–7.33 (m, 5H, arom. H).

^{1}H NMR of **26** in CDCl$_3$ + DMSO-d$_6$: δ (ppm) = 1.28–1.55 (t, 3H, C**H**$_3$-CH$_2$-); 2.38–2.77 (m, 9H, C**H**$_3$ + **-H**$_2$C-N-(C**H**$_2$)$_2$-); 2.82–3.20 (m, 4H, -(**H**$_2$C)$_2$-N-Ph); 3.65–3.82 (distorted d, 3H, Hα of propyl + O**H**); 3.96–4.34 (m, 1H, -C**H**-); 4.43–4.77 (q, 2H, -C**H**$_2$-CH$_3$); 6.78–7.63 (m, 5H, arom. H).

General method for synthesis of 2-[3-(4-aryl-1-piperazinyl)]propyl derivatives of 4-methoxy- and 4-ethoxy-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-diones (**19**, **20**).

0.022 mol of potassium was dissolved in 120 mL of anhydrous ethanol and to this solution 0.01 mol of 4-methoxy- or 4-ethoxy-6-methyl-1H-pyrrolo[3,4-c]pyridine-1,3(2H)-dione (**10**, **11**) was added. The reaction mixture was stirred for 10 min and next, to the obtained suspension (imide **10**) or clear solution (imide **11**) 0.012 mol of 1-(3-chlorophenyl)-4-(3-chloropropyl)piperazine monohydrochloride (**13** × HCl) (Aldrich Chemical Company) was added. The mixture was stirred at first at room temperature (about 2 h) then boiled. The course of the reaction was controlled by TLC (eluant chloroform:methanol (15:1 v/v)), Rf = 0.77 (**19**) and 0.85 (**20**).

Then, after filtration, ethanol was evaporated to a small volume and the residue was left to crystallize. The separated crystals (**19**, **20**) were collected on a filter and purified using the solvent given in Table 1.

The properties of the obtained compounds **19** and **20** are shown in Table 1, but the assignments of their ^{1}H NMR spectra are presented below:

^{1}H NMR of **19**: δ (ppm) = 1.66–2.08 (m, 2H, Hβ of propyl); 2.23–2.74 (m, 9H, C**H**$_3$ + **-H**$_2$C-N-(C**H**$_2$)$_2$-); 2.86–3.31 (m, 4H, -(C**H**$_2$)$_2$-N-Ph); 3.61–3.96 (t, 2H, Hα of propyl; 4.12 (s, 3H, OC**H**$_3$); 6.58–7.42 (m, 5H, arom. H).

^{1}H NMR of **20**: δ (ppm) = 1.28–1.60 (t, 3H, C**H**$_3$-CH$_2$-); 1.65–2.10 (m, 2H, Hβ of propyl) 2.24–2.66 (m, 9H, C**H**$_3$ + **-H**$_2$C-N-(C**H**$_2$)$_2$-); 2.89–3.18 (m, 4H, -(**H**$_2$C)$_2$-N-Ph); 3.56–3.90 (t, 2H, Hα of propyl); 4.44–4.73 (q, 2H, -C**H**$_2$-CH$_3$); 6.58–7.29 (m, 5H, arom. H).

2-Hydroxy-3-[4-(3(4)-chlorophenyl)-1-piperazinyl]propyl chloride (**12**, **14**).

Sodium (0.02 mol) was dissolved in 50 mL of anhydrous ethanol and to this solution 0.02 mol of 3(4)-chlorophenylpiperazine monohydrochloride

was added. The mixture was stirred at room temperature for 2 h. The inorganic substance was collected on a filter and then ethanol was evaporated to a small volume under diminished pressure. To the obtained solution 0.02 mol of epichlorohydrin was added and stirred at room temperature for 5 h. The separated solid substance was collected on a filter and washed with ethanol and then with distilled water.

M.p.'s 82–84°C (yield 85%) (**12**), 91–92°C (yield 70%) (**14**).

Compounds **12** and **14** were insoluble in water and gave no chloride ions test with silver nitrate.

12: $C_{13}H_{18}N_2OCl_2$, M_w = 289,20 (C, H, N determinations)

^{1}H NMR of **12** (300 MHz): δ (ppm) = 2.58–2.90 (m, 6H, **-H**$_2$C-N(C**H**$_2$)$_2$-); 3.18–3.35 (m, 4H, -(**H**$_2$C)$_2$-N-Ph); 3.55–3.66 (distorted d, 3H, -C**H**$_2$Cl + O**H**); 3.97–4.08 (m, 1H, -C**H**-), 6.75–7.28 (m, 4H, H of phenyl).

14: $C_{13}H_{18}N_2OCl_2$, M_w = 289,20 (C, H, N determinations)

^{1}H NMR of **14**: δ (ppm) = 2.43–2.97 (m, 6H, **-H**$_2$C-N(C**H**$_2$)$_2$-); 3.04–3.33 (m, 4H, -(**H**$_2$C)$_2$-N-Ph); 3.49–3.74 (distorted d, 3H, -C**H**$_2$Cl + O**H**); 3.81–4.17 (m, 1H, -C**H**-), 6.68–7.33 (m, 4H, H of phenyl).

Chloride of 2-hydroxy-7-(3(4)-chlorophenyl)-7-aza-4-azoniaspiro[3,5]nonane (**12a**, **14a**).

The synthesis of compounds **12a**, **14a** was performed similarly to procedure described for chlorides **12**, **14** but the reagents were stirred at 40°C (5 h). A few milliliters of diethyl ether were added to a cold solution and the mixture was left overnight. The resulting solid substance was collected on a filter and washed with small amount of ethanol. The analytical sample was obtained after crystallization from ethanol.

M.p.'s 137–139°C (yield 60%) (**12a**), 127–128°C (yield 61%) (**14a**).

12a and **14a** were water-soluble and gave positive reaction for chloride ions with silver nitrate.

12a: $C_{13}H_{18}N_2OCl_2$, M_w = 289,20 (C, H, N determinations)

^{1}H NMR of **12a** in DMSO-d_6 (300 MHz): δ (ppm) = 3.39–3.48 (m, 4H, **-**(**H**$_2$C)$_2$-N-Ph); 3.54–3.62 and 3.65–3.72 (2 distorted t, 4H, 2 × C**H**$_2$ of piperazine fragment); 4.15–4.24 (dd, 2H, -C**H**$_2$-); 4.49–4.58 (m, 3H, -C**H**$_2$- + O**H**), 4.61–4.72 (m, 1H, -C**H**-); 6.52–7.27 (m, 4H, H of phenyl).

14a: $C_{13}H_{18}N_2OCl_2$, M_w = 289,20 (C, H, N determinations)

^{1}H NMR of **14a** in DMSO-d_6 (300 MHz): δ (ppm) =

Table 1. Properties of the investigated compounds.

				IR absorptions in KBr (cm^{-1})		
Compound	formula (molecular weight)	M.p. (°C) (solvent)	Yield %	C=O	OH	Disubstituted benzene
17	$C_{22}H_{25}ClN_4O_4$ (444.91)	178–180 (ethyl acetate)	63	1730, 1775	3110–3200	755, 780
18	$C_{23}H_{27}ClN_4O_4$ (458.93)	177–178 (ethyl acetate)	50	1725, 1768	3100–3200	748, 770
19	$C_{22}H_{25}ClN_4O_4$ (428.91)	95–98 (ethanol)	60	1725, 1773		750, 780
20	$C_{23}H_{27}ClN_4O_3$ (442.93)	76–78 (ethanol)	72	1715, 1765		742, 770
21	$C_{22}H_{25}ClN_4O_4$ (444.91)	224–226 (ethanol)	78	1720, 1773	3090–3180	820
22	$C_{23}H_{27}ClN_4O_4$ (458.93)	222–224 (ethyl acetate)	87	1720, 1770	3105–3180	820
23	$C_{22}H_{25}FN_4O_4$ (428.45)	176–178 (chloroform/ether, 19:1, v/v)	72	1730, 1775	3090–3175	750
24	$C_{23}H_{27}FN_4O_4$ (442.48)	166–168 (ethanol)	55	1715, 1770	3075–3165	760
25	$C_{22}H_{25}FN_4O_4$ (428.45)	221–223 (ethyl acetate)	82	1720, 1770	3090-3190	822
26	$C_{23}H_{27}FN_4O_4$ (442.480)	219–220 (ethyl acetate)	75	1720, 1770	3085–3105	815

3.39–3.48 (m, 4H, **-**($\mathbf{H}_2$C)$_2$-N-Ph); 3.55–3.61 and 3.65–3.72 (2 distorted t, 4H, 2 × C$\mathbf{H}_2$ of piperazine fragment); 4.15–4.24 (dd, 2H, -C$\mathbf{H}_2$-); 4.49–4.58 (m, 3H, -C$\mathbf{H}_2$- +O**H**), 4.61–4.72 (m, 1H, -C**H**-); 6.52–7.28 (m, 4H, H of phenyl).

2-Hydroxy-3-[4-(3(4)-chlorophenyl)-1-piperazinyl]propyl chloride (**15**, **16**).

Epichlorohydrin (0.02 mol) was added to a vigorously stirred solution of the appropriate substituted N-phenylpiperazine (0.02 mol) in 50 mL of anhydrous ethanol. The mixture was stirred at room temperature for 5 h. The separated solid substance was collected on a filter and washed with ethanol, then with distilled water.

M.p.'s 65–67˚C (yield 75%) (**15**), 79–82˚C (yield 85%) (**16**).

Compounds **15** and **16** were insoluble in water and gave no chloride ions test with silver nitrate.

15: $C_{13}H_{18}N_2OFCl$, M_w = 272,75 (C, H, N determinations)

^{1}H NMR of **15:** δ (ppm) = 2.34–2.89 (m, 6H, **-**$\mathbf{H}_2$C-N(C$\mathbf{H}_2$)$_2$-); 2.95–3.32 (m, 4H, -($\mathbf{H}_2$C)$_2$-N-Ph); 3.42–3.72 (distorted d, 3H, -C$\mathbf{H}_2$Cl + O**H**); 3.80–4.18 (m, 1H, -C**H**-), 6.70–7.21 (m, 4H, H of phenyl).

16: $C_{13}H_{18}N_2OFCl$, M_w = 272,75 (C, H, N determinations)

^{1}H NMR of **16:** δ (ppm) = 2.36–2.84 (m, 6H, **-**$\mathbf{H}_2$C-N(C$\mathbf{H}_2$)$_2$-); 2.96–3.24 (m, 4H, -($\mathbf{H}_2$C)$_2$-N-Ph); 3.40–3.62 (distorted d, 3H, -C$\mathbf{H}_2$Cl + O**H**); 3.70–4.01 (m, 1H, -C**H**-), 6.68–7.04 (m, 4H, H of phenyl).

Chlorides of 2-hydroxy-7-(2(4)-fluorophenyl)-7-aza-4-azoniaspiro[3,5]nonane (**15a**, **16a**).

Epichlorohydrin (0.02 mol) was added to a solution of N-(2(4)-fluorophenyl)piperazine in 50 mL of anhydrous ethanol. The mixture was stirred at 40˚C for 5 h. A few milliliters of diethyl ether were added to a cold solution and the mixture was left overnight. The resulting solid substance was collected on a filter and washed with a small amount of ethanol. The analytical sample was obtained after crystallization from ethanol.

M.p.'s 198–200˚C (yield 68%) (**15a**), 123–125˚C (yield 62%) (**16a**).

15a and **16a** were water-soluble and gave positive reaction for chloride ions with silver nitrate.

15a: $C_{13}H_{18}N_2OCl_2$, M_w = 272,75 (C, H, N determinations)

^{1}H NMR of **15a** in DMSO-d_6 (300 MHz): δ (ppm) = 3.20–3.50 (m, 4H, **-**($\mathbf{H}_2$C)$_2$-N-Ph); 3.60–3.68 and 3.72–3.79 (2 distorted t, 4H, 2 × C$\mathbf{H}_2$ of piperazine fragment); 4.17–4.26 (dd, 2H, -C$\mathbf{H}_2$-); 4.52–4.59 (m, 3H, -C$\mathbf{H}_2$- + O**H**), 4.59–4.72 (m, 1H, -C**H**-); 6.98–7.22 (m, 4H, H of phenyl).

16a: $C_{13}H_{18}N_2OCl_2$, M_w = 272,75 (C, H, N determinations)

^{1}H NMR of **16a** in DMSO-d_6 (300 MHz): δ (ppm) = 3.25–3.40 (m, 4H, **-**($\mathbf{H}_2$C)$_2$-N-Ph); 3.55–3.65 and 3.70–3.80 (2 distorted t, 4H, 2 × C$\mathbf{H}_2$ of piperazine fragment); 4.18–4.29 (dd, 2H, -C$\mathbf{H}_2$-); 4.50–4.58 (m, 3H, -C$\mathbf{H}_2$- + O**H**), 4.62–4.72 (m, 1H, -C**H**-); 6.67–7.13 (m, 4H, H of phenyl).

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 were according to the Animal Care and Use Committee Guidelines, and 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 as the suspension in 0.5% methylcellulose in constant volume of 10 mL/kg.

Statistical analysis

The statistical significance was calculated using the Student't 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 morality of mice after 24 hours.

"Writhing" syndrome in mice according to Hendershot and Forsaith (5). Different doses of the tested compounds ranging from 0.78 mg/kg to 100 mg/kg were administered intraperitoneally (*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 per cent:

Table 2. Influence of the compounds investigated on the pain reaction in the "writhing syndrome" test in mice.

Compound	X/LD_{50}	Dose mg/kg	Mean no. of writhings ± SEM	ED_{50} [mg/kg]	LD_{50}/ED_{50} Therap. index
Control	–	–	29.7 ± 3	–	–
17	1/80 1/160 1/320 1/640	25 12.5 6.25 3.125	6.5 ± 3.2**** 3.7 ± 1**** 5.2 ± 1.3**** 18.2 ± 4	3.51 (2.9–4.2) –	> 569.8 –
Control	–	–	21.3 ± 2.4	–	–
18	1/40 1/80 1/160 1/320	50 25 12.5 6.25	1.8 ± 0.7**** 4.1 ± 2** 6.2 ± 1.1** 11.4 ± 3.1	6.4 (5.3–7.6)	> 312.5
19	1/40 1/80 1/160 1/320	50 25 12.5 6.25	0.8 ± 0.1**** 2.4 ± 0.2**** 8.6 ± 2.1** 13.1 ± 1.8	8.8 (6.7–11.4)	> 227.2
20	1/40 1/80 1/160 1/320	50 25 12.5 6.25	1.1 ± 0.8**** 4.2 ± 1.1*** 6.8 ± 1.3** 13.6 ± 2.3	8.7 (7.2–10.4)	> 229.8
21	1/40 1/80 1/160 1/320	50 25 12.5 6.25	1.9 ± 0.8**** 5.1 ± 1.5*** 8.4 ± 1.4** 13.9 ± 2.8	9.5 (7.8–9.8)	> 222.0
22	1/40 1/80 1/160	50 25 12.5	6.1 ± 1.8** 8.9 ± 1.1** 14.8 ± 2.7	8.0 (7.3–8.8)	> 250.0
Control	–	–	29.7 ± 3	–	–
23	1/40 1/160 1/640	50 12.5 3.125	9.4 ± 2.7*** 14.5 ± 4.3** 24 ± 4.8	16.4 (12.1–21.2)	> 124.7
Control	–	–	21.3 ± 2.4	–	–
24	1/40 1/80 1/160 1/320	50 25 12.5 6.25	0.3 ± 0.1**** 4.2 ± 0.8*** 7.2 ± 3.1* 12.8 ± 2.1	8.6 (7.8–9.4)	> 232.5
25	1/40 1/80 1/160 1/320	50 25 12.5 6.25	2.0 ± 0.9**** 5.8 ± 1.8*** 10.4 ± 1.2* 14.2 ± 2.1	11.3 (8.7–14.7)	> 176.9
26	1/40 1/80 1/160 1/320	50 25 12.5 6.25	1.8 ± 1.1**** 3.9 ± 2.2*** 5.4 ± 0.9** 11.4 ± 2.2	6.2 (5.1–7.4)	> 322.5
Control	–	–	19.2 ± 3.2	–	–
Acetylsali–cylic acid	– – –	100 50 30	3.2 ± 1.2**** 8.5 ± 1.3** 11.2 ± 2.1	39.15 (29.1–48.4)	–
Morphine	– – –	10 3 1	1.2 ± 0.8**** 7.5 _ 2.9** 16.2 ± 3.5	2.44 (1.18–5.02)	–

Each group consisted of 6–8 aimals. **** $p < 0.001$; *** $p < 0.01$; ** $p < 0.02$; * $p < 0.05$

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

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 (s) 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 25, 50, 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 intraperitoneally at a dose-range of 1.56–50 mg/k. Thirty minutes after the injection of the investigated compounds, 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 the investigated compounds were not toxic (LD_{50} > 2000 mg/kg). All tested imides, given at dose of 2000 mg/kg, caused sedation and decrease of the locomotor activity.

"Writhing syndrome" test in mice.

All compounds tested showed analgesic activity in this test. The most potent effect was produced by compound **17**, which was significantly effective up to a dose of 6.25 mg/kg. Imides **18–21** and **23–26** decreased the pain sensitivity in this test up to a dose of 12.5 mg/kg while **22** was active up to a

Table 3. Influence of the compounds investigated on the pain reaction in the "hot plate" test in mice.

Compound	Dose		Time of reaction to pain stimulus ± SEM (s)
	fraction of LD_{50}	mg/kg	
Control	–	–	19.5 ± 2.6
17	1/10 1/20	200 100	32.7 _ 13 32.2 ± 10
Control	–	–	16.75 ± 2
18	1/10 1/20	200 100	19.2 ± 3.3 16.3 ± 3.7
19	1/10 1/20	200 100	39.2 ± 10.4* 23.2 ± 7.3
20	1/10 1/20 1/40 1/80	200 100 50 25	37.7 ± 7* 28.8 ± 3.2* 28.7 ± 6.7* 11.2 ± 0.6
21	1/10 1/20	200 100	19.9 ± 2.7 17.6 ± 1.2
22	1/10 1/20	200 100	19.8 ± 3.9 17.3 ± 5.7
Control	–	–	19.5 ± 2.6
23	1/10 1/20	200 100	21.3 ± 2.2 30.5 ± 3.4
Control	–	–	16.75 ± 2
24	1/10 1/20	200 100	24.7 ± 2.9 11 ± 1.2
25	1/10 1/20	200 100	25.8 ± 6.6 11.7 ± 3.8
26	1/10 1/20	200 100	19 ± 3.4 8.3 ± 2.7

Each group consisted of 6–8 animals. * $p < 0.05$

dose of 25 mg/kg. All derivatives studied displayed stronger analgesic activity than ASA but weaker one than morphine. The summarized data are shown in Table 2.

"Hot plate" test.

In the "hot plate" test only two compounds (**19** and **20**) were active in a dose of 200 mg/kg (**19**) and up to a dose of 50 mg/kg (**20**) (Table 3).

Locomotor activity.

All compounds tested significantly suppressed the spontaneous locomotor activity of mice during a 30 min observation period. Imide **23** produced a significant decrease of the locomotor activity up to a dose of 12.5 mg/kg whereas compounds **17–20** and **24–26** acted up to a dose of 25 mg/kg. Derivative **22** inhibited spontaneous locomotor activity in mice up to a dose of 50 mg/kg while **21** was active in this test up to a dose of 100 mg/kg. The ED_{50} values and therapeutic indexes for the compounds investigated are presented in Table 4.

From the data presented above it can be seen that all compounds tested **17–26** displayed signifi-

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

Compound	Dose		No. of impulses	ED_{50} (mg/kg)	Therap. index
	X/LD_{50}	mg/kg			
Control	–	–	451 ± 68	–	–
17	1/40 1/80 1/160	50 25 12.5	146.2 ± 48.4*** 205 ± 62.4** 272 ± 81.2	20.5 (17.1–24.5)	> 97.6
Control	–	–	425 ± 62	–	–
18	1/40 1/80 1/160	50 25 12.5	180 ± 28*** 255 ± 69** 389 ± 38**	35.8 (25.5–50.1)	> 55.8
19	1/40 1/80 1/160	50 25 12.5	178 ± 48*** 254 ± 58* 396 ± 47**	37.7 (29–49)	> 53
20	1/40 1/80 1/160	50 25 12.5	162 ± 41*** 189 ± 58** 310 ± 54**	27.4 (22.8–32.8)	> 72.9
21	1/10 1/20 1/140	200 100 50	173 ± 29*** 294 ± 48** 399 ± 28	153.5 (127–183)	> 13.0
22	1/10 1/20 1/40 1/80	200 100 50 25	140 ± 40*** 208 ± 78** 268 ± 40* 389 ± 69	93 (71.5–121)	> 21.5
Control	–	–	451 ± 68	–	–
23	1/40 1/80 1/160 1/320	50 25 12.5 6.25	102 ± 38**** 119 45.6**** 210 ± 74** 386 ± 79	161 (12.3–20.9)	> 124.4
Control	–	–	425 ± 62	–	–
24	1/40 1/80 1/160	50 25 12.5	109 ± 26**** 221 ± 71** 342 ± 74	27.5 (25–30.2)	> 72.7
25	1/40 1/80 1/160	50 25 12.5	191 ± 40*** 252 ± 54* 381 ± 37	39.4 (34.2–45.3)	> 50.7
26	1/40 1/80 1/160	50 25 12.5	168 ± 41*** 224 ± 45** 352 ± 38	33.7 (28–40.4)	> 59.3

Each group consisted of 6–8 aimals. **** $p < 0.001$; *** $p < 0.01$; ** $p < 0.02$; * $p < 0.05$

cant antinociceptive properties in the "writhing syndrome" test and were non-toxic. The most active substance in this test was imide **17**, containing methoxy group in position 2 of the pyridine ring and *m*-chlorophenyl substituent at N-4 atom of piperazine. The replacement of methoxy group by ethoxy one in **17** (imide **18**) caused a considerable decrease of the analgesic activity. It was in accordance with our earlier statement (1, 2). Elimination of the hydroxy group in the side-alkyl chain in **17** and **18** (imides **19** and **20**) also weakened the antinociceptive effects. Worthy of notice is a fact that in this case both methoxy and ethoxy homologues displayed almost the same analgesic properties. Displacement of chlorine atom in **17** and **18** from *meta* to *para* position (derivatives **21** and **22**) was unprofitable. *Meta*-chloroisomers (**17**, **18**) acted in this test stronger than *para*-chloroisomers (**21**, **22**). The replacement of chlorine atom by fluorine in **21** and **22** (imides **25** and **26**) caused an increase of the analgesic action only in the case of ethoxy derivative **26**. Methoxyimide with *p*-chlorophenyl substituent at N-4 atom of piperazine ring (**21**) was more active in this test than *p*-fluoro analogue **25**. The situation of fluorine atom in *ortho* position of the phenyl (imides **23** and **24**) was less advantageous than that in *para*-position (substances **25** and **26**). In the case of compounds **23** and **24** containing *o*-fluorophenyl substituent, ethoxy homologue **24** was more active as anti-writhing agent than methoxy one.

In the "hot plate" test only compounds **19** and **20** characterized by a lack of the OH group in the side-alkyl chain produced the analgesic effect. Both imides contained *m*-chlorophenyl substituent at N-4 atom of piperazine. Compound **20** with OC_2H_5 group displayed in this test stronger analgesic properties than methoxy derivative **19**.

Similarly as previously (1, 2), in all the cases antinociceptive effects were associated with the significant suppression of the spontaneous locomotor activity of mice. The most active substance in this test was compound **23**, possessing OCH_3 and *o*-fluorophenyl substituents. The weakest inhibition of the locomotor activity was produced by compounds **21** and **22**, containing *p*-chlorophenyl at N-4 atom of piperazine. The imide with OC_2H_5 group (**22**) was more active in this test than methoxy derivative (**21**).

Previously (1) synthesized imides **1**, **2** displayed analgesic properties in the "writhing" syndrome test up to a dose of 0.39 mg/kg (ED_{50} = 0.4 mg/kg) (**1**), 1.56 mg/kg (ED_{50} = 1.4 mg/kg) (**2**). These data indicate that in this test all the compounds studied were weaker analgesic agents than the above mentioned imides **1**, **2**. Substances **19** and **20** were also less active in this test than their analogues **6** and **7** (0.78 mg/kg (ED_{50} = 1.03 mg/kg) (**6**), 1.56 mg/kg (ED_{50} = 2.59 mg/kg) (**7**)) (2).

In the "hot plate" test **1**, **2** and **6** displayed analgesic properties up to a dose of 12.5 mg/kg (ED_{50} = 11.9 mg/kg((**1**), 25 mg/kg (ED_{50} = 17.6 mg/kg) (**2**), 100 mg/kg (**6**). Compound **7** was inactive in this test similarly as most of the newly synthesized imides. Two active compounds **19** and **20** showed considerably weaker analgesic activity in this test than **1** and **2**. Antinociceptive effects of **19** were also weaker than those of **6** whereas imide **20** was more active than **6**. Introduction of chlorine atom into the phenyl at N-4 atom of piperazine in **7** (compound **20**) produced analgesic action.

Compounds **2**, **6** and **7** suppressed the spontaneous locomotor activity in mice at a dose of 200 mg/kg (ED_{50} = 107.9 mg/kg) (**2**), up to a dose of 12.5 mg/kg (**6**, **7**) (ED_{50} = 15.17) (**6**) and 21.04 mg/kg (**7**). Imide **1** was inactive in this test. A majority of the newly synthesized substances were more active in this test than compound **2**.

The data presented above indicate that the described modifications in the structure of imides **1**, **2**, **6** and **7** did not influence the toxicity. All the tested compounds had LD_{50} > 2000 mg/kg similarly to the parent substances. At the same time the performed modifications did not increase the analgesic activity in relation to imides **1**, **2**, **6**, **7** in the "writhing" syndrome test. In the "hot plate" test most of the newly synthesized compounds were inactive alike as **7**.

CONCLUSION

The analgesic activities of the compounds obtained in this study were measured using the phenylbenzoquinone-induced writhing assays and "hot-plate" test. From the pharmacological and toxicological point of view, all imides tested possessed significant antinociceptive effects in the phenylbenzoquinone-induced writhing test with ED_{50} values ranging from 3.51 to 16.04 mg/kg. All the investigated compounds displayed more potent analgesic activity than ASA. The most active derivatives (**17–22**, **24, 26**) had a low toxicity (LD_{50} > 2000 mg/kg, *i.p.*) and showed some sedative effects from a dose of 6.25–12.5 mg/kg. Two of them (**19** and **20**) were also active in the test of "hot plate".

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