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Synthesis, Pharmacological Properties and SAR of New 1,4-Disubstituted Piperazine Derivatives with Hypnotic-Sedative Activity *

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Synthesis, chromatographic behaviour, pharmacological data and structure activity relationship (SAR) studies of some 1-(pyrimidin-2-yl)piperazine derivatives **1**–**13** are reported. The hydrophobic indices and chromatographic retention factors of the compounds exhibited statistically significant linear correlation with the calculated lipophilicities. The highest hypnotic-sedative activity as measured in loss of the righting reflex, rota-rod and spontaneous locomotor activity tests exhibited compound **6** possessing *n*-hexyl R substituent. The hypnotic activity of compounds **1**–**13** measured in loss of the righting reflex test could be described with the aid of hydrophobic indices in terms of Hansch parabolic relationship for structurally related group of compounds (separately for compounds with R = alkyl or R = cycloalkyl). The necessity to describe the pharmacological activity for aliphatic and alicyclic series with 2 different equations corresponded well to the observed difference in the pharmacological properties between the both groups of compounds.

Key words: hypnotic-sedative agents, synthesis, SAR

Study of a relationship between chemical structure and pharmacological properties is an often used tool in a drug research. The scope of the drug research covers such area as a search for a new drug as a new chemical entity (NCE), a better understanding of drug action mechanisms, approaches to overcome serious problems involving a well known in chemotherapy multidrug resistance (MDR) of microrganisms and cancer tissues [1–5].

It is known that correlation between biological and physicochemical properties for a group of compounds may be assessed with the aid of different structural descriptors. Lipophilicity is one of the most important and widely used parameter since it strongly influences on a compound distribution between biological compartments [6]. Lipophilicity (originally defined as an octanol-water partition coefficient)

* Dedicated to Prof. E. Borowski on the occasion of his 75th birthday.

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may be measured experimentally by a shake-flask method or calculated theoretically. It can also be evaluated on the basis of chromatographic parameters either directly as logarithm of retention index or a so-called hydrophobic index, that is a retention index extrapolated to 100% water [7–9].

Hypnotic-sedative agents form a large group of compounds commonly used to treat sleep disorders. According to WHO data *ca*. 1/3 adults in USA suffer from insomnia [10]. Hypnotic-sedatives produce sedative effects, at higher doses causing hypnosis end even general anaesthesia and their mechanism of action is sometimes unclear. Two main classes of hypnotic-sedative agents are benzodiazepines and barbiturates. The benzodiazepines, which are used as hypnotics, sedatives and anxiolytics bind to the benzodiazepine site of $GABA_A$ receptor complex and enhance the binding of its neurotransmitter – gamma-aminobutyric acid [11–14]. Barbiturates, the other class of hypnotic-sedatives, also modulate the GABA neurotransmission [15].

In our previous papers [16–19] we described preparation, pharmacological and SAR studies of several 1,4-disubstituted piperazine derivatives, a new class of hypnotic-sedatives with mechanism of activity different [19] from that observed for known hypnotic-sedative zopiclone acting through $GABA_A$ receptor [20]. It has been found that the examined 1,4-disubstituted piperazines reduced the 5-HT turnover in the mouse brain after the systemic administration, exhibiting resemblance to zopiclone in that experimental paradigm [19].

In the present paper we report preparation, chromatographic behaviour, pharmacological data and structure activity relationship (SAR) studies of some new 1-(pyrimidin-2-yl)piperazine derivatives **1**–**13**, where compounds **1**–**8** form an aliphatic and compounds **9**–**12** an alicyclic homologous series (Fig. 1).

EXPERIMENTAL

Chemistry

Melting points (uncorrected values) were determined with Büchi 535 apparatus. ^{1}H NMR and ^{13}C NMR spectra were obtained using Varian Gemini 2000 spectrometer (200 MHz) with Me_4Si as internal standard; chemical shifts were reported in ppm (δ) and signals were quoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). IR spectra were recorded on Perkin-Elmer 1724X spectrophotometer in 4000–400 cm^{-1} range in KBr pellet or $CHCl_3$ film. GC/MS spectra were recorded on a Hewllet-Packard GC model 5890 with 5970 mass detector by the electron impact (EI) method. Elemental analyses were within 0.4% of the theoretical value. For some compounds making not stoichiometric salts elemental analysis were not supplied. Reagents and solvents were purchased from common commercial suppliers and were used as received except ethanol, which was dried prior to use. For column chromatography Merck silica gel 70–230 meshes were used.

1-(4-Pyrimidin-2-yl-piperazin-1-yl)-pentan-1-one (4). The mixture of ethyl 3-[4-(pyrimidin-2-yl)piperazin-1-yl]-2-propyl-3-oxopropanoate (**14**) [18] (28 mM) and 5 NaOH solution (40 mM) was stirred for 2 h at room temperature, acidified with 50% H_2SO_4 to pH 2 and stirred under reflux for an additional 2 h. The reaction mixture was cooled, extracted 3 times with diethyl ether

and dried over $MgSO_4$. The solvent was evaporated under reduced pressure (130 Pa) to give an oily product which was purified by flash chromatography on silica gel with chloroform as an eluent to give 9.7 mM of **4** (2.4 g, 48%). **4 · HCl** (hydrochloride was obtained as described in the procedure for compounds **1**–**3**, **5**, **7**–**13**). Yield 84%, M.p. 135–136°C, ^{1}H NMR ($CDCl_3$), δ: 0.94 (3H, t, J = 7.1Hz, CH_2-$\underline{C}H_3$), 1.30–1.48 (2H, m, $\underline{C}H_2$-CH_3), 1.58–1.74 (2H, m, $\underline{C}H_2$-CH_2-CO), 2.38 (2H, t, J = 7.3 Hz, CO-$\underline{C}H_2$), 3.50–3.90 (8H, m, 4N-$\underline{C}H_2$), 6.53 (1H, t, J = 4.8 Hz, $\underline{C}$H<CH=N), 8.32 (2H, d, J = 4.7 Hz, CH<$\underline{C}$H=N), ^{13}C NMR ($CDCl_3$, 200 MHz), δ: 158.55, 111.15, 46.05, 44.47, 44.23, 41.97, 33.78, 28.07, 23.20, 14.51, IR ($CHCl_3$) ν_{max} 3429, 2961, 2874, 2549, 1630, 1611, 1460, 1347, 1296, 980 cm^{-1}, MS m/z (%): 248 (M^+, 30), 163 (25), 134 (64), 108 (100), 80 (26), 56 (62), 41 947). Anal. Calc. for $C_{13}H_{20}N_4OHCl$ (284.80): C, 54.8; H, 7.4; N, 19.7. Found: C 54.8; H 7.7; N 19.8.

1-[4-(Pyrimidin-2-ylo)piperazin-1-ylo]heptan-1-on (6). The compound was obtained as described above from ethyl 3-[4-(pyrimidin-2-yl)piperazin-1-yl]-2-pentyl-3-oxopropanoate (**15**).Yield 82% (4.6 g). **6 · HCl** (hydrochloride was obtained as described in the procedure for compounds **1**–**3**, **5**, **7**–**13**). Yield 97%, M.p. 132.5–134.5°C, ^{1}H NMR ($CDCl_3$), δ: 0.89 (3H, t, J = 6.6 Hz, $CH_2\underline{C}H_3$), 1.24–1.42 (6H, m, $\underline{C}H_2$-$\underline{C}H_2$-$\underline{C}H_2$-CH_3), 1.58–1.74 (2H, m, $\underline{C}H_2$-CH_2-CO), 2.40 (2H, t, J = 7,3 Hz, CO-$\underline{C}H_2$), 3.50–3.90 (8H, m, 4N-$\underline{C}H_2$), 6.55 (1H, t, J = 4.8 Hz, $\underline{C}$H<CH=N), 8.34 (2H, d, J = 4.7 Hz, CH<$\underline{C}$H=N), ^{13}C NMR ($CDCl_3$, 200 MHz), δ: 172.78, 162.28, 158.53, 111.14, 46.02, 44.44, 44.20, 41.96, 34.05, 32.23, 29.76, 25.92, 23.13, 14.63, IR ($CHCl_3$) ν_{max} 3428, 2958, 2860, 2556, 1630, 1613, 1469, 1435, 1346, 1296, 980 cm^{-1}, MS m/z (%): 276 (M^+, 38), 208 (13), 163 (32), 134 (71), 122 (92), 108 (100), 80 (21), 56 (51), 43 (47). Anal. Calc. for $C_{15}H_{24}N_4OHCl$ 0.25 H_2O (312.85): C 56.8; H 8.1; N 17.5. Found: C 56.8; H 8.1; N 17.4.

*General procedure for the synthesis of compounds **1–3**, **5** and **7–13**.* To the solution of 24 mM of 1-(pyrimidin-2-yl)piperazine (**16**) in 80 ml of triethylamine 29 mM of appropriate acid chloride was slowly added keeping room temperature and a mixture was stirred for 8–48 hours. Than mixture was poured to 150 ml of water and extracted with chloroform (2×60 ml), combined organic layers were washed with water and dried over $MgSO_4$. After an evaporation of the solvent under reduced pressure (130 Pa) the crude product was purified by column chromatography on silica gel with hexane/ethyl acetate gradient (0, 1, 3, 5, 10%) (compounds **1**–**3**, **5, 7**, **8**, **12** and **13**, 40–73% yield) or by crystallization from ethyl acetate (compounds **9**–**11**, 52–72% yield).

Hydrochlorides preparation: 0.01 M of a compound was dissolved in 10 ml of anhydrous ethanol followed by an addition of the equimolar amount of 2.6 M hydrogen chloride in ethanol, with stirring and cooling the mixture. After 2 h at room temperature the reaction mixture was concentrated to half of the initial volume, hydrochlorides were precipitated with ether, filtered off and dried on the air to give hydrochlorides with 70–98% yield (as calculated to the free base).

1-[4-(Pyrimidin-2-yl)piperazin-1-yl]ethan-1-on (1). 1·HCl. M.p. 117–119°C, ^{1}H NMR ($CDCl_3$), δ: 2.10 (1H, s, CO-$\underline{C}H_3$), 3.60–4.36 (8H, m, 4N-$\underline{C}H_2$), 6.85 (1H, t, J = 4.7 Hz, $\underline{C}$H<CH=N), 8.62 (2H, d, J = 4.7 Hz, CH<$\underline{C}$H=N), ^{13}C NMR (D_2O), δ: 166.41, 159.33, 155.77, 112.77, 47.85, 47.06, 46.28, 41.93, IR (KBr) ν_{max} 3423, 3059, 2932, 2722, 1645, 1604, 1534, 1433, 1407, 1349, 1283, 1225, 1064, 995 cm^{-1}, MS m/z (%): 206 (78 M^+), 163 (9), 108 (100), 80 (36), 56 (28), 42 (19). Anal. Calc. for $C_{10}H_{14}N_4OHCl$ (242.71): C, 49.5; H, 6.2; N, 23.1. Found: C 49.4; H 6.2; N 23.1.

1-[4-(Pyrimidin-2-yl)piperazin-1-yl]propan-1-on (2). 2·HCl. M.p. 158–159°C, ^{1}H NMR ($CDCl_3$), δ: 1.18 (3H, t, J = 7.4 Hz, CH_2-$\underline{C}H_3$), 2.21 (2H, q, J = 7.4 Hz, CO-$\underline{C}H_2$), 3.62–4.38 (8H, m, 4N-$\underline{C}H_2$), 6.90 (1H, t, J = 4.7 Hz, $\underline{C}$H<CH=N), 8.60 (2H, d, J = 4.7 Hz, CH <$\underline{C}$H=N), ^{13}C NMR ($CDCl_3$), δ: 172.36, 161.37, 157.62, 110.29, 45.03, 43.60, 43.40, 41.21, 26.44, 9.33, IR (KBr) ν_{max} 3407, 2456, 2385, 1609, 1629, 1541, 1463, 1433, 1346, 1229, 978 cm^{-1}, MS m/z (%): 220 (88 M^+), 205 (32), 163 (36), 134 (100), 108 (100), 80 (15), 56 (30), 42 (8). Anal. Calc. for $C_{11}H_{16}N_4OHCl$ (256.74): C, 51.5; H, 6.7; N, 21.8. Found: C 51.5; H 6.6; N 21.7.

1-[4-(Pyrimidin-2-yl)piperazin-1-yl]butan-1-on (3). 3·HCl. M.p. 133–135°C, ^{1}H NMR ($CDCl_3$), δ: 1.00 (3H, t, J = 7.4 Hz, CH_2-$\underline{C}H_3$), 1.68 (2H, m, $\underline{C}H_2$-CH_3), 2.37 (2H, t, J = 7.5 Hz, CO-CH_2), 3.62–4.38 (8H, m, 4N-$\underline{C}H_2$), 6.90 (1H, t, J = 4.7 Hz, $\underline{C}$H<CH=N), 8,60 (2H, d, J = 4,7Hz, CH<$\underline{C}$H=N), ^{13}C NMR ($CDCl_3$), δ: 171.56, 152.55, 109.50, 45.77, 45.15, 44.22, 40.33, 34.82, 18.22, 13.63, IR (KBr) ν_{max} 3058,

2965, 2432, 1659, 1604, 1540, 1434, 1332, 1222, 980 cm^{-1}, MS m/z (%): 234 (57 M^+), 219 (24), 163 (16), 134 (70), 108 (100), 80 (24), 56 (54), 43 (39). Anal. Calc. for $C_{12}H_{18}N_4OHCl$ (270.77): C, 53.2; H, 7.1; N, 20.7. Found: C 53.1; H 7.1; N 20.6.

1-[4-(Pyrimidin-2-yl)piperazin-1-yl]hexan-1-on (5). 5·HCl. M.p. 160–161°C, ^{1}H NMR ($CDCl_3$), δ: 0.85–1.0 (3H, m, CH_2-CH_3), 1.25–1.45 (4H, m, CH_2-CH_2-CH_3), 1.68 (2H, m, CO-CH_2-CH_2), 2.38 (2H, m, CO-CH_2), 3.55–3.95 (8H, m, 4N-CH_2), 6.60 (1H, t, J = 4.8 Hz, CH<CH=N), 8.36 (2H, d, J = 4.8 Hz, CH<CH=N), ^{13}C NMR ($CDCl_3$), δ: 172.07, 157.67, 110.29, 45.30, 44.18, 43.84, 41.25, 33.38, 31.63, 25.01, 22.47, 13.95, IR (KBr) ν_{max} 3395, 2175, 1616, 1634, 1545, 1445, 1423, 1339, 1210, 981 cm^{-1}, MS m/z (%): 262 (66 M^+), 247 (22), 219 (4), 194 (15), 163 (33), 134 (73), 123 (92), 108 (100), 80 (26), 56 (60), 43 (57). Anal. Calc. for $C_{14}H_{22}N_4O2$ HCl (335.28): C, 50.2; H, 7.2; N, 16.7. Found for **5**·1.8 HCl: C 51.3; H 7.3; N 16.9.

1-[4-(Pyrimidin-2-yl)piperazin-1-yl]octan-1-on (7). 7·HCl. M.p. 129–131°C, ^{1}H NMR ($CDCl_3$), δ: 0.75–0.95 (3H, m, CH_2-CH_3), 1.10–1.40 (8H, m, CH_2-CH_2-CH_2-CH_2-CH_3), 1.50–1.60 (2H, m, CO-CH_2-CH_2), 2.30 (2H, m, CO-CH_2), 3.55–4.20 (8H, m, 4N-CH_2), 6.82 (1H, t, J = 4.8 Hz, CH<CH=N), 7.70 (1H, m, NH^+), 8.56 (2H, d, J = 4.7 Hz, CH<CH=N), ^{13}C NMR ($CDCl_3$), δ: 171.69, 161.31, 157.54, 110.20, 45.17, 43.61, 43.36, 41.09, 33.19, 31.48, 29.21, 28.88, 25.12, 22.38, 13.08, IR (KBr) ν_{max} 3416, 2928, 2124, 1626, 1542, 1440, 1347, 1256, 982 cm^{-1}, MS m/z (%): 290 (100 M^+), 275 (35), 222 (28), 163 (54), 134 (73), 122 (89), 108 (67), 80 (8), 56 (14). Anal.: not stoichiometric hydrated salt.

1-[4-(Pyrimidin-2-yli)piperazin-1-yl]nonan-1-on (8). 8·HCl. M.p. 177–179°C, ^{1}H NMR ($CDCl_3$), δ: 0.80–0.95 (3H, m, CH_2-CH_3), 1.08–1.45 (10H, m, CH_2-CH_2-CH_2-CH_2-CH_2-CH_3), 1.50–1.62 (2H, m, CO-CH_2-CH_2), 2.38 (2H, m, CO-CH_2), 3.65–4.40 (8H, m, 4N-CH_2), 6.90 (1H, t, J = 4.8 Hz, CH<CH=N), 8.60 (2H, d, J = 4.8 Hz, CH<CH=N), ^{13}C NMR ($CDCl_3$), δ: 171.91, 161.37, 157.65, 110.31, 45.28, 43.71, 43.45, 41.21, 33.31, 31.69, 29.35, 29.27, 29.03, 25.23, 22.52, 13.98, IR (KBr) ν_{max} 3504, 3442, 2925, 2854, 1609, 1537, 1448, 1342, 1261, 1208, 981 cm^{-1}, MS m/z (%): 304 (100 M^+), 289 (29), 236 (33), 163 (51), 134 (71), 122 (100), 108 (74), 96 (15), 56 (17). Anal. Calc. for $C_{17}H_{28}N_4OHCl$ (340.92): C, 59.9; H, 8.6; N, 16.4. Found: C 59.9; H 8.4; N 16.4.

Cyclopropyl-[4-(pyrimidin-2-yl)piperazin-1-yl]methanon (9). **9·HCl**. M.p. 152–154°C, ^{1}H NMR ($CDCl_3$), δ: 0.75–0.86 (2H, m, cyclopropyl), 0.98–1.08 (2H, m, cyclopropyl), 1.73–1.87 (1H, m, CO-CH), 3.62–4.00 (8H, m, 4N-CH_2), 6.55 (1H, t, J = 4.8 Hz, CH<CH=N), 8.60 (2H, d, J = 4.8 Hz, CH<CH=N), ^{13}C NMR ($CDCl_3$), δ: 172.21, 161.46, 157.71, 110.28, 45.00, 43.41, 41.66, 10.84, 7.35, IR (KBr) ν_{max} 3084, 3001, 2858, 1624, 1589, 1551, 1501, 1441, 1363, 1239, 1041, 982 cm^{-1}, MS m/z (%): 232 (37 M^+), 217 (11), 163 (22), 147 (19), 134 (84), 122 (70), 108 (100), 96 (12), 69 (55), 56 (75), 41 (93). Anal. Calc. for $C_{12}H_{16}N_4OHCl$ (268.75): C, 53.6; H, 6.4; N, 20.8. Found: C, 53.5; H, 6.2; N, 20.9.

Cyclobutyl-[4-(pyrimidin-2-yl)piperazin-1-yl]methanon (10). **10·HCl**. M.p. 133–135°C, ^{1}H NMR ($CDCl_3$), δ: 1.80–2.50 (6H, m, cyclobutyl), 3.22–3.40 (1H, m, CO-CH), 3.40–3.85 (8H, m, 4N-CH_2), 6.54 (1H, t, J = 4.8 Hz, CH<CH=N), 8.32 (2H, d, J = 4.8 Hz, CH<CH=N), ^{13}C NMR ($CDCl_3$), δ: 173.32, 161.54, 157.76, 110.35, 44.64, 43.79, 43.44, 41.36, 37.09, 24.94, 17.81, IR (KBr) ν_{max} 2977, 2863, 1634, 1587, 1549, 1489, 1446, 1358, 1244, 1012, 986 cm^{-1}, MS m/z (%): 246 (33 M^+), 217 (7), 163 (32), 147 (21), 134 (80), 122 (77), 108 (100), 80 (28), 69 (10), 56 (92), 41 (30). Anal. Calc. for $C_{13}H_{18}N_4OHClH_2O$ (300.79): C, 51.9; H, 7.0; N, 18.6. Found: C, 51.3; H, 7.0; N, 18.6.

Cyclopentyl-[4-(pyrimidin-2-yl)piperazin-1-yl]methanon (11). 11·HCl. M.p. 145–147°C, ^{1}H NMR ($CDCl_3$), δ: 1.50–1.92 (8H, m, cyclopentyl), 2.95 (1H, quin., CO-CH), 3.56–3.90 (8H, m, 4N-CH_2), 6.55 (1H, t, J = 4.8 Hz, CH<CH=N), 8.36 (2H, d, J = 4.8 Hz, CH<CH=N), ^{13}C NMR ($CDCl_3$), δ: 174.84, 161.58, 157.79, 110.37, 45.18, 43.86, 43.51, 41.54, 41.01, 30.02, 25.95, 22.47, 13.95, IR (KBr) ν_{max} 2948, 2866, 1634, 1590, 1549, 1445, 1361, 1311, 1230, 984 cm^{-1}, MS m/z (%): 260 (43 M^+), 245 (16), 192 (20), 163 (41), 134 (77), 122 (100), 108 (100), 96 (23), 69 (66), 56 (69), 41 (80). Anal. Calc. for $C_{14}H_{20}N_4OHCl0.5\ H_2O$ (305.81): C, 55.0; H, 7.2; N, 18.3. Found: C, 54.5; H, 7.3; N, 18.2.

Cyclohexyl-[4-(pyrimidin-2-yl)piperazin-1-yl]methanon (12). 12·HCl. M.p. 162–164°C, ^{1}H NMR ($CDCl_3$), δ: 1.15–1.95 (10H, m, cyclohexyl), 2.40–2.60 (1H, m, CO-CH), 3.65–4.45 (8H, m, 4N-CH_2), 6.90 (1H, t, J = 4.8 Hz, CH<CH=N), 8.60 (2H, d, J = 4.8 Hz, CH<CH=N), ^{13}C NMR ($CDCl_3$), δ: 174.67, 161.37, 157.59, 110.24, 44.99, 43.89, 43.46, 41.22, 40.31, 29.24, 25.66, IR (KBr) ν_{max} 3409,

2935, 2857, 1629, 1608, 1434, 1346, 1246, 978 cm^{-1}, MS m/z (%): 274 (77 M^+), 259 (30), 206 (28), 163 (73), 134 (84), 122 (100), 108 (71), 96 (20), 56 (25). Anal. Calc. for $C_{15}H_{22}N_4OHClH_2O$ (328.84): C, 54.8; H, 7.6; N, 17.0. Found: C 54.9; H 7.3; N 17.0.

Adamantyl-[4-(pyrimidin-2-yl)piperazin-1-yl]methanon (13). 13·HCl. M.p. 117–119°C, ^{1}H NMR ($CDCl_3$), δ: 1.75–1.80 (6H, m, adamantyl), 2.00–2.18 (9H, m, adamantyl), 3.60–3.85 (8H, m, 4N-$\underline{C}H_2$), 6.53 (1H, t, J = 4.8 Hz, $\underline{C}H$<CH=N), 8.32 (2H, d, J = 4.8 Hz, CH<$\underline{C}H$=N), ^{13}C NMR ($CDCl_3$), δ: 175.72, 157.50, 110.15, 44.89, 43.77, 41.49, 38.87, 36.40, 28.24, IR ($CDCl_3$) ν_{max} 3412, 2912, 2855, 2458, 1629, 1608, 1542, 1409, 1346, 1259, 1230, 1013, 978 cm^{-1}, MS m/z (%): 326 (9 M^+), 235 (10), 163 (13), 147 (17), 135 (100), 122 (17), 107 (10), 93 (14), 56 (3), 41 (6). Anal. Calc. for $C_{19}H_{26}N_4OHCl$ (362.90): C, 62.9; H, 7.5; N, 15.4. Found: C 62.7; H 7.6; N 15.4.

Chromatographic conditions. Liquid chromatograph (LabAlliance) equipped with two Series III Pumps, Model 525 Dual-wavelength UV-VIS Detector), Rheodyne Model 7725i injection valve controlled with Data Ally system was used. All chromatograms were obtained with the aid of Phenomenex Luna C18 column, 5 μ, 250×4.8 mm ID, flow rate 1 ml/min, λ = 244 nm. Mixtures of a phosphate buffer (KH_2PO_4/Na_2HPO_4) pH = 7.4 and methanol were used as mobile phases. Dead volumes were determined with 10^{-3} M KNO_3 solution at 220 nm. HPLC grade methanol was purchased from Merck, Warsaw. Water was purified with the aid of Milli-Q combined filters (Millipore, El Passo, TX, USA).

Hydrophobic index determination. Hydrophobic indices log k_w was determined as described in [8] using four different organic modifier concentrations. For each compound three capacity factor/methanol concentration points were obtained (Table 1).

Pharmacology

In vivo experiments. The Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland, approved all the experimental procedures. The experiments were conducted on male Albino-Swiss mice (20–28 g). The animals were kept at room temperature of 20–21ºC on a natural day – night cycle; they were housed in plastic boxes (55×35×20 cm) in groups of 20, with free access to food and water before the experiment. Experimental and control groups consisted of 10 animals each. The tested compounds were administered intraperitoneally (*ip*) as suspensions in a 1% Tween 80 (compounds **1, 2, 3** and **5**) or as solutions in physiological saline (compounds **4, 6, 7, 8** and **9**) in a volume of 10 ml/kg. The control groups of animals received the same amounts of the solvent. ED_{50} (effective dose) values were calculated on the basis of the effect of at least 3 doses, according to the Litchfield & Wilcoxon method [21].

The spontaneous locomotor activity of a single mice was measured for 30 min in photoresistor actometers (circular cages, 25 cm in diameter, two light sources, two photoresistors) at 1 h after administration of the tested compounds. ED_{50} values, *i.e.* the doses inhibiting the spontaneous locomotor activity by 50%, for each compound was calculated.

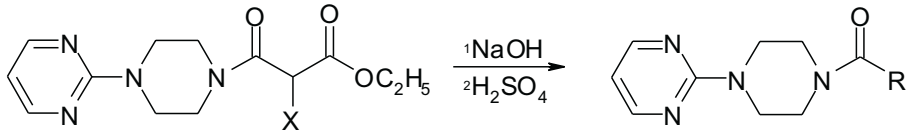
Rota-rod test. The preselected mice (*i.e.* the animals keeping on a rotating rod for 2 min) were placed on a rotating rod (1 cm in diameter, 6 rpm) and observed for 2 min. The number of animals falling from the rota-rod was recorded. The studied compounds were administered 1 h before the test. ED_{50} values, *i.e.* the doses that made 50% of animals falling from the rota-rod within 2 min, for each compound were calculated.

Righting reflex. The loss of the righting reflex for at least 15 sec was accepted as a criterion of a sedative or general anaesthetic action. Observations were conducted immediately after the administration of the tested compounds. ED_{50} values, *i.e.* the doses necessary to abolish the righting reflex in 50% mice, for each compound were calculated.

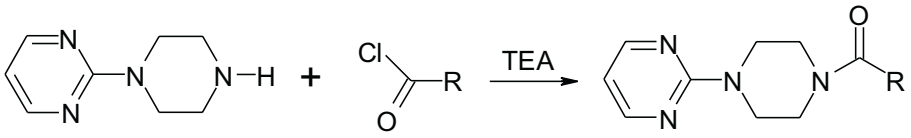
RESULTS AND DISCUSSION

Chemistry

Synthesis. Compounds **1**–**3**, **5** and **7**–**12** were obtained with high yields by the acylation of 1-(pyrimidin-2-yl)piperazine **16** with appropriate alkyl or cycloalkyl acid chlorides (Fig. 1). Compounds **4** and **6** were obtained by a hydrolysis and subsequent decarboxylation of ethyl 3-[4-(pyrimidin-2-yl)piperazin-1-yl]-2-propyl-3-oxopropanoate (**14**) and ethyl 3-[4-(pyrimidin-2-yl)piperazin-1-yl]-2-pentyl-3-oxopropanoate (**15**), respectively. For pharmacological studies all compounds were used as hydrochlorides.



14 X=C_3H_7 **4**, **6**
15 X=C_5H_{11}



16 **1**–**3**, **5**, **7**–**13**

R=

1	CH_3	9	
2	CH_2CH_3	10	
3	$(CH_2)_2CH_3$	11	
4	$(CH_2)_3CH_3$	12	
5	$(CH_2)_4CH_3$	13	
6	$(CH_2)_5CH_3$		
7	$(CH_2)_6CH_3$		
8	$(CH_2)_7CH_3$		

Figure 1. Schemes of synthesis of the compounds **1**–**13**.

Hydrophobicity evaluation. As physicochemical descriptors for SAR analysis chromatographic retention factors and hydrophobic indices (log k_w, [7,8]) were employed. The retention factors for compounds **1**–**13** were obtained by means of partition chromatography on octadecylsilane stationary phase. The hydrophobic indices, being a measure of compounds partition between water and organic (octadecylsililic) stationary phase, were calculated using linear extrapolation [7] of isocratic retention factors to 100% water according to equation (1) (Table 1).

$$\log k = \log k_w - S\varphi \quad (1)$$

φ – volume fraction of methanol

Table 1. Isocratic and extrapolated retention factors of compounds **1**–**13** obtained with methanol as an organic modifier. [a]Linear correlation coefficient; [b]Calculated with the aid of Hyperchem 6 program.

Compd.	log $k_{0.55}$	log $k_{0.60}$	log $k_{0.65}$	log $k_{0.70}$	log k_w	S	r[a]	log P[b]
1	–0.116	–0.273	–0.378	–	1.316	2.62	0.9934	0.45
2	0.163	0.018	–0.114	–	1.684	2.77	0.9996	1.08
3	0.425	0.249	0.098	–	2.219	3.27	0.9990	1.47
4	0.713	0.519	0.350	–	2.705	3.63	0.9992	1.87
5	1.031	0.794	0.587	–	3.468	4.44	0.9992	2.27
6	–	1.086	0.840	0.674	3.544	4.12	0.9937	2.66
7	–	1.407	1.097	0.880	4.553	5.27	0.9948	3.06
8	–	1.687	1.348	1.104	5.169	5.83	0.9956	3.46
9	0.244	0.126	–0.039	–	1.808	2.83	0.9954	1.14
10	0.553	0.399	0.209	–	2.451	3.44	0.9981	1.53
11	0.776	0.611	0.393	–	2.891	3.83	0.9968	1.93
12	0.978	0.790	0.555	–	3.312	4.23	0.9979	2.32
13	–	1.429	1.046	0.924	4.415	5.05	0.9582	2.97

The retention factors within the examined series of compounds **1**–**13** grew both for aliphatic (**1**–**8**) and alicyclic (**9**–**13**) series with the growing number of carbon atoms in R substituent (Table 1). The average methylene group increment $\tau_{methylene}$ ($\tau_X = \log k_{R\text{-}X} - \log k_{R\text{-}H}$, [22–25]) for compounds **1**–**12** (compound **13**, R = adamantyl was not included in the analysis) was found to be $\tau_{CH_2} = 0.26\pm0.04$ and 0.23 ± 0.04 for retention factors obtained with 60% and 65% methanol, respectively (τ values calculated separately for aliphatic and alicyclic substituents). Similar value calculated for hydrophobic indices was 0.53 ± 0.15.

The obtained hydrophobic indices (as well as retention factors obtained with 60% and 65% methanol) exhibited statistically significant linear correlation (eq. 2) with the calculated lipophilicities (Hyperchem 6.0) for the whole set of compounds **1**–**13**:

$$\log P = -0.23(\pm0.11) + 0.74(\pm0.03)*\log k_w \quad (2)$$

$r = 0.9880$, $s = 0.142$, $F = 450.13$, $p < 0.01$

Pharmacology

The potential hypnotic-sedative activity of compounds **1**–**13** was assessed in behavioural tests in mice such as abolition of the righting reflex (being an indirect measure of hypnotic activity), disturbance of motor coordination (myorelaxant activity) and inhibition of spontaneous locomotor activity (manifestation of compounds sedative effects). The obtained results (Table 2) compared to those obtained for zopiclone [19] – a known hypnotic sedative of new generation [20] – indicate that the tested compounds produced a general depressive action on the central nervous system. It has been observed that compounds **1**–**13** (with ED_{50} from 100 mg/kg for compound **10** to more than 400 mg/kg for compound **13**), like zopiclone – a well known hypnotic-sedative drug (ED_{50} = 225 mg/kg) – induced (a few minutes after their administration) the abolition of the righting reflex which lasted up to 30–40 min (data not shown). The most potent in that test (ED_{50} < 200 mg/kg) appeared to be compounds **6**, **10** and **11** possessing hexyl, cyclobutyl and cyclopentyl substitutent, respectively. The sedative effect of **1**–**13** (measured 1 h after their administration) manifested itself by the inhibition of the spontaneous locomotor activity in mice.

Table 2. Results of behavioural experiments for compounds **1**–**13** in mice. * Data taken from ref. [19].

Compound	**ED_{50} mg/kg**		
	Abolition of the righting reflex	Disturbance of motor coordination (rota-rod test)	Inhibition locomotor activity
1	400 (296.2–540.0)	>400	80 (51.6–124.0)
2	265 (230.4–304.8)	>400	235 (189.5–291.4)
3	230 (198.2–266.8)	>400	150 (108.7–207.0)
4*	200 (181.8–222.0)	260 (244.4–276.6)	73 (48.0–111.0)
5	210 (175.0–252.0)	305 (278.5–334.0)	87 (60.0–126.2)
6*	185 (156.8–218.3)	195 (169.1–224.8)	65 (45.8–92.3)
7	280 (273.2–330.4)	275 (229.2–330.0)	66 (42.6–102.3)
8	275 (233.1–324.5)	280 (247.8–316.4)	77 (49.7–119.4)
9	275 (261.9–288.8)	>400	90 (62.1–130.5)
10	100 (80.0–125.0)	330 (259.8–419.0)	92 (65.7–128.8)
11	175 (162.8–188.1)	265 (240.9–291.0)	137 (107.9–174.0)
12	200 (169.5–236.0)	>400	98 (67.6–142.1)
13	>400	360 (313.0–414.0)	210 (107.1–210.0)
zopiclone*	225	51	12

It was produced by the compounds at doses 1.5–5 times lower than those abolishing the righting reflex; the ED_{50} values ranged from 65 (**6**) to 232 (**2**) mg/kg. In that test zopiclone was active at 18 times lower dose than that inducing the hypnotic effect [19]. The examined compounds – like zopiclone – disturbed motor coordination in mice in the rota-rod test, the effect connected usually with myorelaxant properties. The ataxia – *i.e*. disturbances of motor coordination – (tested 1 h after the compounds administration), appeared after the high doses of **1**–**13**, comparable to those that evoked the abolishing of the righting reflex but 2–5 times higher than those inducing the decrease in locomotor activity. Zopiclone induced such effects at doses 4–5 times lower than those affecting the righting reflex, and at the same time 5 times higher to that, which decreased the exploratory activity.

The obtained results also indicate that despite similar pharmacological properties the studied 1-(2-pyrimidyl)piperazines are not a uniform group of compounds as regards their central depressant activity. Indeed, the compounds **1**, **7** an **8**, belonging to the aliphatic series (R = methyl, heptyl and octyl, respectively) produce a sedative effect in preference to ataxic and hypnotic effects, whereas compounds **10** and **11** (alicyclic series, R = cyclobutyl and cyclopentyl, respectively) induce a sedative and hypnotic effects at the same doses with a some preference to an ataxic effect.

The origin of the central depressant activity evoked by compounds **1**–**13** is unknown, but a common feature of the compounds and zopiclone (benzodiazepine receptor agonist) is that after their systemic administration sedative-hypnotic effects were observed.

SAR studies. In the aliphatic series the highest pharmacological activity in the loss of the righting reflex test has been obtained for compound **6** possessing six-membered aliphatic side chain (ED_{50} = 185 mg/kg) and in the alicyclic series for compound **10** possessing cyclobutyl substituent (ED_{50} = 100 mg/kg). The highest pharmacological activity in the inhibition of spontaneous locomotor activity and disturbances of motor coordination was obtained for compound **6** with ED_{50} 195 mg/kg and 65 mg/kg, respectively.

Activity of compounds **1**–**13** in the loss of the righting reflex test (an indirect measure of hypnotic activity, taken as log ($1/ED_{50}$)) exhibited Hansch parabolic correlation [10,11] with the hydrophobic indices both for aliphatic (Fig. 2, eq 3, compounds **1**–**8**) and alicyclic (Fig. 3, eq. 4, compounds **9**–**13**) series, but not for the whole group of compounds. The statistically significant parabolic correlations within the both group of compounds could also be obtained when retention factors obtained for 60% and 65% methanol were employed as a lipophilicity measure. However, it should be noted that the parabolic equation (4) calculated for compounds **9**–**13** was based on its ascending site on one experimental point only what would make the statistical validity of the equation less convincing.

$$\log (1/ED_{50}) = -2.99(\pm 0.12) + 0.41(\pm 0.08)*\log k_w - 0.061(\pm 0.013)*\log k_w^2 \quad (3)$$

$r = 0.9092$, $p < 0.01$

$$\log (1/ED_{50}) = -3.73(\pm 0.33) + 1.07(\pm 0.22)*\log k_w - 0.19(\pm 0.03)*\log k_w^2 \quad (4)$$

$r = 0.9790$, $p < 0.05$

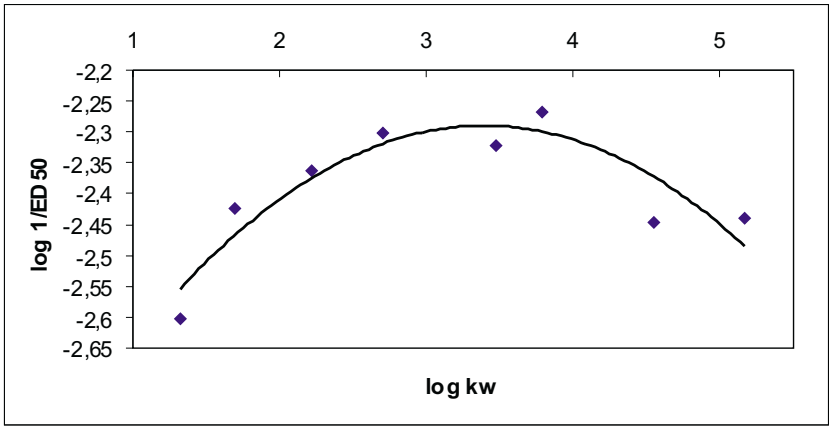


Figure 2. Relationship between hydrophobic index and in the inducing loss of the righting reflex in mice for compounds **1**–**8**.

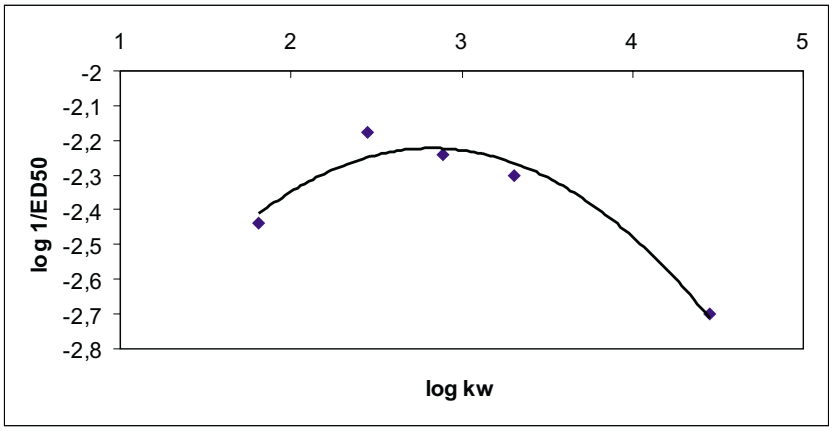


Figure 3. Relationship between hydrophobic indices and activity in the inducing loss of the righting reflex in mice for compounds **9**–**13**.

The necessity to describe the pharmacological activity for aliphatic and alicyclic compounds with 2 different equations corresponds well to the discussed earlier difference in the pharmacological properties between the both groups of compounds – aliphatic producing a sedative effect in preference to ataxic and hypnotic effects whereas alicyclic giving a sedative and hypnotic-ataxic effects at the same doses with a some preference to an ataxic effect.

The above results also clearly indicate that within both groups of compounds (aliphatic and alicyclic) maximum hypnotic-sedative activity has been obtained. The hypnotic activity for the most active compounds **4**–**6** and **10**–**12** was comparable to that obtained for zopiclone, a commonly used hypnotic-sedative drug. Therefore, at that stage of research they seem to be promising drug candidates for further pharmacological evaluation.

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