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Research Article

Synthesis and Evaluation of Antidepressant-like Activity of Some 4-Substituted 1-(2-methoxyphenyl) Piperazine Derivatives

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A series of new derivatives of *N*-(2-methoxyphenyl) piperazine have been synthesized for their affinity toward serotonergic receptors and for their potential antidepressant-like activity. They have been evaluated toward receptors 5-HT_{1A}, 5-HT_6, and 5-HT_7, as well as *in vivo* in the tail suspension, locomotor activity, and motor co-ordination tests. All the tested compounds proved very good affinities toward 5-HT_{1A} and 5-HT_7 receptors. The most promising compound was 1-[(2-chloro-6-methylphenoxy)ethoxyethyl]-4-(2-methoxyphenyl)piperazine hydrochloride, exhibiting affinity toward receptors K_i <1 nM (5-HT_{1A}) and K_i = 34 nM (5-HT_7). Antidepressant-like activity (tail suspension test) was observed at 2.5 mg/kg b.w. (mice, i.p.), and the effect was stronger than that observed for imipramine (5 mg/kg b.w.). Sedative activity was observed at ED_{50} (locomotor test, mice, i.p.) = 17.5 mg/kg b.w. and neurotoxicity was observed at TD_{50} (rotarod, mice, i.p.) = 53.2 mg/kg b.w.

Key words: (2-methoxyphenyl)piperazine, 5-HT, antidepressant-like activity, chimney test, locomotor test, neurotoxicity, rotarod, tail suspension test

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Depression is a prevalent psychiatric disorder, concerning about 21% of human world population. It is characterized by anhedonia or loss of interest or pleasure in normal activity. It affects quality of life and productivity and is a significant factor limiting length of life (1). Despite many antidepressant drugs, still their effectiveness is unsatisfactory. Among many types of depression, major depressive disorder or unipolar depression is predominant and is a leading indication in the interest of drug development efforts.

Serotonin metabotropic receptors are known to influence mood and cognitive performance of the central nervous system, especially 5-HT_{1A}, 5-HT_6, and 5-HT_7 receptors have been well described for their role in depression, anxiety, memory deficits, as well as other disorders characterized by change of cholinergic neurotransmission (2). Moreover, it has been reported that 5-HT_{1A} antagonists may serve as adjunctive therapies for selective serotonin reuptake inhibitors (SSRIs), due to their potential of reducing the delay of onset of SSRIs activity. Moreover, 5-HT_{1A} ligands may present antidepressant activity themselves (3). As anxiety is often concomitant to depression, the pharmacological effect of anxiolytics may be observed also in antidepressant-like activity in animal models.

Among selective 5-HT_{1A} ligands, one can find derivatives of 1-alkyl-4-aryl-piperazine. Typical examples are lecozotan (antagonist), BMY 7378, or buspirone (partial agonists) (Figure 1) (4). Moreover, aroxyalkyl derivatives of *N*-(2-methoxyphenyl)piperazine have been in the field of our interest in the recent years (5,6). Some of them, for example, 1-[(2,6-dimethylphenoxy)propyl]-4-(2-methoxyphenyl) piperazine hydrochloride (Figure 1), exhibited satisfactory affinity toward α_1 versus α_2-adrenoreceptors, K_i = 2.4 and 341.1 nM, respectively (6). As a continuation of our studies on phenylpiperazine derivatives, four new substituted phenoxypropyl derivatives of *N*-(2-methoxyphenyl)piperazine have been synthesized for their affinity toward serotonergic receptors as well as antidepressant-like activity.

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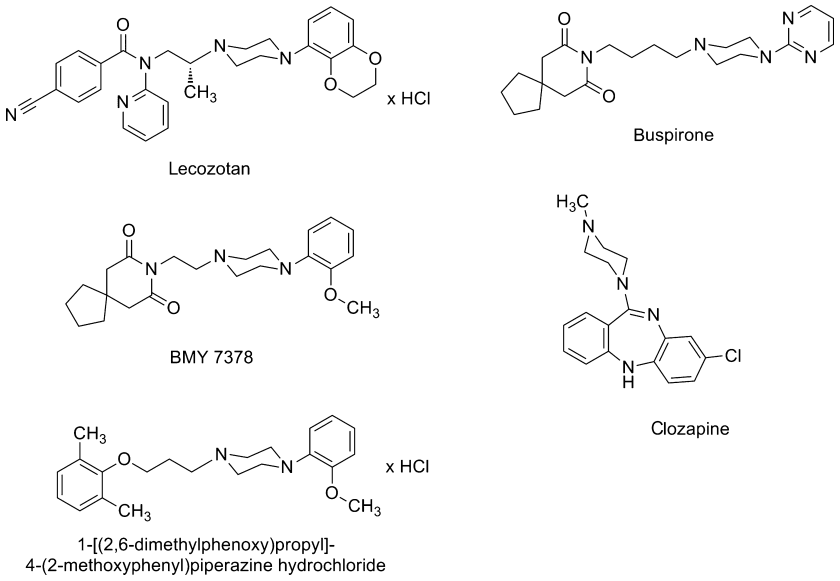


Figure 1: Chemical structures of some derivatives of piperazine - reference compounds (4–6).

The choice of substituents and their positions in the phenyl ring was based on our former experience—the position 2 and additional position 3, 5, or 6 have specifically proved to be beneficial. Therefore, our aim was to continue research among these derivatives, comparing them together with other parameters such as length of the linker between phenoxyl and *N*-(2-methoxyphenyl)piperazine groups.

The linker, as in our former studies, usually had the length of two or three methylene groups, and the bonds were single. Use of either propyl (compounds **1**-**4**) or ethoxyethyl (compounds **5**-**6**) linker was a modification that could substantially change pharmacological properties of our compounds, especially that the alkyl linker in reference compounds has 2-4 methylene groups (Figure 1).

Methods and Materials

Chemistry

All reagents were purchased from Alfa Aesar (Karlsruhe, Germany). Solvents were commercially available materials of reagent grade. Melting points (mp) are uncorrected and were determined using a Büchi SMP-20 apparatus (Büchi Labortechnik, Flawil, Switzerland). The infrared spectra were recorded on potassium bromide pellets using a Jasco FT/IR 410 spectrometer (Jasco Inc., Easton, MD, USA). ^{1}H NMR and ^{13}C NMR spectra for compounds were recorded on a Bruker AVANCE III 600 (Bruker, Karlsruhe, Germany) (resonance frequencies 600.20 MHz for ^{1}H and 150.94 MHz for ^{13}C) equipped a 5-mm probehead: PABBO with z-gradient or TBI with XYZ gradients. The ^{1}H spectra were recorded with 16 scans, 1 second relaxation delay, 4 seconds acquisition time, 128 kW FID size, and 16234 Hz spectral width. The ^{13}C spectra were recorded with WALTZ-16 ^{1}H broadband decoupling, a few thousand scans, 2 seconds relaxation delay, 0.9 second acquisition time, 64 kW FID size, and 36 057 Hz spectral width. Standard pulse sequences from Bruker library were used for 2D spectra. Gradient-enhanced sequences were used for the homo- and heteronuclear 2D experiments. All processing and analysis were performed using BRUKER'S TOPSPIN 3.0 software suite. Results are presented in the following format: chemical shift δ in ppm, multiplicity, coupling constant J in Hertz (Hz), number of protons, and proton's position. Multiplicities are showed as the abbreviations: s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublets of doublets), t (triplet), and m (multiplet). Elemental analyses were performed on an Elementar Vario EL III (Elementar Analysansysteme, Hanau, Germany). Analyses of percentage content of carbon, hydrogen, and nitrogen were within 0.4% of the theoretical values. Purity was checked with use of LC-MS system, which consisted of a Waters Acquity UPLC, coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). All the analyses were carried out using an Acquity UPLC BEH C18, 1.7 lm, 2.1 _ 100 mm column. A flow rate of 0.3 mL/min and a gradient of (5–95)% B over 10 min and then 100% B over 2 min were used. Eluent A: water/0.1% HCOOH; eluent B: acetonitrile/0.1% HCOOH. LC/MS data were obtained by scanning the first quadrupole in 0.5 second in a mass range from 50 to 1000 Da; eight scans were summed up to produce the final spectrum.

General procedure for preparation of the tested compounds

0.02 mole of *N*-(2-methoxyphenyl)piperazine was added to a solution of 0.02 mole of appropriately (2-chloro-5-methylphenoxy)-, (2,5-dimethylphenoxy)-, (2,3,5-trimethylphenoxy)-, or (2,4,6-trimethylphenoxy)propyl, or (2,6-dimethyl- or (2-chloro-6-methylphenoxy)ethoxyethyl bromide in 50 mL of toluene, and the reaction mixture was refluxed in the presence of 0.02 mole (excess) anh. K_2CO_3 for 12 h.

Inorganic salts were filtered off from the hot mixture and washed with hot toluene (5 mL). The solvent was distilled from the filtrate under reduced pressure. After addition of acetone saturated with gaseous HCl to the residue, the mixture was refluxed and cooled. The crystals formed were collected by filtration and dried. Recrystallization was performed from mixture acetone/ethanol 1:1 (v/v).

In case of compounds **1-4**, the base product was recrystallized from acetone.

1-[(2-chloro-5-methylphenoxy)propyl]-4-(2-methoxyphenyl)piperazine hydrochloride (1)

$C_{21}H_{28}N_2O_2Cl_2$; white solid, mp 200–202 °C (base: 83–85 °C (acetone)); el. anal. $^{calcd}/_{found}$ $C^{61.31}/_{60.90}$; $H^{6.86}/_{7.16}$; $N^{6.81}/_{6.65}$; IR (KBr, cm^{-1}) *v*: 3480, 3059, 3010, 2979, 2963, 2928, 2879, 2837, 2621, 2497, 2305, 2205, 1487, 1260, 1065, 760; ^{1}H NMR (DMSO-d_6, δ ppm): 11.30 (bs, 1H, CH_2-NH^+); 7.29 (d, J = 8.0, 1H, Ar); 7.07–6.88 (m, 5H, Ar); 6.79 (ddd, J = 8.0, J = 1.9, J = 0.7, 1H, Ar); 4.16 (t, J = 6.2, 2H, Ar-O-CH_2); 3.80 (s, 3H, Ar-O-CH_3); 3.65–3.44 (m, 4H, N-CHH-pip(a)); 3.33–3.27 (m, 2H, -CH_2-NH^+); 3.27–3.09 (m, 4H, N-CHH-pip(e)); 2.30 (s, 3H, Ar-CH_3); 2.33–2.25 (m, 2H, -CH_2-CH_2-CH_2); $[M+H]^+$ 375.36, 98.97%.

1-[(2,5-dimethylphenoxy)propyl]-4-(2-methoxyphenyl)piperazine hydrochloride (2)

$C_{22}H_{31}N_2O_2Cl$; white solid, mp 208–210 °C (base: 70–72 °C (acetone)); IR (KBr, cm^{-1}) *v*: 3439, 3056, 2980, 2953, 2922, 2842, 2680, 2584, 2522, 2474, 1502, 1247, 750; ^{1}H NMR (DMSO-d_6, δ ppm): 11.07 (bs, 1H, CH_2-NH^+); 7.08–6.88 (m, 5H, Ar); 6.77 (d, J = 1.7, 1H, Ar); 6.66 (ddd, J = 7.5, J = 1.7, J = 0.9, 1H, Ar); 4.05 (t, J = 6.1, 2H, Ar-O-CH_2); 3.80 (s, 3H, Ar-O-CH_3); 3.63–3.47 (m, 4H, N-CHH-pip(a)); 3.34–3.27 (m, 2H, -CH_2-NH^+); 3.27–3.07 (m, 4H, N-CHH-pip(e)); 2.26 (s, 3H, Ar-CH_3(5)); 2.33–2.25 (m, 2H, -CH_2-CH_2-CH_2); 2.13 (s, 3H, Ar-$(CH_3)_2$); $[M+H]^+$ 355.42, 99.69%.

1-[(2,3,5-trimethylphenoxy)propyl]-4-(2-methoxyphenyl)piperazine hydrochloride (3)

$C_{23}H_{33}N_2O_2Cl$; white solid, mp 224–226 °C; IR (KBr, cm^{-1}) *v*: 3427, 2976, 2359, 1610, 1485, 1263, 1213, 1022, 1455, 1307; ^{1}H NMR (DMSO-d_6, δ ppm): 11.43 (bs, 1H, CH_2-NH^+); 7.04 (ddd, J = 8.1, J = 7.1, J = 1.7, 1H, H-5′); 7.00 (dd, J = 8.1, J = 1.6, 1H, H-6′); 6.98 (dd, J = 7.8, J = 1.7, 1H, H-3′); 6.92 (ddd, J = 7.8, J = 7.1, J = 1.6, 1H, H-4′); 6.63 (d, J = 2.3, 1H, H-6); 6.59 (d, J = 2.3, 1H, H-4); 4.01 (t, J = 6.1, 2H, Ar-O-CH_2); 3.80 (s, 3H, Ar-O-CH_3); 3.64–3.46 (m, 4H, N-CHH-(pip)a); 3.42–3.26 (m, 2H, CH_2-NH^+); 3.26–3.14 (m, 4H, N-CHH-(pip)e); 2.31–2.23 (m, 2H, CH_2-CH_2-CH_2); 2.22 (s, 3H, Ar-CH_3(5)); 2.17 (s, 3H, Ar-CH_3(3)); 2.05 (s, 3H, Ar-CH_3(2)); $[M+H]^+$ 369.44, 99.29%.

1-[(2,4,6-trimethylphenoxy)propyl]-4-(2-methoxyphenyl)piperazine hydrochloride (4)

$C_{23}H_{33}N_2O_2Cl$; white solid, mp 234–236 °C (base: 95–97 °C (acetone)); el. anal. $^{calcd}/_{found}$ $C^{62.57}/_{62.32}$; $H^{7.96}/_{8.08}$; $N^{6.34}/_{6.49}$; IR (KBr, cm^{-1}) *v*: 3436, 3061, 2988, 2919, 2873, 2838, 2666, 2591, 2520, 2450, 1608, 1501, 1463, 1246, 749; ^{1}H NMR (DMSO-d_6, δ ppm): 11.49 (bs, 1H, CH_2-NH^+); 7.05 (ddd, J = 8.1, J = 7.1, J = 1.7, 1H, H-5′); 7.00 (dd, J = 8.1, J = 1.6, 1H, H-6′); 6.99 (dd, J = 7.9, J = 1.7, 1H, H-3′); 6.92 (ddd, J = 7.9, J = 7.1, J = 1.6, 1H, H-4′); 6.82 (s, 2H, H-3, H-5); 3.81 (s, 3H, Ar-O-CH_3); 3.77 (t, J = 5.9, 2H, Ar-O-CH_2); 3.65–3.47 (m, 4H, N-CHH-pip(a)); 3.40–3.33 (m, 2H, -CH_2-NH^+); 3.28–3.14 (m, 4H, N-CHH-pip(e)); 2.32–2.22 (m, 2H, CH_2-CH_2-CH_2); 2.19 (s, 6H, 2* Ar-CH_3 (2,6)); 2.18 (s, 3H, Ar-CH_3(4)); $[M+H]^+$ 369.44, 100%.

1-[(2,6-dimethylphenoxy)ethoxyethyl]-4-(2-methoxyphenyl)piperazine hydrochloride (5)

$C_{23}H_{33}N_2O_3Cl$; white solid, mp 146–148 °C; IR (KBr, cm^{-1}) *v*: 3501, 3444, 3015, 2978, 2952, 2925, 2625, 2491, 2296, 2200, 1609, 1453, 1262, 1205, 766; ^{1}H NMR (DMSO-d_6, δ ppm): 11.25 (bs, 1H, NH^+); 7.06–6.90 (m, 7H, Ar); 3.98 (ddd, J = 6.0, J = 4.0, J = 2.0, 2H, Ar-O-CH_2); 3.91 (ddd, J = 4.6, J = 3.3, J = 2.8, 2H, -O-CH_2-CH_2-NH); 3.78 (ddd, J = 6.0, J = 4.0, J = 2.0, 2H, Ar-O-CH_2-CH_2-O); 3.78 (s, 3H, -O-CH_3); 3.62–3.56 (m, 2H, Ar-N-CHH-$(pip)_a$); 3.50–3.45 (m, 2H, CH_2-N-CHH-$(pip)_a$); 3.39 (ddd, J = 4.6, J = 3.3, J = 2.8, 2H, -O-CH_2-CH_2-NH); 3.32–3.24 (m, 2H, Ar-N-CHH-$(pip)_e$); 3.17–3.09 (m, 2H, -CH_2-N-CHH-$(pip)_e$); 2.24 (s, 6H, Ar-$(CH_3)_2$). ^{13}C NMR: 155.26 (C-1); 151.70 (C-2′); 139.17 (C-1′); 130.26 (C-2); 128.60 (C-3); 123.60 (C-5′); 123.43 (C-4′); 120.74 (C-4); 118.15 (C-3′); 111.86 (C-4); 70.77 (Ar-O-CH_2-CH_2-O); 69.74 (Ar-O-CH_2-CH_2-O); 64.89 (-O-CH_2-CH_2-NH^+); 55.26 (O-CH_3); 54.79 (-O-CH_2-CH_2-NH^+); 51.64 (Ar-N-CH_2-(pip)); 46.74 (-CH_2-N-CH_2-(pip)); 15.84 (Ar-$(CH_3)_2$); $[M+H]^+$ 385.40; 99.22%.

1-[(2-chloro-6-methylphenoxy)ethoxyethyl]-4-(2-methoxyphenyl)piperazine hydrochloride (6)

$C_{22}H_{30}N_2O_3Cl_2$, white solid, mp 155–157 °C; IR (KBr, cm^{-1}) *v*: 3387, 3007, 2966, 2614, 2401, 1707, 1610, 1457, 1263, 1018, 765; ^{1}H NMR (DMSO-d_6, δ ppm): 11.65 (bs, 1H, NH^+); 7.31 (ddd, J = 8.0, J = 1.7, J = 0.7, 1H, H-4); 7.20 (ddd, J = 7.6, J = 1.7, J = 0.8, 1H, H-6); 7.08–6.99 (m, 4H, H-5, H-3′, H-5′, H-6′); 6.92 (ddd, J = 8.7, J = 7.2, J = 1.6, 1H, H-4′); 4.06 (t, J = 4.6, 2H, Ar-O-CH_2-); 4.00 (t, J = 5.0, 2H, -O-CH_2-CH_2-NH-); 3.82–3.80 (m, 2H, Ar-O-CH_2-CH_2-O-); 3.80 (s, 3H, -O-CH_3); 3.65–3.58 (m, 2H, Ar-N-CHH- $(pip)_a$); 3.52–3.46 (m, 2H, CH_2-N-CHH- $(pip)_a$); 3.43–3.37 (m, 2H, -O-CH_2-CH_2-NH-); 3.37–3.29 (m, 2H, Ar-N-CHH- $(pip)_e$); 3.29–3.21 (m, 2H, -CH_2-N-CHH- $(pip)_e$); 2.30 (s, 3H, CH_3-Ar (1)); ^{13}C NMR: 152.60 (C-2); 151.71(C-2′); 138.46 (C-1′);

133.23 (C-3); 129.89 (C-4); 127.69 (C-6); 126.62 (C-1); 124.92 (C-5); 123.94 (C-5′); 120.75 (C-4′); 118.43 (C-3′); 111.99 (C-6′); 71.46 (Ar-O-CH_2-CH_2-O-); 69.48 (Ar-O-CH_2-CH_2-O-); 64.91 (-O-CH_2-CH_2-NH^+-); 55.31 (-O-CH_3); 54.76 (-O-CH_2-CH_2-NH^+-); 51.43 (Ar-N-CH_2-(pip)); 46.82 (-CH_2-N-CH_2- (pip)); 15.99 (CH_3-Ar (1)). $[M+H]^+$ 405.34; 99.89%.

Pharmacology

Cells preparation

HEK293 cells with stable expression of human serotonin 5-HT_{1A}R, 5-HT_6, or 5-HT_{7b}R (prepared with the use of Lipofectamine 2000) were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were grown in Dulbeco's modified Eagle's medium containing 10% dialyzed fetal bovine serum and 500 μg/mL G418 sulfate. For membranes preparation, cells were subcultured in 10-cm-diameter dishes, grown to 90% confluence, washed twice with a phosphate-buffered saline (PBS) prewarmed to 37 °C, and were pelleted by centrifugation (200 × *g*) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol. Prior to membrane preparation, the pellets were stored at −80 °C.

Radioligand binding assays

Cell pellets were thawed and homogenized in 20 volumes of assay buffer using an Ultra Turrax tissue homogenizer and centrifuged twice at 35 000 × *g* (20 000 r.p.m.) for 20 min at 4 °C, with incubation for 15 min at 37 °C in between. The composition of the assay buffers was as follows: for 5-HT_{1A}R: 50 mM Tris–HCl, 0.1 mM EDTA, 4 mM $MgCl_2$, 10 μM pargyline, and 0.1% ascorbate; for 5-HT_6R: 50 mM Tris–HCl, 0.5 mM EDTA, and 4 mM $MgCl_2$; and for 5-HT_{7b}R: 50 mM Tris–HCl, 4 mM $MgCl_2$, 10 μM pargyline, and 0.1% ascorbate.

All assays were incubated in a total volume of 200 μL in 96-well microtiter plates for 1 h at 37 °C, except for 5-HT_{1A}R which were incubated at room temperature for 1 h. The process of equilibration is terminated by rapid filtration through Unifilter plates with a 96-well cell harvester, and radioactivity retained on the filters was quantified on a Microbeta plate reader. For displacement studies, the assay samples contained as radioligands: 1.5 nM [^{3}H]8-OH-DPAT (187 C_i/mM) for 5-HT_{1A}R; 2 nM [^{3}H]LSD (85.2 C_i/mM for 5-HT_6R or 0.6 nM [^{3}H]5-CT (39.2 C_i/mM) for 5-HT_7R.

Non-specific binding was defined with 10 μM of 5-HT in 5-HT_{1A}R and 5-HT_7R binding experiments, whereas 10 μM methiothepin was used in 5-HT_6R assays. Each compound was tested in triplicate at 7–8 concentrations (10^{-11}–10^{-4} M). The inhibition constants (K_i) were calculated from the Cheng-Prusoff equation (7). Results were expressed as means of at least two separate experiments.

Membrane preparation and general assay procedures for cloned receptors were adjusted to 96-microwell format based on protocols described by us previously (8,9).

Tail suspension test in mice

The experiments were conducted on Swiss albino (Krf: CD-1) mice according to the method described by Steru *et al.* (10). Each animal was fastened with medical adhesive tape 50 cm below the surface. The total time of immobility was measured during the entire 6 min of the testing session. Immobility was scored manually and was defined when the animals hung passively without limb movements. The compounds were injected at the doses 1.25–10 mg/kg b.w. If the compound given at the lowest dose was activated in the tail suspension test (TST), the dose was reduced by half, until the disappearance of the antidepressant-like activity, or statistically insignificant results.

Spontaneous locomotor activity

The locomotor activity was measured with photoresistor actometers (Ugo Basile, Italy) connected to a counter for the recording of light-beam interruptions, and the number of light-beam crossings was counted during the session (either 6-min or 30-min). The number of crossings of the light beams was then recorded as the locomotor activity. The studied compounds were administered at the doses active in TST to determine whether the observed effect is specific, and for the statistical analysis, the data obtained in the 6th min of the observation were used. To evaluate whether compounds possess the ability to induce CNS depression, the data obtained in the 30th min of the observation were used and median effective doses (ED_{50}) were calculated. To determine ED_{50} values, the compounds were administered at the lowest doses active in the TST, and then, the dose was gradually increased until the appearance of sedative effects.

Rotarod test

The test was performed according to the method described by Sałat *et al.* (11) with some minor modifications. Mice were trained for three consecutive days on the rotarod apparatus (rotarod apparatus, May Commat RR0711, Turkey; rod diameter: 2 cm), rotating at a constant speed of 24 r.p.m. During each training session, the animals were placed on a rotating rod for 3 min with an unlimited number of trials. The proper experiment was conducted at least 24 h after the final training trial. On the test day, mice were given the test compound or vehicle and tested on the rotarod, revolving at 24 r.p.m. Motor impairment was defined as the inability to remain on the rotating rod for 1 min. It was measured and expressed as the number of animals that fell off the rotating rod. ED_{50} values were then calculated. To determine

ED_{50} values, the compounds were administered at the lowest doses active in the TST, and then, the dose was gradually increased until the appearance of neurotoxic effects.

Chimney test

The chimney test was performed according to a method described by Boissier *et al.* (12). Previously trained and selected animals were placed in a 25 cm long and 2.5 cm in diameter, horizontally located tube, which was reversed in such a way that the mice were able to leave it only climbing backward up until they reached another end. The inability of mice to perform the test within 60 seconds indicated motor impairment. The number of animals that were unable to climb backwards was recorded, and ED_{50} values were calculated. To determine ED_{50} values, the compounds were administered at the dose active in the TST, and then, the dose was gradually increased until the appearance of neurotoxic effects.

Data analysis

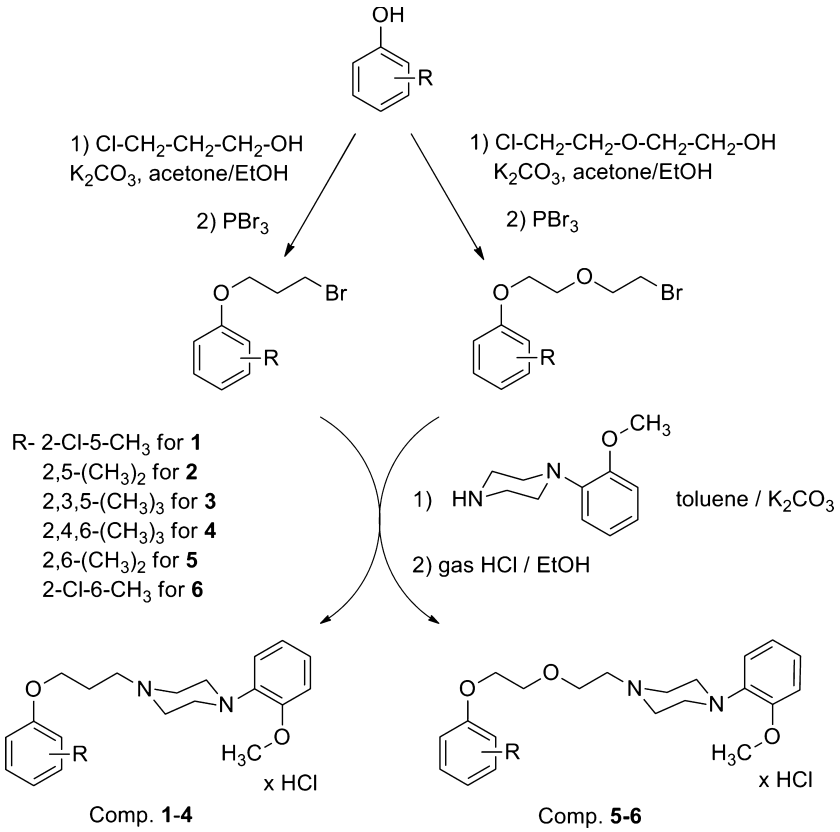
The data obtained were presented as means ± SEM and evaluated using Student's *t*-test or one-way analysis of variance (ANOVA), followed by Dunnett's test. Differences between groups were considered significant if $p < 0.05$.

The log-probit method described by Litchfield and Wilcoxon (13) was used to determine ED_{50} for compounds in locomotor activity, rotarod, and chimney tests. In locomotor activity test, ED_{50} is defined as the dose of the tested compound that decreases spontaneous locomotor activity in 50% of mice compared with vehicle-treated group. In rotarod and chimney tests, TD_{50} is defined as the dose of investigated compound that impairs motor co-ordination in 50% of mice compared with vehicle-treated group.

Results

Chemistry

Compounds **1-6** were obtained by *N*-alkylation of *N*-(2-methoxyphenyl)piperazine using, respectively, phenoxypropyl or phenoxyethoxyethyl bromide. The reaction was performed in the presence of K_2CO_3 as a proton acceptor in toluene solution. The yield of alkylation was in the range 40–55%. Appropriate phenoxypropyl or phenoxyethoxyethyl bromides were achieved according to earlier-published methodology (14), and crude products were used for further alkylation. All amines received as oily products were converted into hydrochlorides upon treatment with excess of ethanolic solution of gaseous HCl. The raw hydrochlorides were recrystallized from the mixture of acetone/ethanol 1:1 (v/v). The synthesis and chemical structures of the tested compounds are shown in Scheme 1.



Scheme 1: Synthesis and structures of compounds **1–6**.

Pharmacology

Compounds **2**, **4**, and **6** showed very high affinity toward 5-HT_{1A} receptor (K_i <1 nM). The results are superior to both serotonin and buspirone. Compounds **2**, **3**, and **6** showed high affinity toward 5-HT_7 receptor (K_i <35 nM), and the results are comparable to clozapine. None of the tested compounds proved nanomolar affinity toward 5-HT_6 receptor (Table 1).

Compounds **1**, **2**, and **4** decreased immobility time in mice at the dose 2.5 mg/kg b.w. by 47.0%, 60.3%, and 53.4%, respectively, but no effect was detectable using either lower or higher concentrations of the compounds. The reason for this fact may be a possible characteristic of antidepressants—U-shape of effect–dose curve in such tests. Compound **3** did not influence immobility time at the doses 2.5–10 mg/kg b.w. Compound **5** at the doses 2.5–10 mg/kg b.w. statistically significantly decreased immobility time in mice by 56.8% to 59.2%. Compound **6** decreased immobility time in mice by 41.6% and 54.5% at the doses 2.5 and 5 mg/kg b.w., respectively. Imipramine at the doses 5 and 10 mg/kg b.w. statistically significantly decreased immobility time in mice by 52.4% and 83.5%, respectively. The results of TST in mice are summarized in Table 2.

None of the compounds affected locomotor activity in mice at the doses active in TST in mice (Table 3). Compounds **1**, **2**, **4**, and **6** statistically significantly decreased locomotor activity in mice at the doses 30 and 60 mg/kg b.w. by 94.8% and 99.7% ($F(4,41) = 11.170$, $p < 0.0001$), 72.3% and 98.3% ($F(3,29) = 8.413$, $p < 0.001$), 64.9% and 97.5% ($F(3,30) = 8.750$, $p < 0.001$), and 51.4% and 87.8% ($F(4,45) = 26.750$, $p < 0.0001$), respectively. Compound **5** statistically significantly decreased locomotor activity in mice at the doses 20 and 40 mg/kg b.w. by 37.5% and 94.8%, respectively ($F(3,36) = 12.420$, $p < 0.0001$). ED_{50} values for all compounds are shown in Table 3.

Table 1: Results of binding to serotoninergic receptors of tested and reference compounds

	K_i [nM]		
Compd.	5-HT_{1A}	5-HT_6	5-HT_7
1	15	–	–
2	<1	1809	23
3	6	4465	25
4	<1	8058	157
5	41	9967	156
6	<1	9617	34
Serotonin	3	–	–
Buspirone	12	–	–
Imipramine	>10 000	190	1000
Clozapine	143	4	18

Inhibition constants (K_i) were calculated according to the equation of Cheng and Prusoff (7). Radioligand binding assays to rats brain tissues using [^{3}H]8-OH-DPAT for 5-HT_{1A}, [^{3}H]-LSD for 5-HT_6, [^{3}H]-5-CT for 5-HT_7; $n = 3$.

Table 2: Effect of the studied compounds and imipramine on the duration of immobility time in tail suspension test in mice

Treatment	Dose [mg/kg b.w.]	Immobility time [s]
Vehicle (0.5% MC)	–	131.0 ± 11.6
1	1.25	95.7 ± 12.4
	2.5	69.4 ± 12.1**
	5	99.3 ± 12.1
	10	125.2 ± 15.4
		$F(4,41) = 3.741$, $p < 0.05$
Vehicle (0.5% MC)	–	128.7 ± 14.5
2	1.25	121.6 ± 15.5
	2.5	51.1 ± 14.7**
	5	131.5 ± 20.1
	10	122.6 ± 15.2
		$F(4,41) = 4.011$, $p < 0.01$
Vehicle (0.5% MC)	–	126.9 ± 14.4
3	2.5	106.7 ± 14.3
	5	132.5 ± 12.4
	10	127.2 ± 18.1
		$F(3,36) = 0.561$, NS
Vehicle (0.5% MC)	–	129.7 ± 14.8
4	1.25	84.9 ± 12.1
	2.5	59.1 ± 13.7**
	5	91.8 ± 14.9
	10	111.8 ± 11.5
		$F(4,45) = 3.980$, $p < 0.01$
Vehicle (0.5% MC)	–	138.1 ± 12.4
5	1.25	96.0 ± 14.8
	2.5	59.6 ± 10.1***
	5	59.4 ± 9.4***
	10	56.3 ± 11.6****
		$F(4,43) = 8.650$, $p < 0.0001$
Vehicle (0.5% MC)	–	147.3 ± 7.7
6	1.25	125.6 ± 14.3
	2.5	86.0 ± 18.4**
	5	67.0 ± 11.4***
	10	110.9 ± 12.5
		$F(4,42) = 4.292$, $p < 0.01$
Vehicle (DW)	–	140.2 ± 7.4
Imipramine	2.5	116.0 ± 11.2
	5	78.2 ± 10.9***
	10	34.4 ± 10.3****
		$F(3,34) = 20.830$, $p < 0.0001$

All compounds were administered i.p. 30 min before the test. The values are expressed as mean ± SEM, $n = 9$–10 mice per group. Statistical analysis: one-way ANOVA (Dunnett's post hoc) **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ versus respective vehicle-treated group. NS, non significant; MC, methylcellulose; DW, distilled water.

None of the compounds affected motor co-ordination in rotarod and chimney tests at the doses active in TST in mice (Table 4).

Table 3: The influence of the studied compounds on locomotor activity in mice

Treatment	Dose active in the TST [mg/kg b.w.]	Number of crossings	ED_{50} [mg/kg b.w.] (CI)
Vehicle (0.5% MC)	–	349 ± 58	–
1	2.5	377 ± 48	12.6 (5.4–29.5)
		t(18) = 0.376, NS	
2	2.5	383 ± 63	15.0 (6.8–33.0)
		t(18) = 0.405, NS	
Vehicle (0.5% MC)	–	345 ± 46	–
4	2.5	337 ± 47	34.6 (21.4–56.1)
		t(18) = 0.123, NS	
Vehicle (0.5% MC)	–	347 ± 27	–
5	2.5	356 ± 28	21.8 (16.3–29.3)
	5	342 ± 24	
	10	355 ± 51	
		F(3,33) = 0.034, NS	
Vehicle (0.5% MC)	–	387 ± 30	–
6	2.5	322 ± 32	17.5 (11.9–25.8)
	5	342 ± 39	
		F(2,27) = 0.972, NS	

All compounds were administered i.p. 30 min before the test. The values are expressed as mean ± SEM, n = 9–10 mice per group. Statistical analysis: Student's t-test or one-way ANOVA (Dunnett post hoc). ED_{50} values were calculated using the log-probit method described by Litchfield and Wilcoxon [18]; NS, non significant; MC, methylcellulose. Compounds **1** and **2** were evaluated versus the same control (vehicle); CI, confidence interval; TST, Tail suspension test.

Table 4: The influence of the studied compounds on motor coordination in rotarod and chimney tests

		Rotarod test		Chimney test	
Treatment	Dose active in the TST [mg/kg b.w.]	% of animals that fell from rotating rod	TD_{50} [mg/kg b.w.] (CI)	% of animals that did not climb out the chimney	TD_{50} [mg/kg b.w.] (CI)
1	2.5	0	46.0 (34.5–61.3)	0	18.7 (13.1–26.8)
2	2.5	0	106.0 (72.0–156.1)	0	30.5 (23.3–39.8)
4	2.5	0	19.6 (10.6–36.1)	0	14.8 (8.9–24.8)
5	2.5	0	46.6 (41.2–52.7)	0	22.4 (11.5–43.6)
	5	0		0	
	10	0		0	
6	2.5	0	53.2 (44.1–62.2)	0	20.1 (13.2–30.5)
	5	0		0	

All compounds were administered i.p. 30 min before the test. n = 10 mice per group. TD_{50} values were calculated using the log-probit method described by Litchfield and Wilcoxon [17]; CI, confidence interval; TST, Tail suspension test.

Compound **1** at the doses 20, 30, and 40 mg/kg statistically significantly increased time spent in the chimney compared with the vehicle-treated animals by 84%, 111%, and 136%, respectively ($F(4,41) = 6.642$, $p < 0.001$). Compound **2** at the doses 30, 40, and 50 mg/kg statistically significantly increased time spent in the chimney compared with control group by 122%, 134%, and 161%, respectively ($F(4,40) = 5.520$, $p < 0.01$). Compound **4** at the doses 20 and 40 mg/kg statistically significantly increased time spent in the chimney compared with control group by 223% and 296%, respectively ($F(3,36) = 14.230$, $p < 0.0001$). In comparison with vehicle-treated group, compound **5** at the doses 40 and 50 mg/kg b.w. statistically significantly increased time spent in the chimney by 218% and 304%, respectively ($F(3,36) = 4.434$, $p < 0.01$). Compound **6** at the doses 25 and 40 mg/kg b.w. statistically significantly increased time spent in the chimney compared with control group by 210% and 260%, respectively ($F(3,36) = 7.789$, $p < 0.001$). ED_{50} values in rotarod and chimney tests for both compounds are presented in Table 4.

Discussion

It is commonly known that 5-HT_{1A} receptors have been implicated in many CNS disorders, including anxiety and depression. Numerous researches show that 5-HT_{1A}

receptor ligands possess antidepressant-like properties (15,16). Given the fact that all studied compounds showed high affinity for serotonergic 5-HT_{1A} receptors, and compounds **2**, **3**, and **6** exhibit favorable 5-HT_7 binding properties, their antidepressant-like activity was investigated in tail suspension test in mice, which is widely used to evaluate antidepressant properties. In this test, all compounds except for **3** showed significant activity, stronger than that of imipramine (Table 2). From *in vivo* observations, compounds **5** and **6** seem the most promising, as they were active also at other doses apart from 2.5 mg/kg (Table 2). This is very interesting taking into account receptor-binding properties, as activity of compound **5** does not correlate with its receptor binding to 5-HT_{1A} or 5-HT_7. This can be explained by other possible mechanism(s) of action.

As not only antidepressants but also psychostimulants show activity in TST, the influence on locomotor activity in mice was also evaluated. None of the compounds affected locomotor activity in mice at the doses active in TST, and this fact suggests that the observed antidepressant-like effect was specific. It has been shown that arylpiperazines produce antidepressant-like activity in many behavioral tests and models of depression (17–21). The research results are convergent with our findings.

The results of our study clearly show that all active compounds possess sedative properties. In patients suffering from depression with agitation or insomnia, sedation can be desirable. On the other hand, excessive sleepiness during antidepressant therapy is regarded as side-effect and is the most common reason for drug discontinuation. Taking into account that studied compounds showed sedative properties at the doses around five to fourteen times higher than the lowest dose active in TST (Table 3), the risk of excessive sedative effect seems to be low. Nevertheless, this requires further investigation.

It is well known that drugs acting within CNS may have negative influence on brain function. None of the compounds impaired motor co-ordination in mice at antidepressant-like doses, and this observation suggests that none of the compounds possesses neurotoxic properties at active doses (Table 4). The neurotoxic effect was observed at much higher doses (rotarod—around 19–42 times as high, chimney test—around 8–12 times as high) (Table 4).

Structure–activity relationship

Among the synthesized group of compounds, **1**-**4** and **5**-**6** represent variously substituted (phenoxy)propyl or (phenoxy)ethoxyethyl derivatives of 1-(2-methoxyphenyl)piperazine, respectively. It can be observed that compounds **5**-**6** are at least as active as **1**-**4** (Tables 1–3), but less neurotoxic (rotarod and chimney test, Table 4).

In terms of various substitution of the phenyl ring, compounds **2**, **4**, and **5**-**6** exhibit the most favorable pharmacological properties, which suggest that substitution with methyl in position 2, as a common feature of all compounds may be significant, as well as use of at least 2 lipophilic substituents (methyl, chloro).

As the reference compound constituted 4-[(2,6-dimethylphenoxy)propyl]-1-(2-methoxyphenyl)piperazine hydrochloride (Figure 1), the modifications were made in terms of changing position of methyl in the phenyl ring from 6 to 5 (compound **2**) as well as addition of an extra methyl group in the phenyl ring in various positions—2,3,5 as well as 2,4,6 (as in compounds **3**-**4**). These changes resulted in most favorable results for 2,5-dimethyl isomer (**2**) of the reference compound—its affinities toward 5-HT_{1A} and 5-HT_7 remain below 1 nM (Table 1), its ED_{50} in locomotor activity is equal to 15 mg/kg b.w. (mice, i.p., Table 3), and the compound is the least neurotoxic of all the synthesized compounds, revealing TD_{50} (mice, i.p.) = 106 mg/kg b.w. (rotarod) and 30.5 mg/kg b.w. (chimney test).

Then, substitution in compound **2** of one methyl into chloro in the phenyl ring resulted in compound **1**–4-[(2-chloro-6-methylphenoxy)propyl]-1-(2-methoxyphenyl)piperazine hydrochloride with locomotor activity ED_{50} = 12.6 mg/kg b.w.

Compound **5** is ethoxyethyl analog of the reference compound (Figure 1) (6). Its antidepressant-like activity was comparable to imipramine (Table 2). Therefore, similar to modifications in compounds **1** and **2**, compound **6** is chloro analog of compound **5**. The affinities of **5** and **6** toward 5-HT_{1A} receptor can be compared—compound **6** exhibits K_i <1 nM and **5** K_i = 41 nM. Compound **6** also exhibits more favorable antidepressant-like activity than both **5** and imipramine—the observed doses at a significant effect are 2.5 mg/kg b.w. for **6** and 5 mg/kg b.w. for imipramine (Table 2). Its ED_{50} in locomotor activity is 17.5 mg/kg b.w. (Table 3) and TD_{50} = 53.2 and 20.1 mg/kg b.w. in rotarod and chimney test, respectively (Table 4).

Conclusions

As a continuation of our former studies, it has been revealed that variously substituted aroxypropyl derivatives of 1-(2-methoxyphenyl)piperazine can present very good affinities toward 5-HT_{1A} and 5-HT_7 receptors. The *in vivo* results reflect the *in vitro* activity, which may suggest some favorable ADME properties. However, this should be further explored. As an unobvious result of the performed studies, exchange of the propyl linker into ethoxyethyl resulted in achievement of both favorable *in vitro* and *in vivo* characteristics. As a conclusion, the title group of compounds is promising in terms of antidepressant-like activity, and some further research in terms of *in vivo* properties should be performed, especially for ethoxyethyl derivatives of 1-(2-methoxyphenyl)piperazine. Moreover,

the observed affinities toward 5-HT_7 receptors suggest that it is probable to find cognitive activity, and this possibility also should be explored.

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Conflict of Interest

There is no conflict of interests among the authors.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Compound 1.

Figure S2. Compound 2.

Figure S3. Compound 3.

Figure S4. Compound 4.

Figure S5. Compound 5.

Figure S6. Compound 6.