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POTENTIAL ANXIOLYTIC, BUT NOT ANTIDEPRESSANT, ACTIVITY OF NEW 7-ARYLPIPERAZINYLBUTYL-8-MORPHOLINYL--PURINE-2,6-DIONE ANALOGS IN MICE

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On the basis of our earlier studies with serotonin receptor ligands in the group of long-chain arylpiperazine derivatives of purine-2,6-dione, a series of new 7-arylpiperazinylbutyl-8-morpholinyl-purine-2,6-dione analogs (GR-26/10, GR-27/10, GR-28/10) was designed, synthesized and studied in *in vitro* assays for the affinity of these compounds for 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 receptors. We have shown that the introduction of a methoxy moiety at the 2-position or a chloride atom at the 3-position of the phenylpiperazine structure of 8-morpholin-4-yl-purin-2,6-dione modified the affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 receptors as compared with the unsubstituted parent compound (GR-26/10). Then the selected compounds were pharmacologically evaluated in mouse models of depression and anxiety, i.e. the forced swim and the four-plate tests, respectively. Among them, the GR-28/10 compound with a chloride substituent at the 3-position of phenylpiperazine revealed specific anxiolytic, but not antidepressant, activity in mice.

Key words: 5-HT_{1A}; 5-HT_{2A}; 5-HT_7 receptor ligands; long-chain arylpiperazines; purine-2,6-diones; anxiolytic activity; antidepressant activity; mice

INTRODUCTION

The serotonin (5-hydroxytryptamine, 5-HT) neurotransmitter system regulates complex sensory, motor, affective, and cognitive functions. During the past several years a large amount of information about 5-HT receptors has been collected, but the role of some of them is still unclear. The specific localization of 5-HT receptors in the central nervous system as well as high affinity of some antidepressant and/or antipsychotic drugs for these receptors may suggest their involvement in

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affective disorders (Carr and Lucki, 2011; Hirano et al., 2009; Mattson et al., 2005). Among 5-HT receptors, particularly the 5-HT_{1A}, 5-HT_{2A}, 5-HT_6 and/or 5-HT_7 types are associated with anxiety, depression, and schizophrenic symptoms, and learning and memory disorders (Carr and Lucki, 2011; Wesołowska, 2010; Wesołowska and Nikiforuk, 2008; Wesołowska, 2007; Wesołowska et al., 2007; Wesołowska et al., 2006). Postsynaptic 5-HT_{1A} receptors are essential for producing antidepressant- and/or anxiolytic-like effects of 5-HT_{1A} receptor agonists and possibly selective 5-HT reuptake inhibitors. 5-HT_{2A} receptor antagonists may also produce potential antidepressant/antipsychotic activity. Furthermore, studies have shown that by reducing the function of 5-HT_7 receptors, antidepressant-like behavioral effects can be evoked (Carr and Lucki, 2011).

Arylpiperazine is a core fragment of many bioactive compounds exhibiting a variety of pharmacological effects. Previously, it has been shown that their pharmacological activity can be mediated by different subpopulations of 5-HT, dopamine and adrenergic receptors (Lopez-Rodriguez et al., 2002; Jurczyk et al., 2004). The most thoroughly studied group of arylpiperazine derivatives is the one called long-chain arylpiperazines (LCAPs) which have been recognized as 5-HT ligands, particularly 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 ligands (Jurczyk et al., 2004; Chłoń-Rzepa et al., 2007). Their general chemical structure contains an alkyl chain (two to four methylene units) attached to the N4 atom of the piperazine moiety and a terminal amide or an imide fragment. Some of our previous structure-affinity and structure-intrinsic activity studies were concerned with chemical modifications in a group of compounds containing a theophylline fragment and an arylpiperazine moiety. These compounds, in functional in vivo models, behaved like postsynaptic 5-HT_{1A} antagonists (Chłoń-Rzepa et al., 2007). To continue our research with the class of LCAP derivatives of theophylline, we designed a novel series of 7-arylpiperazinylbutyl-8-morpholinyl-purine-2,6-dione analogs (GR-26/10, GR-27/10, GR-28/10). The synthesis of these compounds was presented earlier (Chłoń-Rzepa et al., 2010). Their structures were confirmed by ^{1}H NMR spectra and an elementary analysis. All the new compounds were tested for their affinity for 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 receptors. On the basis of binding studies, the compounds GR-27/10 and GR-28/10 as hydrochloride salts were evaluated in preclinical models of anxiety and depression in mice.

MATERIAL AND METHODS

Drugs

The investigated compounds GR-26/10 **(**1,3-di-methyl-8-morpholin-4-yl-7-[4-(4-phenyl-piperazin-1-yl)-butyl]-3,7-dihydro-purine-2,6-dione hydrochloride), GR-27/10 (7-{4-[4-(2-methoxy-phenyl)-piperazin-1-yl]-butyl}-1,3-dimethyl-8-morpholin-4-yl-3,7-dihydro-purine-2,6-dione hydrochloride), GR-28/10 (7-{4-[4-(3-chloro-phenyl)-piperazin-1-yl]-butyl}-1,3-dimethyl-8-morpholin-4-yl-3,7-dihydro-purine-2,6-dione hydrochloride) were synthesized at the Department of Pharmaceutical Chemistry, Jagiellonian University, Medical College in Cracow (Chłoń-Rzepa et al., 2010). Diazepam (Polfa), imipramine hydrochloride (Polfa), Tween 80 (Tween 80, Sigma Aldrich). The following radioligands were used: [^{3}H]-5-CT (34.5 Ci/mmol, NEN Chemicals), [^{3}H]-8-OH-DPAT (170 Ci/mmol, NEN Chemicals) and [^{3}H]-ketanserin.

In *in vivo* experiments, all the compounds were administered intraperitoneally (i.p.) at a volume of 10 ml/kg body weight 60 (diazepam) or 30 min (the remaining compounds) before the test. Imipramine hydrochloride was dissolved in distilled water, whereas diazepam, GR-27/10, and GR-28/10 were suspended in 1% aqueous solution of Tween 80 immediately before administration.

Animals

The experiments were performed on male Albino Swiss mice (22-26 g) and were approved by the Local Ethics Commission for Animal Experiments of Jagiellonian University in Cracow. The animals were kept in groups of 12-20 mice in type III-1290 cages (26.5 x 42.0 x 15.0 cm) at a room temperature of 22 ± 2°C, under 12/12 h light/dark cycle, and had free access to food and water before the experiments. Each experimental group consisted of 6-9 animals/dose, and all the animals were used only once. The experiments were performed between 8 a.m. and 3 p.m.

Statistical analysis

All the data are presented as the mean ± S.E.M. The statistical significance of drug effects was evaluated using a separate analysis of variance (ANOVA), followed by Bonferroni's post-hoc test; $p<0.05$ was considered statistically significant.

In vitro radioligand binding assays

All the assays were carried out on rat brain tissues; inhibition constants (K_i) were determined from at least three separate experiments in which 8-10 drug concentrations, run in triplicate, were used. The binding effect was terminated by rapid filtration through Whatman GF/B filters, followed by three 4 mL washes with the ice-cold incubation buffer. The radioligand concentration used in competition assays was equal to the K_d values obtained in the respective saturation experiment, that is 1, 0.6, and 0.5 nM for [^{3}H]-8-OH-DPAT, [^{3}H]-ketanserin and [^{3}H]-5-CT, respectively.

The radioligand retained on the filters was measured by liquid scintillation counting (Beckman LS 6500 apparatus) in 4mL scintillation FLUID (Akwascynt, BioCare). Binding isotherms of the tested compounds were analyzed by nonlinear regression (Prism, GraphPad Software Inc., San Diego, USA), using the Cheng-Pursoff equation to calculate K_i values.

Serotonin 5-HT$_{1A}$ and 5-HT$_{2A}$ binding assays

Radioligand studies with native 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors were conducted according to the methods described previously (JURCZYK at al., 2004). Briefly, the following were used: for 5-HT$_{1A}$ assays, rat hippocampal membranes, [^{3}H]-8-OH-DPAT (170 Ci/mmol, NEN Chemicals), and 5-HT (10 μM) for non-specific binding; for 5-HT$_{2A}$ assays [^{3}H]-ketanserin (88.0 Ci/mmol, NEN Chemicals) and metysergide (1 μM) for non-specific binding.

Serotonin 5-HT$_7$ binding assays

The 5-HT$_7$ receptor binding assay was performed using rat hypothalamic membranes, according to the method described by Aguirre et al. (1998) with a minor modification described previously (Chłoń-Rzepa et al., 2007). In brief, the membrane aliquots were incubated in the presence of 3 μM (±)-pindolol (to eliminate the binding to 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors) with 0.5 nM [^{3}H]-5-CT (34.5 Ci/mmol, NEN Chemicals) and 10 μM of 5-HT for non-specific binding.

In vivo experiments

Forced swim test in mice

The experiment was carried out according to the method of Porsolt et al. (1978). Mice were individually placed in a glass cylinder (25 cm high; 10 cm in diameter) containing 6 cm of water maintained at 23-25°C, and were left there for 6 min. A mouse was regarded as immobile when it remained floating on the water, making only small movements to keep its head above it. The total duration of immobility was recorded during the last 4 min of the 6-min test session.

Four-plate test in mice

The four-plate apparatus (BIOSEB) consists of a cage (25 x 18 x 16 cm) floored with four identical rectangular metal plates (8 x 11 cm) separated from one another by a gap of 4 mm. The top of the cage is covered with a transparent Perspex lid that prevents escape behaviour. The plates are connected to a device that can generate electric shocks. Following a 15-s habituation period, the animal's motivation to explore a novel environment is suppressed by an electric foot shock (0.8 mA, 0.5 s) every time it moves from one plate to another during a 1-min test session. This action is referred to as a 'punished crossing', and is followed by a 3- s shock interval, during which the animal can move across plates without receiving a shock.

Spontaneous locomotor activity in mice

The locomotor activity was recorded with an Opto M3 multi-channel activity monitor (MultiDevice Software v.1.3, Columbus Instruments). The mice were individually placed in plastic cages (22 x 12 x

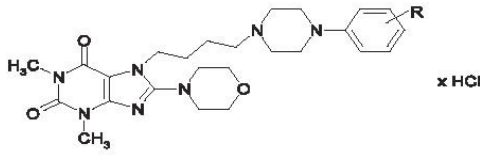
13 cm) for a 30-min habituation period, and then the crossings of each channel (ambulation) were counted during the first 1-min (i.e. the time equal to the observation period in the four-plate test) and 5-min experimental sessions. The cages were cleaned up with 70% ethanol after each mouse.

RESULTS AND DISCUSSION

It is well known that central 5-HT receptors play an essential role in a number of psychiatric disorders such as anxiety, depression and schizophrenia. Apart from the well-established role of 5-HT_{1A} and 5-HT_{2A} receptors, the most recently identified 5-HT_7 receptors are also implicated in the control of mood diseases. It has been recently reported that differently substituted arylpiperazine moieties are often incorporated into the structure of 5-HT_7 receptor ligands (Raubo et al., 2006). Hence, all purine-2,6-diones were evaluated for their in vitro affinity, not only for 5-HT_{1A} and 5-HT_{2A} receptors, but also for 5-HT_7 sites. The newly synthesized derivatives of 7-arylpiperazinylbutyl-8-morpholinyl-purine-2,6-dione analogs showed a diversified level of affinity for the investigated 5-HT receptors, with K_i values ranging from 7 to 1277 nM. The receptor binding data of new compounds are presented in Table 1. Generally, in comparison with the previously reported butyl derivatives with the 8-alkoxy moiety (Chłoń-Rzepa et al., 2007), the replacement of the ethoxy or propoxy moiety by morpholinyl in the group of purine-2,6-diones resulted in a decrease in the 5-$HT_{1A/7}$ affinity and in a slight increase in the affinity for 5-HT_{2A} sites. However, the obtained results have proven that the introduction of a methoxy moiety in the 2-position or a chloride atom in the 3-position of the phenylpiperazine structure of 8-morpholin-4-yl-purin-2,6-dione modified the affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 receptors as compared with the unsubstituted parent compound (the GR-26/10 compound). The affinity of the 2-methoxyphenylpiperazine derivative (compound GR-27/10) significantly increased for 5-HT_{1A} and 5-HT_7 receptors, and at the same time it decreased about three times for 5-HT_{2A} sites. However, the affinity of the GR-28/10 compound with a 3-chloro substituent in the phenylpiperazine structure highly (4-11 times) increased for 5-HT_{1A} and 5-HT_{2A} receptors. Our results have also confirmed that, as compared with the previous report (Chłoń-Rzepa et al., 2007), the introduction of a chloride substituent into the phenylpiperazine derivative group resulted in an almost 10-fold increase in 5-HT_7 affinity in the case of GR-28/10 vs. GR-26/10 compounds. It was also found that the modification in the arylpiperazine moiety by introducing the OCH_3 group in the 2-position (GR-27/10) slightly increased (about three times) the affinity for 5-HT_7 receptor vs. the GR-26/10 compound (Table1).

The *in vitro* binding studies have indicated that two of the newly synthesized derivatives of

TABLE 1. The structure and binding affinity of the investigated 7-arylpiperazinylbutyl-8-morpholin-4-yl-purine-2,6-dione analogs for serotonin receptors.



Compound	R	K_i (nM) ± SEM		
		5-HT_{1A}	5-HT_{2A}	5-HT_7
GR-26/10	H	138 ± 12	77 ± 6	1277 ± 76
GR-27/10	2-OCH3	7 ± 1	205 ± 26	335 ± 15
GR-28/10	3-Cl	22 ± 2	21 ± 2	112 ± 10

TABLE 2. The effects of the compounds GR-27/10 and GR-28/10 in the forced swim test in mice

Treatment	Dose (mg/kg)	Immobility time (s) Mean ± SEM
Vehicle	-	154.9 ± 8.3
GR-27/10	10	149.9 ± 12.0
	20	139.6 ± 18.6
	30	105.1 ± 18.0
		F(3,28)=2.278 ns
GR-28/10	10	188.0 ± 9.3
	20	178.3 ± 8.1
	30	200.6 ± 11.8[a]
		F(3,30)=4.001 p<0.05
Vehicle	-	162.7 ± 6.8
Imipramine	5	170.4 ± 10.9
	10	77.8 ± 12.2[b]
	20	119.6 ± 13.0[a]
		F(3,36)=16.7570 p<0.0001

All the compounds were administrated i.p. 30 min before the test; n=8-9 mice per group.
[a] p<0.05, [b]p<0.001 versus the respective vehicle group

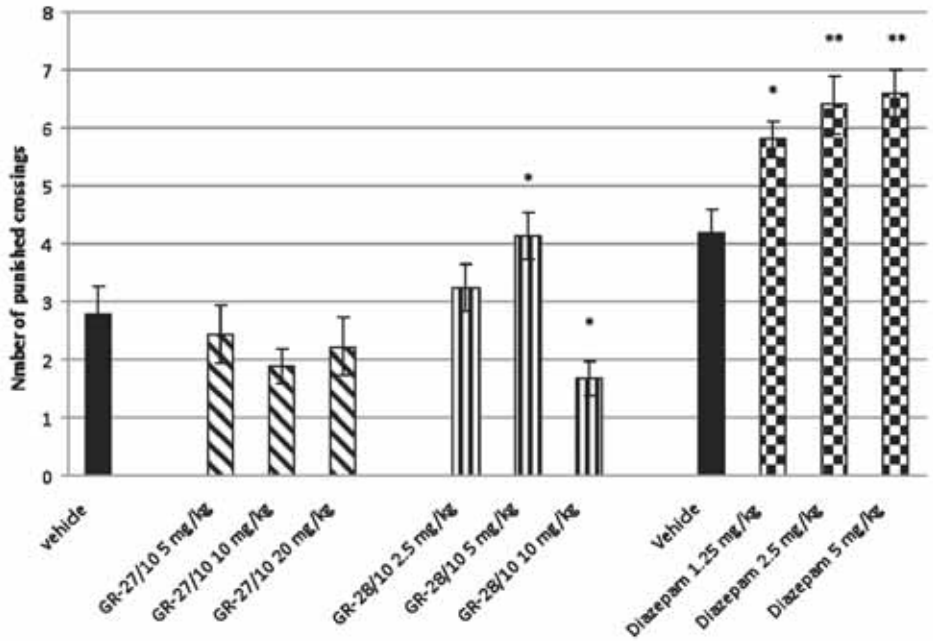
7-arylpiperazinylbutyl-8-morpholin-4-yl-purine-2,6-dione (GR-27/10 and GR-28/10) display interesting affinity for the above-mentioned 5-HT receptors. Hence, their potential anxiolytic and antidepressant activity was examined in two screening behavioral models in mice.

The results obtained previously indicated that the series of 7-phenylalkyl-purine-2,6-diones exerted anxiolytic- and/or antidepressant-like effects (Chłoń-Rzepa et al., 2007). The forced swim test in mice was carried out to investigate antidepressant-like activity of GR-27/10 and GR-28/10 analogs and their effects were compared with those of the reference compound imipramine. The studied antidepressant drug significantly reduced the immobility time of mice when applied at doses of 10 mg/kg (a 52% reduction) and 20 mg/kg (a 26.5% reduction), but not at a dose of 5 mg/kg. The investigated compound GR-27/10 dose-dependently decreased the immobility time; however, it did not reach the statistically significant level. GR-28/10 did not produce an antidepressant-like effect; on the contrary, it significantly (at a dose of 30 mg/kg) increased the immobility time of mice in Porsolt's test (Table 2).

To investigate anxiolytic-like activity, the four-plate test was carried out in mice. The reference compound diazepam at doses of 1.25, 2.5 and 5 mg/kg dose-dependently increased (by 38%, 52% and 57%, respectively) the number of punished crossings in mice. The GR-28/10 compound administered at a dose of 5 mg/kg significantly (by 46%) increased the number of punished crossing in that test. The GR-27/10 compound showed no anxiolytic-like effect in that test; it did not change the number of punished crossing as compared with the vehicle group (Fig.1).

The influence of the effective doses recorded only in the four-plate test was studied in the case of spontaneous locomotor activity in mice in order to exclude the possibility of competing behaviors such as general locomotor activity. The spontaneous locomotor activity was determined in separate groups of mice. During the 1- or 5-minute observation period diazepam diminished spontaneous locomotor activity but it did not evoke the statistically significant effect, while the investigated compound GR-28/10 produced a significant reduction in mouse activity (Table 3). Since both the tested compounds do not stimulate spontaneous locomotor activity, their anxiolytic-like effect observed in the four-plate test in mice seems to be specific.

In conclusion, we have shown that the replacement of the ethoxy or propoxy moiety with morpholinyl in the group of purine-2,6-diones resulted in a decrease in the 5-$HT_{1A/7}$ affinity and in a slight



The compounds Gr-27/10 and GR-28/10 were administrated 30 min, while diazepam – 60 min before the test; n=9-10 mice per group. * p<0.05, **p<0.01 versus the respective vehicle group

Fig. 1. The effects of the investigated compounds and diazepam in the four-plate test in mice.

TABLE 3. The effects of the GR-28/10 compound and diazepam on the locomotor activity of mice

Treatment	Dose (mg/kg)	Number of crossings during 1 min Mean ± SEM	Number of crossing during 5 min Mean ± SEM
Vehicle	-	17.7 ± 7.3	139.9 ± 50.0
GR-28/10	5	0.63 ± 0.3	19.7 ±5.4[a]
		F(1,16)=4.347 ns	F(1,16)=4.5331 p<0.05
Vehicle	-	18.3 ± 6.2	160.3 ± 76.5
Diazepam	1.25	14.1 ± 3.3	126.7 ± 12.7 ns
	2.5	11.8 ± 2.5	123.9 ± 50.8 ns
	5	10.8 ± 2.2	100.7 ± 42.2 ns
		F(3,34)=1.056 ns	F(3,34)=1.314 ns

The GR-28/10 compound was administered 1815 sec (30 min + 15 sec) before the test and diazepam was given 3615 sec (60 min + 15 sec) before the test; locomotor activity was measured every 1 min for 5 min (5 intervals), [a] p<0.05

increase in the affinity for 5-HT_{2A} sites. Moreover, the obtained results have proven that the introduction of a methoxy moiety in the 2-position or a chloride atom in the 3-position of the phenylpiperazine structure of 8-morpholin-4-yl-purin-2,6-dione modified the affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 receptors as compared with the unsubstituted parent compound (the GR-26/10 compound). Among them, the GR-28/10 compound with the chloride substituent in the 3-position of phenylpiperazine revealed activity in the screening animal model of anxiety in mice. However, its sedative effect practically excluded this ligand from being regarded as a potential drug.

REFERENCES

Aguirre, N., S. Ballaz, B. Lasheras and J. Del Rio. 1998. MDMA (`Ecstasy') enhances 5-HT_{1A} receptor density and 8-OH-DPAT-induced hypothermia: blockade by drugs preventing 5-hydroxytryptamine depletion. *Eur. J. Pharmacol.* 346: 181-188.

Carr, G.V. and I. Lucki. 2011. The role of serotonin receptor subtypes in treating depression: a review of animal studies. *Psychopharmacology* 213: 265-287.

Chłoń-Rzepa, G., M. Harłacz, P. Żmudzki, M. Pawłowski, A.J. Bojarski, B. Duszyńska and A. Kozioł. 2010. Synthesis of long-chain arylpiperazine theophylline derivatives as potential 5-HT7 and 5-HT6 receptor ligands. The seventh multidisciplinary conference on drug research, 10-12 May 2010, Zakopane, Poland.

Chłoń-Rzepa, G., P. Żmudzki, P. Zajdel, A.J. Bojarski, B. Duszyńska, A. Nikiforuk, E. Tatarczyńska and M. Pawłowski. 2007. 7-Alkylpiperazinylalkyl and 7-tetrahydroisoquinolinylalkyl derivatives of 8-alkoxy-purine-2,6-dione and some of their purine-2,6,8-trione analogs as 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 serotonin receptor ligands. *Bioorg. Med. Chem.* 15: 5239-5250.

Hirano, K., T.M. Piers, K.L. Searle, N.D. Miller, A.R. Rutter and P.F. Chapman. 2009. Procognitive 5-HT_6 antagonists in the forced swimming test: Potential therapeutic utility in mood disorders associated with Alzheimer's disease. *Life Sci.* 84: 558-562.

Jurczyk, S., M. Kołaczkowski, E. Maryniak, P. Zajdel, M. Pawłowski, E. Tatarczyńska, A. Kłodzińska , E. Chojnacka-Wójcik, A .J. Bojarski, S. Charakchieva-Minol, B. Duszyńska, G. Nowak and D. Maciąg. 2004. New Arylpiperazine 5-HT_{1A} receptor ligands containing the pyrimido[2,1-*f*]purine fragment: synthesis, in vitro, and in vivo pharmacological evaluation. *J. Med. Chem.* 47: 2659-2666.

Lopez-Rodriguez, M.L., D. Ayala, B. Benhamu, M.J. Morcillo and A. Viso. 2002. Arylpiperazine derivatives acting at 5-HT_{1A} receptors. *Curr. Med. Chem.* 9: 443-469.

Mattsson, C., C. Sonesson, A. Sandahl, H.E. Greiner, M. Gassen, J. Plaschke, J. Leibrock and H. Böttcher. 2005. 2-Alkyl-3(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles as novel 5-HT_6 receptor agonists. *Bioorg. Med. Chem. Lett.* 15: 4230-4234.

Porsolt, R.D., G. Anton, N. Blavet and M. Jalfre. 1978. Behavioural despair in rats: a new model sensitive to antidepressant treatments. *Eur. J. Pharmacol.* 47: 379-391.

Raubo, P., M. S. Beer, P.A. Hunt, I.T. Huscroft, C. London, J.A. Stanton and J.J. Kulagowski. 2006. Aminoalkyl phenyl sulfones—a novel series of 5-HT_7 receptor ligands. *Bioorg. Med. Chem. Lett.* 16 (5): 1255-1258.

Wesołowska, A., A. Nikiforuk, K. Stachowicz and E. Tatarczyńska. 2006 Effect of the selective 5-HT_7 receptor antagonist SB 269970 in animal models of anxiety and depression. *Neuropharmacology* 51: 578-586.

Wesołowska, A., A. Nikiforuk, E. Tatarczyńska and E. Chojnacka-Wójcik. 2007. Enhancement of the anti-immobility action of antidepressants by selective 5-HT_7 receptor antagonist in the forced swimming test in mice. *Eur. J. Pharmacol.* 555: 43-47.

Wesołowska, A. 2007. The anxiolytic-like effect of selective 5-HT_6 receptor antagonist SB-399885 in rats. *Pharmacol. Rep.* 59: 664-671.

Wesołowska, A. 2010. Potential role of the 5-HT_6 receptor in depression and anxiety: an overview of preclinical data. *Pharmacol. Rep.* 62: 564-577.

Wesołowska, A. and A. Nikiforuk. 2008. The selective 5-HT_6 receptor antagonist SB-399885 enhances anti-immobility action of antidepressants in rats. *Eur. J. Pharmacol.* 582: 88-93.