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HBK-7 — A new xanthone derivative and a 5-HT_{1A} receptor antagonist with antidepressant-like properties

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ABSTRACT

Xanthone derivatives possess many biological properties, including neuroprotective, antioxidant or antidepressant-like. In this study we aimed to investigate antidepressant- and anxiolytic-like properties of a new xanthone derivative – 6-methoxy-4-[4-(2-methoxyphenyl)piperazin-1-yl]-9H-xanthen-9-one (HBK-7), as well as its possible mechanism of action, and the influence on cognitive and motor function. HBK-7 in our earlier studies showed high affinity for serotonergic 5-HT_{1A} receptor. We determined the affinity of HBK-7 for CNS receptors and transporters using radioligand assays and examined its intrinsic activity towards 5-HT_{1A} receptor. We evaluated antidepressant- and anxiolytic-like activity of HBK-7 in the mouse forced swim test, and four-plate test, respectively. We examined the influence on locomotor activity in mice to determine if the effect observed in the forced swim test was specific. We used step-through passive avoidance and rotarod tests to evaluate the influence of HBK-7 on cognitive and motor function, respectively. HBK-7 showed moderate affinity for dopaminergic D_2 receptor and very low for serotonergic 5-HT_{2A}, adrenergic α_2 receptors, as well as serotonin transporter. Functional studies revealed that HBK-7 was a 5-HT_{1A} receptor antagonist. HBK-7 (10 mg/kg) decreased immobility time in the forced swim test. Combined treatment with sub-effective doses of HBK-7 and fluoxetine reduced immobility of mice in the forced swim test. Pretreatment with *p*-chlorophenylalanine and WAY-100,635 antagonized the antidepressant-like effect of HBK-7. Neither of the treatments influenced locomotor activity of mice. HBK-7 at antidepressant-like dose did not impair memory or motor coordination in mice. We demonstrated that HBK-7 was a 5-HT_{1A} receptor antagonist with potent, comparable to mianserin, antidepressant-like activity. HBK-7 mediated its effect through serotonergic system and its antidepressant-like action required the activation of 5-HT_{1A} receptors. At active dose it did not influence cognitive and motor function. Since 5-HT_{1A} receptor antagonists may accelerate the occurrence of antidepressant effect, our findings highlight their potential as future antidepressants.

1. Introduction

Major depression is one of the most common mental disorders and a leading cause of disability worldwide. Despite numerous available antidepressants, over one-third of patients do not respond to pharmacotherapy (Al-harbi, 2012). Therefore, the scientists are still searching for new compounds with increased efficacy.

One group of compounds that attract scientists' attention are xanthones: a class of oxygen containing heterocycles found in higher plant families, fungi, and lichen. Xanthones present multiple biological activities (Jindarat, 2014; Wezeman et al., 2015). In preclinical studies xanthone derivatives showed antiarrhythmic (Rapacz et al., 2014, 2015), hypotensive (Szkaradek et al., 2015), antitumor (Wu et al., 2015), neuroprotective (Phyu and Tangpong, 2014), antioxidant (Fouotsa et al., 2015) or antidepressant-like properties (Jastrzębska-Więsek et al., 2003; Pytka et al., 2015b, 2015c). We previously reported antidepressant-like effects of two xanthone derivatives in the forced swim test and tail suspension test in mice (Pytka et al., 2015c; 2015b). Their activity was stronger or comparable to that of the reference antidepressants (i.e. reboxetine, fluoxetine, wenlafaxine).

In this study we aimed to investigate antidepressant- and anxiolytic-like properties of a new xanthone derivative: 6-methoxy-4-[4-(2-methoxyphenyl)piperazin-1-yl]-9H-xanthen-9-one (HBK-7), as well as its possible mechanism of action and the influence on cognitive and motor function. Since HBK-7 in our earlier experiments showed high

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affinity for serotonergic 5-HT_{1A} receptors (Waszkielewicz et al., 2013), we also determined its intrinsic activity at 5-HT_{1A} receptors. Moreover, we examined the affinity of HBK-7 for a broader spectrum of central nervous system receptors and transporters.

2. Materials and methods

2.1. Animals

Adult male mice (stock's name: CD-1, 18–21 g), purchased from Animal House at the Faculty of Pharmacy, Jagiellonian University Medical College, Cracow, Poland were used in each experiment. The groups of 15 mice were kept to a plastic cage (60 cm × 38 cm × 20 cm) at a room temperature (22 ± 2 °C), on 12 h light/dark cycles (the lights turned on at 7:00 a.m., and off at 19:00 p.m.). The rodents always had free access to standard laboratory food, as well as tap water. All experiments were conducted between 9 a.m. and 2 p.m. Each experimental group consisted of 10 randomly selected animals. The animals were used only once in each test, and killed by cervical dislocation immediately after the experiment. All experimental procedures were carried out in accordance with EU Directive 2010/63/EU and approved by the I Local Ethics Committee for Experiments on Animals of the Jagiellonian University in Krakow, Poland (approval numbers: 78/2013, 52/2014, 124/2014 and 102/2015).

2.2. Drug administration

The studied compound: 6-methoxy-4-[4-(2-methoxyphenyl) piperazin-1-yl]-9H-xanthen-9-one (HBK-7, Fig. 1) was synthesized in the Department of Bioorganic Chemistry, Chair of Organic Chemistry, Pharmaceutical Faculty, Jagiellonian University (Waszkielewicz et al., 2013). HBK-7, fluoxetine (Sigma, Germany), reboxetine (Sigma, Germany), bupropion (Sigma, Germany), mianserin (Organon, Netherlands), medazepam (Polfa Tarchomin, Poland), and scopolamine (Sigma, Germany) were dissolved in saline, and administered intraperitoneally (i.p.) at a volume of 10 ml/kg 30 min before each test. WAY-100635 was dissolved in saline and administered subcutaneously (s.c.) at a volume of 10 ml/kg 15 min before the studied compound. *p*-Chlorophenylalanine (pCPA, Sigma, Germany) was suspended in 1% Tween. The control groups were given saline (i.p.) 30 min before the test.

2.3. Radioligand binding assays

2.3.1. Serotonergic 5-HT_{2A} receptor

Radioligand binding was performed on membranes from CHO-K1 cells, which were stably transfected with the human 5-HT_{2A} receptor. All assays were carried out in duplicates. 50 μl working solution of the tested compounds, 50 μl [^{3}H]-ketanserin (final concentration 0.5 nM, K_D 0.3 nM) and 150 μl diluted membranes (5 μg protein per well) prepared in assay buffer (50 mM Tris, pH 7.4, 4 mM $CaCl_2$, 0.1% ascorbic acid) were transferred to polypropylene 96-well microplate using 96-wells pipetting station Rainin Liquidator (MettlerToledo). Serotonin (100 μM) was used to define nonspecific binding. Microplate was covered with a sealing tape, mixed and incubated for 60 min at 27 °C. The reaction was terminated by rapid filtration through GF/B filter mate presoaked with 0.3% polyethyleneimine for 30 min. Ten rapid washes with 200 μl 50 mM Tris buffer (4 °C, pH 7.4) were performed using automated harvester system Harvester-96 MACH III FM (Tomtec). The filter mates were dried at 37 °C in forced air fan incubator and then solid scintillator MeltiLex was melted on filter mates at 90 °C for 5 min. Radioactivity was counted in MicroBeta2 scintillation counter (PerkinElmer) at approximately 30% efficiency. The inhibitory constant (K_i) was estimated.

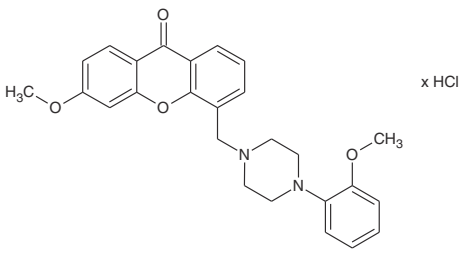

Fig. 1. Chemical structure of HBK-7. 6-Methoxy-4-[4-(2-methoxyphenyl)piperazin-1-yl]-9H-xanthen-9-one.

2.3.2. Serotonin transporter

The experiment was performed according to the method described by (Owens et al., 1997). Rats' cerebral cortices were homogenized in 30 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.7 at 25 °C) containing 150 mM NaCl and 5 mM KCl. Then the homogenate was centrifuged at 20,000 × *g* for 20 min. The resulting supernatant was decanted and the pellet was resuspended in the same quantity of the buffer, and centrifuged as above. The pellet was resuspended and centrifuged for further two times. [^{3}H]-Citalopram (50 Ci/mM, NEN Chemicals) was used for labeling serotonin transporter. The final mixture consisted of 240 ml tissue suspension, 30 ml of a 10 μM imipramine (unspecific binding), 30 ml of 1 nM [^{3}H]-citalopram and 100 ml buffer containing seven concentrations (10^{-10}–10^{-4}M) of the tested compound. The incubation was performed using plates (MAFCNOB 10, Millipore) at 23 °C for 60 min. After that time the incubation was terminated by rapid vacuum filtration over glass filters (Watman GF/B). Next the filters were washed two times with 100 ml of ice-cold buffer (0–48 °C) and placed in scintillation vials with scintillation cocktail. Radioactivity was measured in a WALLAC 1409 DSA – liquid scintillation counter. All assays were done in duplicates. K_i value was estimated.

2.3.3. Adrenergic α_2 receptor

Tissue (rat cortex) was homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer, pH 7.6 using an Ultra Turrax T25B (IKA) homogenizer. The homogenate was centrifuged at 20,000 × *g* for 20 min. The resulting supernatant was decanted and pellet was resuspended in the same buffer and centrifuged again in the same conditions. The final pellet was resuspended in appropriate volume of buffer (10 mg/1 ml). [^{3}H]-Clonidine (25.5 Ci/mmol, α_2-noradrenergic receptor) was used as a ligand. The incubation mixture (final volume of 550 μl) consisted of 450 μl of membrane suspension, 50 μl of a [^{3}H]-clonidine (2 nM) solution and 50 μl of buffer containing seven or eight concentrations (1 nM to 100 μM) of the investigated compounds. The radioactivity was measured in MicroBeta2 scintillation counter (PerkinElmer, USA). All assays were done in duplicates. To measure unspecific binding clonidine at a final concentration of 10 μM was present. K_i value was estimated.

2.3.4. Dopaminergic D_2 receptor

Radioligand binding was performed using membranes from CHO-K1 cells stably transfected with the human D_2 receptor. 50 μl working solution of the tested compounds, 50 μl [^{3}H]-methylspiperon (final concentration 0.4 nM, K_D 0.4 nM) and 150 μl diluted membranes (10 μg protein per well) prepared in assay buffer (50 mM HEPES, pH 7.4, 50 mM NaCl, 5 mM $MgCl_2$, 0.5 mM EDTA) were transferred to polypropylene 96-well microplate using 96-wells pipetting station Rainin Liquidator (MettlerToledo). (+)-Butaclamol (10 μM) was used to define nonspecific binding. Microplate was covered with a sealing tape, mixed and incubated for 60 min at 37 °C. The reaction was terminated by rapid filtration through GF/C filter mate presoaked with 0.3% polyethyleneimine for 30 min. Ten rapid washes with 200 μl 50 mM Tris buffer (4 °C, pH 7.4) were performed using automated harvester system Harvester-96 MACH III FM (Tomtec). The filter mates were dried at 37 °C in forced air fan incubator and then solid scintillator MeltiLex was melted on filter mates at 90 °C