Antidepressant-like and anxiolytic-like activity of a novel indoleamine derivative ADN-2013 in rodents

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Abstract

A novel indolamine derivative, ADN-2013, has been proved to possess a preclinical activity profile appropriate for the treatment of behavioral and psychological symptoms of dementia. Its antidepressant and anxiolytic properties have been examined in rat and mice models. The receptor mechanisms underlying the antidepressant properties of ADN-2013 have also been elucidated. ADN-2013 significantly shortened the immobility time measured in the forced swim test in mice and rats, producing an effect that was abolished by the dopamine D_1-receptor antagonist SCH 23390 in rats. Moreover, ADN-2013 evoked anxiolytic-like activity in both "conditional" and "unconditional" anxiety-like paradigms in mice and rats. From these results, it is likely that direct antagonism toward serotonin 5-HT_6 receptors and an indirect effect of dopamine, acting mostly via D_1-like receptors, may be involved in the antidepressant action of ADN-2013. However, the partial agonist activity of ADN-2013 toward D_2 receptors, observed in in vitro studies, might also contribute to this effect.

Keywords: schizophrenia, antipsychotic, anxiolytic, antidepressant, rats, mice

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Introduction

Schizophrenia is a severe mental disorder with a lifetime prevalence of approximately 1% in the global population [1] that is associated with positive, negative, and cognitive symptoms [2]. A large body of evidence shows that comorbid anxiety and mood disorders are relatively prevalent among patients with schizophrenia (up to 60% of patients) [1], leading to worse prognosis, weaker response to treatment, as well as impairment of psychosocial functioning and quality of life [2].

Anxiety symptoms in patients with schizophrenia may be manifested in different ways, such as active response to external stimuli and conditions, phenomena secondary to the positive symptoms, as well as a concomitant disorders [1]. Based on a meta-analysis, Achim et al. [3] identified the most common co-occurring diseases in schizophrenia as social phobia, posttraumatic stress disorder, and obsessive–compulsive disorder. In most of the research, comorbidity of anxiety disorder and schizophrenia has been associated with worse outcomes, poorer quality of life, and higher risk of suicide. According to epidemiological data, 13% to 80% of patients with schizophrenia suffer from depressive symptoms that might take a one of four following forms: 1) depression as a prodromal symptom; 2) depressive symptoms as an important part of an acute episode; 3) early post psychotic depression; and 4) late post-psychotic depression [4]. The most frequent diseases occurring concomitantly with schizophrenia are major depressive disorder, post-psychotic depression, and schizoaffective disorder [5]. Affective symptoms in schizophrenia might affect treatment course and efficacy, and may be linked with feelings of helplessness and hopefulness as well as a higher incidence of suicidal thoughts, tendencies, and suicides [6, 7].

Antipsychotics that are currently available are often used off-label in elderly patients for the treatment of behavioral and psychological symptoms of dementia (BPSD). This is probably attributable to the fact that psychosis, physical aggression, depression, anxiety, and cognitive deficits develop in 90% of patients with dementia, whereas affective symptoms occur in 50% of patients with Alzheimer's disease [8"ISSN" : "1784-3286 (Print].

Despite relevant advances in pharmacotherapy with antipsychotics, the available treatments are still unsatisfactory [9, 10], with resultant investigative efforts directed toward developing novel, safer, and more effective antipsychotics. Compounds with multireceptor profiles of

 Article submitted: 21.03.2017, Accepted: 11.06.2017

action, that is, antagonism/partial agonism to D_2 and 5-HT_{1A}, antagonism to 5-HT_{2A}, 5-HT_6, and 5-HT_7, display fewer side effects (extrapyramidal dysfunction, memory disruption, etc.) and adjuvant antidepressant, anxiolytic, and/or cognition improvement benefits [11, 12]. Aripiprazole, an antipsychotic drug approved by the Food and Drug Administration Agency (FDA) for the treatment of schizophrenia and bipolar affective disorder, possesses the above-mentioned multireceptor profile [13].

ADN-2013 is an indolamine analog of aripiprazole that acts as a preferential 5-HT_6 antagonist and D_2 partial agonist; the K_i values for these receptors are 1 and 6.3 nM, respectively. Furthermore, ADN-2013 displays high affinity toward 5-HT_{1A}, D_3, D_4, and 5-HT_{2A} receptors (>90% binding in 1.0E-06 M) as well as binding to 5-HT_7, α_{1A}, α_{2C}, and H_1 receptors (>50% binding in 1.0E-06 M), in addition to favorable selectivity over muscarinic and hERG sites [14]. ADN-2013 reversed the hyperlocomotion induced by d-amphetamine in mice—a screening test employed to detect potential antipsychotic activity with a minimum effective dose (MED) of 10 mg/kg (data not shown). In rats, ADN-2013 demonstrated antidepressant-like activity in the forced swim test (MED=1 mg/kg), decreased anhedonia-like behavior (MED=1 mg/kg), and increase flexibility in operant saccharin self-administration tests (MED=3 mg/kg). Furthermore, ADN-2013 manifested anti-conflict activity in the conflict drinking test in 3-month old rats (MED=1 mg/kg) as well as in the open-field test conducted in 3-month-old (MED=1 mg/kg) and aged rats (MED=3 mg/kg) [14].

In the present study, which continues the examination of central activity of ADN-2013, we aimed to assess its extend potential antidepressant and anxiolytic activity in mice and rats. Moreover, the receptor mechanism underlying antidepressant properties of ADN-2013 was examined.

Materials and methods

Subjects

Male Swiss Albino mice weighing 21-23 g upon arrival from a licensed dealer (Staniszewska; Ilkowice, Poland) and male CD-1 mice (22-30 g), purchased from accredited animal facility Jagiellonian University Medical College (Kraków, Poland), were group-housed for 3-4 day period in polycarbonate Makrolon type 3 cages (dimensions 26.5 x 15 x 42 cm) in an environmentally controlled, experimental room (ambient temperature 21±1°C; relative humidity 50-60%; 12:12 light:dark cycle, lights on at 8:00), in groups of 15. Male Wistar rats weighing 205-225 g upon arrival from accredited animal facility Jagiellonian University Medical College (Kraków, Poland) were group-housed for 6 day period in polycarbonate Makrolon type 3 cages (dimensions 26.5×15×42 cm) in an environmentally controlled room (ambient temperature 22±2°C; relative humidity 50-60%; 12:12 light:dark cycle, lights on at 8:00), in groups of 4. Standard laboratory food (LSM-B) and filtered water were freely available. On the day before experiments the equipment produces "white noise" was turned on for 30 min and mice or rats were weighted exact to 1 g. Animals were assigned randomly to treatment groups. All the experiments were performed by two observers unaware of the treatment applied between 9:00 and 14:00 on separate groups of animals. All animals were used only once and were killed immediately after the experiment. All the experimental procedures were approved by the I Local Ethics Commission at the Jagiellonian University in Kraków.

Drug treatment

ADN-2013 (hydrochloride salt) provided by Adamed Ltd. was suspended in 1% Tween 80 (Sigma-Aldrich) and administered 30 before an experimental procedure, intraperitoneally (i.p.) in a volume of 2 ml/kg (rats) and 10 ml/kg (mice). Reference drugs SCH-23390 (Abcam, UK) and WAY-100635 (Tocris, UK) were dissolved in water and administered 60 min. i.p. and 45 min. subcutaneously (s.c.), respectively. Idazoxan (Sigma Aldrich, USA) and prazosin (Ascent, UK) were dissolved in 0.9 % NaCl and administered 60 min i.p. Sulpiride was suspended in 1% Tween (Abcam, UK) and administered i.p. 60 min before he experiment. All solutions were prepared immediately prior to use and protected from the light.

Forced swim test in Albino Swiss mice

The experiment was conducted according to the method of Porsolt et al. [15]. Mice were individually placed in a glass cylinder (25 cm high; 10 cm in diameter) containing 10 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 a 6-min test session.

Spontaneous locomotor activity in Albino Swiss and CD-1 mice

The locomotor activity was recorded with an Opto M3 multichannel activity monitor (MultiDevice Software v.1.3, Columbus Instruments). Albino Swiss mice or CD-1 mice were individually placed in plastic cages (22x12x13 cm) for 30 min habituation period, and then the ambulation were counted from 2 to 6 min or for 30 min, that is the time equal to the observation period in the forced swim test or the marble burying test, respectively. The cages were cleaned up with 70% ethanol after each mouse.

Forced swim test in rats

The procedure used to determine antidepressant-like activity was based on the technique described previously [16]. Briefly, rats were individually placed in glass cylinders (40 cm in height, 17 cm in diameter) filled with water (temperature: 23 ± 1°C) at a height that made it impossible to reach the bottom with hind paws (25 cm). There were two swimming sessions separated by 24 h: an initial 15 min pretest and a 5 min test. The duration of immobility in the test session was recorded by a blind observer located in an adjacent room with the aid of a video camera. A rat was considered immobile when it floated without moving except to keep its head above the water surface.

Vogel conflict drinking test in rats

The testing procedure based on a method described by Vogel et al. [17] was performed using Anxiety Monitoring System "Vogel test" produced by TSE Systems. The apparatus consisted of a polycarbonate cage (dimensions 26.5x15x42 cm), equipped with a grid floor made from stainless steel bars and a drinking bottle containing tap water. Experimental chambers (two) were connected to PC software by control chassis and a device that generates electric shocks. In this "conditional " model an electric shock as noxious stimulus is applied. The testing procedure consisted of two-day habituation/adaptation and an exact test. On the first day of the experiment, the rats were adapted to the test chamber for 10-min adaptation period during which they had free access to the drinking bottle followed by a 24 h water deprivation period. Afterwards, they were allowed a 30-min free-drinking session in their home cages. This protocol of 24-hour deprivation and adaptation period was repeated on the second day. On the third day animals were place again in the test chamber 30 min after administration of vehicle/ ADN-2013 and were given free access to drinking bottle during 5 min. Recording data started immediately after the first lick and every 20 licks rats were punished with an electric shock (0.5 mA, lasting 1 s). The impulses were released *via* the spout of the drinking bottle. The number of licks and the number of shocks received throughout a 5-min experimental session were recorded automatically.

Hot plate and free-drinking tests in rats

To exclude possible drug-induced changes in shock sensitivity or an increasing influence on thirst drive which can lead to false positive results in the Vogel conflict drinking test, stimulus threshold and water consumption during a free-drinking session were determined in separate groups of rats. In either of those two studies, the rats were manipulated similarly to the Vogel conflict drinking test, including two 24-h water deprivation periods separated by 10-min adaptation session in experimental cages and 30-min of water availability in their home cages. In the free-drinking test, each animal was allowed to freely drink from the drinking bottle and the amount of water (g) consumed during 5 min was recorded for each rat. The pain threshold was evaluated using the hot plate test (Commat Ltd, Turkey) in rats [18]. The plate was enclosed with a transparent Plexiglass cylinder (35 cm high) to keep the animal on the heated surface of the plate. The latency to pain reaction (lick a hind paw or jumping) when the rat was placed on a hot plate (52.5 ± 0.5°C, 19 cm diameter) was measured. The rat was removed from the plate immediately upon visible pain reaction or if no response occurred within 30 s.

Elevated plus-maze (EPM) test in rats

The testing procedure was based on a method described by Pellow and File [19]. Plus-maze apparatus (an automated device produced by Campden Instruments Ltd. (United Kingdom)) made of durable, high density, non-porous black plastic, elevated to a height of 50 cm, consisted of two open arms (50×10 cm) and two closed arms (50×10 cm, and 30 cm high walls), arranged so that the two arms of each type were opposite each other. The floor of the plus-maze was made of infrared transparent material what means that there are no visible sensors. The plus-maze apparatus was connected to PC software by control chassis. The experiments were conducted in a darkened room, only the center of the maze was illuminated with low-intensity light (30 lux measured on the maze level). Each rat was gently placed in the center of the plus-maze, facing one of the closed arms, immediately after a

5 min adaptation period in a plastic black box (60×60×35 cm), to increase the overall activity in the EPM. During a 5 min test period, automated Motor Monitor System recorded the number of entries into the closed and open arms and the time spent in either type of the arms. The device counted an effective arm-entry when the four paws of a rat were into any arm. The maze was thoroughly cleaned after each trial.

Exploratory activity measured in the EPM in rats

To assess an influence of the tested compound on general exploratory activity of rats and control possible changes within, total ambulation (the total distance covered by a rat, and ambulation along X and Y axis) and total number of entries (into open and closed arms) was taken during a 5 min test period (i.e. the time equal to the observation period in the EPM test). The experiment was performed using EPM apparatus (details see above).

Marble burying test (MBT) in CD-1 mice

MBT was carried out on the basis of the procedure described by Broekkamp et al. [20]. Mice were placed individually into plastic cages that were identical to their home cage containing 20 glass marbles (1.5 cm in diameter) evenly spaced on sawdust 5 cm deep, without food and water. After 30 minutes the number of marbles at least 2/3 buried/1/3 uncovered was recorded. The percent of marbles left uncovered was used as an indication of anxiolytic-like activity.

Statistical analysis

All the data are presented as the mean±SEM. The statistical significance of the results was evaluated by a one-way ANOVA (when one drug was given) or two-way ANOVA (when two drugs were administered), followed by Bonferroni's Comparison Test.

Results

Forced swim test in Albino Swiss mice

As shown in Fig. 1, ADN-2013 administered at doses of 1.25 and 2.5 mg/kg showed antidepressant-like activity, reducing immobility time by 17% and 29%, respectively.

WAY-100635 administered simultaneously with ADN-2013 significantly attenuated the antidepressant-like effect produced by the tested compound; however the interaction effect was not found (Fig. 2).

Spontaneous locomotor activity in Albino Swiss mice

Table 1 shows that ADN-2013 administered at antidepressant doses did not change locomotor activity of mice measured during 4 min, i.e. at the time equal to the test session in the forced swim test.

Forced swim test in rats

As shown in Fig. 3, ADN-2013 displayed antidepressant-like activity in rats given at a dose of 3 mg/kg only, decreasing immobility time by 25%. Its potential antidepressant activity was completely reversed by D_1 selective antagonist – SCH 233900 (0.063 mg/kg), but not by sulpiride (D_2 antagonist), WAY-100635 (5-HT_{1A} antagonist), prazosin (α_1 antagonist) and idazoxan (α_2 antagonist).

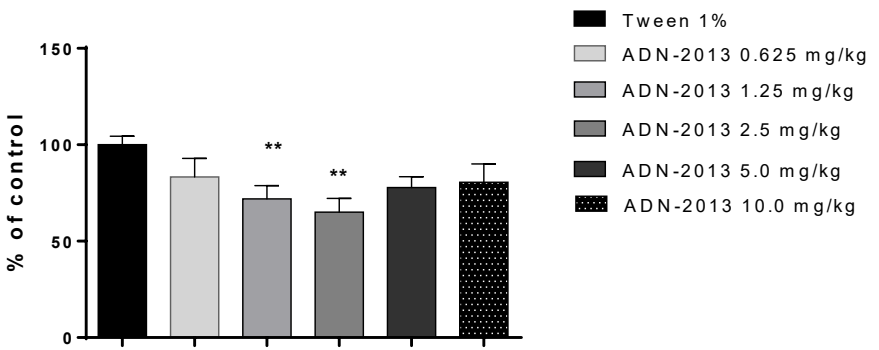

Fig. 1. Effect of ADN-2013 in the forced swim test in Albino Swiss mice. Each bar represents the mean ± SEM for 7-9 mice. One-way ANOVA revealed the significant effect: [$F(5,47)=4.468$, $p<0.05$]. **p<0.01 *vs* control group (Bonferroni's test).

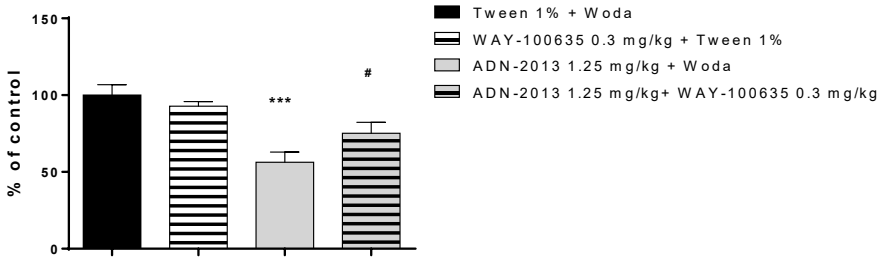

Fig. 2. Effect of WAY-100635 on ant-immobility activity of ADN-2013 in the forced swim test in Albino Swiss mice. Each bar represents the mean ± SEM for 7-9 mice. Two-way ANOVA revealed the significant main effect of ADN-2013, the interaction effect had not been found [$F(1,32)=3.0260$, ns], ***$p<0.001$ *vs* control group; $^{\#}p<0.05$ *vs* ADN-2013 alone (Bonferroni Test)

Tab. 1. Effect of ADN-2013 on spontaneous locomotor activity in Albino Swiss mice

Compound	Dose [mg/kg]	Ambulation Mean ± SEM
Tween 1%	–	157.3 ± 84.3
ADN-2013	1.25	231.1 ± 48.9
	2.5	326.5 ± 86.9

The results are presented for 6 mice. One-way ANOVA revealed no significant effect: [$F(1,18)=3.881$, ns].

Tab. 2. Effect of ADN-2013 in the Vogel conflict drinking test in rats

Compound	Dose [mg/kg]	The number of accepted shocks Mean ± SEM	The number of licks Mean ± SEM
Tween 1%	-	8.5 ± 3.5	181.3 ± 74.0
ADN-2013	0.3	14.5 ± 3.5	306.3 ± 73.0
	1.0	27.3 ± 3.9*	561.1 ± 80.9*
	3.0	32.3 ± 6.0**	669.6 ± 120.5**

The result are presented for 6-8 rats. One-way ANOVA revealed the significant effect: the number of accepted shocks [$F(3,24)=6.028$, $p<0.01$] and the number of licks [$F(3,24)=6.134$, $p<0.01$] induced by ADN-2013, *$p<0.05$; **$p<0.01$ *vs* control group (Bonferroni's test).

Tab. 3. Effect of ADN-2013 in the hot plate and free--drinking tests in rats

Compound	Dose [mg/kg]	The reaction time [s] Mean ± SEM	The amount of drunk water [g/5 min] Mean ± SEM
Tween 1%	-	9.5 ± 1.1	5.4 ± 0.6
ADN-2013	1.0	4.6 ± 0.3	9.2 ± 0.8
	3.0	4.7 ± 0.5	9.3 ± 0.7

The result are presented for 6-8 rats. One-way ANOVA did not show the significant effect: the reaction time [$F(2,15)=0.0845$, ns] and the amount of water [$F(2,15)=1.906$, ns].

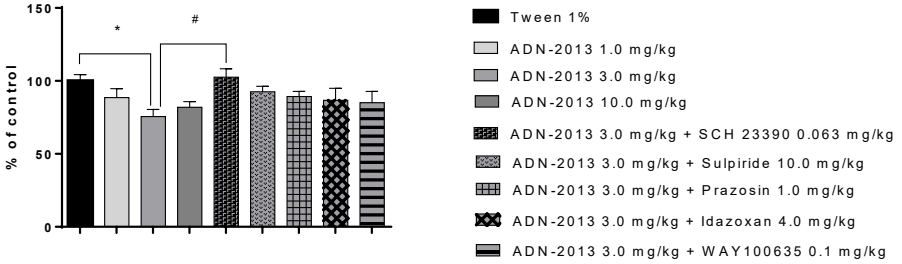

Fig. 3. Effect of ADN-2013 alone or administered with antagonists in the forced swim test in rats. Each bar represents the mean ± SEM for 7-12 rats. One-way ANOVA revealed the significant effect for ADN-2013 alone: [$F(3,26)=4,1808$, $p<0,05$]. The obtained effect was significantly reversed by SCH 23390: [$F(2,26)=4,4223$, $p<0.05$]. *$p<0.05$ *vs* control group; #$p<0.05$ *vs* ADN-2013 alone (Bonferroni's test).

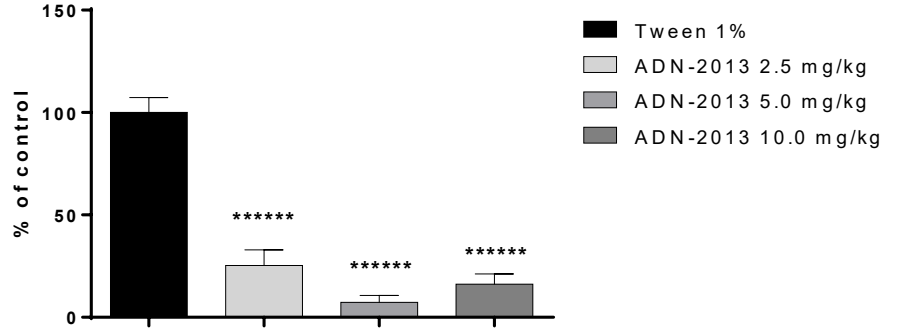

Fig. 4. The influence of ADN-2013 on the number of buried marbles in mice. Each bar represents the mean ± SEM for 8-10 mice. One-way ANOVA revealed the significant effect: the number of buried marbles [$F(3,29)=44,134$; $p=0.00000$]. ******$p=0.0$ (Bonferroni's test).

Tab. 4. Effect of ADN-2013 in the elevated plus-maze test in rats

The compound	Dose [mg/kg]	The time spent in open arms Mean ± SEM	% of the time spent in open arms Mean ± SEM	The number of open arms entries Mean ± SEM	% of open arms entries Mean ± SEM
Tween 1%	-	30.5 ± 10.7	13.70 ± 5.1	6.9 ± 1.5	21.60 ± 3.8
ADN-2013	0.03	61.0 ± 4.4 *	28.70 ± 1.7 *	13.4 ± 1.0 **	30.90 ± 1.7
	0.1	41.4 ± 7.7	18.70 ± 4.2	10.2 ± 1.9	28.80 ± 5.0
Tween 1%	-	77.9 ± 26.4	31.50 ± 10.7	10.5 ± 3.3	30.80 ± 8.8
ADN-2013	0.3	109.0 ± 22.3	43.00 ± 8.5	18.7 ± 3.3	54.0 ± 7.1
	1.0	105.3 ± 21.0	42.00 ± 8.4	14.8 ± 2.8	45.00 ± 7.0
	3.0	75.5 ± 15.7	30.60 ± 6.9	12.9 ± 1.3	42.80 ± 5.2

The result are presented for 6-8 rats. One-way ANOVA revealed the significant effect for the dose of 0.03 mg/kg: the time spent in open arms [$F(2,17)=3.836$, $p<0.05$], the percentage of the time spent in open arms [$F(2,17)=4.003$, $p<0.05$] and the number of open arms entries [$F(3,24)=6.134$, $p<0.01$]. There is no significant effect for lower doses: the percentage of open arms entries [$F(2,17)=1.889$, ns] and for higher doses: the time spent in open arms ($F(3,22)=0.716$, ns], the percentage of time spent in open arms [$F(3,22)=0.60$, ns], the number of open arms entries [$F(3,22)=1.621$, ns], the percentage of entries into open arms [$F(3,22) = 1.774$, ns]. *$p<0.05$; **$p<0.01$ *vs* control group (Bonferroni's test).

Tab. 5. Effect of ADN-2013 on exploratory activity in EMP in rats

Compound	Dose [mg/kg]	The total number of entries Mean ± SEM	Total distance [cm] Mean ± SEM	X Ambulation Mean ± SEM	Y Ambulation Mean ± SEM
Tween 1%	-	34.0 ± 5.2	4010.0 ± 458.1	138.3 ± 20.5	65.1 ± 13.8
ADN-2013	0.03	43.3 ± 2.1	4939.0 ± 128.4	162.4 ± 9.0	95.6 ± 7.1
	0.1	34.5 ± 3.7	4621.3 ± 234.7	174.0 ± 14.4	87.0 ± 8.3

The result are presented for 6-8 rats. One-way ANOVA revealed no significant effect: the total number of entries [$F(2,17)=1.830$, ns], total distance [$F(2,17)=2.369$, ns], X ambulation [$F(2,17)=1.369$, ns] and Y ambulation [$F(2,17)=2.394$, ns].

Vogel conflict drinking test in rats

As shown in Tab. 2, ADN-2013 administered at doses of 1 and 3 mg/kg increased, in the significant manner, the number of accepted shocks and the number of licks. The Bonferroni *post hoc* comparison revealed significance increment in both measured parameters: the number of accepted shocks (321 and 388%, respectively) and the number of licks (309.5 and 369.3%, respectively).

Hot Plate and Free-drinking Tests in rats

Anti-conflict doses of ADN-2013, designated in the Vogel test, had no influence on the reaction time measured in the hot plate test and on the amount of water drunk by deprived rats during 5 min test session (Tab. 3).

Elevated plus-maze test in rats

As demonstrated in Tab. 4 ADN-2013 given at only one dose of 0.3 mg/kg displayed anxiolytic-like activity in EPM increasing the time spent in the open arms (of 200 %), percentage of the time spent in the open arms (of 209 %) and also the number of entrance into them (of 143 %).

Exploratory activity measured in the EPM in rats

As shown in Tab. 5 ADN-2013 administered at an active dose of 0.3 mg/kg had no influence on all parameters measured in the EPM.

Marble burying test in CD-1 mice

As shown in Fig. 4 ADN-2013 given at doses of 2.5-10.0 mg/kg significantly, but in a dose-independent manner, reduced the number of buried marbles (76-85%, respectively).

Spontaneous locomotor activity in CD-1 mice

ADN-2013, administered at the smallest dose only, did not affect the locomotor activity (Tab. 6).

Tab. 6. Effect of ADN-2013 on spontaneous locomotor activity in CD-1 mice

Compound	Dose [mg/kg]	Ambulation Mean ± SEM
Tween 1%	-	882.7 ± 279.5
ADN-2013	2.5	999.2 ± 458.2

The results are presented for 8-10 mice. One-way ANOVA revealed no significant effect: [$F(1,18)=0.218$, ns].

Discussion

By applying a molecular modeling-assisted "design in" strategy, we managed to obtain a promising leading hybrid molecule ADN-2013 that acts as a preferential 5-HT_6 receptor antagonist/D_2 receptor partial agonist with significant selectivity for important anti-targets, namely M_1 muscarinic receptors and the hERG channel. This compound was significantly active in tests for antidepressant and anxiolytic activity in rat models, even at lower doses than the reference compounds, that is, aripiprazole and SB-271046. Furthermore, when ADN-2013 was tested in operant conditioning tests, it demonstrated a procognitive effect [14]. ADN-2013 displayed a multireceptor profile in its *in vitro* activity tests. This molecule is characterized by strong antagonism of 5-HT_6 receptors and partial agonism of D_2 sites. Despite possessing substantial affinity at 5-HT_{1A} and 5-HT_{2A} receptors, the functional responses to ADN-2013 were considerably weaker than those determined at 5-HT_6 and D_2 receptors. Thus, our experiments were conducted to broaden the knowledge base of the pharmacological *in vivo* activity of ADN-2013 and establish the mechanism of its antidepressant action.

Antidepressant-like activity

ADN-2013 was active in a classic test of antidepressant-like activity [16, 21],both in mice (described in this paper) and rats [14]. At low doses of 0.625 and 1.25 mg/kg in mice and 3 mg/kg in

rats, ADN-2013 significantly decreased immobility time, with higher doses not showing significant differences in activity. Because ADN-2013 presents strong antagonism at 5-HT_6 sites [14] and as preclinical studies have shown that selective 5-HT_6 receptor antagonists, such as SB-399885, exert an antidepressant-like effect in the forced swim and tail suspension tests in rodents (rats and mice) [22], it can be assumed that blockade of 5-HT_6 binding sites may be associated with the observed antidepressant-like activity of ADN-2013. The tested compound presents U-shaped effects in both species similar to the antidepressant activity of antipsychotics such as aripiprazole, risperidone, lurasidone [23], and the 5-HT_6 receptor antagonist SB-399885 [22]. The present findings also demonstrate that the antidepressant activity of ADN-2013 was abolished by a selective D_1 receptor antagonist SCH 23390 in rats, but not by antagonists of D_2 (sulpiride), α_1 (prazosin), and α_2 (idazoxan) receptors. However, sulpiride attenuated the anti-immobility action of ADN-2013, although the result did not reach significance. Moreover, WAY 100635, a silent 5-HT_{1A} receptor antagonist, attenuated anti-immobility activity in mice, but not in rats; in this evaluation as well, two-way ANOVA did not reveal a significant level of this effect in mice.

Based on the obtained results, it is likely that direct antagonism toward 5-HT_6 receptors and indirect effect of dopamine, acting mostly *via* D_1-like receptors, maybe involved in the observed antidepressant action of ADN-2013. Firstly, a dopaminergic mechanism may be involved in the functional effects of SB-399885, although this compound does not bind to dopamine receptors [24]. SB-399885 increased the basal extracellular dopamine concentration in the rat hippocampus and prefrontal cortex, and enhanced the haloperidol – and risperidone-induced increases in dopamine efflux in both these regions [255-HT6 receptors are widely expressed and highly enriched in the basal ganglia. However, in the mouse brain, only very low levels of 5-HT6 receptor mRNA and receptor protein, measured by TaqMan reverse transcriptase-polymerase chain reaction and selective radioligand binding, could be detected, with no evidence of enrichment in the basal gangla. The mouse receptor was cloned and transiently expressed in human embryonic kidney 293 cells to characterize its pharmacological profile. Despite significant sequence homology between human, rat, and mouse 5-HT6 receptors, the pharmacological profile of the mouse receptor was significantly different from the rat and human receptors. Four amino acid residues, conserved in rat and human and divergent in mouse receptors, were identified, and various mutant receptors were generated and their pharmacologies studied. Residues 188 (tyrosine in mouse, phenylalanine in rat and human, 26]. Secondly, other 5-HT_6 antagonists can potentiate amphetamine-evoked behavioral effects and increase the extracellular levels of dopamine in the rat frontal cortex, nucleus accumbens, and striatum [27,28, 29]. Thirdly, the anti-immobility effect of SB-399885 was abolished by the preferential D_1 – and D_2-like receptor antagonists SCH 23390 and sulpiride, respectively [22]. Fourthly, the antidepressant activity of antipsychotic drugs might be attributable to the influence on dopamine receptors, namely partial agonism toward D_1 and/or D_2 receptors [30]. Such partial activation of dopamine receptors reduces dopamine output in the midbrain limbic system, which leads to fewer positive symptoms; but this is not sufficient to influence feelings of pleasure and satisfaction. Because the dopamine output in the cerebral cortical pathways may be too low, partial agonists increase dopamine release in this area, which leads to improvement in mood [30]. Thus, the partial agonist activity of ADN-2013 toward the D_2 receptors, as observed in *in vitro* studies, might contribute to its antidepressant action.

Anxiolytic-like activity of ADN-2013

Acutely administered ADN-2013 also displayed anxiolytic properties in rodent behavioral models that were based on different paradigms and when using distinct threatening stimuli. In two independent rat models used for evaluating potential anxiolytic action [17, 19, 31] ADN-2013 evoked anxiolytic activity that was not task specific because its effects were observed across "unconditional" (EPM) and "conditional" (Vogel) anxiety-like paradigms. In the Vogel conflict drinking test, the tested compound at doses of 1 and 3 mg/kg significantly increased the number of licks and accepted shocks without any influence on pain threshold, thirst, and water intake. Furthermore, ADN-2013, when administered at a dose of 0.3 mg/kg, increased the time spent in the open arms, percentage of the time spent in the open arms, and the number of open-arm entries without affecting the exploratory activity measured in the EPM. Thus, the observed anxiolytic properties of ADN-2013 in both tests are specific.

Finally, ADN-2013 at a dose of 2.5 mg/kg reduced the number of buried marbles without affecting locomotor activity that reflects the potential and specific ability to attenuate anxiety/compulsive-like behavior.

Increasingly, clinical data are suggesting the moderate efficacy of atypical antipsychotics in treatment of primary or concomitant anxiety disorders (27–71% of patients on antipsychotic monotherapy or adjunctive therapy). However, their use is limited by severe and unpleasant side effects which result in therapy discontinuation, and this has been reported particularly in aripiprazole, olanzapine, risperidone, and quetiapine [32].

Olanzapine and risperidone, when tested in rat EPM, displayed anxiolytic-like activity at doses of 0.3 and 1 mg/kg without any influence on motor activity [33]. ADN-2013, tested in the same paradigm, was effective as an anxiolytic at the same dose as risperidone. Acute and chronic administration of both aripiprazole (1.5 mg/kg) and olanzapine (0.5 mg/kg) showed an anxiolytic effect that produced a significant increase in the number of entries into the white compartment of the two-compartment exploratory test in non-stressed rats. However, only aripiprazole displayed anxiolytic properties in prenatally stressed rats (an animal model of schizophrenia) [34]. Slot et al. [35] examined the potential anxiolytic properties of 36 compounds, including both typical and atypical antipsychotics, in a marble burying assay in mice. The significant reduction in the number of buried marbles has been shown for clozapine (0.16–10 mg/kg), aripiprazole (at the highest dose of 10 mg/kg), partially for risperidone (0.16–0.63 mg/kg), but not for olanzapine. In another study using a different strain of mice, aripiprazole (1 mg/kg) reduced the number of buried marbles without affecting locomotor activity in mice, and this effect was reversed by WAY-100635.

Neurobiological mechanisms that mediate the anxiolytic action of ADN-2013 are not clear. As was mentioned earlier, ADN-2013 acts preferentially as a 5-HT_6 antagonist/partial D_2 agonist. Thus, it seems that 5-HT_6 receptor antagonism is most probably involved in this effect. Indeed, Wesołowska et al. [36] showed that SB-399885 displayed pronounced anxiolytic-like activity in various models, both in mice and rats (i.e., the four-plate test, the Vogel conflict drinking test, and the EPM test). In comparison, MEDs of ADN-2013 are 0.03 and 1.0 mg/kg, which are smaller than the MEDs of SB-399885 (1 and 0.3 mg/kg), tested in the Vogel conflict drinking and the EPM tests, respectively [22]. Furthermore, the above-described effects of 5-HT_6 receptor blockade were suggested to be connected with indirect enhancement of GABA-ergic transmission [37]. Furthermore, the differences in MED values might indicate that, in the case of ADN-2013, other mechanisms could be involved. For example, 5-HT_{1A} receptors are considered to be involved in the modulation of anxiolytic activity [38]. Thus, 5-HT_{1A} receptor knockout mice present anxious behavior that is observed with a reduction of the time spent in the open arms of the EPM, the elevated zero maze, and the center of the open field—the time of novel object exploration [39]. In preclinical and clinical studies, buspirone, a 5-HT_{1A} partial agonist [40],has been shown to possess anxiolytic properties. Jahromy et al. [41] showed that the test drug when administered for 10 days significantly increased the percentage of open-arm entries of mice in the EPM.

Conclusion

ADN-2013 displays antidepressant – and anxiolytic-like properties in common preclinical animal models of rats and mice. These data, together with results obtained by Kołaczkowski et al. [14] showing the procognitive activity of ADN-2013, indicate that the unusual combination of 5-HT_6 antagonism/partial D_2 agonism of ADN-2013, together with its action at additional target receptors, might be desirable in a novel therapeutic agent for BPSD and/or mood disorders. Future research directed at clarifying potential therapeutic possibilities of ADN-2013 is required.

Acknowledgments

The study was financially supported by Adamed Ltd, Poland and funds for Statutory Activity of the Jagiellonian University Medical College, Cracow, Poland (K/ZDS/006133).

Resumo

Nova indolaminderivaĵo, ADN-2013, posedas preklinike pruvitan aktivecprofilon, kiu verŝajne taŭgus por la kuracado de kondutaj kaj psikologiaj simptomoj de demenco. Ĉia antidepresiaj kaj antianksiecaj ecoj estas esploritaj ĉe rataj kaj musaj modeloj. La receptormekanismoj, kiuj respondecas por la antidepresiaj ecoj de ADN-2013 same estis esploritaj. ADN-2013 rimarkinde

mallongigis la senmoviĝantan tempon mezuritan en la kompulsa naĝtesto ĉe musoj kaj ratoj, produktante efikon, kiu estis maligita per la Dopamino-D_1-receptorantagonisto SCH 23390 ĉe ratoj. Plie, ADN-2013 kaŭzis antianksiecsimilan aktivecon kaj en kondiĉita kaj en nekondiĉita anksiecsimilaj paradigmoj ĉe musoj kaj ratoj. Pro tiuj rezultoj verŝajnas, ke rekta antagonismo kontraŭ serotoninaj 5-HT_6 receptoroj kaj nerekta efiko de dopamino, ĉefe pere de D_1-similaj receptoroj, eble partoprenas la antidepresian efikon de ADN-2013. Tamen la parte agonista aktiveco de ADN-2013 rilate al D_2-receptoroj, kiu estis notita dum esploroj *in vitro*, eble ankaŭ kontribuas al tiu efiko.

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