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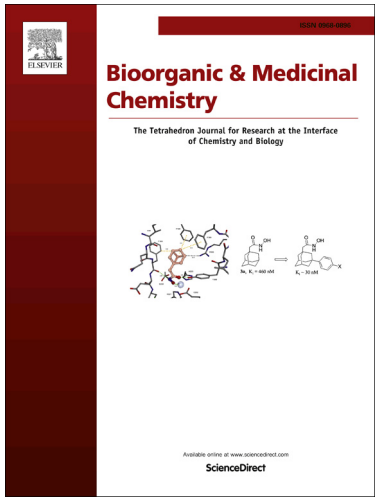

Novel Spirohydantoin Derivative as a Potent Multireceptor-Active Antipsychotic and Antidepressant Agent

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Novel Spirohydantoin Derivative as a Potent Multireceptor-Active Antipsychotic and Antidepressant Agent.

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Abstract

A series of novel spirohydantoin derivatives with arylpiperazinylbutyl moiety were synthesized and evaluated for serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_7 and dopamine D_2 receptors. Based on these data, four compounds were selected for further binding affinity assays on dopamine D_1, D_3, D_4, and 5-HT_{2C}, 5-HT_6 as well as adrenergic α_1 and α_{2C} receptors, which are involved in various CNS diseases such as schizophrenia, anxiety and/or depression. The compound **14**, 1-{4-[4-(2-metoxyphe-nyl)piperazin-1-yl]butyl}-3',4'-dihydro-2H,2'H,5H-spiro[imidazolidine-4,1'-naphthalene]-2,5-dione, with the most promising functional profile, mixed 5-HT_{2A}/D_2 antagonist and 5-HT_{1A} partial agonist, was selected. In the mouse d-amphetamine-induced locomotor hyperactivity model, compound **14** produced antipsychotic-like activity, which is devoid of cataleptogenic effects and in the forced swim test in mice, it showed a significant antidepressant-like effect unlike the reference drug aripiprazole.

Keywords: Antidepressant activity, Antipsychotic activity, Imidazolidine-2,4-dione Spirohydantoines, D_2/5-HT_{2A} antagonist, 5-HT_{1A} partial agonist

1. Introduction

Schizophrenia is considered one of the most serious mental illnesses, having a considerable social and economic impact, and globally, it affects approximately 1% of the world population [1]. This psychotic illness is clinically described by the occurrence of positive (hallucinations, disorganized thoughts, delusions, and irrational fears) and negative (social withdrawal, diminished affect, poverty of speech, lack of energy, anhedonia) symptoms, as well as cognitive impairments [2,3]. The pathophysiology of schizophrenia is believed to be associated with an imbalance of dopamine neurotransmission, manifested by mesolimbic hyperactivity and mesocortical hypoactivity [1,4,5]. Thus, introduction of drugs known as classical antipsychotics, which antagonize dopamine D_2 receptors, was the first advance in schizophrenia treatment in the mid-twentieth century [1,6]. These drugs are still used for treatment of positive symptoms, but they have failed to manage the negative symptoms of schizophrenia [7]. The undesirable side effects of classical antipsychotics, such as tardive dyskinesia and extrapyramidal symptoms, have led to development of atypical antipsychotic agents, which are generally effective for both positive and negative symptoms of schizophrenia [8].

A prototype of the entire class of atypical antipsychotic drugs was clozapine, which was introduced in the 1990s as the next breakthrough in treatment of schizophrenia [9]. The atypical antipsychotic agents exhibit antagonism towards serotonin 5-HT_{2A} and D_2 receptors, and they show less severe side effects than the classical ones. Furthermore, due to modulation of the central serotonin neurotransmission, atypical antipsychotic drugs may also show anxiolytic and/or antidepressant activity [8,10]. Although clozapine and other atypical antipsychotic agents, e.g. risperidone, olanzapine, and quetiapine have brought improvements in treatment of negative symptoms of schizophrenia, they can still cause substantial weight gain, the metabolic syndrome, blood dyscrasias, and some movement disorders [11,12]. Moreover, they have not fully improved the cognitive impairments and negative symptoms of schizophrenia [11]. It seemed that a solution would be provided by introduction on the market of an atypical drug, starting a new generation of antipsychotics – aripiprazole, which acts as a D_2 and 5-HT_{1A} partial agonist and blocks 5-HT_{2A}/5-HT_7 receptors [13]. Perhaps due to such unusual mechanism of action among atypical antipsychotic agents, aripiprazole shows a favorable safety and tolerability profile referring to the relatively low potential for causing extrapyramidal symptoms, weight gain, QTc prolongation and sedation [14,15]. Despite improvement of the safety profile, aripiprazole may cause other potentially serious side effects such as agitation, akathisia or insomnia [15]. Therefore, development of more effective

antipsychotic compounds with less side effects remains a challenge and a goal for researchers nowadays.

We previously reported on synthesis and biological evaluation of 5-(cyclo)alkyl-5-phenyl- and 5-spiroimidazolidine-2,4-dione derivatives connected by three methylene chain with the arylpiperazinylpropyl moiety. Among them, the most interesting compound I, (1-{3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl}-3',4'-dihydro- 2'H-spiro[imidazolidine-4,1'-naphthalene]-2,5-dione), behaved as a full, pre- and postsynaptic 5-HT_{1A} receptor agonist and displayed antidepressant- and anxiolytic-like activity in behavioral in vivo tests without any unfavorable motor effects [16].

As a part of our ongoing studies on a group of 5-substituted imidazolidine-2,4-dione derivatives of long-chain arylpiperazines (LCAPs), a new series of 4-arylpiperazinylalkyl moiety with mono(bi)cyclic group spiro-connected to hydantoin ring was designed. The structural modification in position 5 of the hydantoin core involved changing of the cycloalkane ring size and position of the aromatic ring. To evaluate the influence of length of spacer between hydantoin ring and arylpiperazine moiety on binding affinity, it was extended from three to four methylene units, in comparison with the previously reported compounds [16,18]. Herein we describe synthesis of new spirohydantoin derivatives and their biological evaluation for 5-$HT_{1A/2A/7}$ and D_2 receptors. For the promising compounds, we present an extended pharmacological in vitro profile, and for the most attractive one **14** – the in vivo activity in common animal models of schizophrenia and affective disorders, i.e. depression and anxiety. In these studies, aripiprazole was used as a reference drug. Additionally, based on molecular modeling results, interactions between compound **14** and 5-HT_{1A} and D_2 receptors are discussed.

2. Chemistry and crystallographic study

New spirohydantoin derivatives **9** – **23** were prepared according to the synthetic route shown in Scheme 1. Imidazolidine-2,4-diones (**1**-**4**) were prepared from the corresponding ketones by means of the Bucherer-Berg reaction with modifications described by Goodson et al. [16,17,18], and after purification good yields were achieved (70-86%). Alkylation of the above at N3 position with 1-bromo-4-chlorobutane resulted in obtaining compounds **5** – **8** in satisfactory yields (65-85%). The following coupling with differently substituted phenylpiperazines gave the final compounds **9**-**23** in moderate yields (53-84%). All the final products were obtained as racemic mixtures and for further pharmacological studies they were transformed into water-soluble hydrochloride salts.

The structure of the final compounds **9**-**26** was established on the basis of the results of elemental (C, H, N) and spectral (^{1}H NMR, ^{13}C NMR, ^{19}F NMR) analyses. The detailed spectral data for each compound is listed in the experimental section.

Additionally, the molecular structure of selected compound, **14**, has been crystallographically confirmed. Hydrochloride salt of compound **14** crystallises as a racemic mixture, with centre of chirality localised at the atom C10 of the molecule (Figure 1). The interesting feature of the presented crystal structure is the mutual orientation of both rings of the arylpiperazine moiety, which is highly conserved in the crystal structures of the 2-OCH_3 substituted arylpiperazine derivatives. This preferential orientation is related to the intramolecular hydrogen bond, formed between equatorial hydrogen atom of piperazine and the oxygen atom of the 2-OCH_3 group (C23-H23A...O31, Figure 1 and Supplementary materials). Single molecule of the presented structure forms two strong hydrogen bonds N-H...Cl^-, (from which one is charge-assisted $N19^+$-H19...$Cl1^-$), creating a zig-zag chain $C^1_2(10)$ [19] along [010] axis.

3. Pharmacology

3.1. In vitro evaluation

Radioligand binding assays were used for determining the affinity of all synthesized compounds for native 5-$HT_{1A/2A/7}$, and D_2 receptors. This was accomplished by displacement of [^{3}H]-8-OH-DPAT from rat hippocampus for 5-HT_{1A} receptors, [^{3}H]-ketanserin from rat cortex for 5-HT_{2A} receptors, metylsergide from rat hypothalamus for 5-HT_7 receptors, and [^{3}H]-spiperone from rat striatum for D_2 receptors. Each compound was tested in triplicate at 7-8 concentrations (10^{-11}-10^{-4}) and inhibition constants (K_i, Table 1) were calculated. Subsequently, the extended screening for receptor binding profile of compounds **10**, **11**, **14**, **15** was determined at Cerep (Le Bois l'Eveque, 86600 Celle L'Evescault, France) according to the previously published methods (Table 2) [20-22]. Briefly, screening procedure was accomplished by displacement of [^{3}H]-8-OH-DPAT, [^{3}H]-ketanserin, [^{3}H]-mesulergine and [^{3}H]-pyrilamine from cloned human receptor stably expressed in HEK-293 cells for 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and H_1 receptors, respectively. Displacement of [^{3}H]-SCH23 390, [^{3}H]-prazosine, [^{3}H]-RX 821 002 and [^{3}H]-4-DAMP evaluated in CHO cells which stably expressed the human D_1, α_1, α_{2C} and M_3 receptors. In case of human 5-$HT_{6/7}$ and $D_{2/3/4}$ receptors stably expressed in CHO cells, [^{3}H]-LSD and [^{3}H]-metyl-spiperone were used, respectively [20-22].

3.2. Functional in vitro and in vivo evaluation

The in vitro functional activity of two selected compounds **10** and **14** on intracellular cAMP/IP1/Ca^{2+} release levels, studied in CHO or HEK-293 cells which stably expressed the

human 5-$HT_{1A/2A/2C/6/7}$, $\alpha_{1/2C}$, H_1 and $D_{1/2/3/4}$ receptors, was determined at Cerep (Le Bois l'Eveque, 86600 Celle L'Evescault, France) according to the previously published methods (Table 3) [20-23]. Comparing affinity and in vitro obtained functional results, compound **14** has been subjected to further functional in vivo studies on selected receptors, i.e. D_2, 5-HT_{2A} and 5-HT_{1A}. To determinate D_2 receptor antagonistic effect, its ability to abolish the apomorphine-induced climbing in mice (Table 4) was tested. Antagonistic $5HT_{2A}$ properties were assessed in the (±)DOI-induced head twitch responses in mice (Table 4) [24]. To examine pre- and postsynaptic $5HT_{1A}$ receptor activity, the ability to induce hypothermia in mice and lower lip retraction (LLR) in rats, respectively, or to inhibit 8-OH-DPAT-induced effects was tested (Table 4) [24].

3.3. Behavioral evaluation

In the successive phase of our investigations the derivative **14** was tested in vivo in behavioral tests commonly used to predict antipsychotic-, antidepressant- and anxiolytic-like activity. To evaluate potential antipsychotic activity in mice and cataleptogenic properties, the hyperactivity induced by d-amphetamine and the bar test, respectively, were employed. Antidepressant properties were established using the forced swim test (FST) in mice [25]. Anxiolytic-like action was investigated in the four-plate test in mice [26]. The influence of the effective doses recorded in above-mentioned models were studied in spontaneous locomotor activity in mice in order to exclude the possibility of competing behaviors such as general locomotor activity and to determine specificity of the observed effects. Aripiprazole was used as a reference antipsychotic drug in behavioral experiments.

4. Results and discussion

4.1. Structure-activity relationship studies and pharmacological in vitro evaluation

The series of spirohydantoin derivatives connected by a tetramethylene spacer with arylpiperazine moiety was evaluated in radioligand binding assays to measure their affinity for 5-HT_{1A}, 5-HT_{2A}, 5-HT_7, and D_2 receptors. The majority of compounds display high affinity (K_i < 100 nM) for 5-HT_{1A} and 5-HT_{2A} receptors and diversified affinity for 5-HT_7 and D_2 receptors, ranging from 27 nM to 1000 nM (Table 1). In general, in comparison with the previously reported derivatives [16], elongation of the space length from three to four methylene units between the spirohydantoin fragment and the arylpiperazine moiety significantly increased the affinity for 5-HT_{1A}, 5-HT_7 and D_2 receptors. At the same time, the affinity of novel compounds for 5-HT_{2A} sites was also slightly improved.

It seems that both the imide/amine fragments and the type of substituent in phenylpiperazine system have a varying impact on the affinity of synthesized compounds for the receptors

examined. Analogues containing the indan or tetralin moiety connected to the hydantoin ring by "spiro carbon" (**9**-**16**), were more active at 5-$HT_{1A/2A/7}$ receptor sites than compounds **17**-**23** with 2/4-phenyl-cyclohexane fragment. Interestingly, the combination of amide core with 2-phenyl-cyclohexane group and 2-OCH_3- or 3-Cl-phenylpiperazine (**22**, **23**) resulted in stronger binding affinity for the 5-HT_7 receptors and weaker for the 5-HT_{2A} ones. Introduction of 3-Cl into the phenylpiperazine moiety increased the affinity for both 5-HT_{1A} and 5-HT_{2A} receptors (**11**, **15**, **19**, **23**), while 2-OCH_3 (**10**, **14**, **18**, **22**) or 3-CF_3 (**12**, **16**, **20**) group favored only binding to 5-HT_{1A} sites. Among the tested compounds, derivatives **11** and **15** showed the most balanced in vitro profile for serotonin 5-$HT_{1A/2A/7}$ receptors. It was also found that 2-methoxyphenylpiperazine moiety increased affinity for D_2 receptors (**10**, **14**, **18**). Among the structural counterparts, the unsubstituted phenylpiperazines were less active for both 5-HT_{1A} and 5-HT_{2A} receptors, while the compounds containing 3-CF_3-phenylpiperazine moiety (**12**, **16**, **20**) showed lower affinity only for the 5-HT_{2A} ones. The obtained affinity results indicated that compounds **10**, **14** and **18** were the most selective for 5-HT_{1A} receptors, whereas compounds **11** and **14** exhibited sustainable affinity for serotonin 5-$HT_{1A/2A/7}$ and dopamine D_2 receptors.

Based on similarity in structure and in vitro results, four promising compounds (**10**, **11**, **14**, **15**) were selected for the extended in vitro screening evaluation. The two compounds (**10**, **14**) possessed required high affinity for serotonin and dopamine receptors necessary for antipsychotic action, and the other two (**11**, **15**), their counterparts, showed promising affinity for antidepressant activity. The above compounds were tested in an independent laboratory of Cerep (France) (Table 2), and the obtained results confirm their strong binding affinities for serotonin 5-$HT_{1A/7}$ receptors, as well as significant affinity for dopamine $D_{3/4}$, adrenergic $\alpha_{1/2C}$ and histaminic H_1 receptors. Moreover, the tested compounds (**10**, **11**, **14**, **15**) exhibited moderate-to-high affinity for $D_{2/1}$ and 5-HT_{2A} receptors. Such multi-receptor profile may indicate the need for further research in the direction of potential antipsychotic or antidepressant effect. Moreover, the lowest percent of displacement for 5-HT_{2C} receptors, presented by compounds **10** and **14**, may also be beneficial in this aspect due to a decreased tendency for inducing weight gain [11]. Thus both of the selected compounds **10** and **14** were tested in vitro for intrinsic activity at a set of monoaminergic receptors 5-HT_{1A}, 5-HT_{2A}, 5-HT_7, D_2-D_4, α_1, α_{2C} and H_1 (Table 3). The in vitro findings display a similar intrinsic activity of both of the compounds studied, i.e. antagonist one at 5-HT_{1A}, $D_{2/4}$ and α_1 receptors with poorly marked agonist activity toward 5-HT_{1A} and D_2 sites. Moreover, despite significant binding to 5-HT_{2A} and 5-HT_7 receptors, the antagonistic activity for these sites measured in

vitro was much weaker than it could be expected. A molecular characterization of new ligands should be based on several observations made in different expression systems and using different read-outs [27]. In vitro model systems are very useful to differentiate between functional properties of compounds, but some caution should be taken when extrapolating results from recombinant systems to in vivo integrated ones. This fact is particularly important for compounds with even low partial agonist activity, where detection could be dependent on the method employed [28,29]. Hence compound **14** has been tested in in vivo functional models for assessing/confirming its inner activity toward 5-HT_{1A}, 5-HT_{2A} and D_2 receptors.

4.2. Functional in vivo evaluation

Compound **14** (5-10 mg/kg) given alone evoked a decrease in body temperature in mice and its effect was significantly attenuated by co-administration with the selective 5-HT_{1A} receptor antagonist, WAY-100635. However, it did not induce LLR per se but, at the same time, significantly decreased the measured parameter produced by a 5-HT_{1A} receptor agonist, 8-OH-DPAT (Table 4). The above results allow us to classify compound **14** as a partial agonist of 5-HT_{1A} sites. Compound **14** did not reveal agonistic properties toward 5-HT_{2A} and D_2 receptors, lacking ability to induce head-twitch and climbing responses, respectively. However, the tested agent (10-40 mg/kg) dose-dependently inhibited the (±)DOI-induced head twitches in mice obtaining ID_{50}=19.65 (18.6-20.7) mg/kg together with reduction of the apomorphine-induced climbing behavior of mice after administration in doses of 1.25-60 mg/kg; ID_{50} dose for compound **14** was 37.93 (14.2-61.65) mg/kg (Table 4). Summarizing, the results of our in vitro and in vivo functional study show that **14** behaves as a partial agonist of the 5-HT_{1A} receptor and displays D_2/5-HT_{2A} receptor antagonist activity.

4.3. Behavioral evaluation

There is a large body of evidence that agonist/antagonist of serotonin receptors produce behavioral activity relevant to depression and anxiety [30,31]. Moreover, several atypical antipsychotic drugs with affinity for serotonin receptors (i.e. risperidone, olanzapine, quetiapine, aripiprazole) have been used alone in clinical treatment of bipolar depression or in combination with selective serotonin re-uptake inhibitors to augment their clinical efficacy [10,32,33].

Considering the premises described above, as well as in vitro and in vivo data obtained for **14**, we undertook to examine its potential antipsychotic, antidepressant and anxiolytic activity in behavioral models commonly used in mice, i.e. d-amphetamine-induced hyperactivity, the forced swim test (FST) and the four-plate test, respectively. The tested compound, at doses of

0.156-0.625 mg/kg, significantly decreased hyperactivity induced by d-amphetamine. By comparison, aripiprazole was active in doses of 0.125-0.5 mg/kg, significantly and dose-dependently attenuating the effect studied (Figure 2). These effects of both agents seem to be specific, since minimum sedative doses for **14** and aripiprazole, investigated in the spontaneous locomotor activity model in mice, were 2.5 mg/kg and 1 mg/kg, respectively (Table 5). Our present results show that **14** (2.5 and 5 mg/kg) revealed antidepressant-like properties, shortening significantly by 37% and 45%, respectively, the immobility time of mice, while aripiprazole (0.01-0.1 mg/kg) was inactive in that test (Figure 3). Effective antidepressant doses of the investigated agent did not stimulate the spontaneous locomotor activity in mice during the 2-6-min session (i.e. at the time identical to the observation period in the FST) (data not shown). The results obtained in the four-plate test indicate that neither **14** (0.312-5 mg/kg) nor aripiprazole (0.06-0.5 mg/kg) produced antianxiety-like effects in mice (Table 6).

As enormous efforts have been made to develop novel antipsychotics that would not produce extrapyramidal side effects (EPS), we also examined the cataleptogenic potential of **14** (3-30 mg/kg) in the bar test in mice, which is viewed as an important rodent model for predicting EPS liability in humans [34]. The minimum cataleptogenic dose of the investigated agent was 30 mg/kg; that is 50-200-fold higher than its effective antipsychotic doses and 30-fold higher than minimal cataleptogenic dose of aripiprazole (1 mg/kg) (Table 7).

The results of pharmacological studies with **14** targeting D_2 and 5-HT_{1A}/5-HT_{2A} receptors show that the investigated agent, as well as aripiprazole, displayed antipsychotic-like activity in d-amphetamine-induced locomotor hyperactivity test, which is believed to reflect the potential efficacy of drugs in treating psychotic symptoms. Additionally, **14** produced catalepsy in a much higher dose than aripiprazole. Furthermore, the compound examined, but not aripiprazole, was effective in a mouse model of depression, i.e. FST. Similarly to our study, the lack of aripiprazole's antidepressant-like activity in FST was reported by Bourin et al. [35]. In contrast, Sarkisyan et al. [36] showed that aripiprazole was active in that test, emphasizing its 5-HT_7 receptor antagonistic properties as the potential mechanism of antidepressant efficacy. These discrepancies may be caused by different strains of mice used by both teams, i.e. Swiss albino at home and C57BL/6J by Sarkisyan et al. The results obtained for derivative **14** are promising; however, they need to be corroborated by further pharmacological studies in order to determine the detailed mechanism of its potential antidepressant activity, as well as its activity in other species.

4.4. The molecular modeling study

The ligand binding mode of compound **14** in the most important biological targets was determined using molecular docking studies. To this end, the previously described homology models of 5-HT_{1A} and D_2 receptors were used [37,38,39,40]. The compound was flexibly docked to the binding sites of the model assemblies of both receptors, mimicking the conformational flexibility of the proteins. The best scored complexes were analyzed to capture the proper binding modes, characterized by favorable ligand-receptor interactions. Since the tested compound was synthesized in a racemic form, both the enantiomers were considered in computational studies. Due to better scores and a higher number of favorable interactions in both receptors, the S enantiomer was regarded preferable and its binding mode was further described.

The ligand binding mode in the both receptors proved to be consistent with the common one for monoaminergic receptor ligands, which stands for reliability of the method [41,42]. The main anchoring interaction was a charge reinforced hydrogen bond between protonated nitrogen atom of the ligand and carboxylic group of Asp3.32, together with the CH-π interactions of arylpiperazine and aromatic aminoacid cluster, mainly Phe6.52 (Figure 4). The geometry of **14** molecule in both receptors was linear, extending from the deeper cavity formed by transmembrane helices (TMHs) 3-6, to the second interaction area, located between TMHs 1, 2 and 7. The latter one is occupied by the spirohydantoin fragment, which finds both the favorable hydrophobic, and polar contacts there. Besides the common interactions described above, there are also additional contacts present, specific for each receptor type. In the case of the 5-HT_{1A} receptor, the o-methoxy group substituted at phenylpiperazine fragment interacts with Lys191 from the second extracellular loop (ECL), carbonyl oxygen of hydantoin forms an h-bond with the amide NH_2 group of Asn7.39, while the aromatic ring of tetraline interacts with the phenyl ring of Tyr2.64 (π-π stacking, Figure 4A). Indeed, in the D_2 receptor, the latter fragment forms a CH-π interaction with Tyr1.35, but the complex is lacking additional favorable interactions of h-bond nature, which may contribute to a relatively lower affinity of **14** for this receptor type (Figure 4B).

5. Conclusions

Summing up, a number of spirohydantoin derivatives with differently substituted 4-arylpiperazinylalkyl moiety have been developed. The applied modification, such as the diversified bicyclic group spiro-connected to the hydantoin ring and the nature of substituent in 4-arylpiperazine moiety, as well as elongation of the alkyl spacer between them, influenced the affinity for 5-HT_{1A}, 5-HT_{2A}, 5-HT_7, and D_2 receptors. Among the derivatives developed, the most promising compound **14** acts as a 5-HT_{1A} receptor partial agonist, and a D_2/5-HT_{2A}

receptor antagonist. This multireceptor profile revealed the unique activity of compound **14** in behavioural in vivo tests. It showed specific antipsychotic-like activity in an animal model of schizophrenia without producing side effects, i.e. catalepsy and sedation in active doses. Furthermore, compound **14**, unlike the antipsychotic reference drug, behaved as a potential antidepressant in a mouse model of depression. It should be underlined that in spirohydantoin derivative, such combination of pharmacological activity - antipsychotic and antidepressant effect - is unique and could be beneficial in treatment of schizophrenia.

6. Experimental Part

6.1. Chemistry

All chemicals and solvents were purchased from commercial suppliers (Aldrich and Chempur) and were used without further purification. Melting points were determined in open capillaries on an Electrothermal 9300 apparatus and were uncorrected.

Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} aluminium sheets (Merck; Darmstadt, Germany), using the following mixtures of solvents: (S_1) benzene/ethyl acetate/acetone (10:5:1), (S_2) acetone/isopropanol/chloroform (20:10:1), (S_3) methanol/ammonium hydroxide (10:3drops), (S_4) ethyl acetate/methanol (8:2), (S_5) chloroform/methanol (9:1).

Analytical HPLC were conducted on a Waters HPLC instrument with Waters 485 Tunable Absorbance Detector UV, equipped with a Symetry column (C18, 3.5 µm, 4.6 x 30 mm) using water/acetonitrile gradient with 0.1% TFA as mobile phase at a flow rate of 5 ml/min. The purity of the investigated compounds (**9**-**26)** ranged from 96 to 99%. Additionally, the liquid chromatography/mass spectrometry (LC/MS) analysis was performed on Waters Acquity TQD system, with a Waters TQD quadrupole mass spectrometer with detection by UV (DAD) using an Acquity UPLC BEH C18 column (1.7µm, 2.1mm x 100mm). Water/acetonitrile gradient with 0.1% TFA was used as a mobile phase at a flow rate of 0.3 ml/min.

NMR spectra were recorded on Varian Mercury 300 MHz spectrometer (Varian Inc., Palo Alto, CA, USA); chemical shifts are expressed in parts per million (ppm), using the solvent ($CDCl_3$ or DMSO-d_6) signal as an internal standard. Signal multiplets are represented by the following abbreviations: s (singlet), brs (broad singlet), d (doublet), t (triplet), m (multiplet). Elemental analyses for C, H, N were carried on an Elementar Vario EL III apparatus (Hanau, Germany). The results of elemental analyses were within 0.4% of the theoretical values.

6.1.1. General procedure for the synthesis of compounds 1-4

A solution of a suitable ketone (0.33 M) and ammonium carbonate (1.00 M) in 50% ethanol was warmed to 50°C, at this point potassium cyanide (0.35 M), previously dissolved in 50 ml of water was dropped in over a period of 15 min. The mixture was refluxed at 56-60°C for more than 20 h. After that time a water condenser was then replaced by an air condenser and the temperature was raised to 80°C for 1h to remove the excess of ammonium carbonate. Then the reaction solution was cooled to room temperature and acidified to pH of 6 with 16% sulphuric acid. The precipitated solids were filtered off, washed with water, and recrystallized from a mixture of ethanol and water, giving an appropriate hydantoin.

Spectroscopic and analytical data matched those reported in the literature for **2',3'-Dihydro-2H,5H-spiro[imidazolidine-4,1'-indene]-2,5-dione (1) and 3',4'-Dihydro-2H,2'H,5H-spiro[imidazolidine-4,1'-naphthalene]-2,5-dione (2)**, see reference [16,17].

6.1.1.2. 8-Phenyl-1,3-diazaspiro[4.5]decan-2,4-dione (3)

The free base was obtained in 70% yield as white powder; mp 287-288°C; TLC: R_f = 0.29 (S_1); Anal. calcd for $C_{14}H_{16}N_2O_2$: C 68.83, H 6.60, N 11.47. found: C 68.90, H 6.90, N 11.57.

6.1.1.3. 6-Phenyl-1,3-diazaspiro[4.5]decan-2,4-dione (4)

The free base was obtained in 86% yield as white powder; mp 257-260°C; TLC: R_f = 0.26 (S_1); Anal. calcd for $C_{14}H_{16}N_2O_2$: C 68.83, H 6.60, N 11.47; found: C 68.64, H 6.52, N 11.73.

6.1.2. General procedure for the synthesis of compounds 5-8

A suitable imidazolidine-2,4-dione (**1**-**4**) (20 mmol) and anhydrous K_2CO_3 (150 mmol) were refluxed in acetone (120 ml) under intensive stirring for 30 min. Then a solution of 1-bromo-3-chloropropane (55 mmol) in acetone (30 ml) was added in drops over the period of 30 min. The reaction was refluxed for 7-20 h. The inorganic salts were filtered off, the solvent was evaporated and the oily residue was purified by crystallization from 60% or 96% ethanol.

6.1.2.1. 1-(4-Chlorobutyl)-2',3'-dihydro-2H,5H-spiro[imidazolidine-4,1'-indene]-2,5-dione (5)

Obtained from **1**, in 85% yield, as white powder; mp 97 – 99°C; TLC: R_f = 0.60 (S_1), 0.68 (S_4); Anal. calcd for $C_{15}H_{17}N_2O_2Cl$: C 61.54, H 5.58, N 9.57, found: C 61.36, H 5.96, N 9.64.

6.1.2.2. 1-(4-Chlorobutylo)-3',4'-dihydro-2H,2'H,5H-spiro[imidazolidine-4,1'-naphthalene]-2,5-dione (6)

Obtained from **2**, in 78% yield, as white powder; mp 105 – 107°C; TLC: R_f = 0.77 (S_1), 0.71 (S_4); Anal. calcd for $C_{16}H_{19}N_2O_2Cl$: C 62.64, H 6.24, N 9.13, found: C 62.54, H 6.37, N 8.99.

6.1.2.3. 3-(4-Chlorobutylo)-8-phenylo-1,3-diazaspiro[4,5]dekan-2,4-dione (7)

Obtained from **3**, in 65% yield, as white powder; mp 204 – 207°C; TLC: R_f = 0.64 (S_1), 0.74 (S_4); Anal. calcd for $C_{18}H_{23}N_2O_2Cl$: C 64.57, H 6.92, N 8.37, found: C 64.47, H 6.96, N 8.34.

6.1.2.4. 3-(4-Chlorobutylo)-6-phenylo-1,3-diazaspiro[4,5]dekan-2,4-dion (8)

Obtained from **4**, in 66% yield, as white powder; mp 195 – 197°C; TLC: *Rf* = 0.62 (S_1), 0.80 (S_4); Anal. calcd for $C_{18}H_{23}N_2O_2Cl$: C 64.57, H 6.92, N 8.37, found: C 64.43, H 7.18, N 8.25.

6.1.3. General procedure for the synthesis of final compounds 9-23

An intermediate 1-(3-chlorobutyl)-spirohydantoin (2.5 – 5 mmol) and the substituted 1-phenylpiperazine (5 – 10 mmol), potassium carbonate (7.5 – 15 mmol) and a bit of potassium iodide in acetonitrile (**9**-**20**) or 2-methoxyethanol (**21-23**) were refluxed for 20 – 48 h separately. After cooling, the solvent was evaporated and the hydantoin derivatives were separated by column chromatography using SiO_2 and a mixture of EtOAc/MeOH = 9/1 or 8/2, as eluting system. Then obtained products were recrystallized (96% ethanol) and converted into the hydrochloride salts by passing of their solution in anhydrous ethanol with hydrochloric gas.

6.1.3.1. 1-[4-(4-Phenylpiperazin-1-yl)butyl]-2',3'-dihydro-2H,5H-spiro[imidazolidine-4,1'-indene]-2,5-dione (9)

White powdery crystals. Yield: 55%; mp 141 – 143°C; TLC: R_f = 0.64 (S_2), 0.69 (S_5); t_R = 2.08; MS calcd for $[M + H]^+$: $C_{25}H_{30}N_4O_2$ *m/z*: 418.53, found: 419.50; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.44 – 1.69 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.13 – 2.23 (m, 1H, indane), 2.33 – 2.38 (t, *J* = 7 Hz, 2H, CH_2N1pip$(CH_2)_2$), 2.50 – 2.54 (t, *J* = 5 Hz, 4H, N1pip$(CH_2)_2$), 2.55 – 2.63 (m, 1H, indane), 2.93 – 3.02 (m, 1H, indane), 3.10 – 3.13 (m, 5H, indane, $(CH_2)_2$N4pip), 3.47 – 3.52 (t, *J* = 7 Hz, 2H, N3CH_2), 6.75 – 6.87 (m, 4H, Ph, N*H*), 7.03 – 7.25 (m, 6H, Ph); Anal. calcd for $C_{25}H_{30}N_4O_2$: C 71.74, H 7.22, N 13.39; found: C 71.73, H 7.15, N 13.28. Compd **9**·HCl: 246 – 248°C; Anal. calcd for $C_{25}H_{30}N_4O_2 \cdot H_2O \cdot HCl$: C 63.48, H 7.03, N 11.84; found: C 63.68, H 7.04, N 11.89.

6.1.3.2. 1-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}-2',3'-dihydro-2H,5H-spiro[imidazolidine-4,1'-indene]-2,5-dione (10)

White powdery crystals. Yield: 64%; mp 132 – 134°C; TLC: R_f = 0.57 (S_2); 0.86 (S_5); t_R = 2.07; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.53 – 1.75 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.18 – 2.28 (m, 1H, indane), 2.41 – 2.46 (t, *J* = 7.45 Hz, 2H, CH_2N1pip$(CH_2)_2$), 2.64 – 2.76 (m, 5H, indane, N1pip$(CH_2)_2$), 2.99 – 3.09 (m, 5H, indane, $(CH_2)_2$N4pip), 3.20 – 3.47 (m, 1H, indane), 3.55 – 3.59 (t, *J* = 7.18 Hz, 2H, N3CH_2), 3.85 (s, 3H, OCH_3), 5.83 (s, 1H, N*H*), 6.84 – 7.02 (m, 4H, Ph), 7.10 – 7.32 (m, 4H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 23.82, 26.16, 30.18, 36.98, 38.51, 50.51, 53.32, 55.31, 58.02, 71.02, 111.09, 118.20, 120.94, 122.61, 122.89, 125.46, 127.33, 129.54, 140.09, 141.25, 144.10, 152.21, 156.97, 175.58; Anal. calcdfor $C_{26}H_{32}N_4O_3$: C 69.62, H 7.19, N 12.49; found: C 69.53, H 7.13, N 12.36. Compd **10**·HCl: mp 235 – 236°C;

Anal. calcd for $C_{26}H_{32}N_4O_3 \cdot 2$ HCl: C 59.88, H 6.57, N 10.74; found: C 59.85, H 6.92, N 10.70.

6.1.3.3. 1-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl}-2',3'-dihydro-2H,5H-spiro[imidazolidine-4,1'-indene]-2,5-dione (11)

White powdery crystals. Yield: 55%; mp 88 – 90°C; TLC: R_f = 0.70 (S_2); 0.91 (S_5); t_R = 2.41; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.52 – 1.75 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.19 – 2.29 (m, 1H, indane), 2.39 – 2.44 (t, J = 7.40 Hz, 2H, CH_2N1pip$(CH_2)_2$), 2.54 – 2.57 (t, J = 5.00 Hz, 4H, N1pip$(CH_2)_2$), 2.64 – 2.75 (m, 1H, indane), 3.00 – 3.09 (m, 1H, indane), 3.15 – 3.28 (m, 5H, indane, $(CH_2)_2$N4pip), 3.55 – 3.60 (t, J = 7.00 Hz, 2H, N3CH_2), 5.83 (s, 1H, N*H*), 6.75 – 6.86 (m, 3H, Ph), 7.12 – 7. 33 (m, 5H, Ph); Anal. calcd for $C_{25}H_{29}N_4O_2Cl \cdot H_2O$: C 63.75, H 6.63, N 11.90; found C 64.09, H 6.83, N 11.55. Compd **11**·HCl: mp 191 – 193°C; Anal. calcd for $C_{29}H_{25}N_4O_2Cl \cdot H_2O \cdot$HCl: C 59.17, H 6.36, N 11.04; found: C 58.90, H 6.35, N 11.08.

6.1.3.4. 1-{4-[4-(3-Trifluoromethylphenyl)piperazin-1-yl]butyl}-2',3'-dihydro-2H,5H-spiro[imidazolidine-4,1'-indene]-2,5-dione (12)

White powdery crystals. Yield: 60%; mp 103 – 105°C; TLC: R_f = 0.60 (S_2); t_R = 2.44; MS calcd for $[M + H]^+$: $C_{26}H_{29}N_4O_2F_3$ *m/z*: 486.5, found: 487.5; ^{1}H NMR ($CDCl_3$) δ (ppm) 7,20 – 7,36 (m, 4H, Ph), 7,02 – 7,12 (m, 4H, Ph), 6,15 (s, 1H, N*H*), 3,54 – 3,58 (t, J = 7 Hz, 2H, N3CH_2), 3,20 – 3,23 (m, 5H, indane, $(CH_2)_2$N4pip), 2,99 – 3,10 (m, 1H, indane), 2,65 – 2,74 (m, 1H, indane), 2,57 – 2,60 (t, J = 5 Hz, 4H, N1pip$(CH_2)_2$), 2,41 – 2,46 (t, J = 7 Hz, 2H, CH_2N1pip$(CH_2)_2$), 2,18 – 2,36 (m, 1H, indane), 1,50 – 1,75 (m, 4H, $CH_2CH_2CH_2CH_2$); ^{19}F NMR ($CDCl_3$) δ (ppm) -62.72 (s, 3F, CF_3).

6.1.3.5. 1-[4-(4-Phenyopiperazin-1-yl)butyl]-3',4'-dihydro-2H,2'H,5H-spiro[imidazolidine-4,1'-naphthalene]2,5-dione (13)

White powdery crystals. Yield: 59%; mp 147 – 149°C; TLC: R_f = 0.63 (S_4); 0.32 (S_5); t_R = 2.15; MS calcd for $[M + H]^+$: $C_{26}H_{32}N_4O_2$ *m/z*: 432.56, found: 433.6; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.54 – 1.64 (m, 2H, $CH_2CH_2CH_2CH_2$), 1.69 – 1.86 (m, 4H, tetralin, $CH_2CH_2CH_2CH_2$), 2.23 – 2.36 (m, 2H, tetralin), 2.43 – 2.48 (t, J = 7.20 Hz, 2H, CH_2N1pip$(CH_2)_2$), 2.59 – 2.62 (t, J = 5.00 Hz, 4H, N1pip$(CH_2)_2$), 2.84 – 2.94 (m, 2H, tetralin), 3.18 – 3.22 (t, J = 5.00 Hz, 4H, $(CH_2)_2$N4pip), 3.58 – 3.63 (t, J = 7.20 Hz, 2H, N3CH_2), 5.76 (s, 1H, N*H*), 6.82 – 6.94 (m, 3H, Ph), 7.03 – 7.29 (m, 6H, Ph); Anal. calcd for $C_{26}H_{32}N_4O_2$: C 72.19, H 7.46, N 12.95; found: C 71.86, H 7.39, N 12.90. Compd **13**·HCl: mp 252 – 253°C; Anal. calcd for $C_{26}H_{32}N_4O_2 \cdot$HCl: C 66.58, H 7.09, N 11.95; found: C 66.63, H 7.05, N 12.01.

6.1.3.6. 1-{4-[4-(2-Metoxyphenyl)piperazin-1-yl]butyl}-3',4'-dihydro-2H,2'H,5H-spiro[imidazoli-dine-4,1'-naphthalene]-2,5-dione (14)

White powdery crystals. Yield: 53%; mp 108 – 109°C; TLC: R_f = 0.56 (S_3); 0.41 (S_5); t_R = 2.17; MS calcd for $[M + H]^+$: $C_{27}H_{34}N_4O_3$ *m/z*: 462.27, found: 463.22; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.53 – 1.67 (m, 2H, $CH_2CH_2CH_2CH_2$), 1.72 – 1.88 (m, 4H, tetralin, $CH_2CH_2CH_2CH_2$), 2.22 – 2.30 (m, 2H, tetralin), 2.43 – 2.47 (t, *J* = 7.05 Hz, 2H, CH_2N1pip$(CH_2)_2$), 2.62 (br s, 4H, N1pip$(CH_2)_2$), 2.83 – 2.95 (m, 2H, tetralin), 3.07 (br s, 4H, $(CH_2)_2$N4pip), 3.54 – 3.62 (t, *J* = 7.18 Hz, 2H, N3CH_2), 3.85 (s, 3H, OCH_3), 5.74 (s, 1H, N*H*), 6.80 – 6.96 (m, 5H, Ph), 7.05 – 7.27 (m, 3H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 19.03, 23.65, 26.16, 28.81, 34.12, 38.48, 50.33, 53.25, 55.31, 57.96, 62.65, 111.09, 118.23, 120.95, 122.96, 126.42, 126.84, 128.58, 129.75, 133.13, 138.11, 141.13, 152.19, 156.91, 176.30; $C_{27}H_{34}N_4O_3 \cdot H_2O$: C 67.48, H 7.55, N 11.66; found: C 67.71, H 7.77, N 11.53. Compd **14**·HCl: mp 241 – 244°C. Anal. calcd for $C_{27}H_{34}N_4O_3 \cdot 2$ HCl C 60.56, H – 6.78, N – 10.46; found: C 60.26, H 7.03, N 10.27.

6.1.3.7. 1-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl}-3',4'-dihydro-2H,2'H,5H-spiro[imidazolidine-4,1'-naphthalene]-2,5-dione (15)

White powdery crystals. Yield: 68%; mp 88 – 91°C; TLC: R_f = 0.65 (S_2); t_R = 2.39; MS calcd for $[M + H]^+$: $C_{26}H_{31}N_4O_2Cl$ *m/z*: 466.21, found: 467.21; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.54 – 1.66 (m, 2H, $CH_2CH_2CH_2CH_2$), 1.68 – 1.84 (m, 2H, tetralin), 1.92 – 2.04 (m, 2H, $CH_2CH_2CH_2CH_2$), 2.24 – 2.32 (m, 2H, tetralin), 2.46 (br. s., 2H, CH_2N1pip$(CH_2)_2$), 2.60 (br.s., 4H, N1pip$(CH_2)_2$), 2.82 – 2.87 (m, 2H, tetralin), 3.21 (br. s., 4H, $(CH_2)_2$N4pip), 3.55 – 3.61 (t, *J* = 7.05 Hz, 2H, N3CH_2), 5.78 (s, 1H, N*H*), 6.72 – 6.90 (m, 3H, Ph), 7.02 – 7.26 (m, 5H, Ph). Anal. calcd for $C_{26}H_{31}N_4O_2Cl$: C 66.87, H 6.69, N 12.00; found: C 66.48, H 7.00, N – 11.92. Compd **15**·HCl: mp 207 – 209°C; Anal. calcd for $C_{26}H_{31}N_4O_2Cl \cdot H_2O \cdot HCl$: C 59.88, H 6.57, N 10.74; found: C 59.49, H 6.62, N 10.53.

6.1.3.8. 1-{4-[4-(3-Trifluoromethylphenyl)piperazin-1-yl]butyl}-3',4'-dihydro-2H,2'H,5H-spiro[imidazolidine-4,1'-naphthalene]-2,5-dione (16)

White powdery crystals. Yield: 63%; mp 180 – 183°C; TLC: R_f = 0.68 (S_2); 0.64 (S_4); 0.60 (S_3); t_R = 2.52; MS calcd for $[M + H]^+$: $C_{27}H_{31}N_4O_2F_3$ *m/z*: 500.56, found: 501.50; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.59 – 1.66 (m, 2H, $CH_2CH_2CH_2CH_2$), 1.70 – 1.87 (m, 4H, tetralin, $CH_2CH_2CH_2CH_2$), 2.23 – 2.33 (m, 2H, tetralin), 2.48 – 2.52 (t, *J* = 7.20 Hz, 2H, CH_2N1pip$(CH_2)_2$), 2.62 – 2.65 (t, *J* = 5.00 Hz, 4H, N1pip$(CH_2)_2$), 2.84 – 2.91 (m, 2H, tetralin), 3.24 – 3.27 (t, *J* = 5.00 Hz, 4H, $(CH_2)_2$N4pip), 3.59 – 3.64 (t, *J* = 7.10 Hz, 2H, N3CH_2), 5.88 (s, 1H, N*H*), 7.01 – 7.36 (m, 8H, Ph); ^{19}F NMR ($CDCl_3$) δ (ppm) -61.63 (s, 3F, CF_3).

6.1.3.9. 3-[4-(4-Phenylpiperazin-1-yl)butyl]-8-phenyl-1,3-diazaspiro[4,5]dekan-2,4-dione

(17)

White powdery crystals. Yield: 84%; mp 238 – 240°C; TLC: R_f = 0.66 (S_2); 0.68 (S_5); t_R = 2.45; MS calcd for $[M + H]^+$: $C_{28}H_{36}N_4O_2$ *m/z*: 460.61, found: 461.60; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.53 – 1.80 (m, 8H, cyclohexane, $CH_2CH_2CH_2CH_2$), 1.97 – 2.12 (m, 4H, cyclohexane), 2.38 – 2.43 (t, *J* = 7.20 Hz, 2H, CH_2N1pip$(CH_2)_2$), 2.52 – 2.55 (t, *J* = 5.00 Hz, 4H, N1pip$(CH_2)_2$), 2.61 – 2.69 (m, 1H, cyclohexane), 3.14 – 3.17 (t, *J* = 5.00 Hz, 4H, $(CH_2)_2$N4pip), 3.58 –3.62 (t, *J* = 7.00 Hz, 2H, N3CH_2), 6.82 – 6.92 (m, 3H, Ph), 7.23 – 7.34 (m, 7H, Ph), 7.80 (s, 1H, N*H*); Anal. calcd for $C_{28}H_{36}N_4O_2 \cdot H_2O$: C 70.26, H 8.00, N 11.71; found: C 70.30, H 8.06, N – 11.70. Compd **17**·HCl: mp 293 – 294°C; Anal. calcd for $C_{28}H_{36}N_4O_2 \cdot H_2O \cdot HCl$: C 65.29, H 7.63, N 10.88; found: C 65.60, H 7.58, N 10.9.

6.1.3.10. 3-{4-[4-(2-Metoxyphenyl)piperazin-1-yl]butyl}-8-phenyl-1,3-diazaspiro[4,5]dekan-2,4-dione (18)

White powdery crystals. Yield: 72%; mp 209 – 211°C; TLC: R_f = 0.69 (S_2); t_R = 2.47; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.51 – 1.80 (m, 8H, cyclohexane, $CH_2CH_2CH_2CH_2$), 1.97 – 2.12 (m, 4H, cyclohexane), 2.39 – 2.44 (t, *J* = 7.10 Hz, 2H, CH_2N1pip$(CH_2)_2$), 2.58 – 2.69 (m, 5H, cyclohexane, N1pip$(CH_2)_2$), 3.05 (br s, 4H, $(CH_2)_2$N4pip), 3.58 – 3.62 (t, *J* = 7.00 Hz, 2H, N3CH_2), 3.85 (s, 3H, OCH_3), 6.84 – 7.02 (m, 3H, Ph), 7.20 – 7.33 (m, 6H, Ph), 7.84 (s, 1H, N*H*); Anal. calcd for $C_{29}H_{38}N_4O_3$: C 70.99, H 7.81, N 11.42; found: C 70.73, H 7.59, N – 11.21. Compd **18**·HCl: mp 273 – 274°C; Anal. calcd for $C_{29}H_{38}N_4O_3 \cdot H_2O \cdot HCl$: C 63.90, H 7.58, N 10.28; found: C 63.82, H 7.61, N – 10.17.

6.1.3.11. 3-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl}-8-phenyl-1,3-diazaspiro[4,5]dekan-2,4-dione (19)

White powdery crystals. Yield: 63%; mp 227 – 228°C; TLC: R_f = 0.73 (S_4); 0.42 (S_5); t_R = 2.65; MS calcd for $[M + H]^+$: $C_{28}H_{35}N_4O_2Cl$ *m/z*: 494.24, found: 495.19; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.63 –1.81 (m, 8H, cyclohexane, $CH_2CH_2CH_2CH_2$), 1.97 – 2.12 (m, 4H, cyclohexane), 2.43 (br. s., 2H, CH_2N1pip$(CH_2)_2$), 2.50 – 2.71 (m, 5H, cyclohexane, N1pip$(CH_2)_2$), 3.26 (br s, 4H, $(CH_2)_2$N4pip), 3.57 – 3.62 (t, *J* = 6,80 Hz, 2H, N3CH_2), 6.73 – 6.86 (m, 3H, Ph), 7.13 – 7.33 (m, 6H, Ph), 7.66 (s, 1H, N*H*); Anal. calcd for $C_{28}H_{35}N_4O_2Cl$: C 67.93, H 7.13, N 11.32; found: C 67.69, H 7.29, N – 11.24. Compd **19**·HCl: mp 283 – 285°C; Anal. calcd for $C_{28}H_{35}N_4O_2Cl \cdot HCl$: C 63.27, H 6.83, N – 10.54; found: C 62.97, H 6.84, N – 10.53.

6.1.3.12. 3-{4-[4-(3-Trifluoromethylphenyl)piperazin-1-yl]butyl}-8-phenyl-1,3-diazaspiro[4,5]dekan-2,4-dione (20)

White powdery crystals. Yield: 81%; mp 217 – 219°C; TLC: R_f = 0.65 (S_2); 0.74 (S_5); t_R = 2.72; MS calcd for $[M + H]^+$: $C_{29}H_{35}N_4O_2F_3$ *m/z*: 528.61, found: 529.50; ^{1}H NMR ($CDCl_3$) δ

(ppm) 1.59 – 1.80 (m, 8H, cyclohexane, $CH_2CH_2CH_2CH_2$) 1.96 – 2.12 (m, 4H, cyclohexane), 2.36 – 2.40 (m, 2H, CH_2N1pip$(CH_2)_2$), 2.44 – 2.70 (m, 5H, cyclohexane, N1pip$(CH_2)_2$), 3.23 (br s, 4H, $(CH_2)_2$N4pip), 3.58 –3.63 (t, J = 6.80 Hz, 2H, N3CH_2), 7.01 – 7.08 (m, 3H, Ph), 7.35 – 7.20 (m, 6H, Ph), 7.63 (s, 1H, N*H*); ^{19}F NMR ($CDCl_3$) δ (ppm) -62,76 (s, 3F, CF_3).

6.1.3.13. 3-[4-(4-Phenylpiperazin-1-yl)butyl]-6-phenyl-1,3-diazaspiro[4,5]dekan-2,4-dione (21)

White powdery crystals. Yield: 60%; mp 185 – 187°C; TLC: R_f = 0.65 (S_2); 0.57 (S_4); t_R = 2.29; MS calcd for $[M + H]^+$: $C_{28}H_{36}N_4O_2$ *m/z*: 460.61, found: 461.60; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.05 – 1.20 (m, 4H, $CH_2CH_2CH_2CH_2$), 1.41 – 1.56 (m, 3H, cyclohexane), 1.66 – 1.91 (m, 5H, cyclohexane), 2.19 – 2.24 (m, 2H, CH_2N1pip$(CH_2)_2$), 2.56 (br. s., 4H, N1pip$(CH_2)_2$), 3.06 – 3.09 (m, 1H, cyclohexane), 3.15 – 3.32 (m, 6H, $(CH_2)_2$N4pip, N3CH_2), 6.81 – 6.95 (m, 3H, Ph), 7.16 – 7.28 (m, 8H, Ph, N*H*); Anal. calcd for $C_{28}H_{36}N_4O_2$: C 73.01, H 7.88, N 12.16; found: C 73.10, H 7.87, N – 11.92. Compd **21**·HCl: mp 208 – 210°C; Anal. calcd for $C_{28}H_{36}N_4O_2$·2 HCl: C 63.03, H 7.18, N 10.50; found: C 63.18, H 7.53, N 10.53.

6.1.3.14. 3-{4-[4-(2-Metoxyphenyl)piperazin-1-yl]butyl}-6-phenyl-1,3-diazaspiro[4,5]dekan-2,4-dione (22)

White powdery crystals. Yield: 57%; mp 173 – 174°C; TLC: R_f = 0.59 (S_2); 0.57 (S_5); t_R = 2.27; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.07 – 1.22 (m, 4H, $CH_2CH_2CH_2CH_2$), 1.43 – 1.60 (m, 3H, cyclohexane), 1.75 – 1.94 (m, 5H, cyclohexane), 2.21 – 2.26 (t, J = 7.20 Hz, 2H, CH_2N1pip$(CH_2)_2$), 2.57 (br s, 4H, N1pip$(CH_2)_2$), 3.07 (br s, 4H, $(CH_2)_2$N4pip), 3.13 – 3.20 (m, 1H, cyclohexane), 3.27 – 3.31 (t, J = 6.90 Hz, 2H, N3CH_2), 3.85 (s, 3H, OCH_3), 6.83 – 7.01 (m, 3H, Ph), 7.16 – 7.25 (m, 7H, Ph, N*H*); Anal. calcd for $C_{29}H_{38}N_4O_3$: C 70.99, H 7.81, N 11.42; found: C 70.63, H 8.05, N – 11.26. Compd **22**·HCl: mp 218 – 220°C; Anal. calcd for $C_{29}H_{38}N_4O_3$·2 HCl: C 61.81, H 7.15, N 9.94; found: C 61.72, H 6.92, N – 9.73.

6.1.3.15. 3-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl}-6-phenyl-1,3-diazaspiro[4,5]dekan-2,4-dione (23)

White powdery crystals. Yield:65%; mp 108 – 109°C; TLC: R_f = 0.61 (S_4); 0.76 (S_5); t_R = 2.49; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.03 – 1.18 (m, 4H, $CH_2CH_2CH_2CH_2$), 1.42 – 1.58 (m, 3H, cyclohexane), 1.72 – 1.95 (m, 5H, cyclohexane), 2.18 – 2.23 (t, J = 6.90 Hz, 2H, CH_2N1pip$(CH_2)_2$), 2.48 – 2.51 (t, J = 4.90 Hz, 4H, N1pip$(CH_2)_2$), 3.07 – 3.09 (m, 1H, cyclohexane), 3.14 – 3.17 (t, J = 4.90 Hz, 4H, $(CH_2)_2$N4pip), 3.22 – 3.30 (t, J = 6.80 Hz, 2H, N3CH_2), 6.74 – 6.85 (m, 3H, Ph), 7.12 – 7.24 (m, 7H, Ph, N*H*); Anal. calcd for $C_{28}H_{35}N_4O_2Cl$: C 67.93, H 7.13, N 11.32; found: C 67.87, H 7.23, N 11.22. Compd **23**·HCl: mp 215 – 218°C; Anal. calcd for $C_{28}H_{35}N_4O_2Cl$·2 HCl: C 59.21, H 6.57, N 9.86; found: C

59.41, H 6.65, N 9.82.

6.2. X-ray structure determination and refinement

Hydrochloride salt of compound **14** crystallises as a racemic mixture, with centre of chirality localised at the atom C10 of the molecule. A good quality single crystal of compound **14** was obtained from ethanol by slow evaporation of the solvent under ambient conditions. A few drops of water were added in order to increase solubility. X-ray diffraction data were collected at 120(2) K using a SuperNova diffractometer with CuKα radiation (λ=1.54184 Å) and processed with CrysAlisPro software [43]. The phase problem was solved by direct methods with SHELXS-97 [44]. Parameters of the obtained model were refined by full-matrix least-squares on F^2 using SHELXL-97 [44]. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms attached to nitrogen atoms N11 and N19 were found on the difference Fourier map and refined with isotropic displacement parameter $U_{iso}[H] = 1.2\ U_{eq}[N]$. The distance restraints on bond length were applied here.

Positions of hydrogen atoms attached to carbon atoms were calculated with C-H = 0.93 Å for aromatic, C-H = 0.97 Å for methylene, C-H = 0.96 Å for methyl groups and were refined using the riding model with the isotropic displacement parameter $U_{iso}[H] = 1.2\ U_{eq}[C]$ or $U_{iso}[H] = 1.5\ U_{eq}[C]$ (methyl groups only).

All crystallographic data are presented in suppl. materials. WinGX [45] software was used to prepare materials for publication. The figure showing asymmetric unit was obtained with ORTEP-3 for Windows [45].

6.3. Pharmacology.

6.3.1. Serotonin 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 receptors binding assays. Radioligand binding studies with native 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 receptors were conducted according to the methods previously described by us [46]. Briefly: 5-HT_{1A} assays used rat hippocampal membranes, [^{3}H]-8-OH-DPAT (170 Ci/mmol, NEN Chemicals) and 5-HT for non-specific binding; 5-HT_{2A} assays used rat cortical membranes, [^{3}H]-ketanserin (88.0 Ci/mmol, NEN Chemicals) and methysergide for nonspecific binding; 5-HT_7 receptor assay was performed using rat hypothalamic membranes, [^{3}H]-5-CT (34.5 Ci/mmol; NEN) and 5-HT for non-specific binding. Each compound was tested in triplicate at 7–8 concentrations (10^{-11}–10^{-4} M). The radioactivity was measured by liquid scintillation counting (Beckman LS 6500 apparatus) in 4 mL scintillation fluid (Akwascynt, BioCare). Binding isotherms were analyzed by nonlinear regression (Prism, GraphPad Software Inc., San Diego, USA), using the Cheng-Prusoff equation to calculate K_i values. Results were expressed as means of at least two separate experiments.

6.3.2. Other in vitro studies. The extended receptor binding and functional profile with respect to 5-$HT_{1A/2A/2C/6/7}$, $\alpha_{1/2C}$, H_1 and $D_{1/2/3/4}$ receptors was determined at Cerep (Le Bois l'Eveque, 86600 Celle L'Evescault, France). The data is expressed as mean % inhibition of control specific binding at the test concentration 1.0E-06 in duplicates. Methodological details of these studies are available on the company's web site (www.cerep.fr).

6.3.3. In vivo studies. The experiments were performed on male Swiss albino mice (22–26 g) purchased from a licensed breeder Staniszewska (Ilkowice, Poland) or male CD-1 mice (accredited animal facility Jagiellonian University Medical College, Kraków, Poland) and mice were kept in groups of ten to Makrolon type 3 cages (dimensions 26.5 × 15 × 42 cm). The animals were kept in an environmentally controlled rooms (ambient temperature 22±2°C; relative humidity 50–60%; 12:12 light:dark cycle, lights on at 8:00). They were allowed to acclimatize with the environment for one week before commencement of the experiments. Standard laboratory food (Ssniff M-Z) and filtered water were freely available. All the experimental procedures were approved by the I Local Ethics Commission at the Jagiellonian University in Krakow.

All the experiments were conducted in the light phase between 09.00 and 14.00 hours. Depending on the type of the test, each experimental group consisted of: 7-9 animals/dose in the body temperature and forced swim tests, 5-6 animals/dose in the lower lip retraction, head twitch response and apomorphine-induced climbing tests and 8-10 animals/dose in the spontaneous locomotor activity, amphetamine-induced hyperlocomotor activity, catalepsy and four-plate tests .The animals were used only once. The experiments were performed by an observer unaware of the treatment administered.

6.3.3.1. Body temperature in CD-1 mice. Effects of the tested compounds given alone on the rectal body temperature in mice (measured with an Ellab thermometer) were recorded 30, 60, 90, and 120 min after their administration. In a separate experiment, the effect of WAY 100635 (0.3 mg/kg) on the hypothermia induced by compounds **14** or 8-OH-DPAT was tested. WAY 100635 was administered 15 min before the compounds or 8-OH-DPAT and rectal body temperature was recorded 30 and 60 min after injection of the tested compounds. The results were expressed as a change in body temperature (Δt) with respect to the basal body temperature, as measured at the beginning of the experiment.

6.3.3.2. Lower lip retraction (LLR) in Wistar rats. LLR was assessed according to the method described by Berendsen et al. [47]. The rats were individually placed in cages (30 cm × 25 cm × 25 cm) and they were scored three times (at 15, 30 and 45 min) after the administration of the tested compounds or 8-OH-DPAT as follows: 0 = lower incisors not

visible, 0.5 = partly visible, 1 = completely visible. The total maximum scores amounted to 3 for each rat. In a separate experiment, the effect of the tested compounds or WAY 100635 on the LLR induced by 8-OH-DPAT (1 mg/kg) was tested. The compounds **14** and WAY 100635 were administered 45 min and 15 min, respectively before 8-OH-DPAT and the animals were scored 15, 30 and 45 min after 8-OH-DPAT administration.

6.3.3.3. Head twitch response in Swiss albino mice. In order to habituate mice to the experimental environment, each animal was randomly transferred to a 12 (diameter × 20 cm (height) glass cage, lined with sawdust 20 min before the treatment. Head twitches in mice were induced by (±)DOI (2.5 mg/kg). Immediately after treatment, the number of head twitches was counted during 20 min. ID_{50} (the dose inhibiting the head twitches in mice by 50%) was calculated using Graph Pad Prism 5 Software.

6.3.3.4. Apomorphine-induced climbing behavior in Swiss albino mice. For observation, mice were placed in separate cages with walls made of metal bars. Apomorphine (3 mg/kg) was injected 10 min after the drugs. Twenty minutes after injection of apomorphine, time of climbing was determined for 2 min. Climbing time was defined as the period during which the animal held the 2, 3 or 4 paws on the wall. ID_{50} (the dose inhibiting climbing behavior in mice by 50%) was calculated using Graph Pad Prism 5 Software.

6.3.3.5. 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 Swiss albino mice were individually placed in plastic cages (22 × 12 × 13 cm), and then the crossings of each channel (ambulation) were counted from 2 to 6 min, i.e. the time equal to the observation period in the forced swim test and during 30-min experimental sessions. The CD-1 mice were individually placed in plastic cages (22 × 12 × 13 cm) for 30 min habituation period, and then ambulation were counted during 1 h with data recording every 5 min. The cages were cleaned up with 70% ethanol after each mouse.

6.3.3.6. d-Amphetamine-induced hyperlocomotor activity in CD-1 mice. The locomotor activity was recorded according to the method described above.

6.3.3.7. Catalepsy. The investigated compound was administered to 10 mice per treatment group. Animal's forelimbs were draped over a thin, cylindrical horizontal rod elevated 4 cm above the tabletop at 30, 60 and 120 min after administration of a test compound. The length of time the animal touched the bar with both front paws was measured up to a pre-set cut-off time of 60 s. A maximum of three trials was used for each animal.

In the bar test, a scoring system used by Ögren *et al.* [48] was employed. Results of each trial were scored as follows: 0 for holding the position for <15 s, 1 for holding for 15-29.9 s, 2 for

holding for 30-59.9 s, and a maximum score of 3 for staying on the bar for ≥60 s. The minimum cataleptogenic dose was defined as the lowest dose inducing a catalepsy mean score of ≥1 at 30, 60 or 120 min post-treatment.

6.3.3.8. Forced swim test in Swiss albino mice. The experiment was carried out according to the method of Porsolt *et al.* [25]. Briefly, 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 a 6-min test session

6.3.3.8. Four-plate test in Swiss albino mice.

The four-plate apparatus (BIOSEB) consists of a cage (25 x 18 x 16 cm) floored by 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 by a transparent Perspex lid that prevents escape behavior. 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.

Drugs. The following drugs were used: d-amphetamine (sulfate, Sigma-Aldrich), aripiprazole (hydrochloride, Adamed Pharmaceuticals), apomorphine (hydrochloride, Tocris) and tested compound **14**. Apomorphine and d-amphetamine were dissolved in distilled water; remaining compounds were suspended in a 1% aqueous solution of Tween 80 immediately before administration. **14** was administered intraperitoneally (i.p.) 60 min, before the test, apomorphine was injected i.p. 20 min before testing, and d-amphetamine was injected subcutaneously (s.c.) 30 min before the test. All compounds were injected at a volume of 10 ml/kg. Control animals received a vehicle injection according to the same schedule.

Statistics. All the data are presented as the mean ± SEM. The statistical significance of the results was evaluated by a one-way ANOVA, followed by Bonferroni's Comparison Test.

6.4. Molecular modeling

The homology models of human D_2 dopamine and 5-HT_{1A} serotonin receptors used herein were generated and described in previously published papers [37,38]. The D_2 receptor models were built using dopamine D_3 receptor crystal structure as a template (Protein Data Bank (PDB) database ID: 3PBL) [49]. The 5-HT_{1A} receptor models were built on the basis of β_2 adrenergic receptor crystal structure (PDB ID: 2RH1) [50]. Sequence alignments between D_2

and 5-HT_{1A} receptors (UniProt database accession numbers P14416 and P08908 respectively) [51] and their templates were performed by hhsearch tool via GeneSilico Metaserver [52]. The crude receptor models were obtained using SwissModel [53], and were validated by processing in Protein Preparation Wizard [54]. For each receptor type, a set of bioactive compounds was selected for ligand-steered binding site optimization, which was performed using induced fit docking (IFD) workflow [55]. That procedure resulted in a variety of conformational models that served as molecular targets in docking studies.

Ligand structures were optimized using LigPrep tool. Glide docking procedure was carried out using default parameters, setting docking precision XP (extra precision) and flexible docking option retaining original conformations of amide bonds. H-bond constraints, as well as centroid of a grid box (22 x 22 x 22 Å) for docking studies were located on Asp3.32.

Glide, induced fit docking, LigPrep and Protein Preparation Wizard were implemented in Schrödinger Suite 2011, which was licensed for Jagiellonian University Collegium Medicum.

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Figure 1. Molecular geometry in the crystal structure of compound **14** showing the atom labelling scheme. Dashed lines represent a charge-assisted hydrogen bond N^+-H...Cl^- and the weak intramolecular hydrogen bond C-H...O. Displacement ellipsoids of non-hydrogen atoms are drawn at the 30 % probability level. Hydrogen atoms are presented as small spheres with an arbitrary radius.

Figure 2. Effects of compound **14** and aripiprazole on the hyperlocomotor activity induced by d-amphetamine in CD-1 mice.

Figure 3. Effects of compound **14** and aripiprazole on the immobility time in the forced swim test in Swiss albino mice.

Figure 4. Binding modes of **14** in the binding sites of 5-HT_{1A} (A) and D_2 (B) receptors. Amino acid residues engaged in ligand binding (within 4Å from the ligand atoms) are shown as thick sticks. Dotted yellow lines represent H-bonds with polar residues. For the sake of clarity a part of ECL2 was hidden. TMH – transmembrane helix; ECL – extracellular loop**.**

Scheme 1. Synthetic pathways of compounds **9**-**23**.

Table 1. Binding of the spirohydantoin derivatives for serotonin (5-HT) and dopamine D_2 receptors.

Compd	$K_i \pm SD$ [nM][a]			
	D_2	5-HT_{1A}	5-HT_{2A}	5-HT_7
9	NT	75 ± 5	111 ± 12	93 ± 12
10	104 ± 7.5	2.6 ± 0.2	98 ± 6	71 ± 9
11	539 ± 46	12 ± 1	21 ± 3	27 ± 2
12	412 ± 12	6 ± 1	87 ± 6	67 ± 9
13	743 ± 49	13 ± 2	77 ± 8	116 ± 16
14	59 ± 2	2.2 ± 0.2	59 ± 4	47 ± 8
15	1100 ± 100	15 ± 2	27 ± 2	37 ± 4
16	608 ± 17	5.3 ± 0.7	157 ± 17	117 ± 13
17	NT	35 ± 3	48 ± 3	515 ± 65
18	79 ± 6	3 ± 0.4	75 ± 6	130 ± 23
19	>10000	30 ± 2	38 ± 4	768 ± 112
20	>10000	22 ± 2	122 ± 78	379 ± 47
21	NT	306 ± 23	414 ± 32	1504 ± 193
22	NT	42 ± 3	498 ± 63	63 ± 7
23	NT	113 ± 15	171 ± 9	43 ± 5

NT – not tested, [a] Data expressed as the mean ± SD of at least two independent experiment in duplicate.

Table 2. Extended receptor binding profile of the selected **10**, **11**, **14**, **15** spirohydantoin derivatives.

Compd	D_1 [a]	D_2 [a]	D_3 [a]	D_4 [a]	5-HT_{1A} [a]	5-HT_{2A} [a]	5-HT_{2C} [a]	5-HT_6 [a]	5-HT_7 [a]	α_1 [a]	α_{2C} [a]	H_1 [a]	M_3 [a]
10	68	96	96	95	103	100	21	3	98	98	101	94	5
11	91	75	97	90	103	98	61	23	99	96	97	101	5
14	87	97	94	98	98	69	24	29	99	99	101	99	-6
15	95	77	93	93	99	95	61	37	100	94	99	102	3

[a] Screening procedure: % inhibition of control specific binding at 1.0E-06 M

Table 3. Extended functional profile of compounds **10** and **14**.

Compd	Agonistic/Antagonistic activity[a]								
	D_2	D_3	D_4	5-HT_{1A}	5-HT_{2A}	5-HT_7	α_1	α_{2C}	H_1
10	20/85	20/66	27/75	37/102	1/18	6/28	2/95	15/69	-2/58
14	24/91	18/53	15/84	26/101	0/27	6/43	1/94	20/79	-2/63

[a] % of control agonist response / % of inhibition of control agonist response at 1.0E-06 M

Table 4. Functional in vivo profile of the compound **14.**

Receptor		**D_2**	**5-HT_{1A}**						**5-HT_{2A}**
Compd	**Dose (mg/kg)**	Apomorfine-induced climbing behavior in mice	**presynaptic** Body temperature in mice [A]				**postsynaptic** Lower lip retraction (LLR) in rats		(±)DOI-induced head-twitch responses in mice
		Mean ± S.E.M.	Δt ± SEM (°C)				Mean ± S.E.M.		Mean ± S.E.M.
			30 min	60 min	90 min	120 min	B	C	
		101.7±4.8(APO)	-	-	-	-	-	-	25.2±3.4(DOI)
Vehicle	-	NT	-0.1±0.1	-0.1±0.1	-0.1±0.1	-0.1±0.1	0.0±0.0	2.8±0.1	NT
14	5	64.7±14.2[a]	-0.8±0.2[a]	-0.7±0.2[a]	-0.7±0.2[a]	-0.6±0.2[a]	NT	NT	NT
	10	64.0±4.9[a]	-1.9±0.1[b]	-1.3±0.2[b]	-1.3±0.2[b]	-1.2±0.2[b]	0.3±0.2	2.8±0.1	15.8±2.2
	20	63.5±7.0[b]	NT	NT	NT	NT	0.4±0.2	1.7±0.2[b]	8.0±2.4[b]
	40	NT	NT	NT	NT	NT	NT	NT	4.5±1.9[c]
	-	$F(3.25)=4.758$ p<0.01	-	-	-	-	-	-	$F(3.25)=10.368$ p<0.001
WAY100635 + **14**	0.1 + 10	NT	-1.1±0.1[b]	-1.0±0.1[b]	NT	NT	NT	NT	NT

[A] An absolute average value of the initial body temperature of mice was 36.1±0.5°C;
B– LLR induced by compound **14,**
C – effect of compound 14 on 8-OH-DPAT-induced LLR
[a] p<0.05, [b]p<0.01, [c]p<0.001 vs respective vehicle group or compound 14 group (one way ANOVA followed by Bonferroni's post-hoc test)
APO – apomorfine (3 mg/kg), DOI – (±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (2.5 mg/kg), NT – not tested,

Table 5. Effects of compound **14** and aripiprazole on the spontaneous locomotor activity in CD-1 mice.

Compound and dose (mg/kg)	**Number of crossings during 60 min Mean±S.E.M.**
Vehicle + vehicle	1811.9 ± 224.2
14 (0.625) + vehicle	1527.3 ± 467.6
14 (1.25) + vehicle	853.3 ± 188.4
14 (2.5) + vehicle	658.7 ± 167.2 [a]
14 (5) + vehicle	617.6 ± 153.8 [a]
14 (10) + vehicle	552.5 ± 133.7 [b]
14 (20) + vehicle	10.9 ± 5.8 [c]
	F(6.64)=7.7428
	p<0.0001
Vehicle + vehicle	1199.3 ± 233.6
Aripiprazole (0.5) + vehicle	734.0 ± 204.0
Aripiprazole (1.0) + vehicle	384.2 ± 56.7 [a]
Aripiprazole (1.25) + vehicle	507.8 ± 71.8 [a]
Aripiprazole (2.5) + vehicle	519.1 ± 114.3 [a]
Aripiprazole (5.0) + vehicle	305.2 ± 78.6 [a]
	F(5.52)=5.150
	p<0.001

[a] p<0.05, [b]p<0.01, [c]p<0.001 vs respective vehicle + vehicle group (one way ANOVA followed by Bonferroni's post-hoc test)

Table 6. Effects of compound **14** and aripiprazole on the number of punished crossings in the four-plate test in Swiss albino mice.

Compound dose (mg/kg)	Number of punished crossings during 60 s Mean ± S.E.M.
Vehicle	3.1 ± 0.3
14 (0.312)	3.7 ± 0.3
14 (0.625)	3.2 ± 0.3
14 (1.25)	3.1 ± 0.5
14 (2.5)	3.9 ± 0.5
14 (5.0)	3.7 ± 0.4
	F(4.50)=4.4238
	ns
Vehicle	3.3 ± 0.2
Aripiprazole (0.06)	4.0 ± 0.4
Aripiprazole (0.125)	4.0 ± 0.3
Aripiprazole (0.25)	3.9 ± 0.4
Aripiprazole (0.5)	2.4 ± 0.3
	F(5.67)=0.95250
	ns

ns – non significant (one way ANOVA followed by Bonferroni's post-hoc test)

Table 7. Cataleptogenic effect of compound **14** and aripiprazole in CD-1 mice.

Compound and dose (mg/kg)	Catalepsy score		
	30 min	60 min	120 min
Vehicle	0.0	0.0	0.0
14 (3)	0.1	0.0	0.3
14 (10)	0.3	0.0	0.0
14 (30)	0.5	1.1	0.2
Vehicle	0.0	0.2	0.6
Aripiprazole (0.3)	0.1	0.8	0.8
Aripiprazole (1)	0.0	1.0	1.7
Aripiprazole (3)	0.1	1.0	2.0

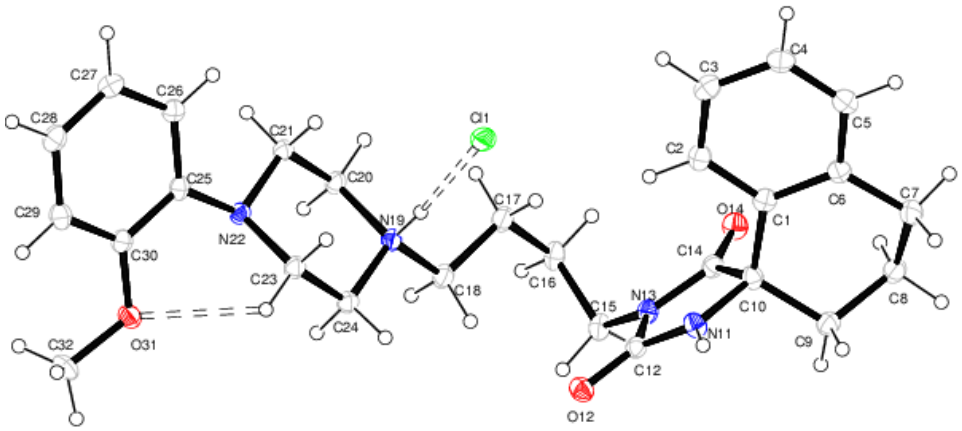
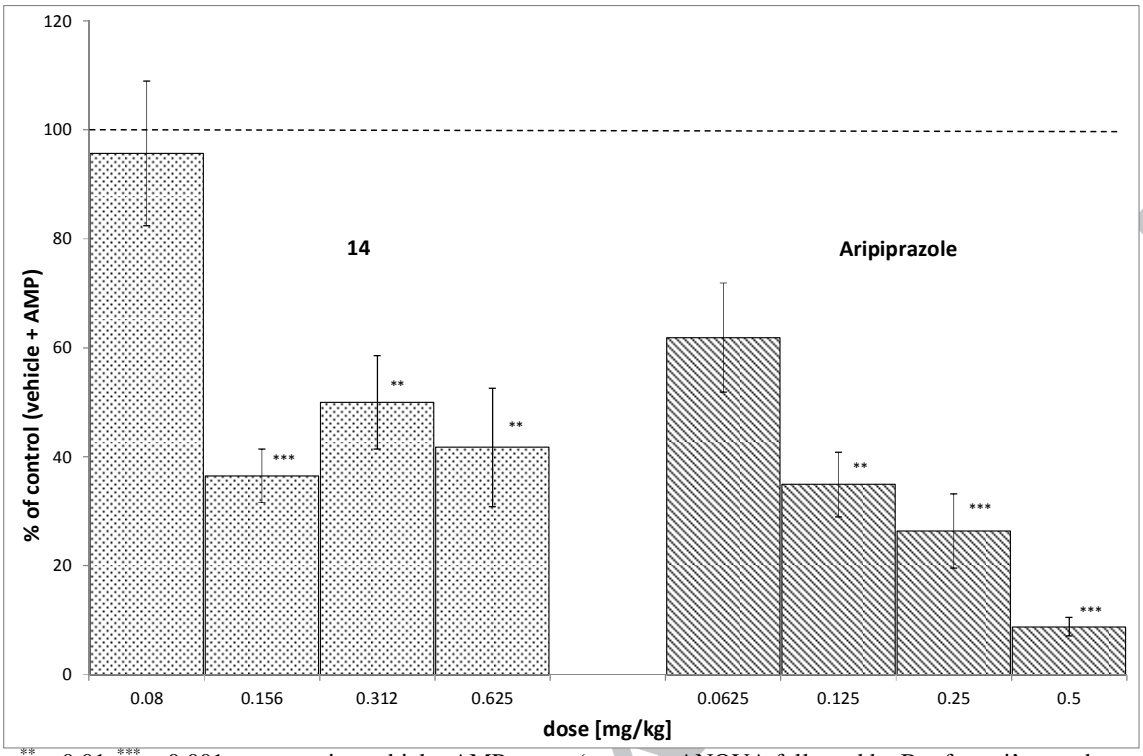
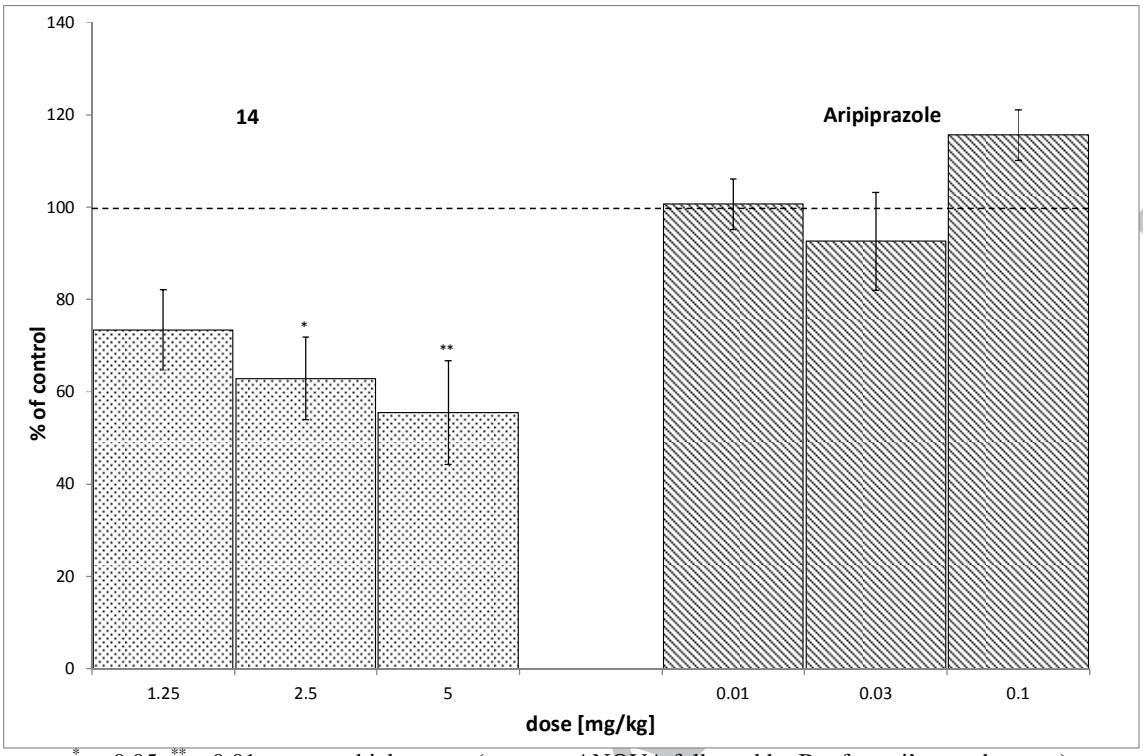


Figure 1.



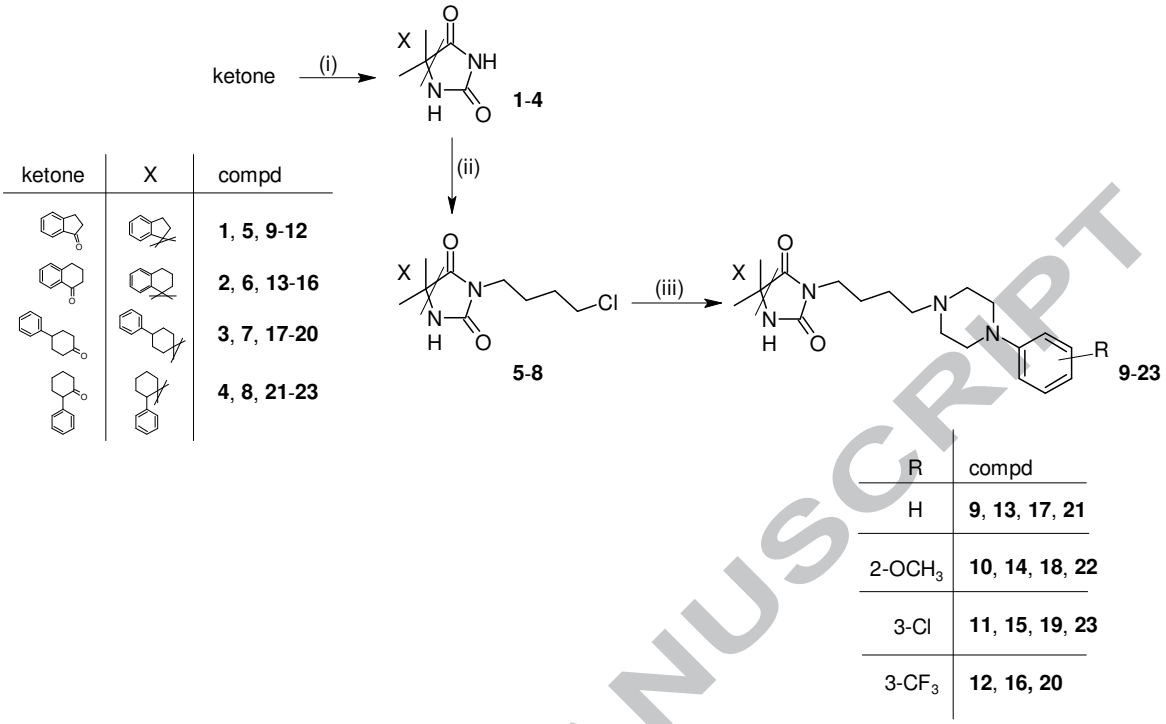
** p<0.01. *** p<0.001 *vs* respective vehicle+AMP group (one-way ANOVA followed by Bonferroni's post-hoc

Figure 2.



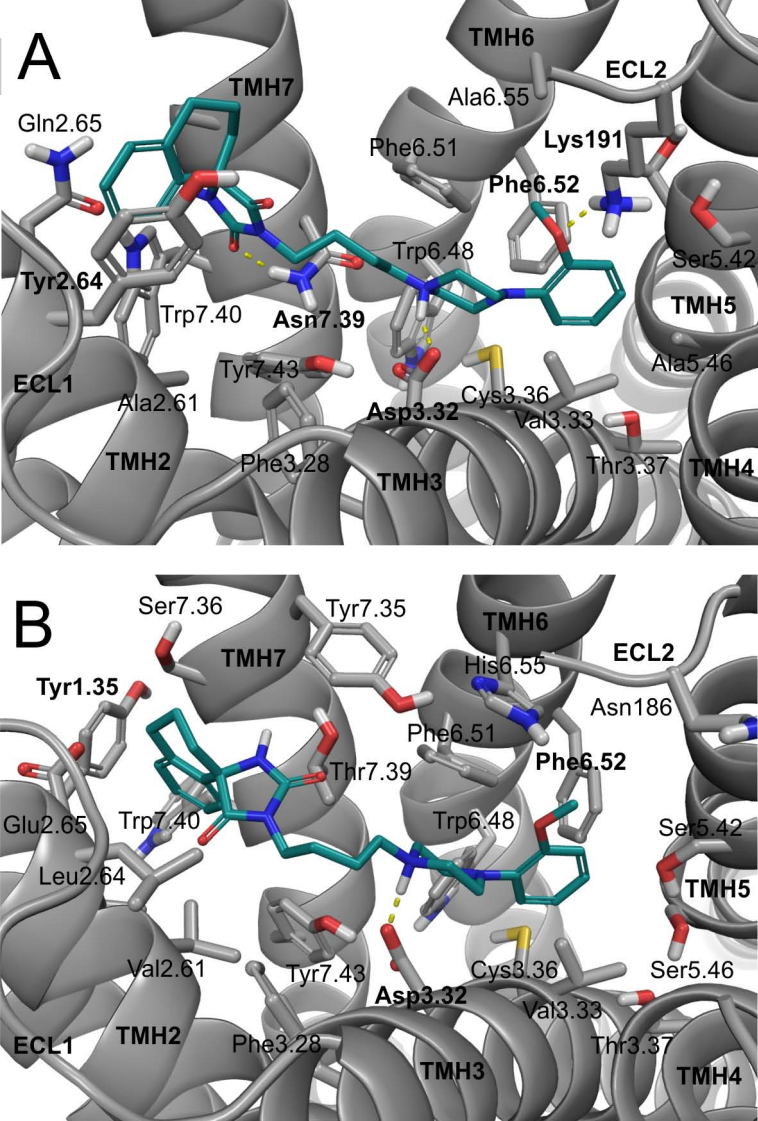
* p<0.05. ** p<0.01 versus vehicle group (one way ANOVA followed by Bonferroni's post-hoc test)

Figure 3.



Scheme 1.

Reagents and conditions: (i) KCN, $(NH_4)_2CO_3$, 50% ethyl alcohol, more than 20h, 56°C; (ii) 1-bromo-4-chlorobutane, K_2CO_3, KJ, acetone, reflux 7-20h (iii), 4-substituted piperazine derivatives, K_2CO_3, KJ, acetonitrile (**9**-20) or 2-methoxyethanol (compd **21**-**23**), reflux 20-40h.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TMH7
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Gln2.65
Phe6.51
Lys191
Phe6.52
Trp6.48
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Tyr2.64
TMH5
Trp7.40
Asn7.39
Ala5.46
Tyr7.43
ECL1
Cys3.36
Ala2.61
Asp3.32
Val3.33
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Phe3.28
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TMH3
B
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Phe3.28
Thr3.37
TMH3
TMH4

Graphical abstract