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Synthesis and biological investigation of new equatorial (β) stereoisomers of 3-aminotropane arylamides with atypical antipsychotic profile.

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Abstract

A series of novel 3*β*-aminotropane derivatives containing a 2-naphthalene or a 2-quinoline moiety was synthesised and evaluated for their affinity for 5-HT_{1A}, 5-HT_{2A} and D_2 receptors. Their affinity for the receptors was in the nanomolar to micromolar range. *p*-substitution (**6c**, **6f**, **6i**, **6l**, **6o**), as well as substitution with chlorine atoms (**6g**, **6h**, **6i**), led to a significant increase in binding affinity for D_2 receptors with compounds **6f** (K_i = 0.6 nM), **6c** and **6i** (K_i = 0.4 nM), having the highest binding affinities. *m*-substituted derivatives were the most promising ligands in terms of 5-HT_{2A} receptor binding affinity whereas 2-quinoline derivatives (**10a**, **10b**) displayed the highest affinity for 5-HT_{1A}R and were the most selective

ligands with K_i = 62.7 nM and K_i =30.5 nM, respectively. Finally, the selected ligands **6b**, **6d**, **6e**, **6g**, **6h**, **6k**, **6n** and **6o**, with triple binding activity for the D_2, 5-HT_{1A} and 5-HT_{2A} receptors, were subjected to *in vivo* tests, such as those for induced hypothermia, climbing behaviour and the head twitch response, in order to determine their pharmacological profile. The tested ligands presented neither agonist nor antagonist properties for the 5-HT_{1A} receptors in the induced hypothermia and lower lip retraction (LLR) tests. All tested compounds displayed antagonistic activity against 5-HT_{2A}, with **6n** and **6o** being the most active. Four (**6b, 6k, 6n** and **6o**) out of eight tested compounds could be classified as D_2 antagonists. Additionally, evaluation of metabolic stability was performed for selected ligands, and introduction of halogen atoms into the benzene ring of **6h**, **6k**, **6n** and **6o** improved their metabolic stability. The project resulted in the selection of the lead compounds **6n** and **6o,** which had antipsychotic profiles, combining dopamine D_2-receptor and 5-HT_{2A} antagonism and metabolic stability.

Keywords: atypical antipsychotics; 3β-aminotropane derivatives; 5-HT_{1A}, 5-HT_{2A}, D_2, dopamine receptor ligands

List of abbreviations: FT-IR, Fourier transform infrared spectroscopy; NMR, nuclear magnetic resonance; HRMS, high resolution mass spectroscopy; EPS, extrapyramidal symptoms; 5-HT_{1A}R, serotonin 5-HT_{1A} receptors; (±)8-OH-DPAT, (±)8-hydroxy-2-(di-*n*-propylamino)tetralin; DMF, dimethylformamide; TEA, triethylamine; DMSO, dimethyl sulphoxide; DCM, dichloromethane; DOI, 2,5-dimethoxy-4-iodoamphetamine; HTR, Head twitch response; LLR, lower lip retraction; APO, apomorphine

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1. Introduction

Schizophrenia was described as a neurobiological disorder almost one hundred years ago. Nevertheless, the precise explanation of its pathogenesis remains unknown.[1] Observations of the antipsychotic efficacy of dopaminergic system inhibitors in the 1950s led to the oldest and the most widely accepted neurochemical theory of schizophrenia pathogenesis, which suggests a considerable role for dopaminergic system disturbances.[2]

First generation antipsychotics (also referred to as typical or classical neuroleptics), such as chlorpromazine and haloperidol, exert their therapeutic effect mainly on the positive

symptoms of schizophrenia and are less effective in alleviation of the negative and cognitive symptoms of this disorder. Moreover, these drugs have numerous adverse effects, among which extrapyramidal symptoms (EPS), such as dystonia, akathisia, parkinsonism and tardive dyskinesia, are of particular importance. Moreover, it is estimated that first generation neuroleptics are ineffective in about 30% of schizophrenic patients.[3] The reversal of positive symptoms of schizophrenia by these antipsychotics is apparently mediated by blockage of D_2 receptors in the mesolimbic region while EPS result from blockage of the corresponding receptors in the basal ganglia.[4]

In 1988 Kane *et. al.* described a new antipsychotic drug — clozapine, which was effective in patients who did not respond to first generation neuroleptic treatment.[5] Moreover, clozapine was effective not only in treatment of the positive symptoms of schizophrenia, but also in reducing negative symptoms and cognitive deficits. At the same time it did not induce the EPS and prolactin; K_i, inhibition constant (PRL) increase commonly seen with first generation neuroleptics. Many of its atypical properties have been attributed to its affinity for serotonin receptors, namely 5-HT_{2A}, 5-HT_{1A}, 5-HT_{2C}, 5-HT_7, and 5-HT_6.[6,7]

The affinity of clozapine for the D_2 dopamine receptor is much lower than that of most first generation neuroleptics. Other second-generation antipsychotic drugs, also known as atypical neuroleptics are: olanzapine, risperidone, zotepine, sertindole, quetiapine and ziprasidone.[3] Meltzer and co-workers found that compounds belonging to this group should possess a pK_i 5-HT_{2A}/D_2 ratio (the so-called Meltzer's index) of ≥ 1.12. Clozapine demonstrates a Meltzer index of 1.19, whereas chlorpromazine has a Meltzer index of 1.02.[8,9]

According to some authors other pK_i ratios, for example D_2/D_3 and D_2/D_4 ,are not relevant in differentiating between classical and atypical antipsychotics, however, according to other studies these ratios play a role.[10] For drugs with remarkably high affinities for D_2 receptors, even those with significant binding to 5-HT_{2A} receptors do not fit the "atypical" pharmacological profile.[11] It is worth mentioning that compounds that do not affect the serotonergic system may belong to the group of atypical neuroleptics as well. An example of such a drug is amisulpride.[12]

The current state of knowledge suggests that drugs simultaneously targeting dopamine D_2 and serotonin 5-HT_{1A} receptors may be beneficial in pharmacotherapy for schizophrenia.[13] However the results of research evaluating the role of the 5-HT_{1A} receptor in the pathophysiology of this disorder are not entirely consistent.[14] An increase in 5-HT_{1A} receptor binding in the prefrontal cortex (PFC) of patients with schizophrenia was reported in post-mortem studies.[15] On the other hand, this observation was not confirmed by molecular

imaging.[16–18] In spite of this, clinical studies have demonstrated that co-administration of 5-HT_{1A} agonists and typical or atypical neuroleptics may enhance the antipsychotic effect of these drugs especially in terms of reduced cognitive deficits (for review see [19]).

A number of studies suggest an important role for 5-HT_{1A} receptors in the control of motor activity and the alteration of dopamine neurotransmission. Chronic administration of 5-HT_{1A} receptor agonists led to a substantial dose-related decrease in striatal 5-HT, and these results suggested that 5-HT has an inhibitory influence on the activity of dopaminergic neurons.[20] The dorsal raphe nuclei mediated serotonergic input is reduced in the substantia nigra in response to the activation of somatodendritic 5-HT_{1A} receptors by selective agonists, which increases dopamine neurotransmission. Moreover, in the striatum, nigrostriatal dopamine neurotransmission is released from the inhibitory influence of 5-HT. Activation of postsynaptic 5-HT_{1A} receptors may also have an important role in improving the negative symptoms and cognitive deficits associated with schizophrenia, because it causes enhancement of mesolimbic and mesocortical dopamine neurotransmission.

For these reasons, in the last few years considerable attention has been given to the new generation of atypical antipsychotics, defined by some researchers as "third-generation neuroleptics".[21] These include aripiprazole, lurasidone, brexpiprazole, perospirone, quetiapine, ziprasidone, and cariprazine, all of which exhibit affinity for the 5-HT_{1A} receptor.[22]

Progress in the development of antipsychotic drugs is achieved mainly by eliminating or diminishing side effects, which are typical for this class of drugs. This process was explicitly presented by Altinbas *et al.*[23] Analysing the binding affinity profiles of the newest antipsychotics, it seems well-founded that the discovery of ligands with high 5-HT_{2A}/D_2 selectivity combined with partial agonistic activity at D_2 receptors is still an important aim in the design of new antipsychotic drugs.[24] The impact of 5-HT_{2A} receptor antagonism on the improved tolerability of atypical antipsychotic drugs is no longer in doubt.[25] 5-HT_{1A} receptor agonism or partial agonism has a considerable effect on the cognitive deficits in schizophrenia.[26] This property is observed in the binding affinity profiles of brexpiprazole and cariprazine, the latest antipsychotic drugs.

The possession of a binding affinity profile such as those discussed above is common for the latest antipsychotics and is essential for their pharmacological activity and reduced side effects.[27]

In a previous publication, we described the synthesis and biological activity of a series of new 3*β*-acylamine derivatives of tropane, among which compound **A** (Ref. [28]) drew our

particular attention as it displayed a very high (in the nM range) affinity for each of the investigated receptors (i.e. 5-HT_{1A}, 5-HT_{2A} and D_2) and an ideal Meltzer index (pKi 5-HT_{2A}/D_2 ratio) of 1.21, confirming its atypical antipsychotic profile.[28] Analysis of the results of *in vitro* radioligand binding assays allowed us to determine the influence of the substituents at the 3*β* and N-8 position of the tropane system on the affinity for 5-HT_{1A}, 5-HT_{2A} and D_2 receptors in the previously obtained series of compounds.[28] A substantial favourable influence of a 2-naphthyl moiety and a benzyl ring respectively (both present in compound **A**) on the binding affinity for 5-HT_{1A}, 5-HT_{2A} and D_2 receptors was observed. This suggested that we should keep the 2-naphtyl moiety unaltered and turn our attention to the modification of the benzyl substituent at N-8 position. The aim of our work was to examine if the presence of *o*-, *m*-, or *p*-substituents (CH_3, OCH_3, Cl, Br, F, CF_3) in the benzyl ring would enhance the biological activity of the investigated analogues of compound **A**. We decided also to introduce an quinoline in the place of naphthalene moiety since it is a heterocyclic analogue to the latter one. This enabled the assessment whether the modification can provide an enhanced affinity for evaluated receptors. Herein, we report the synthesis and *in vitro* biological evaluation of compounds **6a–u** and **10a–b**, as well as the *in vivo* characterisation and studies on the metabolic stability of selected compounds.

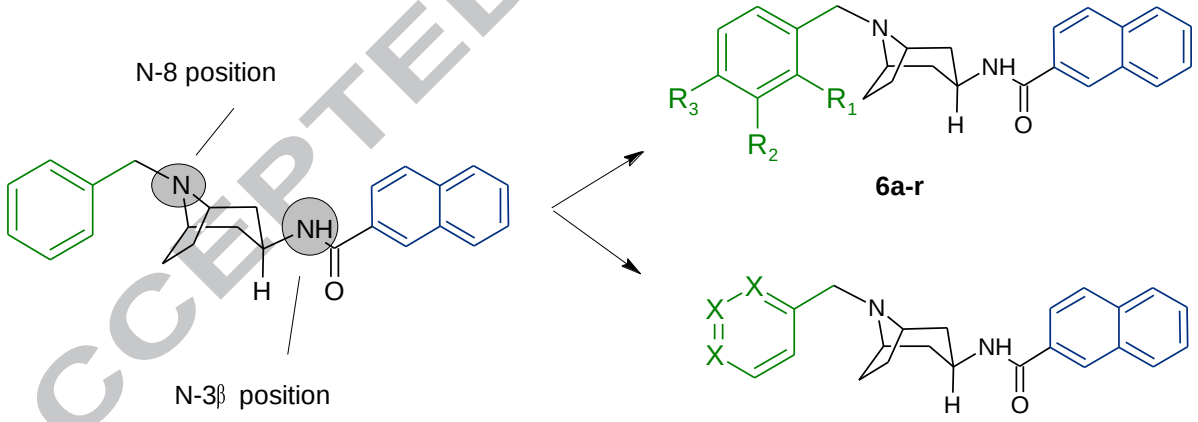


Figure 1. Structure of compound **A** (in Ref. [28]), which was subjected to structural modifications, leading to the 3*β*-acylamine derivatives of tropane described in the present paper (**6a–u**).

2. Results and discussion

2.1. Chemistry

Continuing the search for new dopamine and serotonin receptors ligands in the 3-aminotropane derivatives group, we performed the synthesis of a series of equatorial (*β*) 8-aryl-8-azabicyclo[3.2.1]oct-3-ylamides according to Scheme 1 and Scheme 2.

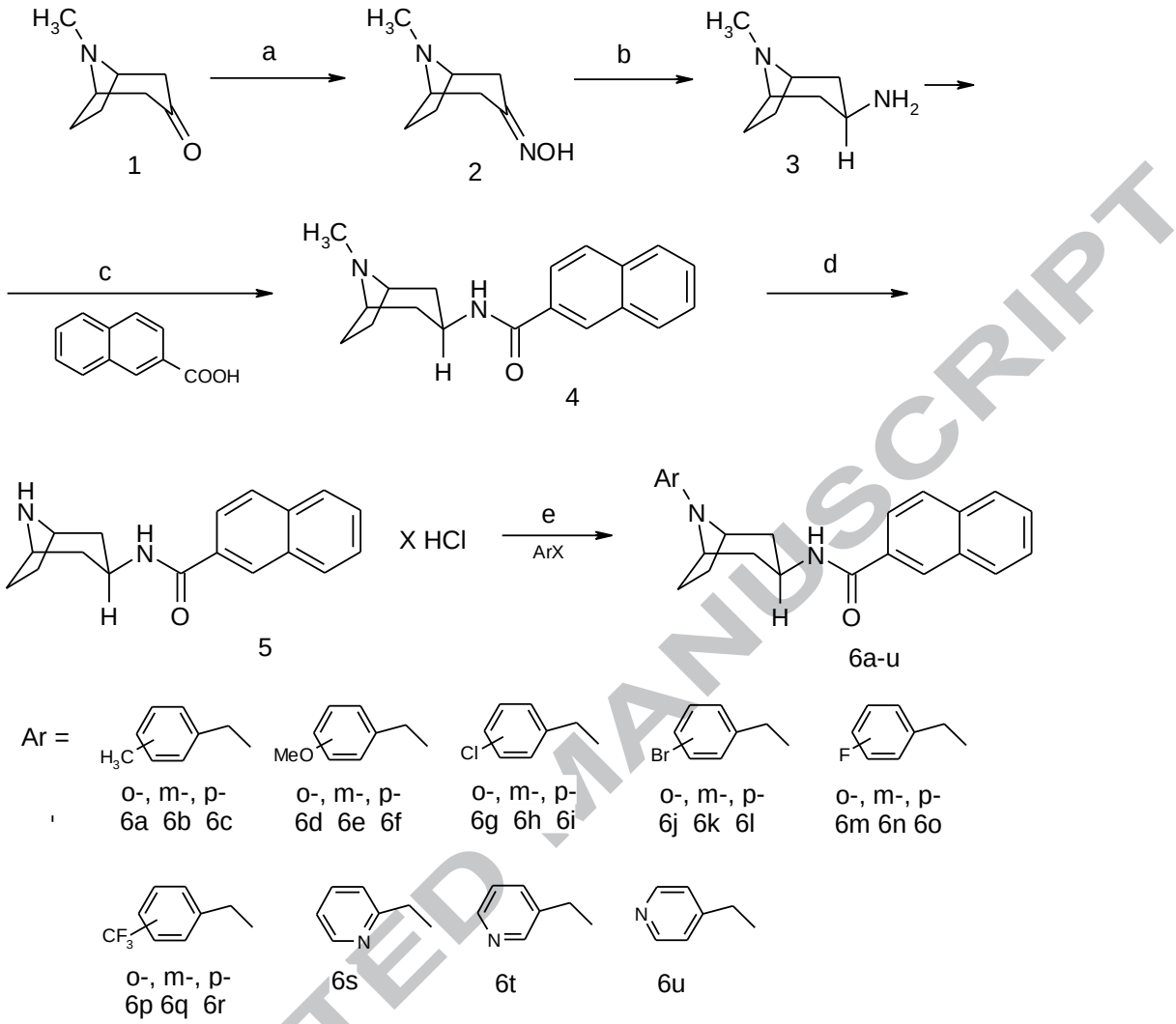
In the first stage, 8-methyl-8-azabicyclo[3.2.1]octan-3-one was converted into an appropriate oxime (**2**) by reaction with hydroxylamine hydrochloride in accordance with the method published in Ref. [29] (see Supporting information for details). The obtained oxime was subsequently subjected to stereoselective reduction to give the corresponding *β*-amine (**3**) using the Bouveault-Blanc method, described in Ref. [30] (see Supporting information for details). However, to isolate this compound from the reaction mixture with a very good yield, it was necessary to use the method of continuous extraction.

In the next stage, the equatorial amine (**3**) was subjected to acylation with naphthalene-2-carboxylic acid, using the mixed anhydride method. The obtained product was *N*-(8-methyl-8-azabicyclo[3.2.1]oct-3*β*-yl)-2-naphthamide (**4**), which has not been described in the literature before. Compound (**4**) was then subjected to demethylation with Olofson's reagent to give N-(8-azabicyclo[3.2.1]oct-3*β*-yl)-2-naphthamide hydrochloride (**5**). Intermediate (**5**) was subsequently alkylated using benzyl chlorides or bromides substituted in the *o*-, *m*- or *p*-position with a methyl, methoxy or trifluoromethyl group, or a chlorine, bromine or fluorine atom to give the final amides (**6a–f**) using Finkelstein protection of KI.

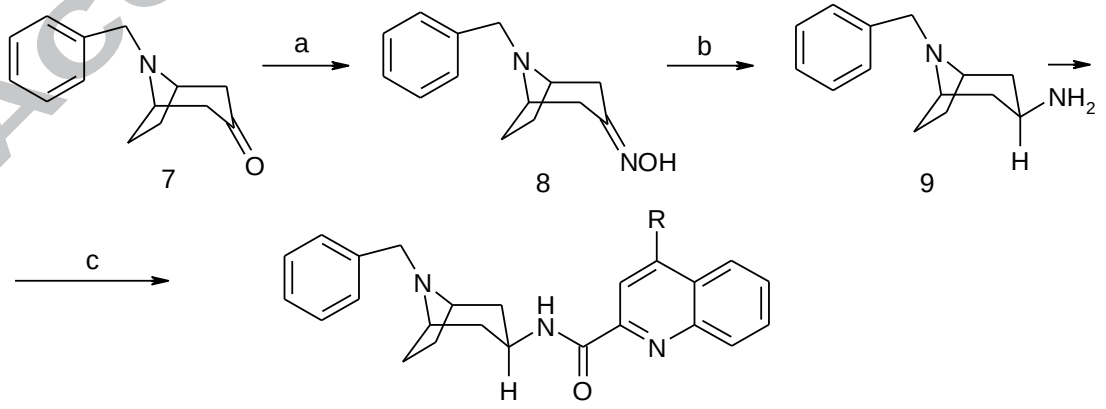
In an analogous manner compounds **6s–u** were synthesised using the corresponding pyridinemethyl chlorides for the alkylation. Reactions **c**–**e** (Scheme 1) were performed retaining the configuration at C3 of the tropane moiety. Therefore they were stereospecific.

In the next step the synthesis of 8-benzyl-8-azabicyclo[3.2.1]octan-3-one (**7**) was performed (Scheme 2). For that purpose the Robinson method was applied. The obtained ketone (**7**) was subsequently converted to an oxime (**8**), which was then subjected to a stereoselective reduction with sodium in butanol to give the equatorial (*β*) 8-benzyl-8-azabicyclo[3.2.1]oct-3*β*-yl-amine (**9**) (see Supporting information for details). The above reactions were carried out using methods described in the literature [31]. In order to obtain the planned *β*-quinolineamides (**10a, b**) the mixed anhydride method was used. This method proved to be stereospecific.

The structure and isomerism of all new intermediates and final compounds was confirmed by analysis of IR ^{1}H NMR and ESI-HRMS spectra. To confirm the composition of the hydrochloride **10a** and oxalate **10b** additional elemental analysis (C, H, N) was performed. The details are presented in the Experimental Section.



Scheme 1. . Synthesis of the new 3*β*-aminotropane derivatives **6a**–**u**. Reagents: (a) NH_2OH x HCl, $NaHCO_3$, EtOH; (b) Na/PrOH; (c) ClCOOEt, TEA, DMF; (d) /I/ $ClCOOCHClCH_3$, DMC, $ClCH_2CH_2Cl$, /II/ MeOH; (e) methyl-, methoxy-, chloro-, bromo-, fluoro-, trifluoromethylbenzyl chlorides or bromides and 2-, 3- or 4-pyridinylmethyl chlorides, anhydrous K_2CO_3, KI, acetone.



10a (R = -H), 10b (R= $-OCH_3$)

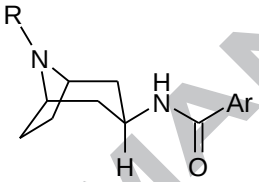
Scheme 2. Synthesis of the new 3*β*-aminotropane derivatives **10a**–**b**. Reagents: (a) NH_2OH x HCl, $NaHCO_3$, EtOH; (b) Na/BuOH; (c) quinoline-2-carboxylic acid or 4-methoxyquinoline-2-carboxylic acid, ClCOOEt, TEA, DMF.

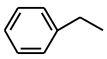
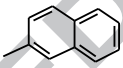
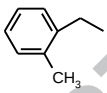
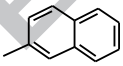
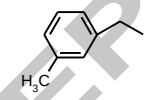
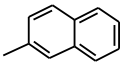
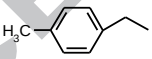
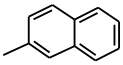
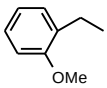
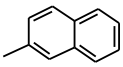
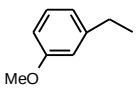
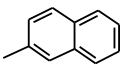
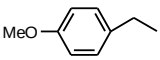
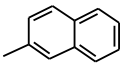
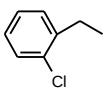
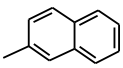
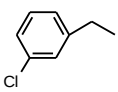
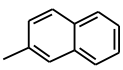
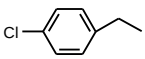
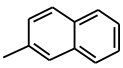
2.2. Biological evaluation

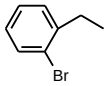
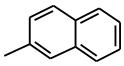
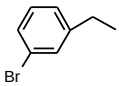
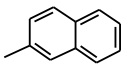
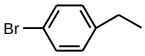
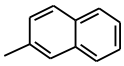
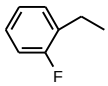
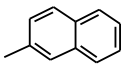
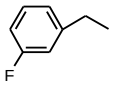
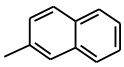
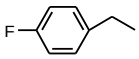
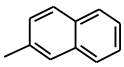
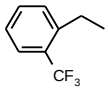
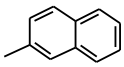
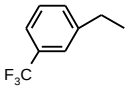
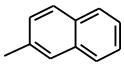
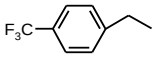
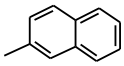
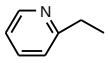
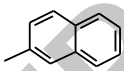
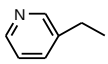
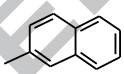
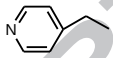
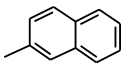
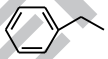
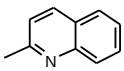
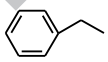
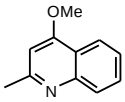
2.2.1 Radioligand binding assay for D_2, 5-HT_{1A} and 5-HT_{2A} receptors

Compounds **6a–u** and **10a–b** were tested for their *in vitro* affinity for the D_2, 5-$HT_{1A,}$ and 5-HT_{2A} receptors using a radioligand binding assay. Competition binding studies were performed according to a previously described procedure in rat brain tissues.[28,32] The results are presented in Table 1.

Table 1
Binding affinities for dopamine D_2 and serotonin 5-HT_{1A} / 5-HT_{2A} receptors.



Compound	R	Ar	K_i [nM] ± S.E.M			pK_i ratio
			D_2	5-HT_{1A}	5-HT_{2A}	5-HT_{2A}/D_2
A[a]			82.4 ± 6.5	303.8 ± 11.4	2.5 ± 0.8	
6a			118.0 ± 14.1	192.7 ± 22.7	854.0 ± 22.4	
6b			3.3 ± 0.2	182.0 ± 1.0	231.0 ± 39.8	0.78
6c			0.4 ± 0.03	728.5 ± 81.0	908.0 ± 37.0	
6d			76.9 ± 3.1	82.5 ± 8.0	919.5 ± 34.5	0.85
6e			25.7 ± 2.3	212.6 ± 23.0	663.0 ± 23.3	0.81
6f			0.6 ± 0.05	2600 ± 300.0	509.0 ± 5.3	
6g			34.8 ± 3.7	158.0 ± 2.0	396.7 ± 4.8	0.86
6h			3.6 ± 0.3	298.0 ± 23.5	135.7 ± 0.9	0.81
6i			0.4 ± 0.05	2500 ± 200.0	571.0 ± 20.4	

6j			22.8 ± 1.1	196.3 ± 28.0	326.5 ± 18.5	
6k			11.6 ± 0.9	199.5 ± 19.7	105.7 ± 5.4	0.88
6l			4.5 ± 0.4	229.7 ± 31.0	298.5 ± 24.0	
6m			714.5 ± 40.5	365.5 ± 22.5	141.0 ± 12.0	
6n			214.0 ± 6.0	210.7 ± 29.0	101.5 ± 0.5	1.05
6o			137.0 ± 15.0	218.0 ± 22.0	209.0 ± 7.8	0.97
6p			536.7 ± 21.5	476.0 ± 50.0	>10000	
6q			217.3 ± 11.0	130.0 ± 12.9	589.0 ± 93.0	
6r			629.0 ± 71.5	2000 ± 200.0	484.0 ± 18.7	
6s			2200.0 ± 600.0	314.8 ± 32.0	1116.0 ± 19.5	
6t			2400.0 ± 200.0	484.5 ± 87.5	502.0 ± 51.0	
6u			2700.0 ± 300.0	2300 ± 200.0	495.0 ± 5.0	
10a			588.6 ± 8.3	62.7 ± 11.8	1291.0 ± 40.5	
10b			>10000	30.5 ± 3.5	2315.0 ± 27.5	
serotonin				9.4		
DPAT				2.3		
Mianserine					18.4	
Clozapine			130[b]	140[b]	8.9[b]	
Quetiapine			180[b]	230[b]	220[b]	

[a] Data from Ref. [28]
[b] Data from Ref. [33]

The influence of the type and location of the substituents in the benzene ring of **6a**–**6r** on the binding affinity for D_2, 5-HT_{1A} and 5-HT_{2A} receptors was analysed.

In terms of their affinity for D_2 receptors, ligands **6a**–**6r** displayed very high (K_i = 0.4 nM for **6c** and **6i**, K_i = 0.6 nM for **6f**) to low affinities for D_2 receptors (K_i = 714.5 nM for **6m**). It is worth noting that *p*-substituted ligands (**6c**, **6f**, **6i**, **6l**, **6o**) proved to be the considerably more promising compounds than their *m*- or *o*-substituted analogues, with compounds **6c** (*p*-CH_3), **6f** (*p*-OCH_3) and **6i** (*p*-Cl) displaying subnanomolar K_i values. The only exception to this rule was ligand **6r** (*p*-CF_3-substituted). We also observed that *m*-substituted compounds have higher affinity for D_2 than their *o*-substituted analogues in all synthesised series of ligands. Analysing the influence of the type of substituent in the benzene ring, we noted that chlorine substitution (**6g**, **6h**, **6i**) had the most favourable effect on D_2 binding affinity, while introduction of fluorine (**6m**, **6n**, **6o**) or a trifluoromethyl substituent (**6p**, **6q**, **6r**) negatively affected D_2 receptor binding affinity. Comparing radioligand binding assay results for compound **A** and its substituted analogues (**6a–6r**), we can conclude that the introduction of a substituent such as a methyl (apart from *o*-methyl substitution in compound **6a**) or methoxy group, or a chlorine or bromine atom led to an increase in the affinity for the D_2 receptor.

As far as affinity for the 5-HT_{1A}R receptor is concerned, *p*-substituted ligands displayed significantly lower affinity than the corresponding *o*- or *m*- substituted compounds. The only exception was compound **6o** (*p*-fluoro-substituted), which turned out to has higher affinity for the 5-HT_{1A}R than its o-substituted (**6m**) analogue and of similar affinity to its *m*-substituted (**6n**) analogue. Comparing K_i values for compound **A** and ligands **6a–6r**, it can be seen that the introduction of substituents in the *o*- (except for –F and $–CF_3$ substituents) or *m*-position proved to be beneficial in terms of 5-HT_{1A}R binding affinity, with the highest affinities observed for compounds **6d** (K_i = 82.5 nM) R_1 = -OCH_3; R_2, R_3 = H and **6q** (K_i = 130 nM) R_2 = -CF_3; R_1,R_3 = H.

Analysing K_i values for 5-HT_{2A} receptors we found that *m*-substituted ligands had the highest binding affinities, with the exception of **6i** and **6q**. The substituted compounds **6a–6r** however displayed significantly lower binding affinities for 5-HT_{2A} receptors (K_i ranging from 101.5 nM to >10 μM) than the unsubstituted compound **A** (K_i = 2.5 nM).

We then studied the effect of replacement of phenyl or naphthyl ring with their heterocyclic analogues, namely 2-, 3- or 4-pyridine (compound **6s**–**6u**) or 2-quinoline (compounds **10a–10b**), respectively.

2-, 3- or 4-pyridine derivatives had significantly lower affinities for D_2 receptors than their benzene substituted analogues, with K_i ranging from 2.2 to 2.7 μM. The highest affinity was observed for a 2-pyridine analogue (**6s**) for both of the receptors D_2 and 5-HT_{1A}. At the same time the 2-pyridine analogue had the lowest affinity for 5-HT_{2A}R. Incorporation of a 2-quinoline moiety (**10a**, **10b**) led to a significant decrease or even a complete loss of affinity for the D_2 and 5-HT_{2A} receptors. Interestingly, at the same time affinity for 5-HT_{1A}R was the highest of all tested compounds with K_i = 62.7 nM for **10a** and K_i =30.5 nM for **10b**.

The *in vitro* binding assay showed that in the investigated series the compounds **6b**, **6d**, **6e**, **6g**, **6h**, **6k**, **6n** and **6o**, displayed triple binding activity for the D_2, 5-HT_{1A} and 5-HT_{2A} receptors. That was the criterion for selecting

6b, **6d**, **6e**, **6g**, **6h**, **6k**, **6n** and **6o** compounds for the behavioural assay. The Meltzer index was calculated for the tested compounds, see Table 1. The compounds with only significant high D_2 receptor affinity was excluded because they do not fit the "atypical" pharmacological profile.

It is worth mentioning that their affinity for these receptors was comparable or higher than those for quetiapine and clozapine, very successful antipsychotic drugs. Clozapine is currently the most powerful antipsychotic drug and has a complex, constantly discussed mechanism of action, being a D_2/5-HT_{2A} receptor antagonist with agonist activity for 5-HT_{1A} receptors.[34] Evidence suggests that the side effects of clozapine are substantial and efforts are still being made to find drugs that would be equally effective and better tolerated.

The mechanism of quetiapine action is also the subject of intense discussion. Some authors suggest it has a distinct mechanism of action.[35] Depending on the dose, quetiapine exerts different effects (hypnotic, sedative or antidepressant and / or antipsychotic). Undoubtedly, the therapeutic effect is the sum of the activity of quetiapine and its active metabolite norquetiapine, a potent norepinephrine reuptake inhibitor and a partial 5-HT_{1A} agonist. The overall pharmacological effect is clearly and precisely explained by Stahl.[36] The antipsychotic activity of quetiapine is closely correlated with D_2 receptor saturation. Because of its moderate binding affinity for D_2 receptors, the antipsychotic effect occurs only at sufficiently high doses. These data suggest the classic mechanism of action of this drug — similar to other antipsychotics. Such a mechanism is also postulated by other authors.[37]

Assuming the mechanism of action proposed by the above-cited Stahl and Meltzer, examination of the *in vivo* properties of the selected derivatives in our study is well justified. As mentioned above, after the rejection of a further search for ligands with outstandingly high D_2 receptor affinity, we focused on compounds having a very similar binding profile to quetiapine.

2.2.2. In vivo studies

2.2.2.1 General

Experiments were carried out on Swiss mice weighing 20-25 g kept in colony cages in standard laboratory conditions. Experimental groups were chosen randomly and each animal was used only once. The compounds studied were suspended in a 1% of Tween 80 (Sigma, St. Louis, MO, USA) and injected intraperitoneally in a volume of 10 ml/kg.

2.2.2.2 Body temperature in mice

(±)8-OH-DPAT induces hypothermia in mice, which is mediated through presynaptic 5-HT_{1A} receptors.[38,39] Its effect is abolished by WAY-100635, as a result of presynaptic 5-HT_{1A} receptor antagonism.[40] The effects of the tested compounds (**6b**, **6d**, **6e**, **6g**, **6h**, **6k**, **6n**, **6o**), given alone at doses of 10 and 20 mg/kg, on the rectal body temperature were recorded 30, 60, 90 and 120 min after administration. None of the studied compounds affected the body temperature of mice (Table 2).

Table 2
Effect of the tested compounds on body temperature in Albino Swiss mice.

Treatment	Dose (mg/kg)	Δt ± SEM (°C)			
		30 min	60 min	90 min	120 min
Vehicle	0	0.33 ± 0.2	0.33 ± 0.2	0.18 ± 0.2	0.15 ± 0.3
6b	10	-0.02 ± 0.3	0.73 ± 0.2	0.35 ± 0.2	0.27 ± 0.2
	20	0.04 ± 0.3	0.47 ± 0.1	0.20 ± 0.3	0.24 ± 0.1
		F(2,16)=0.3041; NS	F(2,16)=1.0350; NS	F(2,16)=0.1131; NS	F(2,16)=0.0787; NS
vehicle	0	0.33 ± 0.2	0.33 ± 0.2	0.18 ± 0.2	0.15 ± 0.3
6d	10	0.23 ± 0.2	0.44 ± 0.1	0.63 ± 0.1	0.26 ± 0.1
	20	-0.34 ± 0.4	0.01 ± 0.3	0.16 ± 0.2	-0.26 ± 0.1
		F(2,17)=1.6290; NS	F(2,17)=1.1221; NS	F(2,17)=2.2471; NS	F(2,17)=1.6841; NS
vehicle	0	0.31 ± 0.3	0.61 ± 0.3	0.63 ± 0.2	1.11 ± 0.2
6e	10	-0.03 ± 0.2	0.03 ± 0.3	0.17 ± 0.3	0.70 ± 0.3
	20	-0.10 ± 0.1	0.57 ± 0.2	0.81 ± 0.3	1.26 ± 0.2
		F(2,18)=1.0227; NS	F(2,18)=1.5303; NS	F(2,18)=1.7871; NS	F(2,18)=1.5018; NS
vehicle	0	0.31 ± 0.3	0.61 ± 0.3	0.63 ± 0.2	1.11 ± 0.2
6g	10	0.00 ± 0.4	0.96 ± 0.2	0.84 ± 0.2	1.34 ± 0.1
	20	-0.36 ± 0.2	0.44 ± 0.2	0.44 ± 0.2	1.39 ± 0.3
		F(2,18)=1.0967; NS	F(2,18)=1.1305; NS	F(2,18)=1.1939; NS	F(2,18)=0.3937; NS
vehicle	0	0.20 ± 0.2	0.50 ± 0.2	0.51 ± 0.2	0.71 ± 0.1
6h	10	0.17 ± 0.4	1.00 ± 0.2	0.84 ± 0.2	1.16 ± 0.2
	20	-0.38 ± 0.2	0.41 ± 0.2	0.41 ± 0.2	1.00 ± 0.2
		F(2,18)=1.3183; NS	F(2,18)=1.8677; NS	F(2,18)=1.2289; NS	F(2,18)=1.7140; NS
vehicle	0	0.17 ± 0.2	0.47 ± 0.2	0.49 ± 0.2	0.97 ± 0.2
6k	10	-0.20 ± 0.3	0.76 ± 0.1	0.64 ± 0.2	1.14 ± 0.3
	20	-0.30 ± 0.2	0.49 ± 0.2	0.36 ± 0.2	1.23 ± 0.3
		F(2,18)=0.9534; NS	F(2,18)=0.8826; NS	F(2,18)=0.4829; NS	F(2,18)=0.2142; NS
vehicle	0	0.17 ± 0.2	0.47 ± 0.2	0.49 ± 0.2	0.97 ± 0.2
6n	10	-0.17 ± 0.2	0.53 ± 0.1	0.47 ± 0.1	0.74 ± 0.2
	20	-0.36 ± 0.1	0.43 ± 0.1	0.20 ± 0.1	0.81 ± 0.2
		F(2,18)=1.5935; NS	F(2,18)=0.0921; NS	F(2,18)=1.0732; NS	F(2,18)=0.3473; NS
vehicle	0	0.16 ± 0.2	0.46 ± 0.2	0.43 ± 0.2	0.90 ± 0.2
6o	10	-0.17 ± 0.2	0.50 ± 0.1	0.44 ± 0.1	0.59 ± 0.2
	20	-0.33 ± 0.1	0.46 ± 0.1	0.23 ± 0.1	0.54 ± 0.1
		F(2,18)=1.8670; NS	F(2,18)=0.0265; NS	F(2,18)=0.6587; NS	F(2,18)=1.2082; NS

The tested compounds were administered i.p. 30 min before the test. The absolute mean body temperatures were within a range of 36.0±0.6°C; n=6-7 mice per group. One-way ANOVA followed by Bonferroni's post hoc test, NS= non-significant.

In separate experiments, the effects of **6b**, **6d**, **6e**, **6g**, **6h**, **6k**, **6n**, and **6o** on (±)8-OH-DPAT-induced hypothermia in mice were studied, since the decrease in body temperature evoked by (±)8-OH-DPAT is attributed to stimulation of presynaptic 5-HT_{1A} receptors and is abolished by WAY-100635, a silent 5-HT_{1A} receptor antagonist (Table 3). In contrast to WAY-100635, none of the tested compounds inhibited the hypothermia induced by 5-HT_{1A} agonist (±)8-OH-DPAT (Table 3).

Table 3
Effect of the tested compounds on (±)8-OH-DPAT (5 mg/kg s.c.)-induced hypothermia in Albino Swiss mice.

Treatment	Dose (mg/kg)	Δt ± SEM (°C)			
		15 min	30 min	45 min	60 min
Vehicle + vehicle	0 + 0	0.28 ± 0.2	0.52 ± 0.3	0.56 ± 0.2	0.70 ± 0.2
Vehicle + (±)8-OH-DPAT	0 + 5	-1.66 ± 0.6[b]	-1.12 ± 0.2[a]	-0.78 ± 0.2	-0.50 ± 0.2
6b + (±)8-OH-DPAT	10 + 5	-0.70 ± 0.2	-0.97 ± 0.3	-0.48 ± 0.4	-0.05 ± 0.3
	20 + 5	-1.90 ± 0.4[c] F(3,18)=11.6730; p<0.001	-2.08 ± 0.4[c] F(3,18)=11.0820; p<0.001	-2.08 ± 0.5[c] F(3,18)=9.1979; p<0.001	-1.50 ± 0.6[b] F(3,18)=6.1883; p<0.01
Vehicle + vehicle	0 + 0	0.28 ± 0.2	0.52 ± 0.3	0.56 ± 0.2	0.70 ± 0.2
Vehicle + (±)8-OH-DPAT	0 + 5	-1.66 ± 0.6[b]	-1.12 ± 0.2[a]	-0.78 ± 0.2[a]	-0.50 ± 0.2[a]
6d + (±)8-OH-DPAT	10 + 5	-1.37 ± 0.5[a]	-1.77 ± 0.5[c]	-1.45 ± 0.5[c]	-1.08 ± 0.4[c]
	20 + 5	-1.47 ± 0.3[a] F(3,18)=5.6742; p<0.01	-1.37 ± 0.2[b] F(3,18)=8.9952; p<0.001	-0.92 ± 0.2[a] F(3,18)=8.0335; p<0.01	-0.65 ± 0.1[a] F(3,18)=8.0719; p<0.01
Vehicle + vehicle	0 + 0	0.28 ± 0.2	0.52 ± 0.3	0.56 ± 0.2	0.70 ± 0.2
Vehicle + (±)8-OH-DPAT	0 + 5	-1.66 ± 0.6[b]	-1.12 ± 0.2[a]	-0.78 ± 0.2[a]	-0.50 ± 0.2[a]
6e + (±)8-OH-DPAT	10 + 5	-1.52 ± 0.2[b]	-1.32 ± 0.2[b]	-0.87 ± 0.2[a]	-0.6 ± 0.1
	20 + 5	-1.25 ± 0.5[a] F(3,18)=6.3865; p<0.01	-1.65 ± 0.5[c] F(3,18)=7.7894; p<0.01	-1.33 ± 0.5[b] F(3,18)=6.6475; p<0.01	-0.97 ± 0.4[b] F(3,18)=5.1691; p<0.01
Vehicle + vehicle	0 + 0	0.27 ± 0.1	0.43 ± 0.2	0.48 ± 0.1	0.58 ± 0.2
Vehicle + (±)8-OH-DPAT	0 + 5	-1.82 ± 0.2[d]	-1.36 ± 0.2[b]	-1.04 ± 0.2[a]	-0.66 ± 0.2

6g + (±)8-OH-DPAT	10 + 5 20 + 5	-0.97 ± 0.2[b] -2.00 ± 0.4[d] F(3,19)=19.6290; p<0.00001	-1.17 ± 0.2[b] -2.12 ± 0.4[c] F(3,19)=14.7720; p<0.0001	-0.62 ± 0.4 -2.18 ± 0.4[d] F(3,19)=12.0470; p<0.001	-0.12 ± 0.3 -1.53 ± 0.5[b] F(3,19)=6.6860; p<0.01
Vehicle + vehicle	0 + 0	0.27 ± 0.1	0.43 ± 0.2	0.48 ± 0.1	0.58 ± 0.2
Vehicle + (±)8-OH-DPAT	0 + 5	-1.82 ± 0.2[d]	-1.36 ± 0.2[b]	-1.04 ± 0.2[a]	-0.66 ± 0.2
6h + (±)8-OH-DPAT	10 + 5 20 + 5	-1.03 ± 0.2[b] -2.12 ± 0.3[d] F(3,19)=24.693; p<0.00001	-1.22 ± 0.2[b] -2.22 ± 0.4[d] F(3,19)=18.088; p<0.0001	-0.85 ± 0.4[a] -2.22 ± 0.4[d] F(3,19)=14.239; p<0.0001	-0.67 ± 0.1 -1.57 ± 0.5 F(3,19)=8.2417; p<0.01
Vehicle + vehicle	0 + 0	0.27 ± 0.1	0.43 ± 0.2	0.48 ± 0.1	0.58 ± 0.2
Vehicle + (±)8-OH-DPAT	0 + 5	-1.82 ± 0.2[d]	-1.36 ± 0.2[b]	-1.04 ± 0.2[a]	-0.66 ± 0.2
6k + (±)8-OH-DPAT	10 + 5 20 + 5	-1.32 ± 0.1[d] -1.42 ± 0.1[d] F(3,19)=49.226; p<0.00001	-1.62 ± 0.1[d] -1.37 ± 0.1[d] F(3,19)=49.226; p<0.00001	-1.00 ± 0.2[c] -1.25 ± 0.1[c] F(3,19)=30.511; p<0.00001	-0.72 ± 0.1[a] -0.58 ± 0.5[a] F(3,19)=17.901; p<0.0001
Vehicle + vehicle	0 + 0	0.28 ± 0.2	0.52 ± 0.3	0.56 ± 0.1	0.70 ± 0.2
Vehicle + (±)8-OH-DPAT	0 + 5	-1.66 ± 0.3[a]	-1.12 ± 0.2[a]	-0.78 ± 0.2[a]	-0.5 ± 0.2[a]
6n + (±) 8-OH-DPAT	10 + 5 20 + 5	-1.60 ± 0.3[b] -1.67 ± 0.4[c] F(3,18)=13.232; p<0.0001	-1.37 ± 0.2[b] -1.83 ± 0.4[c] F(3,18)=10.545; p<0.001	-0.97 ± 0.1[b] -1.53 ± 0.5[c] F(3,18)=10.618; p<0.001	-0.78 ± 0.1[b] -1.08 ± 0.4[c] F(3,18)=8.8983; p<0.001
Vehicle + vehicle	0 + 0	0.28 ± 0.2	0.52 ± 0.3	0.56 ± 0.1	0.70 ± 0.2
6o + (±)8-OH-DPAT	10 + 5 20 + 5	-1.55 ± 0.2[c] -1.62 ± 0.3[c] F(3,18)=13.631; p<0.0001	-1.32 ± 0.1[c] -1.60 ± 0.3[c] F(3,18)=12.558; p<0.001	-0.92 ± 0.1[c] -1.43 ± 0.4[c] F(3,18)=12.381; p<0.001	-0.73 ± 0.1[b] -1.00 ± 0.4[c] F(3,18)=9.2923; p<0.001
Vehicle + vehicle	0 + 0	0.00 ± 0.1	-0.31 ± 0.2	-0.30 ± 0.3	0.00 ± 0.3
Vehicle + (±)8-OH-DPAT	0 + 5	-1.8 ± 0.3[c]	-1.7 ± 0.2[c]	-1.3 ± 0.3	-1.3 ± 0.1[b]
WAY-100635 + vehicle	0.3 + 0	-0.2 ± 0.2	-0.1 ± 0.2	-0.1 ± 0.2	-0.3 ± 0.3
WAY-100635 + (±)8-OH-DPAT	0.3 + 5	-0.6 ± 0.2[C]	-0.8 ± 0.2	-0.5 ± 0.2[A]	-0.2 ± 0.2[B]
		F(3,25)=19.577;	F(3,25)=12.232;	F(3,25)=4.416;	F(3,25)=8.869;

	p<0.0001	p<0.0001	p<0.05	p<0.001

The tested compounds were administered i.p. 45 min prior 8-OH-DPAT; n=6-7 mice per group. The absolute mean initial body temperatures were within a range of 36.0±0.6°C. One-way ANOVA followed by Bonferroni's post hoc test; [a]p<0.05, [b]p<0.01, [c]p<0.001, [d]p<0.0001 vs respective vehicle+vehicle group; [A]p<0.05, [B]p<0.01, [C]p<0.001 vs respective vehicle + (±)8-OH-DPAT group.

2.2.2.3 Lower lip retraction (LLR)

It is commonly accepted that the (±)8-OH-DPAT-induced LLR in normal rats is connected with the stimulation of postsynaptic 5-HT_{1A} receptors.[41] That effect is attenuated by 5-HT_{1A} receptor antagonists, e.g. WAY-100635 (Table 4). The results of our behavioural study presented in Table 4 indicate that the tested compounds neither behave like 5-HT_{1A} receptor agonists nor inhibit LLR produced by (±)8-OH-DPAT, respectively.

Table 4
Induction of lower lip retraction (LLR) by the tested compounds (A) and their effect on the (±)8-OH-DPAT (1 mg/kg s.c.)-induced LLR (B) in rats.

Treatment	Dose (mg/kg)	Mean ± LLR score	
		A	B
Vehicle	0	0.0 ± 0	2.7 ± 0.1
6b	10	0.0 ± 0	2.3 ± 0.3
	20	0.0 ± 0	2.3 ± 0.4
			F(2,11)=0.8540; NS
Vehicle	0	0.0 ± 0	2.8 ± 0.1
6d	10	0.0 ± 0	2.5 ± 0.4
	20	0.0 ± 0	2.0 ± 0.2
			F(2,11)=3.9286; NS
Vehicle	0	0.0 ± 0	2.7 ± 0.1
6e	10	0.0 ± 0	2.4 ± 0.4
	20	0.0 ± 0	2.5 ± 0.4
			F(2,11)=0.3301; NS
Vehicle	0	0.0 ± 0	2.8 ± 0.1
6g	10	0.0 ± 0	2.5 ± 0.3
	20	0.0 ± 0	2.0 ± 0.4
			F(2,11)=2.7500; NS
Vehicle	0	0.0 ± 0	2.8 ± 0.1
6h	10	0.0 ± 0	2.6 ± 0.2
	20	0.0 ± 0	2.1 ± 0.4
			F(2,11)=2.0918; NS
Vehicle	0	0.0 ± 0	2.8 ± 0.1
6k	10	0.0 ± 0	2.4 ± 0.4
	20	0.0 ± 0	2.5 ± 0.3
			F(2,11)=0.8880; NS
Vehicle	0	0.0 ± 0	2.8 ± 0.1
6n	10	0.0 ± 0	2.4 ± 0.4
	20	0.0 ± 0	2.3 ± 0.2

			F(2,11)=1.9082; NS
Vehicle	0	0.0 ± 0	2.7 ± 0.1
6o	10	0.0 ± 0	2.5 ± 0.3
	20	0.0 ± 0	2.4 ± 0.2
			F(2,11)=0.4610; NS
Vehicle	0	0.0 ± 0	2.7 ± 0.2
	0.1	0.0 ± 0	1.4 ± 0.3 p<0.05
WAY 100635	0.2	0.0 ± 0	1.3 ± 0.2 p<0.01
			F(2,15)=9.120; p<0.01

The tested compounds were administered (i.p.) 15 min before the test (A) or 45 min before (±)8-OH-DPAT (s.c.), WAY 100635 was injected s.c., n=4-6 rats per group. One-way ANOVA followed by Bonferroni's post hoc test, NS= non-significant.

2.2.2.4 Induction of head twitches in mice

The HTR occurs after administration of (±)-2,5-dimethoxy-4-iodoamphetamine ((±)-DOI) as a result of the activation of 5-HT_{2A}R.[42]

The obtained results clearly point to **6n** and **6o** as the most potent compounds inhibiting the head-twitch response, produced by (±)-DOI at a dose of 2.5 mg/kg, in mice. Both compounds were still significantly active at 1.25 mg/kg. The weakest potency in this regard was noted for bromo- and chloro-derivatives, which expressed a significant inhibitory action on the head-twitch response at the highest dose of 10 mg/kg. **6b** retained an intermediate inhibitory action, being significantly active in the dose range of 2.5–10 mg/kg (Table 5).

Table 5
Inhibition of (±)-DOI (2.5 mg/kg)-induced head twitches by the investigated compounds **6b**; **6d**; **6e**; **6g**; **6h**; **6k**; **6n**; **6o** given i.p. at various increasing doses in Albino Swiss mice.

Treatment	Dose (mg/kg)	Head twitches (number of episodes)
Vehicle	0	19.88 ± 2.06
6b	1.25	15.63 ± 2.46
	2.5	13.00 ± 1.56[a]
	5	10.00 ± 1.93[b]
	10	2.38 ± 0.86[c]
		F(4,35)=12.550; p<0.0001
Vehicle	0	19.63 ± 2.04
6d	2.5	16.25 ± 1.26
	5	12.25 ± 1.76[a]
	10	8.25 ± 1.56[c]

		F(3,28)=8.595; p=0.0003
Vehicle	0	19.63 ± 2.04
6e	2.5	16.63 ± 1.31
	5	14.25 ± 2.70
	10	9.88 ± 1.30[b]
		F(3,28)=4.560; p=0.0101
Vehicle	0	19.63 ± 2.04
6g	2.5	20.00 ± 2.50
	5	14.75 ± 2.74
	10	10.75 ± 1.80[a]
		F(3,28)=3.646; p=0.0245
Vehicle	0	19.63 ± 2.04
6h	2.5	17.25 ± 1.62
	5	13.13 ± 1.94
	10	8.75 ± 1.88[c]
		F(3,28)=6.490; p=0.0018
Vehicle	0	19.88 ± 2.06
6k	2.5	14.50 ± 2.54
	5	13.88 ± 2.04
	10	11.88 ± 2.14[a]
		F(3,28)=2.404; p=0.0886
Vehicle	0	19.63 ± 2.04
6n	0.625	17.38 ± 2.22
	1.25	2.3 ± 0.2
	2.5	11.38 ± 2.35[a]
	5	3.88 ± 1.51[c]
	10	2.38 ± 1.45[c]
		F(5,42)=12.210; p<0.0001
Vehicle	0	19.63 ± 2.04
6o	0.625	17.63 ± 1.72
	1.25	12.63 ± 1.38[a]
	2.5	5.75 ± 2.22[c]
	5	5.00 ± 1.93[c]
	10	5.38 ± 1.95[c]
		F(5,42)=12.070; p<0.0001

Values represent the number of head twitches (mean ± SEM) during the 20 min test. n=8 mice per group. One-way ANOVA followed by Dunnett's post hoc test; [a]p<0.05, [b]p<0.01, [c]p<0.001 vs.(±)-DOI-treated group.

2.2.2.5 Climbing behaviour

Climbing behaviour induced by apomorphine (APO) results from the stimulation of dopamine D_2 receptors in the striatum.[43]

Out of eight compounds studied, only two (**6k** and **6b**) significantly inhibited the climbing behaviour within the first 30 min of observation. One was active over the period of 40 min (**6n**) and interestingly, one (**6o**) was able to inhibit the climbing behaviour only at the 60 min time point. Probably, pharmacokinetic parameters of this compound are responsible for its peak effect at 60 min and fast decline in its inhibition of climbing behaviour. It is noteworthy that the binding affinities of the tested compounds do not have to be fully correlated with their behavioural effects because their penetration through the blood-brain barrier may significantly differ. A formation of an active metabolite seems unlikely due to the metabolic stability of this compound (see below). The remaining four compounds (**6d**, **6e**, **6g**, and **6h**) were ineffective in this regard (Table 6).

Table 6
Climbing behavior in Albino Swiss mice.

Time (min)	APO + vehicle	APO + **6d**	APO + **6e**	APO + **6g**	APO + **6h**	APO + **6k**	APO + **6b**	APO + **6n**	APO + **6o**
					(10 mg/kg)				
10	8/8	4/8	6/8	4/8	5/8	3/8 [a]	3/8 [a]	3/8 [a]	6/8
20	8/8	5/8	5/8	5/8	5/8	3/8 [a]	2/8 [b]	3/8 [a]	6/8
30	8/8	5/8	5/8	5/8	6/8	3/8 [a]	3/8 [a]	3/8 [a]	5/8
40	8/8	4/8	6/8	5/8	6/8	5/8	4/8	3/8 [a]	5/8
50	8/8	5/8	5/8	5/8	5/8	6/8	7/8	4/8	4/8
60	8/8	4/8	7/8	4/8	5/8	7/8	7/8	6/8	2/8 [b]
120	7/8	6/8	8/8	5/8	7/8	7/8	7/8	7/8	4/8

All investigated compounds were administered at a constant dose of 10 mg/kg, 30 min before testing. APO was administered 10 min before testing. n=8 mice per group. Fisher's exact probability test with Yates' correction for the control group treated with apomorphine (APO) at the appropriate time. [a] $p < 0.05$, [b] $p < 0.01$. vs. APO+vehicle.

2.2.3. Evaluation of metabolic stability

The results incubating the compounds in the presence of pooled human liver microsomes and NADPH are shown in Table 7. Metabolic stability is presented as a half-time value, which allows for easy comparison of the compound structures and their liability to microsomal enzymes, responsible mainly for phase I biotransformation reactions.

Table 7
Experimental $t_{1/2}$ values along with corresponding SD and RSD%.

Compound	substituent	Average $t_{1/2}$ (n=5) [min]	SD	RSD%
6b	3-CH_3	29.955	4.98	16.65
6d	2-OCH_3	41.26	5.93	14.38
6e	3-OCH_3	42.90	6.77	15.80
6g	2-Cl	33.05	8.12	24.59
6h	3-Cl	>45	N/A	N/A
6k	3-Br	>45	N/A	N/A
6n	3-F	>45	N/A	N/A
6o	4-F	>45	N/A	N/A

*SD – standard deviation, RSD% – relative standard deviation, expressed as SD/average*100%.*

The half-time assessment in Table 7 shows that compounds **6h**, **6k**, **6n**, and **6o** are stable under the incubation conditions beyond the end point of the experiment. After 45 minutes of incubation, the compound concentration was still more than 50% of the initial value, preventing a half-time calculation. For compounds **6b**, **6d**, **6e**, and **6g** however, half-time value was easily calculated. Comparison of the structures of these compounds reveals that a halogen moiety might be the factor responsible for increased metabolic stability of **6h**, **6k**, **6n**, and **6o**. **6b** was shown to be the compound most liable to biotransformation of all those studied. The methyl moiety may be responsible for this liability, as **6b** is the only compound among the present derivatives with a methyl group. A methoxyl moiety, present in compounds **6d** and **6e,** seems to increase metabolic stability for the presented group of compounds, yet not in such a significant way as a halogen moiety. Unfortunately, due to the stability of compounds **6h**, **6k**, **6n**, **6o** the direct impact of various halogen groups on metabolic stability cannot be assessed. Compound **6g** is the only halogen derivative, for which the authors were able to evaluate a half-time. It is also the only halogen derivative possessing a chlorine moiety in the *orto*-position. This may lead to the conclusion that not only the type of moiety but also the position of the moiety plays an important role in increasing the metabolic stability, with halogen moieties being the most effective substituents and the meta and para positions affording the most effective substitutions.

Metabolic stability assay was performed on human liver microsomes, as the aim was to compare susceptibility to biotransformation as the potential drug candidates for humans. Metabolic stability of compounds **6h**, **6k**, **6n** and **6o** proved to be of value high enough to be beyond the point of incubation, rendering those compounds metabolically stable when compared to other derivatives in presented set.

3. Conclusions

In summary, our efforts in the synthesis and pharmacological evaluation of new 3*β*-acylaminotropane derivatives have led to the discovery of novel compounds **6n** and **6o** with favourable antipsychotic profiles, combining dopamine D_2-receptor and 5-HT_{2A} antagonism as well as metabolic stability.

SAR studies demonstrate a number of key features of this series: a) The D_2, 5-HT_{1A} and 5-HT_{2A} potencies are sensitive to electronic and stearic modifications on the benzyl ring, and affinities toward these receptors can be tuned by careful choice of substituents. *p*-substituted compounds displayed the highest binding affinities for D_2 of all of the tested series of compounds. However, *o*- or *m*- substituted compounds had considerably higher binding affinities for 5-HT_{1A}R than the corresponding *p*-substituted ligands, whereas *m*-substitution proved to be the most favourable in terms of 5-HT_{2A}R binding affinity. b) Replacement of the benzene ring with 2-, 3- or 4-pyridine led to a significant decrease in binding affinity for the D_2, 5-HT_{1A} and 5-HT_{2A} receptors in the series. c) Owing to the presence of the 2-quinoline moiety, compounds in this series are devoid of activity towards the D_2 and 5-HT_{2A} receptors, but at the same time they display the highest affinity for 5-HT_{1A}R of all of the tested compounds.

The aim of our study was to modify the lead structure (compound A) in order to enhance its affinity for the D_2, 5-HT_{1A} and 5-HT_{2A} receptors. Selected ligands (**6b**, **6d**, **6e**, **6g**, **6h**, **6k**, **6n**, and **6o**) were subjected to *in vivo* biological evaluation, as their affinity for the considered receptors was comparable or better than those for quetiapine and clozapine. The selection of these drugs as a reference was determined by the objective of our work.

The tested compounds neither induced hypothermia in mice nor attenuated the hypothermia induced by the 5-HT_{1A} agonist (±)8-OH-DPAT. None of the ligands induced LLR or inhibited LLR produced by (±)8-OH-DPAT. Based on these results we cannot classify the obtained compounds either as 5-HT_{1A} agonists or as 5-HT_{1A} antagonists. The explanation for these negative results is not straightforward because many factors could influence their behaviour *in vivo*, such as e.g. the moderate affinity of the studied compounds for 5-HT_{1A} receptors, insufficiency of the doses used, affinity of the studied compounds toward other receptors involved in thermoregulation such as α_1-adrenoceptors, different classes of dopamine receptors and/or other biological mechanisms. The affinity of the tested compounds for the 5-HT_{1A} , 5-HT_{2A} and D_2 receptors has been determined, which at this stage of the research restricts a detailed explanation of the lack of positive results. The tested compounds inhibited the HTR, which allows us to classify them as 5-HT_{2A} antagonists, with **6n** and **6o** being the most potent in this regard. Climbing behaviour was inhibited by

compounds **6b** and **6k** (within the first 30 min), **6n** (within 40 min) and **6o** (at 60 min). These results allow us to classify them as D_2 antagonists.

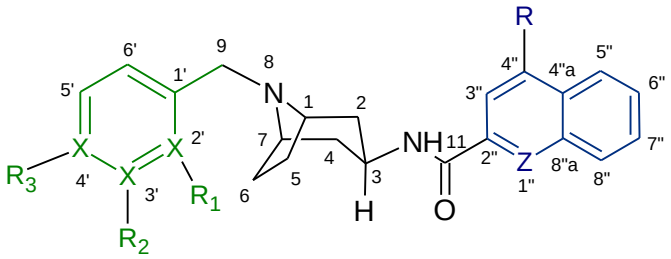
It can be concluded that compounds **6n** and **6o** are the leading compounds in the series with an antipsychotic profile. Moreover it should be noted that compounds **10a** and **10b** displayed the highest affinities for the 5-HT_{1A} receptor. Therefore in the future, structural investigations of adequately designed close analogues could help in the discovery of potent new ligands for the 5-HT_{1A} and D_2 receptors among this group of derivatives.

4. Experimental protocols

4.1. General remarks

All solvents and raw materials were purchased from commercial sources. Column chromatography was carried out using a Merck Silica gel 60A (63–200 µm) column as the stationary phase and chloroform:methanol (9:1 v/v) as eluent. Melting points were determined on an Electrothermal 9100 apparatus with open capillary tubes and were uncorrected. Yields of all carried out reactions were calculated after all purification steps. IR spectra were obtained using a Shimadzu FTIR-8300 spectrometer. NMR spectra were recorded on a Varian Inova 500 (500 MHz) spectrometer. Chemical shifts (δ) were expressed in parts per million (ppm) relative to tetramethylsilane used as the internal reference. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), bs (broad singlet), d (doublet), dd (double doublet), t (triplet), td (triple doublet), ps (pseudo triplet), 4d (quartet of doublets), m (multiplet), q (quartet), qu (quintet), * (conjugation with fluorine nucleus). Coupling constants (*J*) are in hertz (Hz). ESI-HRMS spectra were obtained on an LCT TOF (Micromass) instrument.

Purity (>95%) and homogeneity of the synthesized compounds was confirmed by TLC and ^{1}H NMR spectra. TLC was performed on Merck silica gel 60 F_{254} plates with a mobile phase of toluene, ethanol, and 25% NH_4OH (7.0:3.0:0.1, v/v). Spots were detected by their absorption under UV light (k = 254 nm). Intermediate **2** (Scheme 1) was obtained following the protocol in Ref. [29]. Intermediate **3** (Scheme 1) was obtained following the protocol in Ref. [30]. Intermediates **7**, **8** and **9** (Scheme 1) were obtained according to the protocols in Ref. [31]. The ^{1}H NMR spectra of all considered final compounds are presented in Supporting information.



R_1, R_2, R_3 = H, CH_3, OCH_3, Cl, Br, F, CF_3; X = CH; R = H; Z = CH **6a-r**

R_1, R_2, R_3 = H; X= N, CH; R = H, Z = CH **6s-u**

R_1, R_2, R_3 = H; X = CH; R=H, OCH_3; Z = N **10a-b**

Figure 2. Numbering system for NMR spectra interpretation.

*4.2. (8-Methyl-8-azabicyclo[3.2.1]oct-3β-yl)-2-naphthamide (**4**)*

A solution of naphthalene-2-carboxylic acid (18.9g, 0.11 mol), ethyl chloroformate (10.5 mL, 0.11 mol) and triethylamine (16.5 mL, 0.12 mol) in anhydrous DMF (200 mL) was stirred for 30 min at 0ºC. After this time a solution of amine **3** (16.5g, 0.12 mol) in anhydrous DMF (40 mL) was added dropwise. The cooling bath was removed and stirring was continued for 24 h. The solvent was evaporated *in vacuo* and the residue was dissolved in CH_2Cl_2 (150 mL). The solution was washed with a 5% aqueous solution of Na_2CO_3 (3 x 50 mL), then with 5% NaOH (2 x 50 mL) and once with 100 mL of water. The organic layer was dried with magnesium sulphate, filtered, and concentrated *in vacuo*. The solid residue was purified by crystallisation from ethyl acetate. Yield:14.1g (40.7%), m. p. 200.7–201.8ºC;

IR (KBr) cm^{-1}: ν3247 (NH), 1632 (CO); **ESI-HRMS** m/z calcd for $C_{19}H_{22}N_2OH$ $(M + H)^+$ 295.1810, found: 295.1812.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.22(C1''H, ^4J =1.0; m 7.87 (C4''H, C5''H, C8''H); dd 7.77 (C3''H) ^3J= 8.5, ^4J= 2.0; m 7.53 (C6''H, C7''H); d 6.06 (NH), ^3J= 8.0; m 4.39 (C3H); pt 3.22 (C1H, C5H); s 2.31(C9H); m 2.08 (C2H(E),C4H(E)); m 1.98 (C6H(E),C7H(E)); m 1.77 (C6H(A),C7H(A)); td 1.66 (C2H(A),C4H(A)), $^2J=^3J_1$= 12.5, $^3J_2$= 2.5.

*4.3. N-(8-azabicyclo[3.2.1]oct-3β-yl)-2-naphthamide hydrochloride(**5**)*

To a solution of 10.3g (0.035 mol) (8-methyl-8-azabicyclo[3.2.1]oct-3β-yl)-2-naphthamide (**4**) dissolved in a mixture of 150 mL DCM and 100 mL of 1,2-dichloroethane chilled to 0ºC and maintained at this temperature, 5.25g (4.0 mL, 0.037 mol) 1-chloroethyl chloroformate in 50 mL of 1,2-dichloroethane was added dropwise. The mixture was then

refluxed for 2.5 h. Solvents were removed *in vacuo*, 100 mL of methanol was added to the residue and the mixture was refluxed for 90 min. After removing half of the methanol, the product precipitated. Yield: 8.94g (80.7%), m.p. 160.2–163.4ºC; **IR** (KBr) cm^{-1}: ν3389 (NH), 1653 (CO); **ESI-HRMS** m/z calcd for $C_{18}H_{20}N_2OH$ $(M + H)^+$ 281.1654, found: 281.1658.
^{1}H NMR (500 MHz, $CDCl_3$): d 8.02 (C1"H); m 7.85 (C5"H,C8"H); d 7.80 (C3"H, ^3J=8.5); m 7.57 (C4"H,C6"H,C7"H); m 4.28 (C3H); pt 4.13 (C1H,C5H); m 2.16 (C2H(E),C4H(E)); m 2.06 (C6H(E),C7H(E)); m 2.01 (C6H(A),C7H(A)); td 1.85 (C2H(A),C4H(A)), $^2J=^3J_1$= 14.0, $^3J_2$= 2.5.

*4.4. General procedure for synthesis of amides (**6a-u**)*

N-(8-azabicyclo[3.2.1]oct-3β-yl)-2-naphthamide hydrochloride (**5**) 0.48g (1.5 mmol), 1.7 mmol of an appropriate arylmethyl chloride or bromide, K_2CO_3 0.69g (5.0 mmol) or (0.97g, 7.0 mmol in case of use 2-, 3- and 4-chloromethylpyridine hydrochloride) and 100mg of KI were suspended in 30 mL of acetone. The reaction mixture was refluxed with stirring. The reaction time was determined using TLC. Solvent was removed and the residue was dissolved in a mixture of 30 mL of DCM and 15 mL of water. The organic layer was washed with 10 mL of water, dried with $MgSO_4$ and concentrated. Compounds **6a–o** and **6s–u** were purified by crystallisation. Compounds **6p–r** were purified by column chromatography and subsequently by crystallisation.

*4.4.1. N-[8-(2-methylbenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6a**)*

Crystallisation from acetone. Yield: 0.30g (53.6%), m.p. 180.0-181.0ºC; **IR** (KBr) cm^{-1}: ν3260 (NH), 1625 (CO); **ESI-HRMS** m/z calcd for $C_{26}H_{28}N_2OH$ $(M + H)^+$ 385.2280, found: 385.2266.
^{1}H NMR (500 MHz, $CDCl_3$): d 8.23 (C1''H); m 7.87 (C4''H, C5''H, C8''H); dd 7.80 (C3''H), ^3J=8.5, ^4J=1.5; m 7.53 (C6''H, C7''H); dd 7.36 (C3'H), ^3J=7.0, ^4J=2.5; m 7.16 (C4'H, C5'H, C6'H); d 6.07 (NH), ^3J=8.0; m 4.42 (C3H); s 3.50 (C9H); pt 3.27 (C1H, C5H); s 2,39 (C10H); m 2.10 (C2H(E), C4H(E)); m 1.97 (C6H(E), C7H(E)); m 1.80 (C6H(A), C7H(A)); td 1.65 (C2H(A), C4H(A)), $^2J=^3J_1$=12.0, $^3J_2$=2.5.

*4.4.2. N-[8-(3-methylbenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6b**)*

Crystallisation from ethyl acetate. Yield: 0.44g (78.6%), m.p. 189.5-191.0ºC; **IR** (KBr) cm^{-1}: ν3247 (NH), 1625 (CO); **ESI-HRMS** m/z calcd for $C_{26}H_{28}N_2OH$ $(M + H)^+$ 385.2280, found: 385.2275.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.23 (C1''H); m 7.87 (C4''H, C5''H, C8''H); dd 7.80 (C3''H), ^{3}J=8.5, ^{4}J=2.0; m 7.53 (C6''H, C7''H); m 7.20 (C2'H, C4'H, C5'H); d 7.06 (C6'H, ^{3}J=6.5; d 6.11 (NH), ^{3}J=8.0; m 4.43 (C3H); s 3.53 (C9H); pt 3.28 (C1H, C5H); s 2.35 (C10H); m 2,09 (C2H(E), C4H(E)); m 1.97 (C6H(E), C7H(E)); m 1.80 (C6H(A), C7H(A)); td 1.69 (C2H(A), C4H(A)), $^2J=^3J_1=12.0$, $^3J_2=2.5$.

*4.4.3. N-[8-(4-methylbenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6c**)*

Crystallisation from acetone. Yield: 0.22g (39.3%), m.p. 199.0-199.5ºC; **IR** (KBr) cm^{-1}: ν3257 (NH), 1623 (CO); **ESI-HRMS** m/z calcd for $C_{26}H_{28}N_2OH$ $(M + H)^+$ 385.2280, found: 385.2294.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.23 (C1''H); m 7.87 (C4''H, C5''H, C8''H); dd 7.79 (C3''H), ^{3}J=8.5, ^{4}J=2.0; m 7.53 (C6''H, C7''H); d 7.27 (C3'H, C5'H), ^{3}J=8.5; d 7.13 (C2'H, C6'H), ^{3}J=8.5; d 6.09 (NH), ^{3}J=8.0; m 4.42 (C3H); s 3.52 (C9H); pt 3.27 (C1H, C5H); s 2.34 (C10H); m 2,07 (C2H(E), C4H(E)); m 1.95 (C6H(E), C7H(E)); m 1.79 (C6H(A), C7H(A)); td 1.66 (C2H(A), C4H(A)), $^2J=^3J_1=12.0$, $^3J_2=2.5$.

*4.4.4. N-[8-(2-methoxybenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6d**)*

Crystallisation from ethanol. Yield: 0.42g (61.8%), m.p. 224.1-225.2ºC; **IR** (KBr) cm^{-1}: ν3280 (NH), 1623 (CO); **ESI-HRMS** m/z calcd for $C_{26}H_{28}N_2O_2Na$ $(M + Na)^+$ 423.2052, found: 423.2048.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.23 (C1''H), ^{4}J=1.0; m 7.88 (C4''H, C5''H, C8''H); dd 7.80 (C3''H), ^{3}J=8.5, ^{4}J=1.5; td 7.22 (C4'H), ^{3}J=8.0, ^{4}J=1.5; td 6.97 (C5'H), ^{3}J=7.5, ^{4}J=1.0; dd 6.85 (C3'H), ^{3}J=8.5, ^{4}J=1.0; d 6.10 (NH), ^{3}J=8.0; m 4.44 (C3'H); s 3,82 (C10H); s 3.58 (C9H); pt 3,31 (C1H, C5H); m 2.12 (C2H(E), C4H(E)); m 1.99 (C6H(E), C7H(E)); m 1.80 (C6H(A), C7H(A)); td 1.72 (C2H(A), C4H(A)), $^2J=^3J_1=12.0$, $^3J_2=2.5$.

*4.4.5. N-[8-(3-methoxybenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6e**)*

Crystallisation from acetone. Yield: 0.29g (42.6%), m.p. 181.2-182.1ºC; **IR** (KBr) cm^{-1}: ν3314 (NH), 1622 (CO); **ESI-HRMS** m/z calcd for $C_{26}H_{28}N_2O_2H$ $(M + H)^+$ 401.2229, found: 401.2248.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.23 (C1''H), ^{4}J=1.0; m 7.88 (C4''H, C5''H, C8''H); dd 7.80 (C3''H), ^{3}J=8.5, ^{4}J=2.0; m 7.53 (C6''H, C7''H); t 7.23 (C5'H), ^{3}J=7.5; t 7.00 (C2'H), ^{4}J=2.0; d 6.96 (C6'H), ^{3}J=7.5; d 6.79 (C4'H) , ^{3}J=8.5, $^4J_1$=2.5, $^4J_2$=0.5; d 6.10 (NH), ^{3}J=8.0; m 4.42 (C3H); s 3.81 (C10H); s 3,55 (C9H); pt 3,27 (C1H, C5H); m 2.07 (C2H(E); C4H(E)); m 1.97 (C6H(E), C7H(E)); m 1.79 (C2H(A), C4H(A)); td 1.68 (C2H(A), C4H(A)) $^2J=^3J_1$=12.0, $^3J_2$=2.5.

*4.4.6. N-[8-(4-methoxybenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6f**)*

Crystallisation from ethanol. Yield: 0.32g (47.1%), m.p. 181.3-182.4ºC; **IR** (KBr) cm^{-1}: ν3274 (NH), 1623 (CO); **ESI-HRMS** m/z calcd for $C_{26}H_{28}N_2O_2H$ $(M + H)^+$ 401.2229, found: 401.2232.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.23 (C1''H); m 7.87 (C4''H, C5''H, C8''H); dd 7.79 (C3''H), ^{3}J=8.5, ^{4}J=1.5; m 7.53 (C6''H, C7''H); d 7.29 (C2'H, C6'H), ^{3}J=8.5; dt 6.86 (C3'H, C5'H), ^{3}J=9.0, ^{4}J=2.5; d 6.09 (NH), ^{3}J=8.0; m 4.42 (C3H); s 3.80 (C10H); s 3,49 (C9H); pt 3,26 (C1H, C5H); m 2.07 (C2H(E), C4H(E)); m 1.96 (C6H(E), C7H(E)); m 1.79 (C6H(A), C7H(A)); td 1.66 (C2H(A), C4H(A)), $^2J=^3J_1$=12.0, $^3J_2$=2.0.

*4.4.7. N-[8-(2-chlorobenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6g**)*

Crystallisation from ethanol. Yield: 0.47g (70.2%), m.p. 207.5-210.0ºC; **IR** (KBr) cm^{-1}: ν3249 (NH), 1633 (CO); **ESI-HRMS** m/z calcd for $C_{25}H_{25}ClN_2OH$ $(M + H)^+$ 405.1734, found: 405.1732.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.24 (C1"H); m 7.88 (C4"H, C5"H, C8"H); dd 7.80 (C3"H), ^{3}J=9.0, ^{4}J=2.0; dd 7.65 (C3'H), ^{3}J=8.0, ^{4}J=1.5; m 7.53 (C6"H, C7"H); dd 7.33 (C6'H), ^{3}J =8.0, ^{4}J =1.5; td 7.25 (C5'H), ^{3}J=8.0, ^{4}J=1.5; td 7.17 (C4'H), ^{3}J=8.0, ^{4}J=1.5; d 6.10 (NH), ^{3}J=8.5; m 4.44 (C3H); s 3.65 (C9H); pt 3.29 (C1H, C5H); m 2.12 (C2H(E), C4H(E)); m 2.00 (C6H(E), C7H(E)); m 1.83 (C6H(A), C7H(A)); td 1.71 (C2H(A), C4H(A)), $^2J=^3J_1$=12.0, $^3J_2$=2.5.

*4.4.8. N-[8-(3-chlorobenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6h**)*

Crystallisation from ethanol. Yield: 0.35g (52.2%), m.p. 195.0-201.2ºC; **IR** (KBr) cm^{-1}: ν3241 (NH), 1635 (CO); **ESI-HRMS** m/z calcd for $C_{25}H_{25}ClN_2OH$ $(M + H)^+$ 405.1734, found: 405.1740.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.24 (C1"H); m 7.88 (C4"H, C5"H, C8"H); dd 7.81 (C3"H), ^{3}J=8.5, ^{4}J=2.5; m 7.54 (C6''H, C7''H); ps 7.44 (C2'H); m 7.23 (C4'H, C5'H, C6'H); d 6.10 (NH), ^{3}J=8.5; m 4.42 (C3H); s 3.53 (C9H); pt 3.25 (C1H, C5H); m 2.07 (C2H(E), C4H(E)); m 1.98 (C6H(E), C7H(E)); td 1.68 (C2H(A), C4H(A)), $^2J=^3J_1$=12.5, $^3J_2$=2.5.

*4.4.9. N-[8-(4-chlorobenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6i**)*

Crystallisation from ethanol. Yield: 0.47g (70.2%), m.p. 189.4-191.9ºC; **IR** (KBr) cm^{-1}: ν3245 (NH), 1639 (CO); **ESI-HRMS** m/z calcd for $C_{25}H_{25}ClN_2OH$ $(M + H)^+$ 405.1734, found: 405.1737.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.23 (C1"H); m 7.88 (C4"H, C5"H, C8"H); dd 7.80 (C3"H), ^{3}J=8.5, ^{4}J=1.5); m 7.54 (C6"H, C7"H); dt 7.32 (C3'H, C5'H), ^{3}J=9.0, ^{4}J=2.0; dt 7.28 (C2'H, C6'H), ^{3}J=8.5, ^{4}J=2.0; d 6,10 (NH), ^{3}J=8.0; m 4.42 (C3H); s 3.51 (C9H); pt 3,23 (C1H, C5H); m 2.06 (C2H(E), C4H(E)); m 1.97 (C6H(E), C7H(E)); m 1.80 (C6H(A), C7H(A)); td 1.66 (C2H(A), C4H(A)), $^2J=^3J_1$=12.0, $^3J_2$=2.5.

*4.4.10. N-[8-(2-bromobenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6j**)*

Crystallisation from ethanol. Yield: 0.40g (54.1%), m.p. 216.5-219.5ºC; **IR** (KBr) cm^{-1}: ν3249 (NH), 1637 (CO); **ESI-HRMS** m/z calcd for $C_{25}H_{25}BrN_2OH$ $(M + H)^+$ 449.1229, found: 449.1216.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.24 (C1"H); m 7.88 (C4"H, C5"H, C8"H); dd 7.81 (C3"H), ^{3}J=8.5, ^{4}J=2.0; dd 7.64 (C3'H), ^{3}J=8.0, ^{4}J=2.0; dd 7.56 (C6'H), ^{3}J=8.0,^{4}J=1.5; m 7.53 (C6"H, C7"H); td 7.30 (C4'H) ,^{3}J=7.5, ^{4}J=1.0; td 7.10 (C5'H), ^{3}J=7.5, ^{4}J=1.5; d 6.10 (NH), ^{3}J=8.0; m 4.44 (C3H); s 3.62 (C9H); pt 3.29 (C1H, C5H); m 2.13 (C2H(E), C4H(E)); m 2.00 (C6H(E), C7H(E)); m 1.83 (C6H(A), C7H(A)); td 1.71 (C2H(A), C4H(A)), $^2J=^3J_1$=12.0, $^3J_2$=2.0.

*4.4.11. N-[8-(3-bromobenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6k**)*

Crystallisation from ethanol. Yield: 0.35g (47.3%), m.p. 203.7-204.5ºC; **IR** (KBr) cm^{-1}: ν3231 (NH), 1635 (CO); **ESI-HRMS** m/z calcd for $C_{25}H_{25}BrN_2OH$ $(M + H)^+$ 449.1229, found: 449.1246.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.24 (C1"H); m 7.89 (C4"H, C5"H, C8"H); dd 7.81 (C3"H), ^{3}J=8.5, ^{4}J=1.5; t 7.60 (C2'H); m7.54 (C6"H, C7"H); 4d 7.37 (C4'H), ^{3}J=8.0, $^4J_1$=2.0, $^4J_2$=1.0; dt 7.29 (C6'H), ^{3}J=7.5; t 7.18 (C5'H), J^3=7.5; d 6.08 (NH), ^{3}J=8.5; m 4.43 (C3H); s 3.53 (C9H); pt 3.25 (C1H, C5H); m 2.07 (C2H(E), C4H(E)); m 1.98 (C6H(E), C7H(E)); m 1.81 (C6H(A), C7H(A)); td 1.67 (C2H(A), C4H(A)), $^2J=^3J_1$=12.5, $^3J_2$=2.5.

*4.4.12. N-[8-(4-bromobenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6l**)*

Crystallisation from ethanol. Yield: 0.35g (47.3%), m.p. 185.2-187.6ºC; **IR** (KBr) cm^{-1}: ν3297 (NH), 1634 (CO); **ESI-HRMS** m/z calcd for $C_{25}H_{25}BrN_2OH$ $(M + H)^+$ 449.1229, found: 449.1232.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.23 (C1"H); m 7.88 (C4"H, C5"H, C8"H); dd 7.80 (C3"H), ^{3}J=8.5, ^{4}J=1.5; m 7.54 (C6"H, C7"H); dt 7.43 (C3'H, C5'H); ^{3}J=8.5, ^{4}J=2.0; dt 7.26 (C2'H, C6'H), ^{3}J=9.5, ^{4}J=2.0; d 6.10 (NH), ^{3}J=8.0; m 4.42 (C3H); s 3.50 (C9H); pt 3.23 (C1H, C5H); m 2.06 (C2H(E), C4H(E)); m 1.97 (C6H(E), C7H(E)); m 1.80 (C6H(A), C7H(A)); td 1.65 (C2H(A), C4H(A)), $^2J=^3J_1$=12.5, $^3J_2$=2.5.

*4.4.13. N-[8-(2-fluorobenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6m**)*

Crystallisation from ethanol. Yield: 0.33g (56.6%), m.p. 200.8-202.1ºC; **IR** (KBr) cm^{-1}: ν3253 (NH), 1636 (CO); **ESI-HRMS** m/z calcd for $C_{25}H_{25}FN_2ONa$ $(M + Na)^+$ 411.1851, found: 411.1849.

^{1}H NMR (500 MHz, $CDCl_3$): bs 8.23 (C1"H); m 7.88 (C4"H, C5"H, C8"H); dd 7.79 (C3"H), ^{3}J=8.5, ^{4}J=2.0; m 7.54 (C6'H, C6''H, C7''H); m 7.22 (C4'H), ^{3}J=7.5, $^4J_{H\text{-}F}$=5.5, ^{4}J=2.0; td 7.12 (C5'H), ^{3}J=7.5, ^{4}J=1.0; m 7.02 (C3'H), $^3J_{H\text{-}F}$=10.0, ^{3}J=8.0, ^{4}J=1.0; d 6.08 (NH), ^{3}J=8.5; m 4.44 (C3H); s 3.61 (C9H); pt 3.30 (C1H, C5H); m 2.12 (C2H(E), C4H(E)); m 1.98 (C6H(E), C7H(E)); m 1.82 (C6H(A), C7H(A)); td 1.69 (C2H(A), C4H(A)), $^2J=^3J_1$=12.5, $^3J_2$=2.0.

*4.4.14. N-[8-(3-fluorobenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6n**)*

Crystallisation from ethanol. Yield: 0.33g (56.6%), m.p. 180.1-180.7ºC; **IR** (KBr) cm^{-1}: ν3247 (NH), 1637 (CO); **ESI-HRMS** m/z calcd for $C_{25}H_{25}FN_2OH$ $(M + H)^+$ 389.2027, found: 389.2029.

^{1}H NMR (500 MHz, $CDCl_3$): bs 8.24 (C1''H); m 7.88 (C4''H, C5''H, C8''H); dd 7.80 (C3''H, ^{3}J=8.5, ^{4}J=1.5; m 7.54 (C6''H, C7''H); m 7.26 (C5'H); d 7.18 (C2'H), $^3J_{H-F}$=10.0; d 7.12 (C6'H), ^{3}J=7.5; td 6.93 (C4'H), ^{3}J=8.0, ^{4}J=2.0; d 6.10 (NH), ^{3}J=8.0; m 4.43 (C3H); s 3.55 (C9H); pt 3.25 (C1H, C5H); m 2.07 (C2H(E), C4H(E)); m 1.98 (C6H(E), C7H(E)); m 1.81 (C6H(A), C7H(A)); td 1.68 (C2H(A), C4H(A)), $^2J=^3J_1$=12.0, $^3J_2$=2.5.

*4.4.15. N-[8-(4-fluorobenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6o**)*

Crystallisation from ethanol. Yield: 0.35g (60.1%), m.p. 201.1-203.9ºC; **IR** (KBr) cm^{-1}: ν3245 (NH), 1636 (CO); **ESI-HRMS** m/z calcd for $C_{25}H_{25}FN_2ONa$ $(M + Na)^+$ 411.1866, found: 411.1849.

^{1}H NMR (500 MHz, $CDCl_3$): bs 8.23 (C1''H); m 7.88 (C4''H, C5''H, C8''H); dd 7.80 (C3''H, ^{3}J=8.5, ^{4}J=2.0; m 7.54 (C6''H, C7''H); m 7.34 (C2'H, C6'H), ^{3}J=8.0, $^4J_{H-F}$=5.5, ^{4}J=2.0; m 7.00 (C3'H, C5'H), ^{3}J=7.5, $^4J=^5$J=2.0; d 6.09 (NH), ^{3}J=8.0; m 4.42 (C3H); s 3.52 (C9H); pt 3.25 (C1H, C5H); m 2.07 (C2H(E), C4H(E)); m 1.97 (C6H(E), C7H(E)); m 1.80 (C6H(A), C7H(A)); td 1.66 (C2H(A), C4H(A)), $^2J=^3J_1$=12.5, $^3J_2$=2.5.

*4.4.16. N-[8-(2-trifluoromethylbenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6p**)*

Purified by column chromatography and crystallisation from ethanol. Yield: 0.40g (53.3%), m.p. 202.0-205.1ºC; **IR** (KBr) cm^{-1}: ν3275 (NH), 1636 (CO); **ESI-HRMS** m/z calcd for $C_{26}H_{25}F_3N_2ONa$ $(M + Na)^+$ 461.1809, found: 461.1817.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.25 (C1''H); d 7.99 (C3'H), ^{3}J=7.5; m 7.89 (C4''H, C5''H, C8''H); dd 7.81 (C3''H), ^{3}J=8.5, ^{4}J=1.5; d 7.62 (C6'H), ^{3}J=8.0; m 7.53 (C4'H, C6''H, C7''H); t 7.32 (C5'H), ^{3}J=7.5; d 6.08 (NH), ^{3}J=8.0; m 4.44 (C3H); s 3.73 (C9H); pt 3.26 (C1H, C5H); m 2.10 (C2H(E), C4H(E)); m 2.00 (C6H(E), C7H(E)); m 1.83 (C6H(A), C7H(A)); td 1.70 (C2H(A), C4H(A)), $^2J=^3J_1$=11.5, $^3J_2$=2.5.

*4.4.17. N-[8-(3-trifluoromethylbenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6q**)*

Purified by column chromatography and crystallisation from ethanol. Yield: 0.49g (65.3%), m.p. 167.0-167.6ºC; **IR** (KBr) cm^{-1}: ν3257 (NH), 1636 (CO); **ESI-HRMS** m/z calcd for $C_{26}H_{25}F_3N_2OH$ $(M + H)^+$ 439.1997, found: 439.1999.

^{1}H NMR (500 MHz, $CDCl_3$): bs 8.25 (C1''H); m 7.89 (C4''H, C5''H, C8''H); dd 7.81 (C3''H), ^{3}J=8.5, ^{4}J=1.5; bs 7.71 (C2'H); m 7.48 – 7.58 (C4'H, C6'H, C6''H, C7''H); t 7.42 (C5'H), ^{3}J=8.0; d 6.10 (NH), ^{3}J=8.5; m 4.43 (C3H); s 3.61 (C9H); pt 3.25 (C1H, C5H); m 2.08 (C2H(E), C4H(E)); m 1.99 (C6H(E), C7H(E)); m 1.83 (C6H(A), C7H(A)); td 1.68 (C2H(A), C4H(A)), $^2J=^3J_1$=12.0, $^3J_2$=2.5.

*4.4.18. N-[8-(4-trifluoromethylbenzyl)-8-azabicyclo[3.2.1]oct-3β-yl)]-2-naphthamide (**6r**)*

Purified by column chromatography and crystallisation from ethanol. Yield: 0.46g (61.3%), m.p. 166.4-166.8ºC; **IR** (KBr) cm^{-1}: ν3251 (NH), 1635 (CO); **ESI-HRMS** m/z calcd for $C_{26}H_{25}F_3N_2OH$ $(M + H)^+$ 439.1997, found: 439.1995.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.24 (C1''H); m 7.88 (C4''H, C5''H, C8''H); dd 7.80 (C3''H), ^{3}J=8.5, ^{4}J=1.5; m 7.54 (C2'H, C3'H, C5'H, C6'H, C6''H, C7''H); d 6.08 (NH), ^{3}J=8.0; m 4.43 (C3H); s 3.61 (C9H); pt 3.25 (C1H, C5H); m 2.08 (C2H(E), C4H(E)); m 1.99 (C6H(E), C7H(E)); m 1.82 (C6H(A), C7H(A)); td 1.68 (C2H(A), C4H(A)), $^2J=^3J_1$=12.0, $^3J_2$=2.0.

*4.4.19. N-(8-pyridin-2-ylmethyl-8-azabicyclo[3.2.1]oct-3β-yl)-2-naphthamide (**6s**)*

Purified by crystallisation from acetone. Yield: 0.24g (42.6%), m.p. 207.8-209.7ºC; **IR** (KBr) cm^{-1}: ν3253 (NH), 1638 (CO); **ESI-HRMS** m/z calcd for $C_{24}H_{25}N_3OH$ $(M + H)^+$ 372.2076, found: 372.2088.

^{1}H NMR (500 MHz, $CDCl_3$): dt 8.53 (C6H), ^{3}J=5.0, $^4J=^5$J=1.0; d 8.23 (C1''H); m 7.88 (C4''H, C5''H, C8''H); dd 7.80 (C3''H), ^{3}J=8.5, ^{4}J=2.0; td 7.66 (C4'H), ^{3}J=7.5, ^{4}J=2.0; m 7.54 (C3'H, C6''H, C7''H); td 7.15 (C5'H), ^{3}J=6.5, ^{4}J=1.0; d 6.14 (NH), ^{3}J=8.5; m 4.45 (C3H); s 3.75 (C9H); pt 3.30 (C1H, C5H); m 2.12 (C2H(E), C4H(E)); m 1.97 (C6H(E), C7H(E)); m 1.82 (C6H(A), C7H(A)); td 1.74 (C2H(A), C4H(A)), $^2J=^3J_1$=12.5, $^3J_2$=2.5.

*4.4.20. N-(8-pyridin-3-ylmethyl-8-azabicyclo[3.2.1]oct-3β-yl)-2-naphthamide (**6t**)*

Purified by crystallisation from acetone. Yield: 0.29g (51.5%), m.p. 179.8-180.5ºC; **IR** (KBr) cm^{-1}: ν3260 (NH), 1635 (CO); **ESI-HRMS** m/z calcd for $C_{24}H_{25}N_3OH$ $(M + H)^+$ 372.2076, found: 372.2086.

^{1}H NMR (500 MHz, $CDCl_3$): dd 8.59 (C2'H), $^4J_1$=2.0, $^4J_2$=0.5; dd 8.49 (C6'H), ^{3}J=4.5, ^{4}J=1.5; d 8.25 (C1''H); m 7.85 (C4''H, C5''H, C8''H); dd 7.82 (C3''H), ^{3}J=8.5, ^{4}J=2.0; dt 7.72 (C4'H), ^{3}J=8.0, ^{4}J=2.5; m 7.52 (C6''H, C7''H); 4d 7.24 (C5'H), $^3J_1$=8.0, $^3J_2$=4.4, ^{5}J=0.0; d 6.33 (NH), ^{3}J=8.0; m 4.43 (C3H); s 3.55 (C9H); pt 3.23 (C1H, C5H); m 2.06 (C2H(E), C4H(E)); m 1.95 (C6H(E), C7H(E)); m 1.80 (C6H(A), C7H(A)); td 1.67 (C2H(A), C4H(A)), $^2J=^3J_1$=12.5, $^3J_2$=2.5.

*4.4.21. N-(8-pyridin-4-ylmethyl-8-azabicyclo[3.2.1]oct-3β-yl)-2-naphthamide (**6u**)*

Purified by crystallisation from acetone. Yield: 0.18g (32.0%), m.p. 202.1-204.0ºC; **IR** (KBr) cm^{-1}: ν3245 (NH), 1634 (CO); **ESI-HRMS** m/z calcd for $C_{24}H_{25}N_3OH$ $(M + H)^+$ 372.2076, found: 372.2061.

^{1}H NMR (500 MHz, $CDCl_3$): dt 8.53 (C2'H, C6'H), ^{3}J=6.0, $^4J=^5$J=1.5; d 8.27 (C1''H); m 7.87 (C4''H, C5''H, C8''H); dd 7.83 (C3''H), ^{3}J=8.5, ^{4}J=2.0; m 7.53 (C6''H, C7''H); d 7.33 (C3'H, C5'H), ^{3}J=6.0; d 6.33 (NH), ^{3}J=8.0; m 4.43 (C3H); s 3.56 (C9H); pt 3.23 (C1H, C5H); m 2.06 (C2H(E), C4H(E)); m 1.82 (C6H(A), C7H(A)); td 1.71 (C2H(A), C4H(A)), $^2J=^3J_1$=12.5, $^3J_2$=2.5.

*4.5. General procedure for synthesis of amides (**10a-b**)*

A solution of suitable quinolinecarboxylic acid (5 mmol), ethyl chloroformate (0.5 mL, 5 mmol) and triethylamine (0.75 mL, 5 mmol) in anhydrous DMF (25 mL) was stirred for 30 min at 0ºC. A solution of amine **9** (5 mmol) in anhydrous DMF (15 mL) was added dropwise. The cooling bath was removed and stirring was continued for 24 h. The solvent was evaporated *in vacuo* and to the residue DCM (15 mL) was added.

*4.5.1. N-(8-benzyl-8-azabicyclo[3.2.1]oct-3b-yl)-quinoline-2-carboxamide(**10a**)*

Formed white precipitate of compound **10a** hydrochloride was filtered and washed with DCM. Purification by crystallization from ethyl acetate. Yield: 0.87g (47.0%), m.p. 275.0-275.5ºC; Anal. Calcd for $C_{29}H_{25}N_3O$ x 2HCl x 2.5 H_2O: C 58.90%, H 6.59%, N 8.59%;

Found: C 58.66%, H 6.51%, N 8.42% **IR** (KBr) cm^{-1}: ν3398 (NH), 1685 (CO); **ESI-HRMS** m/z calcd for $C_{29}H_{25}N_3OH$ $(M + H)^+$ 432.2076, found: 432.2081.

^{1}H NMR (500 MHz, $CDCl_3$): bs 12.61 (N^+H); d 8.33 (NH), ^3J=9.0; d 8.29 (C4''H), ^3J=8.5; d 8.22 (C3''H), ^3J=8.5; d 8.20 (C8''H), ^3J=8.5; m 7.87 (C2'H, C6'H, C5''H); m 7.79 (C7''H), $^3J_1$=8.5, $^3J_2$=7.0, ^4J=1.5; m 7.62 (C6''H), $^3J_1$=8.5, $^3J_2$=7.0, ^4J=1.0; m 7.46 (C3'H, C4'H, C5'H); m 4.62 (C3H); s 4.13 and 4.14 (C1H, C5H); bs 3.82 (C9H); t 2.91 (C2H(E), C4H(E)); m 2.35 (C6H(E), C7H(E)); m 2.26 (C6H(A), C7H(A)); m 2.12 (C2H(A), C4H(A)).

*4.5.2. N-(8-benzyl-8-azabicyclo[3.2.1]oct-3b-yl)-4-methoxyquinoline-2-carboxamide(**10b**)*

The solution was washed with a 5% aqueous solution of Na_2CO_3 and water, dried with magnesium sulphate, filtered, and the solvent was evaporated *in vacuo*. The residue was dissolved in acetone and acidified with acetonous solution of oxalic acid.

Obtained oxalate was crystallized from isopropanol/ethanol mixture (8:1.5). Yield: 0.86g (42.9%), m.p. 134.2-138.2ºC; Anal. Calcd for $C_{25}H_{27}N_3O_2$ x $C_2H_2O_4$ x H_2O: C 63.64%, H 6.13%, N 8.25%; Found: C 63.84%, H 6.47%, N 7.92%; **IR** (KBr) cm^{-1}: ν 3383 (NH), 1770 (COO^-), 1670 (CO); **ESI-HRMS** m/z calcd for $C_{25}H_{27}N_3O_2H$ $(M + H)^+$ 402.2182, found: 402.2177.

^{1}H NMR (500 MHz, $CDCl_3$): d 8.75 (NH), ^3J=7.5; dd 8.17 (C8''H), ^3J=8.5, ^4J=1.0; d 8.08 (C5''H), ^3J=8.5; m 7.83 (C7''H), $^3J_1$=8.5, $^3J_2$=6.5, ^4J=1.5; m 7.63 (C2'H ,C6'H, C6''H); s 7.57 (C3''H); m 7.43 (C3'H, C4'H, C5'H); m 4.41 (C3H); ps 4.31 (C1H, C5H); s 4.11 (OCH_3); bs 3.76 (C9H); m 2.29 (C6H, C7H); m 1.98 (C2H, C4H).

4.2 Biological tests

4.2.1 Radioligand binding assay

All compounds were tested for their affinities for 5-HT_{1A}, 5-HT_{2A} and D_2 receptors according to previously described procedures.[32]

4.2.2 In vivo studies

The experiments were performed on male Albino Swiss mice (24–28 g) or Wistar rats (280–300 g) and were approved by the I Local Ethics Commission for Animal Experiments of Jagiellonian University in Cracow Cracow and the I Local Ethics Committee for Animal Experiments of Medical University in Lublin (license no.: 57/2014). The animals were kept in groups of 12–20 for mice and of four for rats, at a room temperature of 22 ± 2ºC, under 12/12

h light/dark cycle, and had free access to food and water before the experiments. Each experimental group consisted of 4–8 animals/dose, and all animals were used only once. The experiments were performed between 8 a.m. and 3 p.m.

The following drugs were used: **6b**, **6d**, **6e**, **6g**, **6h**, **6k**, **6n**, **6o**, WAY 100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-piridinyl) cyclohexanecarboxamide hydrochloride (Tocris Bioscience, UK), apomorphine (*R*-(−)-Apomorphine) hydrochloride hemihydrate (Sigma-Aldrich), (±)-DOI ((±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane) hydrochloride (Sigma-Aldrich), and (±)8-OH-DPAT ((±)8-Hydroxy-2-(di-n-propyloamino)tetralin hydrobromide (Tocris Bioscience, UK). The investigated compounds were suspended in a 1% aqueous solution of Tween 80 (Sigma Aldrich) whereas WAY 100635, apomorphine, (±)-DOI and (±)8-OH-DPAT were used as aqueous solutions.

The obtained data are presented as the mean ± SEM.

4.2.2.1 Body temperature in mice

The experiments were performed on male Albino Swiss mice (24–28 g). The effects of the tested compounds given alone intraperitoneally (*i.p.*) 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 subcutaneously (*s.c.*)) or investigated compounds on the hypothermia induced by (±)8-OH-DPAT (5 mg/kg *s.c.*) was tested. WAY 100635 was administered 15 min before the test compounds or (±)8-OH-DPAT, and rectal body temperature was recorded 15, 30, 45 and 60 min after injection of the tested compounds. The results were expressed as change in body temperature (Δt) with respect to the basal body temperature, as measured at the beginning of the experiment. Comparisons between groups were carried out by one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test (statistical significance set at $p<0.05$).

4.2.2.2 Lower lip retraction (LLR) in Wistar rats

LLR was assessed according to the method described by Berendsen et al. [3040]. 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 *i.p.* administration of the tested compounds or (±)8-OH-DPAT (1 mg/kg *s.c.*) 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 (0.2 mg/kg *s.c.*) on the LLR induced by (±)8-OH-DPAT was tested. The compounds 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. Comparisons between groups were carried out by one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test (statistical significance set at $p<0.05$).

4.2.2.3 Climbing behaviour

All animals were pretreated intraperitoneally (*i.p.*) with the investigated compounds at a constant dose of 10 mg/kg, 30 min prior to the test. APO (2.5 mg/kg, subcutaneously) was injected 10 min before the test. The control group received only APO injection + vehicle. The pretreated mice were individually transferred to Plexiglas cages with metal grids inside. The criterion indicating a positive response was an animal with its four paws on the grid. The experimental groups comprised eight mice and the results were expressed as the number of mice fulfilling the test/number of mice used. The observations were carried out at 10 min intervals up to 60 min in the observation period. The final observation was performed 120 min after the APO injection. All observations were blinded to the observer.

The results from the climbing test were statistically evaluated using Fisher's exact probability test with Yates' correction for the control group.

4.2.2.4 Induction of head twitches in mice

The induction of head twitches was carried out by *i.p.* administration of (±)-DOI at a dose of 2.5 mg/kg, according to Darmani et al. [41]. The evaluated compounds were given *i.p.* 30 min before (±)-DOI at increasing doses of 2.5–10 mg/kg. If the lowest dose of a compound (2.5 mg/kg) was still able to significantly inhibit the head-twitch response, the dose of the compound was lowered to 1.25 or even 0.625 mg/kg. Habituation of mice to experimental conditions was brought about by randomly putting an animal into a cage (12 cm in diameter, 20 cm in height) lined with sawdust, 20 min prior to the first treatment. All observations were performed in a blind fashion.

The results were shown as means ± S.E.M. and compared with one-way analysis of variance, with intergroup comparisons for individual drugs being calculated with Dunnett's test.

4.3 Metabolic stability

Stock solutions of the studied compounds were made at a concentration of 100 µM in a 50/50 acetonitrile/water mixture. Incubation mixes consisted of 2.5 µM of the studied compound, 250 µM of NADPH in phosphate buffer and 1 mg/ml of pooled human liver microsomes (HLM) (Sigma-Aldrich, St. Louis, MO, USA) in potassium phosphate buffer (0.1 M, pH 7.4). Incubations were carried out using a MyBlock mini dry-bath (Benchmark Scientific, NJ, USA) at 37 °C. Incubation mixtures without compound solution were subjected to a 5-minute pre-incubation, and started by addition of 15 µl of compound stock solution. After 5, 10, 15, 30 and 45 min 50 µL samples of incubation reaction were added to an equal volume of ice-cold acetonitrile containing 2.5 µM of IS (atropine sulphate). Control incubations were performed without NADPH to assess possible chemical instability. All samples were immediately centrifuged (10 min. 10,000 rpm) and the resulting supernatant was directly analysed or kept at -80°C until LC-MS analysis.

LC-MS analysis was performed on an Agilent 1260 system coupled to a SingleQuad 6120 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). A Poroshell C18 EC120 column (3.0 x 100 mm, 2.7 µm, Agilent Technologies, Santa Clara, CA, USA) was used in reversed-phase mode with gradient elution starting with 5% of phase A (0.1% formic acid in water) and 95% of phase B (0.1% formic acid in acetonitrile). The gradient elution program was : 0.00–7.00 min - 5%–71.5% B; 7.00–7.50 min - 71.5%–100% B; 7.50–9.00 min 100% B; 9.00–9.50 min - 100%–5% B, 9.50–12.00 min 5% B. The total analysis time was 12.5 min at 40°C, the flow rate was 0.75 mL/min and the injection volume was 10 µL. The mass spectrometer was equipped with an electrospray ionisation source and the ionisation mode was positive. The mass analyser was set individually to each derivative to detect pseudomolecular ions [$M+H^+$]. The MSD parameters of the ESI source were as follows: nebuliser pressure 50 psig (N_2), drying gas 13 L/min (N_2), drying gas temperature 300 °C, capillary voltage 3.5 kV, fragmentor voltage 225 V.

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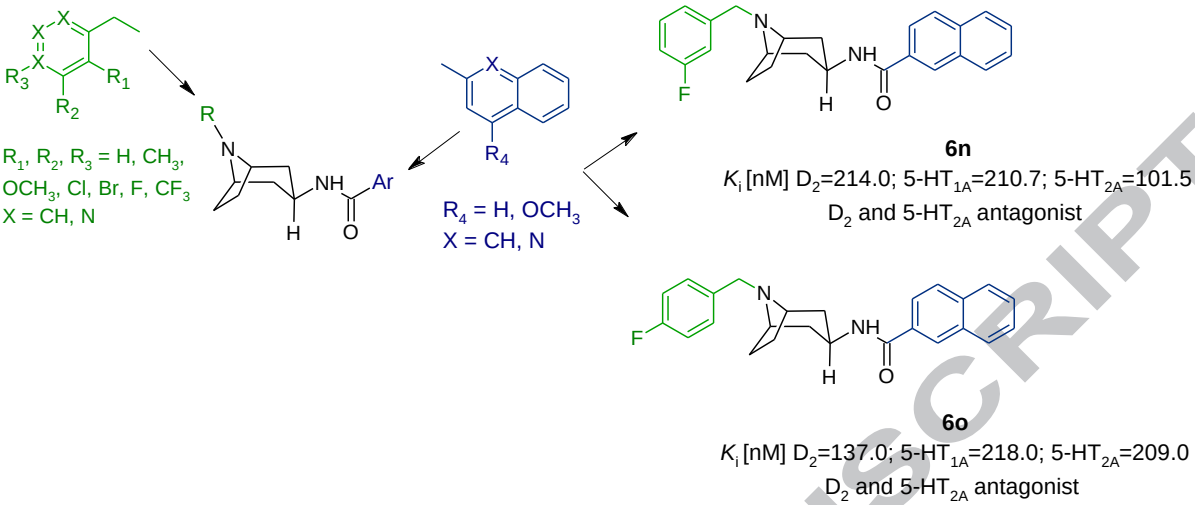
Highlights

- A series of novel 3*β*-aminotropane derivatives was synthesised.
- The ligands had nanomolar to micromolar K_i for 5-HT_{1A}, 5-HT_{2A}, D_2 receptors.
- Affinity for D_2 receptors declined in the series: *p*- < *m*- < *o*-substituted ligands.
- *para*-substitution in the benzene ring decreased affinity for 5-HT_{1A} receptors.
- *meta*-substituted ligands had the highest binding affinities for 5-HT_{2A} receptors.



Graphical Abstract. General structure of synthesized compounds.