Pyridinyl- and pyridazinyl-3,6-diazabicyclo[3.1.1]heptane-anilines: novel selective ligands with subnanomolar affinity for $\alpha_4\beta_2$ nACh receptors.

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ABSTRACT

The cholinergic pathways in the central nervous system (CNS) of animals and humans are important for cognitive and behavioural functions. Until a few years ago, it was thought that the key molecules transducing the cholinergic message were the metabotropic muscarinic receptors, but it is now known that ionotropic neuronal nicotinic receptors (nAChRs) are also involved. Based on recent studies, we prepared a small library of novel 3-substituted-3,6-diazabicyclo[3.1.1]heptanes, whose binding activity and functionality have been assayed.

Among the synthesized compounds, the 3-(anilino)pyridine series resulted in the most interesting compounds with $\alpha_4\beta_2$ K_i values ranging from 0.0225 nM (**12g**) to 2.06 nM (**12o**).

Keywords: 3,6-diazabicyclo[3.1.1]heptanes, synthesis, neuronal nicotinic acetylcholine receptors, $\alpha_4\beta_2$ selectivity, agonism.

1. Introduction

Acetylcholine (ACh) was the first neurotransmitter discovered whose actions are mediated by two different cholinergic receptors, the metabotropic muscarinic acetylcholine receptors (mAChRs) and the ionotropic nicotinic acetylcholine receptors (nAChRs). The former belong to the superfamily of G protein-coupled transmembrane receptors (GPCRs), whose activation triggers a cascade of intracellular reactions mediated by the release of a second messenger, whereas the latter belong to the superfamily of ion channels, and mediate the fast synaptic response following interaction with ACh. To date, nAChR are the best characterized and consist of five subunits arranged symmetrically to delimit a channel through which the flow of cations (Na^+, K^+, Ca^{2+}) occurs. Eleven neuronal nAChR subunits have been identified in mammals: α_2- α_7, α_9, α_{10}; and β_2- β_4 which form pentameric receptors consisting of α_7 or α_9 alone, a combination of α_9 - α_{10} subunits or various combinations of α and β subunits (α_2- α_6 and β_2- β_4, heteromeric receptors) [1,2]. The most expressed subtypes in mammalian brain are the heteromeric $\alpha_4\beta_2$* receptors (*indicate the possibility of additional subunits) and the homomeric α_7 receptors, although some specific areas also contain $\alpha_3\beta_4$* and the $\alpha_6\beta_2$* subtypes [1,2].

At the presynaptic axon terminals, the nAChRs modulate the release of several neurotransmitters (acetylcholine, noradrenaline, dopamine, glutamate and GABA) [3,4]. Since the activation of nAChRs by ACh can modulate a number of physiological processes, nAChRs are involved in several pathological conditions such as inflammation [5], cancer [6] and central nervous system disorders [7].

It's well documented that nAChRs are the target of action of two natural compounds: (*S*)-Nicotine and epibatidine (**Fig. 1**). The former is assumed as the reference $\alpha\square\beta\square$ nAChR agonist in clinical trials and the second showed a high $\alpha_4\beta_2$ affinity (K_i = 0.045 nM). However, due to the lack of selectivity towards receptor subtypes, both are used only as the reference $\alpha\square\beta\square$ nAChR agonists in medicinal chemistry [8,9].

INSERT FIG.1

Bridged piperazines represent an unusual pharmacophore for nicotinic ligands [10]. The introduction of the 3,8-diazabicyclo[3.2.1]octane core provided agonists **1-3** (**Fig. 2**) which possessed a high $\alpha_4\beta_2$ affinity and a powerful analgesic activity due to the activation of central nAChRs [11].

Abbott's investigation of structures typified by the 2,5-diazabicyclo[2.2.1]heptane motif (**Fig. 2**) led to the enantiomers **(*R,R*)-4** and **(*S,S*)-4** and the chlorinated analogue **5**, which is exceptionally powerful and possesses a broad spectrum of analgesic properties in several animal models for acute thermal nociception, in persistent and neuropathic pain. Unfortunately, its inadequate pharmacokinetic properties, and poor penetration into the CNS, have precluded the clinical evaluation for this compound [12]. However, this new series of derivatives allowed us to hypothesize a new pharmacophore model characterized by a nitrogen atom protonated that forms a hydrogen bridge with the Trp-149, an interaction of the CH adjacent to the pyridine nitrogen and a site of the binding pocket of the receptor, cation-π interaction between an aromatic residue (Trp-149) of the binding site of the receptor and the nitrogen atom of the protonated ligand, and a hydrogen bond between the pyridine nitrogen and one molecule of H_2O, held in that position by hydrogen bonds with Leu-119 and Asn-107 [13].

INSERT FIG. 2

The interesting structural variant represented by the 2,5-diazabicyclo[2.2.1]heptane core and the hypothesis of a new pharmacophore model for potential ligands of type nAChR $\alpha_4\beta_2$ and α_7 led us to design and synthesize a series of compounds with a 3,6-diazabicyclo[3.1.1]heptane structure typified by derivatives **6-11** (**Fig. 3**), some of which showed a high affinity and selectivity for the $\alpha_4\beta_2$ subtype receptor [14].

INSERT FIG. 3

Among these new derivatives, compounds bearing a halogen or an aryl ring on pyridine system, showed binding affinity comparable to that of epibatidine and its homologue **5**. Therefore,

we designed the synthesis and biological evaluation of new selective ligands for nAChRs based on the 3,6-diazabicyclo[3.1.1]heptane system.

In this work three different series of 3,6-diazabicyclo[3.1.1]heptanes, reported in **Table 1**, have been synthesized, in which modulation of the substituents on the pyridine ring were evaluated for affinity and selectivity towards nAChRs.

2. Results and discussion

2.1. Chemistry

The chemistry leading from compound **15** to Boc-protected derivatives **16-18**, which serve as starting compounds for next synthetic steps, was previously reported [15]. The syntheses of the derivatives **12a-o, 13a-o** and **14a-o** was performed by a coupling reaction starting from a suitable aniline (**19-33**) and **16**, **17** or **18** followed by *N*-Boc deprotection of aryl-heteroaryl derivatives **34-78** (**scheme 1**).

INSERT SCHEME 1

2.2. nAChR binding affinities

With the aim to evaluate the effect of the introducing a nitrogen spacer in the 3-(5-arylpyridin-3-yl)-3,6-diazabicyclo[3.1.1]heptane series previously reported [15], we synthesized three new series of derivatives: 3-(anilino)pyridines (**12a-o**), 2-(anilino)pyridines (**13a-o**) and 6-(aniline)piperidines (**14a-o**). In **Table 1** are reported the neuronal nicotinic acetylcholine receptor affinities of newly synthesized compounds and, for comparison, K_i values of reference $\alpha_4\beta_2$ ligand [^{3}H]-Epibatidine and α_7 ligand [^{125}I]α-Bungarotoxine. Results are the average of five independent experiments with three replicates at each concentration.

In general all series showed low affinity for α_7 nAChRs with K_i ranging from 26.9 nM (**12a**) to 111 μM (**14f**).

The series of 3-(anilino)pyridines (**12a-o**) provided the most interesting compounds between the aniline derivatives. Among these, derivative **12g**, bearing a 4-NO_2 group on the benzene ring,

showed the best $\alpha_4\beta_2$ receptor profile with a K_i value of 22.5 pM. The compound bearing a 4-methoxyphenyl (**12c**), resulted in 2.6-fold loss in $\alpha_4\beta_2$ affinity (K_i = 59.8 pM) with respect to **12g**. In general the introduction of a chlorine atom has generated compounds **12e** and **12k** with equivalent affinity (K_i $\alpha_4\beta_2$ = 118 pM and K_i $\alpha_4\beta_2$ = 124 pM, respectively), whereas the double substitution as for compound **12m** resulted in 10-fold loss in $\alpha_4\beta_2$ affinity (K_i = 1.25 nM). The *N*-methyl derivative **12o**, showed a 25-fold reduced $\alpha_4\beta_2$ affinity (K_i = 2.06 nM) as compared with the *N*-demethylated analogue **12a**, suggesting that the aniline NH is involved in an interaction with the nAChRs.

Within the isomeric 2-anilino pyridine series (**13a-o**) the introduction on the aniline ring of both EWG and EDG resulted in compounds with $\alpha_4\beta_2$ affinity in the nanomolar range (K_i = 0.569 - 52.1 nM) and a lower α_7 affinity (K_i = 235 nM – 6.15 μM). In particular, compound 4-CF_3-phenyl substituted **13i** resulted the only compound with a subnanomolar $\alpha_4\beta_2$ affinity (K_i = 0.569 nM). The mono- (**13e**,**j**,**k**) or dichlorination (**13l**,**m**) of the aniline ring resulted in derivatives endowed with a comparable $\alpha_4\beta_2$ affinity (K_i = 2.2 – 2.49 nM).

Finally, the series of 6-anilino piridazine (**14a-o**) showed generally lower affinity values towards both nAChR subtypes. Compound **14i**, bearing a 4-CF_3-phenyl was the most active derivative with an $\alpha_4\beta_2$ binding affinity K_i of 6.9 nM, 12-fold less potent with respect to the deaza-analogue **13i** of the previous series, whereas, the methylation of the aniline nitrogen in **14o** resulted in a loss of affinity towards both receptor subtypes (K_i $\alpha_4\beta_2$ = 1.43 μM, K_i α_7 = 101 μM) compared with the *N*-demethylated analogue **14a** (K_i $\alpha_4\beta_2$ = 441 nM, K_i α_7 = 10.4 μM), strengthening the hypothesis of the involvement of the NH group in the receptor interaction.

The α_7 receptors subtype affinities were in line with the analogous pyridines, showing that the affinity for the α_7 receptor subtype is not affected by the presence of a pyridine or piridazine ring, but by the position of the substituent on the heteroaromatic ring.

INSERT TABLE 1

The $\alpha_3\beta_4$ nAChRs affinity for selected compounds was also evaluated (**Table 2**). In general, derivatives with high $\alpha_4\beta_2$ affinity showed $\alpha_3\beta_4$ K_i values in the pM range, as **12a** (K_i $\alpha_3\beta_4$ = 799 pM,

K_i $\alpha_4\beta_2$ = 80.7 pM), **12g** (K_i $\alpha_3\beta_4$ = 684 pM, K_i $\alpha_4\beta_2$ = 22.5 pM), **12h** (K_i $\alpha_3\beta_4$ = 988 pM, K_i $\alpha_4\beta_2$ = 342 pM) and **13i** (K_i $\alpha_3\beta_4$ = 799 pM, K_i $\alpha_4\beta_2$ = 569 pM). Among selected compounds, the 3-chlorine derivative of the the 3-(anilino)pyridine series, **12k**, resulted the most $\alpha_4\beta_2$ selective compounds (K_i $\alpha_3\beta_4$/ K_i $\alpha_4\beta_2$ = 2362.90), whereas the rest showed $\alpha_4\beta_2$ selectivity ranging from 1.23-fold to 36.79-fold.

INSERT TABLE 2

2.3. Functional activity at the nAChRs

According to their binding $\alpha_4\beta_2$ receptors values, compounds **12c** (K_i = 59.8 pM) and **13g** (K_i = 1.76 nM) have been chosen to determine their agonist activity on human recombinant $\alpha_4\beta_2$, α_7 and $\alpha_3\beta_4$ receptors. This last subunit combination was included in the analysis, given that the selected compounds exhibited a binding affinity for $\alpha_3\beta_4$ receptor in the nM range: K_i $\alpha_3\beta_4$, 2.2 nM and 25 nM, for **12c** and **13g**, respectively. None of these two compound was able to evoke detectable whole-cell currents from transiently transfected GH4C1 cells expressing functional human α_7 nAChRs, as assessed by rapid application of 1 mM ACh. Compound **12c** acted as a partial agonist on both $\alpha_3\beta_4$ and $\alpha_4\beta_2$ nAChRs, with EC_{50} values of 2.90±0.03 μM and 1.90±0.8 μM, nH values of 1.79±0.01 μM and 0.9±0.3 μM, and maximal evoked currents of 42.3±0.8 % and 11±1 % of the maximal current amplitude elicited by 1 mM ACh, respectively (**Fig. 4**). By contrast, compound **13g** was not able to evoke significant response from $\alpha_3\beta_4$ expressing cells and displayed a reduced potency for $\alpha_4\beta_2$ nAChRs, with EC_{50} >10 μM (**Fig. 5**). While the lack of effects of both compounds on α_7 nAChRs was expected, due to the high K_i values for this receptor subtype, their partial agonism on heteromeric nAChRs may appear surprising, basing again on the measured binding affinity values. However, the complex mechanisms linking binding, gating of the channel, affinity and efficacy do not allow to draw functional predictions from binding data [16]. It is worth to highlight that partial agonism is a very relevant feature when considering the therapeutic potential of nAChR-interacting compounds, in particular for smoking cessation [17].

INSERT FIG. 4

INSERT FIG. 5

3. Conclusions

Different series of derivatives endowed with a diazabicyclo[3.1.1]heptane core have been synthesized and tested and the small library of 45 compounds synthesized in this study allowed us to consolidate and further investigate SAR studies on the nicotinic pharmacophore.

In general, the introduction of substituted anilines is tolerated for $\alpha_4\beta_2$ receptor affinity, and in minor extent for $\alpha_3\beta_4$, whereas the same modification seems detrimental for α_7 receptor subtype. All new tested compounds showed an affinity and selectivity for $\alpha_4\beta_2$ receptors with K_i values between 22.5 pM, for compound **12g**, and 1.54 μM, for **14k**; within this library, the most $\alpha_4\beta_2$ selective ligands resulted those of 3-(5-anilinopyridin-3-yl)-3,6-diazabicyclo[3.1.1]heptane series, compounds **12a-o**. In particular, the aniline ring (**12a**) or its 4-OMe (**12c**), 4-F (**12f**) and 4-NO_2 (**12g**), resulted the best substituents. On the other hand, in the homologue series, only derivative **13i** showed an $\alpha_4\beta_2$ K_i value of 0.57 nM.

Moreover, the *N*-methylation of aniline nitrogen also resulted in a substantial decrease of affinity, allowing to assume that the aniline NH plays an important role in receptor binding of these novel templates.

4. Experimental section

4.1. General procedures

All reactions involving air or moisture-sensitive compounds were performed under argon atmosphere. Solvents and reagents were obtained from commercial suppliers and were used without further purification. Microwave irradiation experiments were carried out in a Biotage® Microwave Initiator Eight 2.5 in the standard configuration as delivered, including proprietary software. All

experiments were carried out in sealed microwave process vials under normal or low absorption. After completion of the reaction, the vial was cooled down to 25 °C via air jet cooling before opening. Reaction temperatures were monitored by an IR sensor on the outside wall of the reaction. Hydrogenations were carried out in the 4560 Parr Apparatus using a H_2PEM-100 Parker Balston Hydrogen Generator. Flash column chromatography (FC) was performed automatically on Flash-master (Biotage®) with pre-packed Biotage® SNAP silica gel cartridges or manually on silica gel (Kieselgel 60, 0.040−0.063 mm, Merck®). The progress of all reactions was monitored by thin layer chromatography (TLC) performed with Polygram SIL N-HR/HV_{254} pre-coated plastic sheets (0.2 mm) on aluminum sheets (Kieselgel 60 F254, Merck®). Melting points were obtained on a Köfler melting point apparatus and are uncorrected. IR spectra were recorded as nujol mulls on NaCl plates with a Jasco FT/IR 460 plus spectrophotometer and are expressed in ν (cm^{-1}). NMR experiments were run on a Varian Unity 200 spectrometer (200.07 MHz for ^{1}H, and 50.31 MHz for ^{13}C). Spectra were acquired using deuterated chloroform (chloroform-d) as solvent. Chemical shifts (δ) for ^{1}H- and ^{13}C-NMR spectra are reported in parts per million (ppm) using the residual non-deuterated solvent resonance as the internal standard (for chloroform-d: 7.26 ppm, ^{1}H and 77.16 ppm, ^{13}C; for DMSO-d_6: 2.50 ppm, ^{1}H, 39.52 ppm, ^{13}C). Data are reported as follows: chemical shift (sorted in descending order), multiplicity (s for singlet, bs for broad singlet, d for doublet, t for triplet, q for quadruplet, m for multiplet), integration and coupling constants (J) in Hertz (Hz). All final compounds displayed ≥ 95% purity as determined by elemental analysis on a Perkin-Elmer 240-B analyser, for C, H, and N. Unless otherwise specified, all materials, solvents, reagents and precursors **19-33** were obtained from commercial suppliers. Syntheses of compounds **15-18** have been previously reported [14, 15].

*4.1.1. General procedure for the synthesis of anilines derivatives **34 – 78***

To a solution of the haloderivative **16**, **17** or **18** (0.231 mmol) in toluene (3 mL), KO*t*Bu (39 mg, 0.347 mmol), $Pd_2(dba)_3$ (5 mg, 0.005 mmol), Xantophos (8 mg, 0.014 mmol) and the

appropriate aniline **19-33** (0.347 mmol) were added. The mixture was reacted under MW irradiation (0.5-2 h, 130 °C, low abs). The suspension was taken up with EtOAc, washed (H_2O), dried (Na_2SO_4) and concentrated *in vacuum*. The analytically pure product was isolated by FC as indicated below.

*4.1.2. tert-Butyl 3-(5-anilinopyridin-3-yl)-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**34**)*

Title compound was prepared starting from **16** and aniline **19**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **34**. White solid; yield 75% (0.06 g, 0.17 mmol); mp 182-184 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.50 (d, 1H, J = 8.8 Hz), 2.58-2.77 (m, 1H), 3.26 (d, 2H, J =10.2), 3.78-4.07 (m, 2H), 4.38 (d, 2H, J = 5.8 Hz), 5.68 (bs, 1H, NH exch. with D_2O), 6.73 (t, 1H, J = 2.6 Hz), 6.97 (t, 1H, J = 7.2 Hz), 7.10 (d, 2H, J = 7.6Hz), 7.32 (d, 2H, J = 7.2 Hz), 7.70-7.90 (m, 2H). Elemental analysis calculated (%) for $C_{21}H_{26}N_4O_2$: C 68.83, H 7.15, N 15.29. Found: C 68.86, H 7.16, N 15.31.

*4.1.3. tert-Butyl 3-[5-(4-methylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**35**)*

Title compound was prepared starting from **16** and aniline **20**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **35**. White solid; yield 80% (52 mg); mp 172-175 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.49 (d, 1H, J = 8.0 Hz), 2.32 (s, 3H), 2.78-2.94 (m, 1H), 3.25 (d, 2H, J = 10.2 Hz), 3.80-3.98 (m, 2H), 4.20-4.36 (m, 2H), 5.58 (bs, 1H, NH exch. with D_2O), 6.66 (s, 1H), 7.02 (d, 1H, J = 8.6 Hz), 7.11 (d, 2H, J = 8.2Hz), 7.70 (s, 1H), 7.78 (s, 1H). Elemental analysis calculated (%) for $C_{22}H_{28}N_4O_2$: C 69.45, H 7.42, N 14.73. Found: C 69.49, H 7.44, N 14.75.

*4.1.4. tert-Butyl 3-[5-(4-methoxylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**36**)*

Title compound was prepared starting from **16** and aniline **21**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **36**. Pink solid; yield 77% (53 mg); mp 185-187 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.48 (d, 1H, J = 8.6 Hz), 2.52-2.78 (m, 1H), 3.24 (d, 2H, J = 11.0 Hz), 3.81 (s, 3H), 3.82-4.00 (m, 2H), 4.27 (d, 2H, J = 5.6 Hz), 5.47 (bs, 1H, NH exch. with D_2O), 6.52 (s, 1H), 6.88 (d, 2H, J = 9.0 Hz), 7.10 (d, 2H, J = 8.8 Hz), 7.60-7.80 (m, 2H). Elemental analysis calculated (%) for $C_{22}H_{28}N_4O_3$: C 66.64, H 7.12, N 14.13. Found: C 66.67, H 7.13, N 14.15.

*4.1.5. tert-Butyl 3-[5-(4-ethoxylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**37**)*

Title compound was prepared starting from **16** and aniline **22**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **37**. Phink solid; yield 97% (92 mg); mp 179-180 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.42 (s, 3H), 1.48 (d, 1H, J = 8.6 Hz), 2.55-2.78 (m, 1H), 3.23 (d, 2H, J = 10.2 Hz), 3.77-3.90 (m, 2H), 4.03 (q, 2H, J = 7.4 Hz), 4.26 (d, 2H, J = 5.2 Hz), 5.47 (bs, 1H, NH exch. with D_2O), 6.51 (s, 1H), 6.87 (d, 2H, J = 8.8 Hz), 7.08 (d, 2H, J = 8.8Hz), 7.68 (d, 2H, J = 8.8 Hz). Elemental analysis calculated (%) for $C_{23}H_{30}N_4O_3$: C 67.29, H 7.37, N 13.65. Found: C 67.34, H 7.39, N 13.67.

*4.1.6. tert-Butyl 3-[5-(4-chloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**38**)*

Title compound was prepared starting from **16** and aniline **23**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **38**. Beige solid; yield 91% (84 mg); mp 165-170 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.50 (d, 1H, J = 8.4 Hz), 2.60-2.75 (m, 1H), 3.25 (d, 2H, J = 10.2 Hz), 3.80-4.00 (m, 2H), 4.29 (d, 2H, J = 5.6 Hz), 5.68 (bs, 1H, NH exch. with D_2O), 6.67 (s, 1H), 7.02 (d, 2H, J = 8.8 Hz), 7.24 (d, 2H, J = 9.2 Hz), 7.70-7.85 (m, 2H). Elemental

analysis calculated (%) for $C_{21}H_{25}ClN_4O_2$: C 62.91, H 6.29, N 13.98. Found: C 63.98, H 6.31, N 14.27.

4.1.7. tert-Butyl 3-[5-(4-fluoroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate ***(39)***

Title compound was prepared starting from **16** and aniline **24**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **39**. White solid; yield 88% (58 mg); mp 199-202 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.49 (d, 1H, J = 8.6 Hz), 2.56-2.77 (m, 1H), 3.25 (d, 2H, J = 10.2 Hz), 3.73-4.02 (m, 2H), 4.27 (d, 2H, J = 5.4 Hz), 5.57 (bs, 1H, NH exch. with D_2O), 6.58 (s, 1H), 6.87-7.18 (m, 4H), 7.70-7.83 (m, 2H). Elemental analysis calculated (%) for $C_{21}H_{25}FN_4O_2$: C 65.61, H 6.55, N 14.57. Found: C 65.70, H 6.57, N 14.59.

4.1.8. tert-Butyl 3-[5-(4-nitroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate ***(40)***

Title compound was prepared starting from **16** and aniline **25**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **40**. Yellowish solid; yield 82% (78 mg); mp 227-230 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.53 (d, 1H, J = 9.0 Hz), 2.60-2.80 (m, 1H), 3.30 (d, 2H, J = 10.6 Hz), 3.80-4.02 (m, 2H), 4.31 (d, 2H, J = 5.0 Hz), 6.28 (bs, 1H, NH exch. with D_2O), 6.81 (s, 1H), 6.98 (d, 2H, J = 9.0 Hz), 7.97 (s, 2H), 8.15 (d, 2H, J = 9.2 Hz). Elemental analysis calculated (%) for $C_{21}H_{25}N_5O_4$: C 61.30, H 6.12, N 17.02. Found: C 61.39, H 6.13, N 17.09.

4.1.9. tert-Butyl 3-[5-(4-hydroxyanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate ***(41)***

Title compound was prepared starting from **16** and aniline **26**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **41**. Red solid; yield 46% (41 mg); mp 68-70 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.38 (bs, 9H), 1.48 (d, 1H, J = 8.0 Hz), 2.57-2.78 (m, 2H, OH exch.

with D_2O), 3.25 (d, 2H, J = 10.0 Hz), 3.75-4.00 (m, 2H), 4.15-4.37 (m, 2H), 5.50 (bs, 1H, NH exch. with D_2O), 6.46 (s, 1H), 6.85 (d, 2H, J = 8.8 Hz), 7.03 (d, 2H, J = 8.8 Hz), 7.58-7.78 (m, 2H). Elemental analysis calculated (%) for $C_{21}H_{26}N_4O_3$: C 69.95, H 6.85, N 14.65. Found: C 70.47, H 6.88, N 14.69.

*4.1.10. tert-Butyl 3-[5-(4-trifluoromethylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**42**)*

Title compound was prepared starting from **16** and aniline **27**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **42**. Yellowish solid; yield 55% (55 mg); mp 95-97 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.51 (d, 1H, J = 8.6 Hz), 2.60-2.78 (m, 1H), 3.28 (d, 2H, J = 10.6 Hz), 3.77-4.05 (m, 2H), 4.30 (d, 2H, J = 6.2 Hz), 5.89 (bs, 1H, NH exch. with D_2O), 6.77 (s, 1H), 7.08 (d, 2H, J = 8.4 Hz), 7.50 (d, 2H, J = 7.4 Hz), 7.89 (d, 2H, J = 7.0 Hz). Elemental analysis calculated (%) for $C_{22}H_{25}F_3N_4O_2$: C 60.82, H 5.80, N 12.90. Found: C 61.26, H 5.82, N 12.97.

*4.1.12. tert-Butyl 3-[5-(2-chloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**43**)*

Title compound was prepared starting from **16** and aniline **28**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **43**. Beige solid; yield 70% (65 mg); mp 71-72 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.38 (bs, 9H), 1.51 (d, 1H, J = 6.0 Hz), 2.60-2.80 (m, 1H), 3.29 (d, 2H, J = 10.6 Hz), 3.80-4.10 (m, 2H), 4.29 (d, 2H, J = 4.6 Hz), 6.03 (bs, 1H, NH exch. with D_2O), 6.70-6.79 (m, 1H), 6.85 (t, 1H, J = 7.4 Hz), 7.15 (t, 1H, J = 8.4Hz), 7.18-7.45 (m, 2H), 7.80-7.87 (m, 1H), 7.88-7.96 (m, 1H). Elemental analysis calculated (%) for $C_{21}H_{25}ClN_4O_2$: C 62.91, H 6.29, N 13.98. Found: C 63.70, H 6.31, N 14.22.

*4.1.13. tert-Butyl 3-[5-(3-chloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**44**)*

Title compound was prepared starting from **16** and aniline **29**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **44**. Beige solid; yield 98% (91 mg); mp 170-174 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.50 (d, 1H, J = 8.6 Hz), 2.58-2.75 (m, 1H), 3.27 (d, 2H, J = 10.4 Hz), 3.75-4.08 (m, 2H), 4.30 (d, 2H, J = 6.8 Hz), 5.78 (bs, 1H, NH scambia con D_2O), 6.72 (s, 1H), 6.87-7.00 (m, 2H), 7.06 (s, 1H), 7.10-7.24 (m, 1H), 7.83 (d, 2H, J = 4.8 Hz). Elemental analysis calculated (%) for $C_{21}H_{25}ClN_4O_2$: C 62.91, H 6.29, N 13.98. Found: C 63.75, H 6.32, N 14.27.

*4.1.14. tert-Butyl 3-[5-(2,4-dichloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**45**)*

Title compound was prepared starting from **16** and aniline **30**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **45**. Beige solid; yield 82% (82 mg); mp 85-89 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.26-1.55 (m, 10H), 2.60-2.70 (m, 1H), 3.27 (d, 2H, J = 10.6 Hz), 3.75-4.10 (m, 2H), 4.20 (d, 2H, J = 5.6 Hz), 5.97 (bs, 1H, NH exch. with D_2O), 6.72 (s, 1H), 7.05-7-23 (m, 2H), 7.30-7.45 (m, 1H), 7.80-7.98 (m, 2H). Elemental analysis calculated (%) for $C_{21}H_{24}Cl_2N_4O_2$: C 57.94, H 5.56, N 12.87. Found: C 58.15, H 5.58, N 12.95.

*4.1.15. tert-Butyl 3-[5-(3,4-dichloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**46**)*

Title compound was prepared starting from **16** and aniline **31**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **46**. Beige solid; yield 68% (68 mg); mp 147-150 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.51 (d, 1H, J = 9.0 Hz), 2.60-2.75 (m, 1H), 3.27 (d, 2H, J = 10.4 Hz), 3.78-4.07 (m, 2H), 4.29 (d, 2H, J = 5.6 Hz), 5.78 (bs, 1H, NH exch. with D_2O), 6.68 (s, 1H), 6.82-6.97 (m, 1H), 7.15 (s, 1H), 7.33 (s, 1H), 7.84 (s, 2H). Elemental analysis calculated (%) for $C_{21}H_{24}Cl_2N_4O_2$: C 57.94, H 5.56, N 12.87. Found: C 58.19, H 5.59, N 12.99.

*4.1.16. tert-Butyl 3-[5-(3,4-methylendioxyanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**47**)*

Title compound was prepared starting from **16** and aniline **32**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **47**. Red solid; yield 95% (90 mg); mp 178-180 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.48 (d, 1H, J = 8.4 Hz), 2.58-2.75 (m, 1H), 3.24 (d, 2H, J = 10.0 Hz), 3.80-4.00 (m, 2H), 4.20-4.38 (m, 2H), 5.50 (bs, 1H, NH exch. with D_2O), 5.96 (s, 2H), 6.50-6.64 (m, 2H), 6.66-6.80 (m, 2H), 7.60-7.76 (m, 2H). Elemental analysis calculated (%) for $C_{22}H_{26}N_4O_2$: C 64.37, H 6.38, N 13.65. Found: C 64.49, H 6.40, N 13.69.

*4.1.17. tert-Butyl 3-[5-(N-methylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**48**)*

Title compound was prepared starting from **16** and aniline **33**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **48**. White solid; yield 83% (73 mg); mp 92-94 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.48 (d, 1H, J = 8.6 Hz), 2.58-2.75 (m, 1H), 3.23 (d, 2H, J = 10.2 Hz), 3.33 (s, 3H), 3.75-4.02 (m, 2H), 4.27 (d, 2H, J = 5.8 Hz), 6.56 (s, 1H), 6.85-7.18 (m, 3H), 7.32 (d, 2H, J = 8.4 Hz), 7.77 (s, 2H). Elemental analysis calculated (%) for $C_{22}H_{28}N_4O_2$: C 69.45, H 7.42, N 14.73. Found: C 69.63, H 7.44, N 14.77.

*4.1.18. tert-Butyl 3-(6-anilinopyridin-3-yl)-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**49**)*

Title compound was prepared starting from **17** and aniline **19**. The residue was purified by FC (petroleum ether/EtOAc 7:3) to afford **49**. Yellowish solid; yield 48% (29 mg); mp 158-160 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.52 (d, 1H, J = 8.2 Hz), 2.60-2.80 (m, 1H), 3.25 (d, 2H, J = 10.2 Hz), 3.78-4.07 (m, 2H), 4.29 (d, 2H, J = 5.4 Hz), 6.19 (bs, 1H, NH exch. with D_2O), 6.85-7.07 (m, 3H), 7.16-7.40 (m, 4H), 7.70-7.88 (m, 1H). Elemental analysis calculated (%) for $C_{21}H_{26}N_4O_2$: C 68.83, H 7.15, N 15.29. Found: C 68.91, H 7.17, N 15.34.

*4.1.19. tert-Butyl 3-[6-(4-methylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**50**)*

Title compound was prepared starting from **17** and aniline **20**. The residue was purified by FC (petroleum ether/EtOAc 1:1) to afford **50**. Yellowish solid; yield 71% (62 mg); mp 155-158 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.52 (d, 1H, J = 8.2 Hz), 2.30 (s, 3H), 2.58-2.68 (m, 1H), 3.25 (d, 2H, J = 9.6 Hz), 3.77-4.05 (m, 2H), 4.20-4.37 (m, 2H), 6.12 (bs, 1H, NH exch. with D_2O), 6.89 (d, 2H, J = 9.0 Hz), 6.93-7.18 (m, 5H), 7.70-7.87 (m, 1H). Elemental analysis calculated (%) for $C_{22}H_{28}N_4O_2$: C 69.45, H 7.42, N 14.73. Found: C 69.51, H 7.43, N 14.77.

*4.1.20. tert-Butyl 3-[6-(4-methoxylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**51**)*

Title compound was prepared starting from **17** and aniline **21**. The residue was purified by FC (petroleum ether/EtOAc 7:3) to afford **51**. Orange solid; yield 61% (56 mg); mp 144-146 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.52 (d, 1H, J = 8.4 Hz), 2.50-2.70 (m, 1H), 3.24 (d, 2H, J = 10.0), 3.79 (s, 3H), 3.80-4.00 (m, 2H), 4.20-4.40 (m, 2H), 6.03 (bs, 1H, NH scambia con D_2O), 6.76 (d, 1H, J = 8.8 Hz), 6.86 (d, 2H, J = 9.2 Hz), 6.99 (dd, 1H, J_o = 2.8 Hz, J_m = 9.2 Hz), 7.17 (d, 2H, J = 8.8 Hz), 7.65-7.80 (m, 1H). Elemental analysis calculated (%) for $C_{22}H_{28}N_4O_3$: C 66.64, H 7.12, N 14.13. Found: C 66.69, H 7.13, N 14.17.

*4.1.21. tert-Butyl 3-[6-(4-ethoxylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**52**)*

Title compound was prepared starting from **17** and aniline **22**. The residue was purified by FC (petroleum ether/EtOAc 7:3) to afford **52**. Orange solid; yield 70% (66 mg); mp 133-135 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.41 (t, 3H, J = 7.0 Hz), 1.52 (d, 1H, J = 8.4 Hz), 2.55-2.80 (m, 1H), 3.24 (d, 2H, J = 9.78), 3.75-3.90 (m, 2H), 4.01 (q, 2H, J = 7.2 Hz), 4.20-4.26 (m, 2H), 6.00 (bs, 1H, NH exch. with D_2O), 6.76 (d, 1H, J = 8.8 Hz), 6.86 (d, 2H, J = 9.0 Hz), 6.98 (dd,

1H, J_o = 3.2 Hz, J_m = 9.0 Hz), 7.16 (d, 2H, J = 8.8 Hz), 7.73 (d, 1H, J = 3.0 Hz). Elemental analysis calculated (%) for $C_{23}H_{30}N_4O_3$: C 67.29, H 7.37, N 13.65. Found: C 67.35, H 7.40, N 13.68.

4.1.22. tert-Butyl 3-[6-(4-chloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate ***(53)***

Title compound was prepared starting from **17** and aniline **23**. The residue was purified by FC (petroleum ether/EtOAc 8:2) to afford **53**. Red solid; yield 82% (76 mg); mp 176-179 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.36 (bs, 9H), 1.52 (d, 1H, J = 8.2 Hz), 2.60-2.80 (m, 1H), 3.25 (d, 2H, J = 10.4 Hz), 3.80-4.00 (m, 2H), 4.29 (d, 2H, J = 5.2 Hz), 6.19 (bs, 1H, NH exch. with D_2O), 6.86 (d, 1H, J = 9.6 Hz), 7.02 (d, 1H, J = 5.8 Hz), 7.16-7.23 (m, 4H), 7.70-7.85 (m, 1H). Elemental analysis calculated (%) for $C_{21}H_{25}ClN_4O_2$: C 62.91, H 6.29, N 13.98. Found: C 63.91, H 6.30, N 14.15.

4.1.23. tert-Butyl 3-[6-(4-fluoroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate ***(54)***

Title compound was prepared starting from **17** and aniline **24**. The residue was purified by FC (petroleum ether/EtOAc 7:3) to afford **54**. Yellowish solid; yield 88% (78 mg); mp 163-166 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.52 (d, 1H, J = 8.6 Hz), 2.58-2.75 (m, 1H), 3.24 (d, 2H, J = 10.6 Hz), 3.70-4.10 (m, 2H), 4.28 (d, 2H, J = 5.2 Hz), 6.10 (bs, 1H, NH exch. with D_2O), 6.81 (d, 1H, J = 9.0 Hz), 6.90-7.08 (m, 3H), 7.10-7.28 (m, 2H), 7.70-7.85 (m, 1H). Elemental analysis calculated (%) for $C_{21}H_{25}FN_4O_2$: C 65.61, H 6.55, N 14.57. Found: C 65.71, H 6.56, N 14.60.

4.1.24. tert-Butyl 3-[6-(4-nitroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate ***(55)***

Title compound was prepared starting from **17** and aniline **25**. The residue was purified by FC (petroleum ether/EtOAc 7:3) to afford **55**. Red solid; yield 84% (80 mg); mp 218-220 °C. ^{1}H-

NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.45-1.60 (m, 1H), 2.60-2.80 (m, 1H), 3.30 (d, 2H, J = 10.0 Hz), 3.80-4.10 (m, 2H), 4.20-4.40 (m, 2H), 6.73 (bs, 1H, NH exch. with D_2O), 6.95 (d, 1H, J = 9.4 Hz), 7.00-7.20 (m, 1H), 7.32 (d, 2H, J = 9.0 Hz), 7.80-8.00 (m, 1H), 8.15 (d, 2H, J = 9.2 Hz). Elemental analysis calculated (%) for $C_{21}H_{25}N_5O_4$: C 61.30, H 6.12, N 17.02. Found: C 61.37, H 6.13, N 17.08.

*4.1.25. tert-Butyl 3-[6-(4-hydroxyanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**56**)*

Title compound was prepared starting from **17** and aniline **26**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **56**. Red solid; yield 64% (56 mg); mp 94-96 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.52 (d, 1H, J = 8.2 Hz), 2.50-2.80 (m, 2H, OH exch. with D_2O), 3.23 (d, 2H, J = 10.2 Hz), 3.75-4.00 (m, 2H), 4.15-4.35 (m, 2H), 6.07 (bs, 1H, NH exch. with D_2O), 6.75 (d, 2H, J = 8.0 Hz), 6.78-6.85 (m, 1H), 6.90-7.03 (m, 1H), 7.07 (d, 2H, J = 9.0 Hz), 7.60-7.20 (m, 1H). Elemental analysis calculated (%) for $C_{21}H_{26}N_4O_3$: C 69.95, H 6.85, N 14.65. Found: C 70.42, H 6.86, N 14.67.

*4.1.26. tert-Butyl 3-[6-(4-trifluoromethylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**57**)*

Title compound was prepared starting from **17** and aniline **27**. The residue was purified by FC (petroleum ether/EtOAc 7:3) to afford **57**. Yellowish solid; yield 40% (40 mg); mp 156-160 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.52 (d, 1H, J = 8.0 Hz), 2.60-2.80 (m, 1H), 3.27 (d, 2H, J = 10.2 Hz), 3.78-4.10 (m, 2H), 4.20-4.45 (m, 2H), 6.41 (bs, 1H, NH exch. with D_2O), 6.93 (d, 1H, J = 8.8 Hz), 7.00-7.12 (m, 1H), 7.28 (d, 2H, J = 10.2 Hz), 7.48 (d, 2H, J=8.2 Hz), 7.75-7.95 (m, 1H). Elemental analysis calculated (%) for $C_{22}H_{25}F_3N_4O_2$: C 60.82, H 5.80, N 12.90. Found: C 61.30, H 5.83, N 12.98.

*4.1.27. tert-Butyl 3-[6-(2-chloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**58**)*

Title compound was prepared starting from **17** and aniline **28**. The residue was purified by FC (petroleum ether/EtOAc 8:2) to afford **58**. Yellowish solid; yield 65% (60 mg); mp 170-172 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.53 (d, 1H, J = 8.2 Hz), 2.60-2.80 (m, 1H), 3.27 (d, 2H, J = 10.0 Hz), 3.80-4.10 (m, 2H), 4.20-4.40 (m, 2H), 6.60 (bs, 1H, NH exch. with D_2O), 6.28 (t, 1H, J = 6.6 Hz), 6.94 (d, 1H, J = 8.8 Hz), 7.05 (dd, 1H, J_o =2.6 Hz, J_m = 9.2 Hz), 7.18 (t, 2H, J = 7.4 Hz), 7.34 (d, 1H, J = 7.8 Hz), 7.75-7.95 (m, 2H). Elemental analysis calculated (%) for $C_{21}H_{25}ClN_4O_2$: C 62.91, H 6.29, N 13.98. Found: C 63.72, H 6.32, N 14.24.

*4.1.28. tert-Butyl 3-[6-(3-chloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**59**)*

Title compound was prepared starting from **17** and aniline **29**. The residue was purified by FC (petroleum ether/EtOAc 7:3) to afford **59**. Yellowish solid; yield 74% (68 mg); mp 175-178 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.53 (d, 1H, J = 8.2 Hz), 2.60-2.80 (m, 1H), 3.27 (d, 2H, J = 10.4 Hz), 3.80-4.10 (m, 2H), 4.30 (d, 2H, J = 4.4 Hz), 6.55 (bs, 1H, NH exch. with D_2O), 6.87 (d, 1H, J = 9.2 Hz), 7.00-7.22 (m, 2H), 7.28-7.40 (m, 1H), 7.75-7.85 (m, 1H), 7.88 (d, 1H, J = 8.8 Hz). Elemental analysis calculated (%) for $C_{21}H_{25}ClN_4O_2$: C 62.91, H 6.29, N 13.98. Found: C 64.15, H 6.41, N 14.43.

4.1.29. *tert*-Butyl 3-[6-(2,4-dichloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**60**)

Title compound was prepared starting from **17** and aniline **30**. The residue was purified by FC (petroleum ether/EtOAc 7:3) to afford **60**. Yellowish solid; yield 64% (64 mg); mp 142-146 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.26-1.55 (m, 10H), 2.60-2.70 (m, 1H), 3.27 (d, 2H, J = 10.6 Hz), 3.75-4.10 (m, 2H), 4.20 (d, 2H, J = 5.6 Hz), 5.97 (bs, 1H, NH exch. with D_2O), 6.72 (s, 1H), 7.05-7-

23 (m, 2H), 7.30-7.45 (m, 1H), 7.80-7.98 (m, 2H). Elemental analysis calculated (%) for $C_{21}H_{24}Cl_2N_4O_2$: C 57.94, H 5.56, N 12.87. Found: C 58.15, H 5.58, N 12.95.

4.1.30. tert-Butyl 3-[6-(3,4-dichloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**61**)

Title compound was prepared starting from **17** and aniline **31**. The residue was purified by FC (petroleum ether/EtOAc 7:3) to afford **61**. White solid; yield 72% (72 mg); mp 210-213 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.52 (d, 1H, J = 8.6 Hz), 2.60-2.80 (m, 1H), 3.26 (d, 2H, J = 10.2 Hz), 3.80-4.05 (m, 2H), 4.29 (d, 2H, J = 5.6 Hz), 6.22 (bs, 1H, NH exch. with D_2O), 6.84 (d, 1H, J = 9.2 Hz), 7.00-7.15 (m, 2H), 7.28 (d, 1H, J = 8.4 Hz), 7.49 (d, 1H, J = 2.8 Hz), 7.81 (d, 1H, J = 2.4 Hz). Elemental analysis calculated (%) for $C_{21}H_{24}Cl_2N_4O_2$: C 57.94, H 5.56, N 12.87. Found: C 58.32, H 5.61, N 13.64.

*4.1.31. tert-Butyl 3-[6-(3,4-methylendioxyanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**62**)*

Title compound was prepared starting from **17** and aniline **32**. The residue was purified by FC (petroleum ether/EtOAc 1:1) to afford **62**. Beige solid; yield 90% (85 mg); mp 130-132 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.52 (d, 1H, J = 8.6 Hz), 2.58-2.70 (m, 1H), 3.24 (d, 2H, J = 10.0 Hz), 3.80-3.90 (m, 2H), 4.20-4.36 (m, 2H), 5.93 (s, 2H), 6.02 (bs, 1H, NH exch. with con D_2O), 6.62 (dd, 1H, J_o = 2.2 Hz, J_m = 8.4 Hz), 6.70-6.83 (m, 3H), 6.99 (dd, 1H, J_o = 2.6 Hz, J_m = 8.8 Hz), 7.70-7.74 (m, 1H). Elemental analysis calculated (%) for $C_{22}H_{26}N_4O_4$: C 64.37, H 6.38, N 13.65. Found: C 65.47, H 6.51, N 13.78.

*4.1.32. tert-Butyl 3-[6-(N-methylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**63**)*

Title compound was prepared starting from **17** and aniline **33**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **63**. White solid; yield 91% (80 mg); mp 122-124 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.51 (d, 1H, J = 8.4 Hz), 2.60-2.80 (m, 1H), 3.25 (d, 2H, J = 9.4 Hz), 3.43 (s, 3H), 3.80-4.00 (m, 2H), 4.20-4.35 (m, 2H), 6.80 (d, 1H, J = 9.0 Hz), 6.85-7.05 (m, 2H), 7.12 (d, 2H, J = 8.2 Hz), 7.32 (d, 2H, J = 8.4 Hz), 7.80-7.90 (m, 1H). Elemental analysis calculated (%) for $C_{22}H_{28}N_4O_2$: C 69.45, H 7.42, N 14.73. Found: C 70.48, H 7.51, N 15.62.

*4.1.33. tert-Butyl 3-(6-anilinopyridazin-3-yl)-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**64**).*

Title compound was prepared starting from **18** and aniline **19**. The residue was purified by FC (petroleum ether/EtOAc 1:1) to afford **64**. Beige solid; yield 98% (83 mg); mp 122-125 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.52 (d, 1H, J = 8.8 Hz), 2.60-2.80 (m, 1H), 3.38-3.57 (m, 2H), 4.15 (d, 2H, J = 12.0 Hz), 4.31 (d, 2H, J = 4.6 Hz), 6.44 (bs, 1H, NH exch. with D_2O), 6.82 (d, 1H, J = 9.4 Hz), 6.90-7.00 (m, 1H), 7.08 (d, 1H, J = 9.6 Hz), 7.20-7.40 (m, 4H). Elemental analysis calculated (%) for $C_{20}H_{25}N_5O_2$: C 65.37, H 6.86, N 19.06. Found: C 66.52, H 7.08, N 19.64.

*4.1.34. tert-Butyl 3-[6-(4-methylanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**65**)*

Title compound was prepared starting from **18** and aniline **20**. The residue was purified by FC (petroleum ether/EtOAc 1:1) to afford **65**. White solid; yield 54% (35 mg); mp 204-209 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.52 (d, 1H, J = 8.6 Hz), 2.31 (s, 3H), 2.60-2.78 (m, 1H), 3.49-3.66 (m, 2H), 4.16 (d, 2H, J = 12.6 Hz), 4.20-4.37 (m, 2H), 6.34 (bs, 1H, NH exch. with D_2O), 6.81 (d, 1H, J = 9.6 Hz), 7.03 (d, 1H, J = 9.6 Hz), 7.10 (d, 2H, J = 8.0 Hz), 7.20 (d, 2H, J = 8.0 Hz). Elemental analysis calculated (%) for $C_{21}H_{27}N_5O_2$: C 66.12, H 7.13, N 18.36. Found: C 67.51, H 7.33, N 18.55.

*4.1.35. tert-Butyl 3-[6-(4-methoxylanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**66**)*

Title compound was prepared starting from **18** and aniline **21**. The residue was purified by FC (petroleum ether/EtOAc 1:1) to afford **66**. Beige solid; yield 47% (32 mg); mp 164-165 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.51 (d, 1H, J = 8.2 Hz), 2.60-2.80 (m, 1H), 3.40-3.76 (m, 2H), 3.80 (s, 3H), 4.14 (d, 2H, J = 10.2 Hz), 4.20-4.37 (m, 2H), 6.28 (bs, 1H, NH exch. with D_2O), 6.78 (d, 1H, J = 10.0 Hz), 6.87 (d, 2H, J = 9.0 Hz), 6.94 (d, 1H, J = 10.0 Hz), 7.24 (d, 2H, J = 9.0 Hz). Elemental analysis calculated (%) for $C_{21}H_{27}N_5O_3$: C 63.46, H 6.86, N 17.62. Found: C 64.21, H 6.90, N 17.73.

*4.1.36. tert-Butyl 3-[6-(4-ethoxylanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**67**)*

Title compound was prepared starting from **18** and aniline **22**. The residue was purified by FC (petroleum ether/EtOAc 1:1) to afford **67**. Beige solid; yield 53% (50 mg); mp 168-171 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.20-1.60 (m, 13H), 2.60-2.80 (m, 1H), 3.40-3.76 (m, 2H), 4.01 (q, 2H, J = 6.8 Hz), 4.13 (d, 2H, J = 11.2 Hz), 4.25-4.37 (m, 2H), 6.28 (bs, 1H, NH exch. with D_2O), 6.68-7.10 (m, 4H), 7.23 (d, 2H, J = 8.8 Hz). Elemental analysis calculated (%) for $C_{22}H_{29}N_5O_3$: C 64.21, H 7.10, N 17.02. Found: C 64.75, H 7.23, N 17.49.

*4.1.37. tert-Butyl 3-[6-(4-chloroanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**68**)*

Title compound was prepared starting from **18** and aniline **23**. The residue was purified by FC (petroleum ether/EtOAc 1:1) to afford **68**. Beige solid; yield 60% (56 mg); mp 203-206 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.36 (bs, 9H), 1.52 (d, 1H, J = 8.6 Hz), 2.60-2.80 (m, 1H), 3.40-3.76 (m, 2H), 4.16 (d, 2H, J = 11.4 Hz), 4.25-4.37 (m, 2H), 6.38 (bs, 1H, NH exch. with D_2O), 6.83 (d, 1H, J

= 9.4 Hz), 7.98 (d, 1H, J = 9.6 Hz), 7.18-7.45 (m, 4H). Elemental analysis calculated (%) for $C_{20}H_{24}ClN_5O_2$: C 59.77, H 6.02, N 17.43. Found: C 61.13, H 6.23, N 17.75.

*4.1.38. tert-Butyl 3-[6-(4-fluoroanilino)pyridazin-3-yl[-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**69**)*

Title compound was prepared starting from **18** and aniline **24**. The residue was purified by FC (petroleum ether/EtOAc 1:1) to afford **69**. Yellowish solid; yield 46% (41 mg); mp 197-198 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.52 (d, 1H, J = 8.8 Hz), 2.60-2.80 (m, 1H), 3.36-3.70 (m, 2H), 4.14 (d, 2H, J = 11.0 Hz), 4.30 (d, 2H, J = 6.0 Hz), 6.36 (bs, 1H, NH exch. with D_2O), 6.80 (d, 1H, J = 9.6 Hz), 6.90-7.15 (m, 3H), 7.27-7.45 (m, 2H). Elemental analysis calculated (%) for $C_{20}H_{24}FN_5O_2$: C 62.32, H 6.28, N 18.17. Found: C 62.54, H 6.34, N 18.48.

*4.1.39. tert-Butyl 3-[6-(4-nitroanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**70**)*

Title compound was prepared starting from **18** and aniline **25**. The residue was purified by FC (petroleum ether/EtOAc 1:1) to afford **70**. Yellowish solid; yield 65% (62 mg); mp 201-203 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.53 (d, 1H, J = 8.4 Hz), 2.60-2.80 (m, 1H), 3.36-3.60 (m, 2H), 4.17 (d, 2H, J = 11.8 Hz), 4.25-4.40 (m, 2H), 6.38 (bs, 1H, NH exch. with D_2O), 6.84 (d, 1H, J = 9.8 Hz), 7.08 (d, 1H, J = 10.0 Hz), 7.63 (d, 2H, J = 9.0 Hz), 8.14 (d, 2H, J = 8.8 Hz). Elemental analysis calculated (%) for $C_{20}H_{24}N_6O_4$: C 58.24, H 5.87, N 20.38. Found: C 59.23, H 5.93, N 20.67.

*4.1.40. tert-Butyl 3-[6-(4-hydroxyanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**71**)*

Title compound was prepared starting from **18** and aniline **26**. The residue was purified by FC (petroleum ether/EtOAc 3:7) to afford **71**. Beige solid; yield 54% (48 mg); mp 163-166 °C. ^{1}H-

NMR (200 MHz, $CDCl_3$) δ = 1.30-1.47 (m, 10H), 2.60-2.80 (m, 1H), 3.40-3.75 (m, 2H), 3.13 (d, 2H, J = 9.8 Hz), 4.27-4.42 (m, 2H), 6.60 (bs, 1H, NH exch. with D_2O), 6.75-7.10 (m, 2H, OH exch. with D_2O), 7.11-7.45 (m, 4H), 7.57-7.70 (m, 1H). Elemental analysis calculated (%) for $C_{20}H_{25}N_5O_3$: C 62.65, H 6.57, N 18.26. Found: C 63.54, H 6.76, N 18.75.

*4.1.41. tert-Butyl 3-[6-(4-trifluoromethylanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**72**)*

Title compound was prepared starting from **18** and aniline **27**. The residue was purified by FC (petroleum ether/EtOAc 1:1) to afford **72**. Yellowish solid; yield 33% (33 mg); mp 147-150 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.53 (d, 1H, J = 9.0 Hz), 2.58-2.80 (m, 1H), 3.40-3.78 (m, 2H), 4.18 (d, 2H, J = 11.6 Hz), 4.25-4.40 (m, 2H), 6.78 (bs, 1H, NH exch. with D_2O), 6.87 (d, 1H, J = 9.6 Hz), 7.00-7.20 (m, 2H), 7.45-7.65 (m, 3H). Elemental analysis calculated (%) for $C_{21}H_{24}F_3N_5O_2$: C 57.92, H 5.56, N 16.08. Found: C 59.10, H 5.58, N 16.35.

*4.1.42. tert-Butyl 3-[6-(2-chloroanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**73**)*

Title compound was prepared starting from **18** and aniline **28**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **73**. Yellowish solid; yield 58% (54 mg); mp 134-135 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.57 (d, 1H, J = 6.6 Hz), 2.60-2.80 (m, 1H), 3.42-3.68 (m, 2H), 4.18 (d, 2H, J = 11.6 Hz), 4.32 (d, 2H, J = 5.4 Hz), 6.73 (bs, 1H, NH exch. with D_2O), 6.87 (d, 1H, J = 9.4 Hz), 6.93 (s, 1H), 7.03 (d, 1H, J = 9.8 Hz), 7.15-7.23 (m, 1H), 7.38 (d, 1H, J =8.0 Hz), 8.21 (d, 1H, J = 8.4 Hz). Elemental analysis calculated (%) for $C_{20}H_{24}ClN_5O_2$: C 59.77, H 6.02, N 17.43. Found: C 61.13, H 6.23, N 17.75.

*4.1.43. tert-Butyl 3-[6-(3-chloroanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**74**)*

Title compound was prepared starting from **18** and aniline **29**. The residue was purified by FC (petroleum ether/EtOAc 1:1) to afford **74**. Yellowish solid; yield 46% (43 mg); mp 218-220 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.37 (bs, 9H), 1.52 (d, 1H, J = 8.2 Hz), 2.60-2.80 (m, 1H), 3.38-3.65 (m, 2H), 4.17 (d, 2H, J = 11.6 Hz), 4.25-4.40 (m, 2H), 6.70-6.88 (m, 2H, NH exch. with D_2O), 6.90-7.00 (m, 1H), 7.02-7.16 (m, 1H), 7.20-7.46 (m, 3H). Elemental analysis calculated (%) for $C_{20}H_{24}ClN_4O_2$: C 59.77, H 6.02, N 17.43. Found: C 60.85, H 6.17, N 17.69.

*4.1.44. tert-Butyl 3-[6-(2,4-dichloroanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**75**)*

Title compound was prepared starting from **18** and aniline **30**. The residue was purified by FC (petroleum ether/EtOAc 1:1) to afford **75**. Yellowish solid; yield 41% (41 mg); mp 196-200 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.53 (d, 1H, J = 11.0 Hz), 2.60-2.80 (m, 1H), 3.38-3.70 (m, 2H), 4.18 (d, 2H, J = 11.6 Hz), 4.31 (d, 2H, J=6.2 Hz), 6.65 (bs, 1H, NH exch. with D_2O), 6.88 (d, 1H, J = 9.6 Hz), 6.98 (d, 1H, J = 9.4 Hz), 7.18 (d, 1H, J = 9.2 Hz), 7.30-7.46 (m, 1H), 8.36 (d, 1H, J = 8.8 Hz). Elemental analysis calculated (%) for $C_{20}H_{23}Cl_2N_5O_2$: C 55.05, H 5.31, N 16.05. Found: C 55.96, H 5.38, N 16.48.

*4.1.45. tert-Butyl 3-[6-(3,4-dichloroanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**76**)*

Title compound was prepared starting from **18** and aniline **31**. The residue was purified by FC (petroleum ether/EtOAc 1:1) to afford **76**. White solid; yield 44% (44 mg); mp 168-172 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.52 (d, 1H, J = 8.4 Hz), 2.60-2.80 (m, 1H), 3.40-3.70 (m, 2H), 4.17 (d, 2H, J = 11.2 Hz), 4.31 (d, 2H, J = 5.6 Hz), 6.84 (d, 1H, J = 9.6 Hz), 7.00 (d, 1H, J = 9.0 Hz), 7.20-7.46 (m, 2H, NH exch. with D_2O), 7.55-7.80 (m, 2H). Elemental analysis calculated (%) for $C_{20}H_{23}Cl_2N_5O_2$: C 55.05, H 5.31, N 16.05. Found: C 55.78, H 5.36, N 16.31.

*4.1.46. tert-Butyl 3-[6-(3,4-methylendioxyanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**77**)*

Title compound was prepared starting from **18** and aniline **32**. The residue was purified by FC (petroleum ether/EtOAc 4:6) to afford **77**. Yellowish solid; yield 42% (40 mg); mp 165-166 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.36 (bs, 9H), 1.52 (d, 1H, J = 9.4 Hz), 2.60-2.76 (m, 1H), 3.40-3.60 (m, 2H), 4.14 (d, 2H, J = 12.0 Hz), 4.30 (d, 2H, J = 4.8 Hz), 5.95 (s, 2H), 6.27 (bs, 1H, NH exch. with D_2O), 6.68-6.78 (m, 2H), 6.82 (s, 1H), 6.89-7.00 (m, 2H). Elemental analysis calculated (%) for $C_{21}H_{25}N_5O_4$: C 61.30, H 6.12, N 17.02. Found: C 62.06, H 6.14, N 17.35.

*4.1.47. tert-Butyl 3-[6-(N-methylanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**78**)*

Title compound was prepared starting from **18** and aniline **33**. The residue was purified by FC (petroleum ether/EtOAc 1:1) to afford **78**. White solid; yield 60% (53 mg); mp 129-132 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.30-1.58 (m, 10H), 2.60-2.80 (m, 1H), 3.40-3.80 (m, 5H), 4.14 (d, 2H, J = 11.5 Hz), 4.29 (d, 2H, J = 8.8 Hz), 6.67 (d, 1H, J = 9.8 Hz), 6.89 (d, 1H, J = 10.0 Hz), 7.16 (d, 2H, J = 8.8 Hz), 7.20-7.43 (m, 3H). Elemental analysis calculated (%) for $C_{21}H_{27}N_5O_2$: C 66.12, H 7.13, N 18.36. Found: C 67.15, H 7.35, N 18.59.

*4.1.48. General procedure for the preparation of final compounds **12a-o**, **13a-o** and **14a-o**.*

A solution of the appropriate Boc-protected derivative (**34 – 78**) (0.323 mmol) in HCOOH (2 mL) was stirred at room temperature for 20 h and then H_2O was added. The acid solution was extracted with $CHCl_3$, then basified with 10% K_2CO_3 aqueous solution and extracted with $CHCl_3$. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford the analytically pure product.

*4.1.49. 3-(5-Anilinopyridin-3-yl)-3,6-diazabicyclo[3.1.1]heptane (**12a**)*

General procedure was used to prepare title compound starting from **34**. Yellowish solid; yield 97% (83 mg); mp 179-182 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.45-1.80 (m, 2H, NH exch. with D_2O), 2.65-2.87 (m, 1H), 3.53 (bs, 4H), 3.89 (d, 2H, J = 5.2 Hz), 5.69 (bs, 1H, NH exch. with D_2O), 6.74 (s, 1H), 6.97 (t, 1H, J = 7.0 Hz), 7.11 (d, 2H, J = 8.0 Hz), 7.31 (d, 2H, J = 8.0 Hz), 7.70-7.90 (m, 2H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 29.74 (CH_2), 49.52 (CH x 2), 54.11 (CH_2 x 2), 102.57 (CH), 115.97 (CH x 2), 118.92 (CH), 123.40 (C), 126.88 (CH), 127.78 (CH x 2), 138.96 (C), 141.72 (CH), 143.96 (C). IR (nujol) ν: 3431 (NH). Elemental analysis calculated (%) for $C_{16}H_{18}N_4$: C 72.15, H 6.81, N 21.04. Found: C 72.20, H 6.82, N 21.10.

*4.1.50. 3-[5-(4-Methylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**12b**)*

General procedure was used to prepare title compound starting from **35**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **12b**. Pink solid; yield 98% (89 mg); mp 218-221 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.45-1.80 (m, 2H, NH exch. with D_2O), 2.32 (s, 3H), 2.68-2.90 (m, 1H), 3.52 (bs, 4H), 3.88 (d, 2H, J = 4.8 Hz), 5.59 (bs, 1H, NH exch. with D_2O), 6.66 (s, 1H), 6.90-7.24 (m, 4H), 7.72 (s, 1H), 7.79 (s, 1H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 20.71 (CH_3), 21.34 (CH_2), 51.19 (CH x 2), 55.79 (CH_2 x 2), 103.61 (CH), 118.05 (C), 119.13 (CH x 2), 125.33 (CH), 128.47 (CH), 129.96 (CH x 2), 131.87 (C), 139.98 (C), 145.32 (C). IR (nujol) ν: 3431 (NH). Elemental analysis calculated (%) for $C_{17}H_{20}N_4$: C 72.83, H 7.192, N 19.98. Found: C 73.00, H 7.25, N 20.06.

*4.1.51. 3-[5-(4-Methoxylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**12c**)*

General procedure was used to prepare title compound starting from **36**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **12c**. White solid; yield 88% (84 mg); mp 189-191 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.46-1.78 (m, 2H, NH exch. with D_2O), 2.68-2.84 (m, 1H), 4.50 (bs, 4H), 3.81 (s, 3H), 3.87 (d, 2H, J = 5.8 Hz), 5.47 (bs, 1H, NH exch. with D_2O), 6.53 (s, 1H), 6.88 (d, 2H, J = 8.8 Hz), 7.11 (d, 2H, J = 8.8 Hz), 7.68 (d, 1H, J = 2.2 Hz), 7.72 (d, 1H, J = 2.2 Hz). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 31.34 (CH_2), 54.62 (CH x 2), 55.71 (CH_3), 56.42 (CH_2 x 2),

107.31 (CH), 115.15 (CH x 2), 121.73 (CH x 2), 125.41 (CH), 126.75 (CH), 134.12 (C), 138.27 (C), 147.92 (C), 153.32 (C). IR (nujol) ν: 3433 (NH). Elemental analysis calculated (%) for $C_{17}H_{20}N_4O$: C 68.89, H 6.80, N 18.90. Found: C 69.00, H 6.95, N 18.82.

*4.1.52. 3-[5-(4-Ethoxylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**12d**)*

General procedure was used to prepare title compound starting from **37**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **12d**. White solid; yield 97% (97 mg); mp 199-203 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.30-1.52 (m, 4H, NH exch. with D_2O), 1.58 (d, 1H, J = 8.0 Hz), 2.65-2.95 (m, 1H), 3.49 (bs, 4H), 3.87 (d, 2H, J = 6.0 Hz), 4.02 (q, 2H, J = 6.6 Hz), 5.46 (bs, 1H, NH exch. with D_2O), 6.52 (s, 1H), 6.87 (d, 2H, J = 8.8 Hz), 7.09 (d, 2H, J = 8.8 Hz), 7.69 (d, 2H, J = 6.2 Hz). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 15.03 (CH_3), 36.05 (CH_2), 51.16 (CH x 2), 55.79 (CH_2 x 2), 63.79 (O-CH_2), 102.32 (CH), 115.33 (CH x 2), 118.07 (CH), 122.57 (CH x 2), 124.61 (CH), 127.50 (C), 138.29 (C), 141.19 (C), 154.96 (C). IR (nujol) ν: 3433 (NH). Elemental analysis calculated (%) for $C_{18}H_{22}N_4O$: C 69.65, H 7.14, N 18.05. Found: C 69.75, H 7.17, N 18.15.

*4.1.53. 3-(5-(4-chloroanilino)pyridin-3-yl)-3,6-diazabicyclo[3.1.1]heptane (**12e**)*

General procedure was used to prepare title compound starting from **38**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **12e**. Yellowish solid; yield 93% (90 mg); mp 194-197 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.63 (bs, 2H, NH exch. with D_2O), 2.70-2.88 (m, 1H), 3.53 (bs, 4H), 3.89 (d, 2H, J = 5.8 Hz), 5.69 (bs, 1H, NH exch. with D_2O), 6.68 (s, 1H), 7.03 (d, 2H, J = 8.6 Hz), 7.24 (d, 2H, J = 8.6 Hz), 7.80 (d, 2H, J = 4.6 Hz). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 29.81 (CH_2), 49.98 (CH x 2), 54.53 (CH_2 x 2), 112.22 (CH), 117.43 (CH x 2), 123.27 (C), 124.38 (C), 127.96 (CH x 2), 135.71 (CH), 138.71 (C), 141.04 (CH), 144.31 (C). IR (nujol) ν: 3433 (NH). Elemental analysis calculated (%) for $C_{16}H_{17}ClN_4$: C 63.89, H 5.70, N 18.63. Found: C 64.02, H 5.76, N 18.67.

*4.1.54. 3-[5-(4-Fluoroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**12f**)*

General procedure was used to prepare title compound starting from **39**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **12f**. Beige solid; yield 94% (86 mg); mp 118-121 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.50-1.70 (m, 2H, NH exch. with D_2O), 2.70-2.90 (m, 1H), 3.51 (bs, 4H), 3.89 (d, 2H, J = 5.8 Hz), 5.58 (bs, 1H, NH exch. with D_2O), 6.59 (s, 1H), 6.80-7.20 (m, 4H), 7.70-7.95 (m, 2H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 29.27 (CH_2), 49.02 (CH x 2), 53.58 (CH_2 x 2), 101.25 (CH), 113.65 (CH), 114.10 (CH), 117.51 (CH), 117.66 (CH), 122.78 (CH), 125.68 (CH), 137.58 (C), 139.00 (C), 143.62 (C), 152.74 (C). IR (nujol) ν: 3435 (NH). Elemental analysis calculated (%) for $C_{16}H_{17}FN_4$: C 67.59, H 6.03, N 19.70. Found: C 67.63, H 6.09, N 19.77.

*4.1.55. 3-[5-(4-Nitroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**12g**)*

General procedure was used to prepare title compound starting from **40**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **12g**. Yellowish solid; yield 82% (82 mg); mp 255-260 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.57 (bs, 2H, NH exch. with D_2O), 2.75-2.88 (m, 1H), 3.57 (bs, 4H), 3.85-4.05 (m, 2H), 6.20 (bs, 1H, NH exch. with D_2O), 6.82 (s, 1H), 6.99 (d, 2H, J = 9.0 Hz), 7.99 (d, 2H, J = 9.0 Hz), 8.15 (d, 2H, J = 9.0 Hz).^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.83 (CH_2), 59.59 (CH x 2), 54.82 (CH_2 x 2), 107.98 (CH), 113.64 (CH x 2), 126.18 (CH x 2), 128.09 (CH), 130.29 (C), 136.86 (CH), 138.12 (C), 145.48 (C), 150.79 (C). IR (nujol) ν: 3441 (NH); 1652, 1339 (NO_2). Elemental analysis calculated (%) for $C_{16}H_{17}N_5O_2$: C 61.72, H 5.50, N 22.49. Found: C 61.82, H 5.56, N 22.60.

*4.1.56. 3-[5-(4-Hydroxyanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**12h**)*

General procedure was used to prepare title compound starting from **41**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **12h**. Red solid; yield 67% (61 mg). ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.60-2.20 (m, 3H, NH e OH exch. with D_2O), 2.70-2.90 (m, 1H), 3.45-3.63 (m, 4H), 3.92 (d, 2H, J = 5.8 Hz), 5.56 (bs, 1H, NH exch. with D_2O), 6.49 (s, 1H), 6.81 (d, 2H, J = 8.8 Hz), 7.03 (d, 2H, J = 8.8 Hz), 7.60-7.78 (m, 2H). IR (nujol) ν: 3441 (NH). Elemental analysis calculated (%) for $C_{16}H_{18}N_4O$: C 68.06, H 6.43, N 19.84. Found: C 68.16, H 6.50, N 19.91.

*4.1.57. 3-[5-(4-Trifluoromethylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**12i**)*

General procedure was used to prepare title compound starting from **42**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **12i**. Yellowish solid; yield 49% (53 mg); mp 176-180 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.57 1.63 (d, 1H, J = 8.4Hz), 1.66-1.98 (m, 1H, NH exch. with D_2O), 2.70-2.90 (m, 1H), 3.55 (bs, 4H), 3.93 (d, 2H, J = 5.8 Hz), 5.98 (bs, 1H, NH exch. with D_2O), 6.79 (s, 1H), 7.09 (d, 2H, J = 8.0 Hz), 7.50 (d, 2H, J = 7.4 Hz), 7.88 (d, 2H, J = 6.2 Hz). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 29.98 (CH_2), 48.98 (CH x 2), 53.93 (CH_2 x 2), 111.53 (CH x 4), 113.46 (CH x 2), 124.78 (CH), 128.27 (C), 143.66 (C), 146.23 (C), 147.55 (C). IR (nujol) ν: 3432 (NH). Elemental analysis calculated (%) for $C_{17}H_{17}F_3N_4$: C 61.07, H 5.13, N 16.76. Found: C 61.20, H 5.19, N 17.12.

*4.1.58. 3-[5-(2-Chloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**12j**)*

General procedure was used to prepare title compound starting from **43**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **12j**. Yellowish solid; yield 86% (84 mg); mp 205-207 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.62 (d, 1H, J = 8.8 Hz), 1.68 (bs, 1H, NH exch. with D_2O), 2.65-2.90 (m, 1H), 3.55 (bs, 4H), 3.91 (d, 2H, J = 5.6 Hz), 6.03 (bs, 1H, NH exch. with D_2O), 6.70-6.90 (m, 2H), 7.15 (t, 1H, J = 8.0 Hz), 7.20-7.45 (m, 2H), 7.80-7.90 (m, 1H), 7.91-8.05 (m, 1H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 27.51 (CH_2), 4.69 (CH x 2), 53.73 (CH_2 x 2), 104.29 (CH), 119.84 (CH), 121.98 (CH), 125.78 (CH x 2), 127.35 (CH), 128.20 (CH), 129.98 (C), 131.24 (C), 138.28 (C), 143.36 (C). IR (nujol) ν: 3438 (NH). Elemental analysis calculated (%) for $C_{16}H_{17}ClN_4$: C 63.89, H 5.70, N 18.63. Found: C 64.01, H 5.75, N 18.69.

*4.1.59. 3-[5-(3-Chloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**12k**)*

General procedure was used to prepare title compound starting from **44**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **12k**. Beige solid; yield 92% (89 mg); mp 195-198 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.50-1.70 (m, 2H, NH exch. with D_2O), 2.70-2.88 (m, 1H), 3.53 (bs, 4H), 3.90 (d, 2H, J = 5.8 Hz), 5.75 (bs, 1H, NH exch. with D_2O), 6.75 (s, 1H), 6.92 (t, 2H, J =

6.2 Hz), 7.07 (s, 1H), 7.17 (d, 2H, J = 8.2 Hz), 7.70-7.95 (m, 2H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 29.78 (CH_2), 49.53 (CH x 2), 54.09 (CH_2 x 2), 104.03 (CH), 113.29 (CH), 114.75 (CH), 118.03 (CH), 124.47 (C), 127.62 (C), 128.93 (CH), 132.96 (CH), 137.94 (C), 143.76 (CH), 144.00 (C). IR (nujol) ν: 3438 (NH). Elemental analysis calculated (%) for $C_{16}H_{17}ClN_4$: C 63.89, H 5.70, N 18.63. Found: C 63.20, H 5.65, N 18.60.

*4.1.60. 3-[5-(2,4-Dichloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**12l**)*

General procedure was used to prepare title compound starting from **45**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **12l**. Beige solid; yield 84% (91 mg); mp 176-179 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.64 (bs, 2H, NH exch. with D_2O), 2.70-2.90 (m, 1H), 3.55 (bs, 4H), 3.92 (d, 2H, J = 5.6 Hz), 5.98 (bs, 1H, NH exch. with D_2O), 6.73 (s, 1H), 7.00-7.24 (m, 2H), 7.38 (d, 1H, J = 2.2 Hz), 7.90 (s, 2H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.64 (CH_2), 50.51 (CH x 2), 55.16 (CH_2 x 2), 106.92 (CH), 116.78 (CH), 122.30 (CH), 124.27 (C), 127.00 (C), 127.02 (CH), 128.86 (CH), 129.83 (C), 139.90 (C), 138.43 (C), 144.88 (C). IR (nujol) ν: 3428 (NH). Elemental analysis calculated (%) for $C_{16}H_{16}Cl_2N_4$: C 57.33, H 4.81, N 16.71. Found: C 57.41, H 4.83, N 16.80.

*4.1.61. 3-[5-(3,4-Dichloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**12m**)*

General procedure was used to prepare title compound starting from **46**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **12m**. Beige solid; yield 82% (89 mg); mp 217-220 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.59 (bs, 2H, NH exch. with D_2O), 2.70-2.88 (m, 1H), 3.54 (bs, 4H), 3.92 (d, 2H, J = 6.2 Hz), 5.68 (bs, 1H, NH exch. with D_2O), 6.69 (s, 1H), 6.80-6.95 (m, 1H), 7.10-7.20 (m, 1H), 7.21-7.34 (m, 1H), 7.70-7.95 (m, 2H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.29 (CH_2), 50.11 (CH x 2), 54.77 (CH_2 x 2), 104.86 (CH), 115.30 (C), 116.91 (CH), 120.89 (CH), 125.47 (C), 128.56 (C), 129.72 (CH), 131.50 (CH), 138.20 (C), 141.72 (CH), 142.78 (C). IR (nujol) ν: 3428 (NH). Elemental analysis calculated (%) for $C_{16}H_{16}Cl_2N_4$: C 57.33, H 4.81, N 16.71. Found: C 57.40, H 4.87, N 16.73.

*4.1.62. 3-[5-(3,4-Methylendioxyanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**12n**)*

General procedure was used to prepare title compound starting from **47**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **12n**. Beige solid; yield 90% (90 mg); mp 174-175 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.60 (d, 1H, J = 8.8 Hz), 1.77 (bs, 1H, NH exch. with D_2O), 2.70-2.90 (m, 1H), 3.51 (bs, 4H), 3.90 (d, 2H, J = 5.8 Hz), 5.54 (bs, 1H, NH exch. with D_2O), 5.96 (s, 2H), 6.50-6.63 (m, 2H), 6.77-6.81 (m, 2H), 7.65-7.78 (m, 2H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.66 (CH_2), 50.35 (CH x 2), 55.04 (CH_2 x 2), 110.82 (CH_2), 102.16 (C), 108.63 (CH), 110.99 (CH), 112.92 (C), 124.00 (C), 126.65 (C), 137.37 (CH), 141.18 (CH), 141.50 (CH), 145.33 (C), 147.78 (CH). IR (nujol) ν: 3434 (NH). Elemental analysis calculated (%) for $C_{17}H_{18}N_4O_2$: C 65.79, H 5.85, N 18.05. Found: C 65.82, H 5.90, N 18.14.

*4.1.63. 3-[5-(N-Methylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**12o**)*

General procedure was used to prepare title compound starting from **48**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **12o**. White solid; yield 93% (84 mg); mp 110-112 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.62 (bs, 2H, NH exch. with D_2O), 2.68-2.88 (m, 1H), 3.35 (s, 3H), 3.50 (bs, 4H), 3.87 (d, 2H, J = 6.4 Hz), 6.58 (s, 1H), 6.96-7.06 (m, 1H), 7.07 (d, 2H, J = 8.8 Hz), 7.33 (d, 2H, J = 8.8 Hz), 7.9 (s, 2H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 29.96 (CH_2), 42.60 (CH_3), 49.07 (CH x 2), 55.54 (CH_2 x 2), 114.74 (CH), 117.57 (C), 121.82 (CH x 2), 121.96 (CH), 130.10 (CH x 2), 131.11 (CH), 137.32 (C), 148.97 (CH), 150.15 (C). IR (nujol) ν: 3421 (NH). Elemental analysis calculated (%) for $C_{17}H_{20}N_4$: C 72.83, H 7.19, N 19.98. Found: C 72.94, H 7.22, N 20.00.

*4.1.64. 3-(6-Anilinopyridin-3-yl)-3,6-diazabicyclo[3.1.1]heptane (**13a**)*

General procedure was used to prepare title compound starting from **49**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **13a**. Beige solid; yield 95% (82 mg); mp 165-167 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.40-1.88 (m, 2H, NH exch. with D_2O), 2.65-2.85 (m, 1H), 3.40-3.80 (m, 4H), 3.80-4.10 (m, 2H), 6.23 (bs, 1H, NH exch. with D_2O), 6.84-7.18 (m, 3H), 7.20-7.48

(m, 4H), 7.76-7.85 (m, 1H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.01 (CH_2), 50.05 (CH x 2), 54.68 (CH_2 x 2), 110.52 (CH), 115.53 (CH x 2), 117.27 (CH), 118.25 (C), 119.00 (CH), 127.64 (CH x 2), 138.17 (C), 141.92 (CH), 146.03 (C). IR (nujol) ν: 3422 (NH). Elemental analysis calculated (%) for $C_{16}H_{18}N_4$: C 72.15, H 6.81, N 21.04. Found: C 72.20, H 6.85, N 21.11.

*4.1.65. 3-[6-(4-Methylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (**13b**)*

General procedure was used to prepare title compound starting from **50**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **13b**. White solid; yield 95% (86 mg); mp 175-177 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.63 (bs, 2H, NH exch. with D_2O), 2.30 (s, 3H), 2.65-2.85 (m, 1H), 3.51 (q, 4H, J = 10.4 Hz), 3.89 (d, 2H, J = 5.8 Hz), 6.13 (bs, 1H, NH exch. with D_2O), 6.80-7.20 (m, 5H), 7.77 (d, 2H, J = 2.8 HZ). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 20.66 (CH_3), 31.08 (CH_2), 51.40 (CH x 2), 55.93 (CH_2 x 2), 110.07 (CH), 118.03 (CH), 118.44 (CH x 2), 120.01 (CH), 129.68 (CH x 2), 130.58 (C), 139.51 (C), 139.69 (C), 147.43 (C). IR (nujol) ν: 3422 (NH). Elemental analysis calculated (%) for $C_{17}H_{20}N_4$: C 72.83, H 7.19, N 19.98. Found: C 72.92, H 7.15, N 20.02.

*4.1.66. 3-[6-(4-Methoxylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**13c**)*

General procedure was used to prepare title compound starting from **51**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **13c**. Yellowish solid; yield 93% (89 mg); mp 141-143 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.66 (d, 1H, J = 8.8 Hz), 1.94 (bs, 1H, NH exch. with D_2O), 2.65-2.85 (m, 1H), 3.50 (q, 4H, J = 6.8 Hz), 3.80 (s, 3H), 3.87-4.05 (m, 2H), 6.18 (bs, 1H, NH exch. with D_2O), 6.80 (d, 1H, J = 9.0 Hz), 6.86 (d, 2H, J = 9.0 Hz), 7.00 (dd, 1H, J_o = 3.2 Hz, J_m = 9.0 Hz), 7.17 (d, 1H, J = 8.8 Hz), 7.65-7.80 (m, 1H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.52 (CH_2), 50.50 (CH x 2), 54.91 (CH_3), 55.33 (CH_2 x 2), 110.36 (CH), 113.57 (CH x 2), 118.14 (CH x 2), 120.03 (CH), 128.08 (C), 136.23 (CH), 138.25 (C), 147.71 (C), 152.52 (C). IR (nujol) ν: 3427 (NH). Elemental analysis calculated (%) for $C_{17}H_{20}N_4O$: C 68.89, H 6.80, N 18.90. Found: C 69.00, H 6.85, N 18.98.

*4.1.67. 3-[6-(4-Ethoxylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**13d**)*

General procedure was used to prepare title compound starting from **52**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **13d**. Yellowish solid; yield 95% (95 mg); mp 126-128 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.41 (t, 3H, J = 7.0 Hz), 1.65 (d, 1H, J = 8.4 Hz), 1.89 (bs, 1H, NH exch. with D_2O), 2.60-2.80 (m, 1H), 3.50 (q, 4H, J = 7.4 Hz), 3.80-3.97 (m, 2H), 4.01 (q, 2H, J = 7.0 Hz), 6.12 (bs, 1H, NH exch. with D_2O), 6.70-6.90 (m, 3H), 6.99 (dd, 1H, J_o = 2.8 Hz, J_m = 8.8 Hz), 7.15 (d, 2H, J= 9.0 Hz), 7.68-7.80 (m, 1H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 14.89 (CH_3), 31.00 (CH_2), 51.30 (CH x 2), 55.94 (CH_2 x 2), 63.74 (O-CH_2), 109.24 (CH), 115.19 (CH x 2), 120.29 (CH), 121.41 (CH x 2), 129.57 (CH), 135.22 (C), 139.13 (C), 148.50 (C), 154.24 (C). IR (nujol) ν: 3427 (NH). Elemental analysis calculated (%) for $C_{18}H_{22}N_4O$: C 69.65, H 7.14, N 18.05. Found: C 69.79, H 7.21, N 18.13.

*4.1.68. 3-[6-(4-Chloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**13e**)*

General procedure was used to prepare title compound starting from **53**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **13e**. Beige solid; yield 89% (86 mg); mp 156-157 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.58 (bs, 1H, NH exch. with D_2O), 1.65 (d, 1H, J = 8.4 Hz), 2.65-2.85 (m, 1H), 3.52 (q, 4H, J = 5.4 Hz), 3.80-3.95 (m, 2H), 6.18 (bs, 1H, NH exch. with D_2O), 6.89 (d, 2H, J = 8.8 Hz), 7.03 (dd, 1H, J_o = 2.8 Hz, J_m = 9.2 Hz), 7.10-7.40 (m, 3H), 7.70-7.85 (m, 1H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.79 (CH_2), 50.99 (CH x 2), 55.58 (CH_2 x 2), 111.32 (CH), 117.83 (CH x 2), 119.78 (CH), 123.93 (C), 128.46 (CH x 2), 128.95 (CH), 139.42 (C), 141.10 (C), 146.20 (C). IR (nujol) ν: 3425 (NH). Elemental analysis calculated (%) for $C_{16}H_{17}ClN_4$: C 63.89, H 5.70, N 18.63. Found: C 63.97, H 5.76, N 18.60.

*4.1.69. 3-[6-(4-Fluoroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**13f**)*

General procedure was used to prepare title compound starting from **54**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **13f**. Beige solid; yield 93% (85 mg); mp 162-163 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.40-1.58 (bs, 1H, NH exch. with D_2O), 1.64 (d, 1H, J = 8.6 Hz), 2.60-2.88 (m, 1H), 3.50 (q, 4H, J =10.8 Hz), 3.88 (d, 2H, J = 5.4 Hz), 6.30 (bs, 1H, NH exch. with

D_2O), 6.83 (d, 1H, J = 8.8 Hz), 6.87-7.12 (m, 2H), 7.13-7.30 (m, 2H), 7.70-7.90 (m, 2H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 31.07 (CH_2), 51.34 (CH x 2), 55.89 (CH_2 x 2), 110.26 (CH), 115.43 (CH), 115.87 (CH), 119.68 (CH), 119.83 (CH), 120.11 (CH), 129.62 (CH), 138.20 (C), 138.39 (C), 139.66 (C), 147.24 (C). IR (nujol) ν: 3429 (NH). Elemental analysis calculated (%) for $C_{16}H_{17}FN_4$: C 67.59, H 6.03, N 19.70. Found: C 67.93, H 6.11, N 19.78.

*4.1.70. 3-[6-(4-Nitroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**13g**)*

General procedure was used to prepare title compound starting from **55**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **13g**. Red solid; yield 96% (96 mg); mp 205-206 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.55-1.78 (m, 2H, NH exch. with D_2O), 2.70-2.90 (m, 1H), 3.50-3.70 (m, 4H), 3.93 (d, 2H, J = 5.6 Hz), 6.83 (bs, 1H, NH exch. with D_2O), 6.98 (d, 1H, J = 9.9 Hz), 7.08 (dd, 1H, J_o = 3.2 Hz, J_m = 9.0 Hz), 7.29 (d, 2H, J=9.6 Hz), 7.80-7.95 (m, 1H), 8.15 (d, 2H, J=9.2 Hz). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 29.76 (CH_2), 49.68 (CH x 2), 54.20 (CH_2 x 2), 112.39 (CH), 113.22 (CH x 2), 118.47 (CH), 124.07 (CH x 2), 127.32 (C), 136.85 (C), 139.31 (C), 143.48 (C), 148.15 (CH). IR (nujol) ν: 3434 (NH); 1655, 1342 (NO_2). Elemental analysis calculated (%) for $C_{16}H_{17}N_5O_2$: C 61.72, H 5.50, N 22.49. Found: C 61.81, H 5.55, N 22.52.

*4.1.71. 3-[6-(4-Hydroxyanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**13h**)*

General procedure was used to prepare title compound starting from **56**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **13h**. Beige solid; yield 78% (71 mg); mp 218-220 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.30 (bs, 2H, NH e OH exch. with D_2O), 1.70-1.80 (m, 1H), 2.70-2.85 (m, 1H), 3.32 (bs, 4H), 3.37-3.62 (m, 2H), 6.70-6.85 (m, 3H, NH exch. with D_2O), 7.00-7.18 (m, 3H), 7.70-7.92 (m, 2H). IR (nujol) ν: 3445 (NH, OH). Elemental analysis calculated (%) for $C_{16}H_{18}N_4O$: C 68.06, H 6.43, N 19.84. Found: C 68.20, H 6.43, N 19.80.

*4.1.72. 3-[6-(4-Trifluoromethylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**13i**)*

General procedure was used to prepare title compound starting from **57**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **13i**. Beige solid; yield 81% (87 mg); mp 156-157 °C.

^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.49 (bs, 1H, NH exch. with D_2O), 1.65 (d, 1H, J = 9.0Hz), 2.65-2.85 (m, 1H), 3.40-3.80 (m, 4H), 3.91 (d, 2H, J = 5.6 Hz), 6.42 (bs, 1H, NH exch. with D_2O), 6.96 (d, 1H, J = 8.6 Hz), 7.00-7.15 (m, 1H), 7.28 (d, 2H, J = 8.4 Hz), 7.48 (d, 2H, J = 8.6 Hz), 7.80-7.95 (m, 1H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.61 (CH_2), 50.68 (CH x 2), 55.38 (CH_2 x 2), 112.36 (CH), 114.97 (CH x 2), 117.75 (CH x 2), 119.53 (CH), 125.64 (CH), 128.64 (C), 139.62 (C), 145.00 (C), 145.67 (C), 150.23 (C). IR (nujol) ν: 3428 (NH). Elemental analysis calculated (%) for $C_{17}H_{217}F_3N_4$: C 61.07, H 5.13, N 16.76. Found: C 61.19, H 5.20, N 16.66.

*4.1.73. 3-[6-(2-Chloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**13j**)*

General procedure was used to prepare title compound starting from **58**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **13j**. Yellowish solid; yield 89% (86 mg); mp 153-155 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.66 (d, 1H, J = 6.6 Hz), 1.76 (bs, 1H, NH exch. with D_2O), 2.65-2.85 (m, 1H), 3.55 (q, 4H, J = 6.8 Hz), 3.92 (d, 2H, J = 3.0 Hz), 6.60 (bs, 1H, NH exch. with D_2O), 6.82 (t, 1H, J = 5.2 Hz), 6.96 (d, 1H, J = 6.0 Hz), 7.05 (dd, 1H, J_o = 2.0 Hz, J_m = 5.8 Hz), 7.17 (t, 1H, J = 6.0 Hz), 7.35 (dd, 1H, J_o = 1.2 Hz, J_m = 5.8 Hz), 7.70-7.95 (m, 2H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 24.85 (CH_2), 43.97 (CH x 2), 52.07 (CH_2 x 2), 107.93 (CH), 112.90 (CH x 2), 115.66 (CH), 116.56 (CH), 122.29 (CH), 124.29 (CH), 125.15 (C), 133.55 (C), 134.27 (C), 140.92 (C). IR (nujol) ν: 3424 (NH). Elemental analysis calculated (%) for $C_{16}H_{17}ClN_4$: C 63.89, H 5.70, N 18.63. Found: C 63.96, H 5.75, N 18.69.

*4.1.74. 3-[6-(3-Chloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**13k**)*

General procedure was used to prepare title compound starting from **59**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **13k**. Yellowish solid; yield 92% (89 mg); mp 156-158 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ = 1.45 (bs, 1H, NH exch. with D_2O), 1.64 (d, 1H, J = 8.8 Hz), 2.65-2.85 (m, 1H), 3.38-3.60 (m, 4H), 3.90 (d, 2H, J = 4.8 Hz), 6.48 (bs, 1H, NH exch. with D_2O), 6.86 (dd, 1H, J_o = 1.2 Hz, J_m = 7.6 Hz), 6.89-7.08 (m, 2H), 7.10-7.22 (m, 2H), 7.25-7.32 (m, 1H), 7.82 (d, 1H, J = 2.4 Hz). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 31.11 (CH_2), 51.30 (CH x 2), 55.86

(CH_2 x 2), 111.78 (CH), 114.99 (CH), 116.45 (CH), 119.86 (CH), 120.17 (CH), 129.75 (CH), 130.06 (CH), 134.70 (C), 140.23 (C), 143.87 (C), 145.65 (C). IR (nujol) ν: 3424 (NH). Elemental analysis calculated (%) for $C_{16}H_{17}ClN_4$: C 63.89, H 5.70, N 18.63. Found: C 64.00, H 5.73, N 18.68.

*4.1.75. 3-[6-(2,4-Dichloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**13l**)*

General procedure was used to prepare title compound starting from **60**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **13l**. Beige solid; yield 64% (69 mg); mp 105-106 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.53 (bs, 1H, NH exch. with D_2O), 1.65 (d, 1H, J = 8.4 Hz), 2.70-2.90 (m, 1H), 3.40-3.80 (m, 4H), 3.85-4.05 (m, 2H), 6.55 (bs, 1H, NH exch. with D_2O), 6.90 (d, 1H, J = 8.6 Hz), 7.00-7.25 (m, 2H), 7.34 (d, 1H, J = 2.4 Hz), 7.78-8.10 (m, 2H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.99 (CH_2), 51.05 (CH x 2), 55.95 (CH_2 x 2), 112.91 (CH), 116.96 (CH), 119.90 (CH), 121.22 (C), 123.97 (C), 127.40 (CH), 128.75 (CH), 129.55 (CH), 137.77 (C), 140.44 (C), 144.97 (C). IR (nujol) ν: 3422 (NH). Elemental analysis calculated (%) for $C_{16}H_{16}Cl_2N_4$: C 57.33, H 4.81, N 16.71. Found: C 57.40, H 4.88, N 16.69.

*4.1.76. 3-[6-(3,4-Dichloroanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**13m**)*

General procedure was used to prepare title compound starting from **61**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **13m**. Grey solid; yield 83% (90 mg); mp 186-187 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.53 (bs, 1H, NH exch. with D_2O), 1.65 (d, 1H, J = 8.8 Hz), 2.70-2.89 (m, 1H), 3.40-3.70 (m, 4H), 3.91 (d, 2H, J = 4.6 Hz), 6.20 (bs, 1H, NH exch. with D_2O), 6.87 (d, 1H, J = 9.0 Hz), 7.00-7.18 (m, 2H), 7.28 (d, 1H, J = 8.6 Hz), 7.46 (d, 1H, J = 2.8 Hz), 7.82 (d, 1H, J=2.8 Hz). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 29.86 (CH_2), 49.45 (CH x 2), 55.94 (CH_2 x 2), 112.31 (CH), 116.30 (CH), 116.39 (CH), 119.04 (C), 120.90 (CH), 128.34 (C), 130.17 (CH), 130.78 (CH), 139.18 (C), 143.11 (C), 146.32 (C). IR (nujol) ν: 3422 (NH). Elemental analysis calculated (%) for $C_{16}H_{16}Cl_2N_4$: C 57.33, H 4.81, N 16.71. Found: C 57.41, H 4.86, N 16.75.

*4.1.77. 3-[6-(3,4-Methylendioxyanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**13n**)*

General procedure was used to prepare title compound starting from **62**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **13n**. Beige solid; yield 93% (93 mg); mp 172-173 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 11.60-1.80 (m, 2H, NH exch. with D_2O), 2.70-2.85 (m, 1H), 3.50 (q, 4H, J = 10.4 Hz), 3.89 (d, 2H, J = 5.8 Hz), 5.93 (s, 2H), 6.06 (bs, 1H, NH exch. with D_2O), 6.62 (dd, 1H, J_o = 8.4 Hz, J_m = 2.2 Hz), 6.72 (s, 1H), 6.76 (s, 1H), 6.79 (s, 1H), 6.81-6.92 (m, 1H), 7.00 (dd, 1H, J_o = 8.4 Hz, J_m = 3.0 Hz), 7.74 (d, 1H, J = 2.4 Hz). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 31.04 (CH_2), 51.16 (CH x 2), 55.71 (CH_2 x 2), 99.47 (CH), 100.82 (CH), 109.06 (CH), 111.73 (C), 113.40 (CH_2, C), 121.14 (CH), 138.60 (C), 139.38 (C), 140.29 (C), 147.57 (CH), 147.91 (C). IR (nujol) ν: 3430 (NH). Elemental analysis calculated (%) for $C_{17}H_{18}N_4O_2$: C 65.79, H 5.85, N 18.05. Found: C 65.87, H 5.90, N 18.09.

*4.1.78. 3-[6-(N-methylanilino)pyridin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**13o**)*

General procedure was used to prepare title compound starting from **63**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **13o**. White solid; yield 96% (87 mg); mp 94-95 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.55-1.78 (m, 2H, NH exch. with D_2O), 2.70-2.90 (m, 1H), 3.43 (s, 3H), 3.47-3.68 (m, 4H), 3.91 (d, 2H, J = 6.6 Hz), 6.84 (d, 1H, J = 8.8 Hz), 6.87-7.04 (m, 2H), 7.11 (d, 2H, J = 7.6 Hz), 7.31 (d, 2H, J = 8.6 Hz), 7.80-7.95 (m, 1H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 29.56 (CH_2), 41.60 (CH_3), 48.97 (CH x 2), 56.54 (CH_2 x 2), 113.74 (CH), 119.87 (C), 120.42 (CH x 2), 121.32 (CH), 129.10 (CH x 2), 129.91 (CH), 139.32 (C), 147.97 (CH), 150.08 (C). IR (nujol) ν: 3421 (NH). Elemental analysis calculated (%) for $C_{17}H_{20}N_4$: C 72.83, H 7.19, N 19.98. Found: C 72.90, H 7.24, N 19.92.

*4.1.79. 3-(6-Anilinopyridazin-3-yl)-3,6-diazabicyclo[3.1.1]heptane (**14a**)*

General procedure was used to prepare title compound starting from **64**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **14a**. Yellowish solid; yield 94% (81 mg); mp 154-157 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.40-1.80 (m, 2H, NH exch. with D_2O), 2.70-2.90 (m, 1H), 3.60-3.90 (m, 4H), 3.92 (d, 2H, J = 5.8 Hz), 6.47 (bs, 1H, NH exch. with D_2O), 6.83 (d, 1H, J = 9.2

Hz), 6.90-7.05 (m, 1H), 7.10 (d, 1H, J = 9.4 Hz), 7.25-7.45 (m, 4H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 32.19 (CH_2), 49.95 (CH x 2), 54.97 (CH_2 x 2), 114.24 (C), 116.86 (CH x 2), 119.42 (C), 119.70 (CH), 128.51 (CH x 2), 142.16 (CH), 151.22 (CH), 154.94 (CH). IR (nujol) ν: 3388 (NH). Elemental analysis calculated (%) for $C_{15}H_{17}N_5$: C 67.39, H 6.41, N 26.20. Found: C 67.45, H 6.49, N 26.16.

*4.1.80. 3-[6-(4-Methylanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**14b**)*

General procedure was used to prepare title compound starting from **65**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **14b**. Yellowish solid; yield 98% (89 mg); mp 114-116 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.40-180 (m, 2H, NH exch. with D_2O), 2.32 (s, 3H), 2.68-2.90 (m, 1H), 3.76 (bs, 4H), 3.92 (d, 2H, J = 7.6 Hz), 6.41 (bs, 1H, NH exch. with D_2O), 6.81 (d, 1H, J = 9.8 Hz), 7.00-7.25 (m, 5H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 20.68 (CH_3), 31.09 (CH_2), 50.58 (CH x 2), 55.79 (CH_2 x 2), 113.62 (CH), 118.32 (CH), 119.08 (CH x 2), 129.71 (CH x 2), 131.41 (C), 138.65 (C), 151.81 (C), 155.44 (C). IR (nujol) ν: 3388 (NH). Elemental analysis calculated (%) for $C_{16}H_{19}N_5$: C 68.30, H 6.81, N 24.89. Found: C 68.41, H 6.83, N 24.85.

*4.1.81. 3-[6-(4-Methoxylanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**14c**)*

General procedure was used to prepare title compound starting from **66**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **14c**. Yellowish solid; yield 87% (84 mg); mp 145-146 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.45-1.78 (m, 2H, NH exch. with D_2O), 2.60-2.80 (m, 1H), 3.70-3.88 (m, 4H), 3.80 (s, 3H), 3.92 (d, 2H, J = 9.4 Hz), 6.28 (bs, 1H, NH scambia con D_2O), 6.78 (d, 1H, J = 9.6 Hz), 6.87 (d, 2H, J = 9.0 Hz), 6.96 (d, 1H, J = 9.6 Hz), 7.24 (d, 2H, J = 9.0 Hz). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.16 (CH_2), 49.45 (CH x 2), 54.59 (CH_3), 54.90 (CH_2 x 2), 113.14 (CH x 2), 117.45 (C), 118.67 (CH), 119.01 (CH x 2), 134.36 (CH), 151.21 (C), 152.98 (C), 153.90 (C). IR (nujol) ν: 3383 (NH). Elemental analysis calculated (%) for $C_{16}H_{19}N_5O$: C 64.63, H 6.44, N 23.55. Found: C 64.70, H 6.50, N 23.60.

*4.1.82. 3-[6-(4-Ethoxylanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**14d**)*

General procedure was used to prepare title compound starting from **67**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **14d**. Yellowish solid; yield 89% (90 mg); mp 145-147 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.41 (t, 3H, J = 7.0 Hz), 1.50-1.80 (m, 2H, NH exch. with D_2O), 2.60-2.80 (m, 1H), 3.60-3.88 (m, 4H), 3.91 (d, 2H, J = 8.4 Hz), 4.02 (q, 2H, J = 6.8 Hz), 6.30 (bs, 1H, NH exch. with D_2O), 6.78 (d, 1H, J = 9.8 Hz), 6.86 (d, 2H, J = 8.6 Hz), 6.96 (d, 1H, J = 9.8 Hz), 7.23 (d, 2H, J = 8.8 Hz). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 14.85 (CH_3), 31.04 (CH_2), 50.53 (CH x 2), 55.78 (CH_2 x 2), 63.71 (O-CH_2), 113.81 (CH), 115.11 (CH x 2), 118.02 (CH), 121.85 (CH x 2), 134.16 (C), 152.46 (C), 154.62 (C), 155.27 (C). IR (nujol) ν: 3383 (NH). Elemental analysis calculated (%) for $C_{17}H_{21}N_5O$: C 65.57, H 6.80, N 22.49. Found: C 65.53, H 6.85, N 22.56.

*4.1.83. 3-[6-(4-Chloroanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**14e**)*

General procedure was used to prepare title compound starting from **68**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **14e**. Beige solid; yield 85% (83 mg); mp 214-215 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.64 (bs, 2H, NH exch. with D_2O), 2.60-2.80 (m, 1H), 3.70-3.85 (m, 4H), 3.92 (d, 2H, J =11.0 Hz), 6.44 (bs, 1H, NH exch. with D_2O), 6.85 (d, 1H, J = 9.8 Hz), 7.02 (d, 1H, J = 9.8 Hz), 7.18-7.45 (m, 4H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.45 (CH_2), 49.81 (CH x 2), 55.02 (CH_2 x 2), 113.42 (CH), 118.14 (CH x 2), 119.53 (CH), 123.58 (C), 127.76 (CH x 2), 139.97 (C), 150.69 (C), 154.38 (C). IR (nujol) ν: 3392 (NH). Elemental analysis calculated (%) for $C_{15}H_{16}ClN_5$: C 59.70, H 5.34, N 23.21. Found: C 59.83, H 5.36, N 23.18.

*4.1.84. 3-[6-(4-Fluoroanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**14f**)*

General procedure was used to prepare title compound starting from **69**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **14f**. Yellowish solid; yield 85% (78 mg); mp 204-205 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.50-1.90 (m, 2H, NH exch. with D_2O), 2.70-2.90 (m, 1H), 3.60-3.87 (m, 4H), 3.92 (d, 2H, J = 5.2 Hz), 6.44 (bs, 1H, NH exch. with D_2O), 6.82 (d, 1H, J = 9.4 Hz), 7.00-7.22 (m, 3H), 7.26-7.40 (m, 2H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.71 (CH_2), 49.97 (CH x 2), 54.89 (CH_2 x 2), 112.83 (CH), 114.27 (CH), 114.54 (CH), 114.98 (CH), 118.15 (CH), 118.28

(CH), 119.51 (C), 138.51 (C), 151.05 (C), 154.87 (C). IR (nujol) ν: 3387 (NH). Elemental analysis calculated (%) for $C_{15}H_{16}FN_5$: C 63.14, H 5.65, N 24.55. Found: C 63.20, H 5.60, N 24.60.

*4.1.85. 3-[6-(4-Nitroanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**14g**)*

General procedure was used to prepare title compound starting from **70**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **14g**. Orange solid; yield 92% (93 mg); mp 127-128 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.40-1.80 (m, 2H, NH exch. with D_2O), 2.70-2.90 (m, 1H), 3.60-3.90 (m, 4H), 3.92 (d, 2H, J = 5.8 Hz), 6.47 (bs, 1H, NH exch. with D_2O), 6.83 (d, 1H, J = 9.2 Hz), 6.90-7.05 (m, 1H), 7.10 (d, 1H, J = 9.4 Hz), 7.25-7.45 (m, 4H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 32.19 (CH_2), 49.95 (CH x 2), 54.97 (CH_2 x 2), 114.24 (C), 116.86 (CH x 2), 119.42 (C), 119.70 (CH), 128.51 (CH x 2), 142.16 (CH), 151.22 (CH), 154.94 (C). IR (nujol) ν: 3446 (NH); 1656, 1341 (NO_2). Elemental analysis calculated (%) for $C_{15}H_{16}N_6O_2$: C 57.68, H 5.16, N 26.91. Found: C 57.74, H 5.20, N 26.96.

*4.1.86. 3-[6-(4-Hydroxyanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**14h**)*

General procedure was used to prepare title compound starting from **71**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **14h**. Brown solid; yield 68% (62 mg); mp 157-158 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.40-2.10 (m, 3H, NH e OH exch. with D_2O), 2.60-2.90 (m, 1H), 3.65-3.80 (m, 4H), 3.82-4.10 (m, 2H), 6.63 (bs, 1H, NH exch. with D_2O), 6.70-7.15 (m, 2H), 7.23-7.60 (m, 4H). IR (nujol) ν: 3395 (NH). Elemental analysis calculated (%) for $C_{15}H_{17}N_5O$: C 63.59, H 6.05, N 24.72. Found: C 63.64, H 6.09, N 24.79.

*4.1.87. 3-[6-(4-Trifluoromethylanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**14i**)*

General procedure was used to prepare title compound starting from **72**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **14i**. Yellowish solid; yield 75% (81 mg); mp 206-207 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.63 (bs, 2H, NH exch. with D_2O), 2.65-2.90 (m, 1H), 3.70-3.88 (m, 4H), 3.93 (d, 2H, J = 5.6 Hz), 6.80 (bs, 1H, NH exch. with D_2O), 6.88 (d, 2H, J = 9.8 Hz), 7.09 (d, 2H, J = 9.6 Hz), 7.40-7.56 (m, 2H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.61 (CH_2), 50.02 (CH x

2), 55.12 (CH_2 x 2), 113.50 (CH), 116.17 (CH x 3), 119.98 (CH x 2), 125.37 (C x 2), 144.54 (C), 150.52 (C), 154.85 (C). IR (nujol) ν: 3389 (NH). Elemental analysis calculated (%) for $C_{16}H_{16}F_3N_5$: C 57.31, H 4.81, N 20.89. Found: C 57.40, H 4.85, N 20.86.

*4.1.88. 3-[6-(2-Chloroanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**14j**)*

General procedure was used to prepare title compound starting from **73**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **14j**. Beige solid; yield 82% (80 mg); mp 149-150 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.63 (d, 1H, J = 8.8 Hz), 1.86 (bs, 1H, NH exch. with D_2O), 2.65-2.90 (m, 1H), 3.65-3.87 (m, 4H), 3.93 (d, 2H, J = 5.6 Hz), 6.76 (bs, 1H, NH exch. with D_2O), 6.80-6.95 (m, 2H), 7.05 (d, 1H, J = 9.6 Hz), 7.09-7.29 (m, 1H), 7.37 (dd, 1H, J_o = 8.0 Hz, J_m = 1.8 Hz), 8.18 (dd, 1H, J_o = 8.4 Hz, J_m = 1.4 Hz). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.33 (CH_2), 49.35 (CH x 2), 55.48 (CH_2 x 2), 114.21 (CH), 119.91 (CH), 120.65 (CH), 121.77 (C), 127.27 (C), 129.22 (CH), 136.34 (C), 138.29 (CH), 150.93 (C), 155.42 (C). IR (nujol) ν: 3391 (NH). Elemental analysis calculated (%) for $C_{15}H_{16}ClN_5$: C 59.70, H 5.34, N 23.21. Found: C 59.82, H 5.36, N 23.20.

*4.1.89. 3-[6-(3-Chloroanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**14k**)*

General procedure was used to prepare title compound starting from **74**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **14k**. Beige solid; yield 86% (84 mg); mp 188-190 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.64 (d, 1H, J = 8.6 Hz), 1.75 (bs, 1H, NH exch. with D_2O), 2.70-2.90 (m, 1H), 3.78 (bs, 4H), 3.94 (d, 2H, J = 5.6 Hz), 6.83 (bs, 1H, NH exch. with D_2O), 6.90-7.03 (m, 2H), 7.11 (d, 1H, J = 9.2 Hz), 7.14-7.30 (m, 2H), 7.35-7.45 (m, 1H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.12 (CH_2), 49.99 (CH x 2), 55.66 (CH_2 x 2), 112.79 (CH), 114.21 (C), 115.21 (CH), 116.03 (CH), 118.80 (C), 119.95 (CH), 129.54 (C), 133.20 (CH), 143.25 (CH), 150.97 (C). IR (nujol) ν: 3392 (NH). Elemental analysis calculated (%) for $C_{15}H_{16}ClN_5$: C 59.70, H 5.34, N 23.21. Found: C 59.84, H 5.38, N 23.19.

*4.1.90. 3-[6-(2,4-Dichloroanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**14l**)*

General procedure was used to prepare title compound starting from **75**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **14l**. Yellowish solid; yield 87% (94 mg); mp 93-94 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.55-1.80 (bs, 2H, NH exch. with D_2O), 2.70-2.90 (m, 1H), 3.79 (bs, 4H), 3.94 (d, 2H, J = 5.4 Hz), 6.67 (bs, 1H, NH exch. with D_2O), 6.89 (d, 1H, J = 9.2 Hz), 7.00 (d, 1H, J = 9.6 Hz), 7.19 (dd, 1H, J_o =8.8 Hz, J_m = 2.0 Hz), 7.30-7.40 (m, 1H), 8.36 (d, 1H, J = 9.0 Hz). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 29.67 (CH_2), 50.64 (CH x 2), 55.73 (CH_2 x 2), 113.94 (CH), 118.96 (C), 120.07 (C), 121.73 (C), 125.31 (CH), 127.68 (CH), 128.67 (CH), 136.37 (CH), 155.36 (C), 155.84 (C). IR (nujol) ν: 3385 (NH). Elemental analysis calculated (%) for $C_{15}H_{15}Cl_2N_5$: C 63.58, H 4.54, N 20.83. Found: C 63.68, H 4.54, N 20.80.

*4.1.91. 3-[6-(3,4-Dichloroanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**14m**)*

General procedure was used to prepare title compound starting from **76**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **14m**. Yellowish solid; yield 82% (89 mg); mp 143-145 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.50-1.80 (bs, 2H, NH exch. with D_2O), 2.70-2.90 (m, 1H), 3.78 (bs, 4H), 3.93 (d, 2H, J = 5.4 Hz), 6.80-6.92 (m, 2H, NH exch. with D_2O), 7.04 (d, 1H, J = 9.2 Hz), 7.20-7.35 (m, 2H), 7.46-7.68 (m, 1H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 30.22 (CH_2), 49.50 (CH x 2), 54.79 (CH_2 x 2), 113.30 (C), 116.22 (CH), 119.56 (CH), 120.76 (C), 127.28 (C), 128.99 (CH), 130.85 (CH), 140.90 (CH), 150.19 (C), 154.23 (C). IR (nujol) ν: 3385 (NH). Elemental analysis calculated (%) for $C_{15}H_{15}Cl_2N_5$: C 63.58, H 4.54, N 20.83. Found: C 63.66, H 4.56, N 20.79.

*4.1.92. 3-[6-(3,4-Methylendioxyanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**14n**)*

General procedure was used to prepare title compound starting from **77**. The residue was purified by FC ($CHCl_3/CH_3OH$ 8/2) to afford **14n**. Brown solid; yield 82% (82 mg); mp 199-200 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.65 (d, 1H, J = 7.0 Hz), 1.71 (bs, 1H, NH exch. with D_2O), 2.70-2.90 (m, 1H), 3.75 (bs, 4H), 3.90-4.00 (m, 2H), 5.95 (s, 2H), 6.42 (bs, 1H, NH exch. with D_2O), 6.68-6.78 (m, 2H), 6.80 (d, 1H, J = 9.8 Hz), 6.91 (s, 1H), 6.99 (d, 1H, J = 9.8 Hz). IR (nujol)

ν: 3396 (NH). Elemental analysis calculated (%) for $C_{16}H_{17}N_5O_2$: C 61.72, H 5.50, N 22.49. Found: C 61.82, H 5.55, N 22.54.

*4.1.93. 3-[6-(N-Methylanilino)pyridazin-3-yl]-3,6-diazabicyclo[3.1.1]heptane (**14o**)*

General procedure was used to prepare title compound starting from **78**. The residue was purified by FC ($CHCl_3$/CH_3OH 8/2) to afford **14o**. Beige solid; yield 93% (85 mg); mp 83-84 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.63 (d, 1H, J=9.0 Hz), 1.72 (bs, 1H, NH exch. with D_2O), 2.70-2.90 (m, 1H), 3.54 (s, 3H), 3.76 (bs, 4H), 3.86-4.05 (m, 2H), 6.68 (d, 1H, J = 9,8 Hz), 6.91 (d, 1H, J=10.0 Hz), 7.05-7.22 (m, 3H), 7.28-7.45 (m, 2H). ^{13}C-NMR (200 MHz, $CDCl_3$) δ = 29.45 (CH_2), 36.64 (CH_3), 45.77 (CH x 2), 55.38 (CH_2 x 2), 116.74 (CH), 120.87 (C), 121.42 (CH x 2), 130.15 (CH x 2), 132.10 (CH), 144.32 (C), 148.97 (CH), 155.08 (C). IR (nujol) ν: 3422 (NH). Elemental analysis calculated (%) for $C_{16}H_{19}N_5$: C 68.30, H 6.81, N 24.29. Found: C 68.41, H 6.85, N 24.85.

4.2. Binding studies

The affinity (K_i) of the synthetized compounds for the $\alpha_4\beta_2$, α□β□□and α_7 receptors was tested in vitro using [^{3}H]-Epibatidine labeled rat cerebral cortex membranes ($\alpha_4\beta_2$), or membranes of HEK 243 cells transiently trasfected with human α□β□ nAChR, [^{125}I] α-Bungarotoxin labelled rat hippocampus membranes (α_7) according to a previously described experimental protocol [18].

The LIGAND program was used to calculate the K_i values of all the tested compounds using data obtained from at least three independent saturation and competition binding experiments.

4.3. Functional activity.

The electrophysiological measurements were made as previously described [19]. Briefly, cDNAs coding for human neuronal nicotinc subunits were obtained from Janssen Pharmaceuticals (Titusville, NJ, USA). Human neuronal α7, α□β□, α4β2 nAChRs were expressed by transient transfection in the rat anterior pituitary GH4C1 cell line [20] by adding 1 mg of each subunit cDNA to each dish, together with 4 □L lipofectamine. Whole-cell currents were recorded using borosilicate glass patch pipettes (3–6 M□ tip resistance) connected to an Axopatch 200A amplifier

(Molecular Devices, Union City, CA, USA). Data were recorded and analysed using pCLAMP10 software (Molecular Devices). Whole-cell capacitance and patch series resistance (5-15 M□) were estimated from slow-transient compensations, with a series resistance compensation of 70-90%. Cells were voltage clamped at a holding potential of -70 mV and continuously superfused using a gravity-driven perfusion system with independent tubes for standard and agonist-containing solutions, positioned 50-100 □m from the patched cell. A fast exchanger system (RSC-100, BioLogique, Nice, France) allowed complete solution exchange in less than 50 ms. Data are given as means ± standard error of the mean (SEM). Dose-response curves were constructed by sequentially applying different concentrations of agonists, and normalizing the current amplitudes to the value obtained by using 1 mM ACh on the same cell. Data were best-fitted to a Hill equation.

Appendix A. Supplementary data

^{1}H-NMR and ^{13}C-NMR spectra of of representative compounds **12g**, **13g**, and **14g** are available.

Conflict of interest

None of the authors have conflict of interest to declare

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Captions to Figures and Schemes

Fig. 1. Natural nAChRs ligands.

Fig. 2. Diazabicycloalcanes.

Fig. 3. 3,6-Diazabicyclo[3.3.1]heptanes.

Scheme 1. Reagents and conditions: a) Dihalopyridine, BINAP, $Pd_2(dba)_3$, Cs_2CO_3, PhMe, rfx, 20 h (for **16** and **17**); b) DCPD, IPr·HCl, $Pd_2(dba)_3$, Cs_2CO_3, dioxane, rfx, 22 h (**18**); c) Xantphos, $Pd_2(dba)_3$, *t*BuOK, PhMe, 130 °C, 0.5-2 h, MW (normal abs); d) HCOOH, rt, overnight.

Table 1. $\alpha\square\beta\square$ and α_7 nAChR binding affinity for compounds 12a-o, 13a-o and -14a-o. [a]Coefficient of variation on three independent experiments. [b]Not Determined. [c][^{3}H]-Epibatidine. [d][^{125}I]α-Bungarotoxine.

Table 2. $\alpha_3\beta_4$ nAChR binding affinity for compounds 12a-c, e-i, k, 13c, f-i, k, m and -14i. [a]Coefficient of variation on three independent experiments. [b][^{3}H]-Epibatidine.

Fig. 4. Partial agonism of compound **12c** on human heteromeric nAChRs.

PANEL A. Typical whole-cell inward currents elicited by nACh (1 mM) and compound **12c** (100 μM) applied on GH4C1 cells transiently transfected with cDNA for human α_4 and β_2 subunits (left) or human α_3 and β_4 subunits (right). Holding potential, -70 mV. Note the lower current amplitude evoked by compound **12c**, behaving as a partial agonist on these receptors. PANEL B. Dose-response curves obtained by best-fitting the normalized current amplitudes, elicited by compound **12c** applied to GH4C1 cells expressing human $\alpha_4\beta_2$ (□) or human $\alpha_3\beta_4$ nAChRs (●), to the following equation: $I = Imax\ [agonist]^{nH}/(EC_{50}{}^{nH} + [agonist]^{nH})$. For $\alpha_4\beta_2$ and $\alpha_3\beta_4$ nAChRs the EC_{50} values were 2.90±0.03 μM and 1.90±0.8 μM, respectively; nH values were 1.79±0.01 μM and 0.90±0.3 μM, respectively.

Fig. 5. Partial agonism of compound **13g** on human heteromeric nAChRs.

PANEL A. Typical whole-cell inward currents elicited by nACh (1 mM) and compound **13g** (100 μM) applied on GH4C1 cells transiently transfected with cDNA for human α_4 and β_2 subunits

(left) or human α_3 and β_4 subunits (right). Holding potential, -70 mV. Note the absence of response on $\alpha_3\beta_4$ nAChRs. PANEL B. Dose-response relations of the normalized current amplitudes elicited by compound **13g** applied to GH4C1 cells expressing human $\alpha_4\beta_2$ (□) or human $\alpha_3\beta_4$ nAChRs (●). For $\alpha_4\beta_2$ nAChRs the EC_{50} value was > 10 μM.

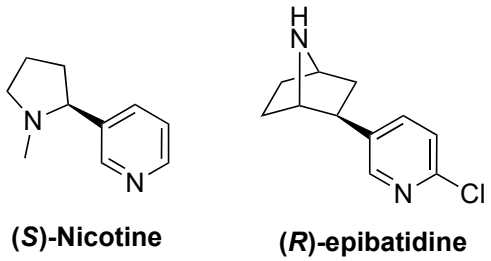


Fig. 1. Natural nAChRs ligands.

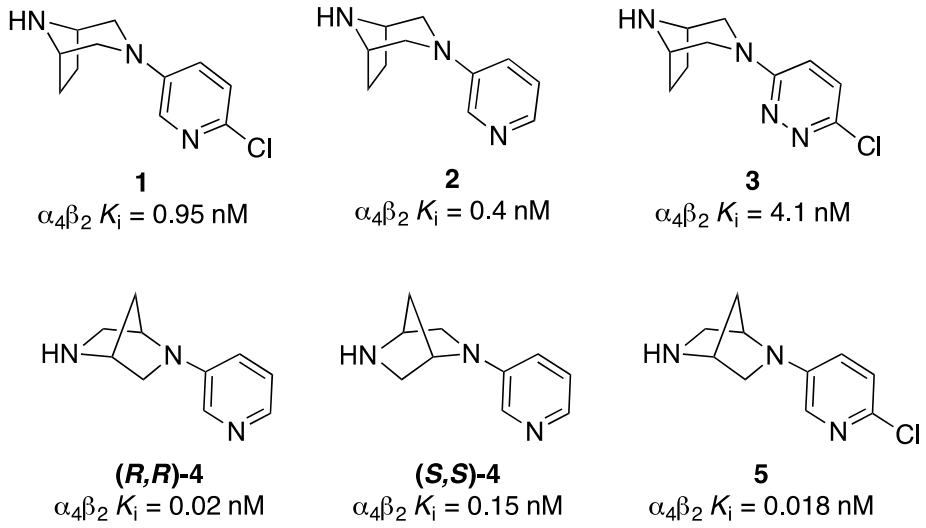
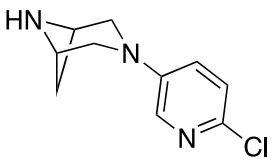
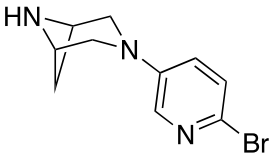


Fig. 2. Diazabicycloalcanes.



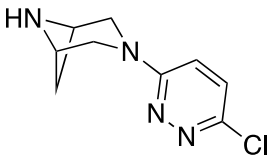
6

$\alpha_4\beta_2$ K_i = 6.98 pM
α_7 K_i = 115 nM
selectivity ($\alpha_7/\alpha_4\beta_2$) = 16,475



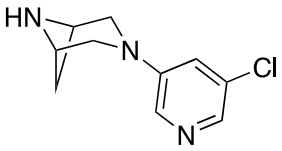
7

$\alpha_4\beta_2$ K_i = 9.67 pM
α_7 K_i = 132 nM
selectivity ($\alpha_7/\alpha_4\beta_2$) = 23,076



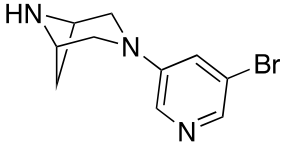
8

$\alpha_4\beta_2$ K_i = 168 pM
α_7 K_i = 25 nM
selectivity ($\alpha_7/\alpha_4\beta_2$) = 149



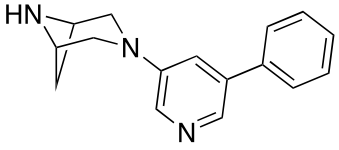
9

$\alpha_4\beta_2$ K_i = 6.32 pM
α_7 K_i = 73 nM
selectivity ($\alpha_7/\alpha_4\beta_2$) = 11,550



10

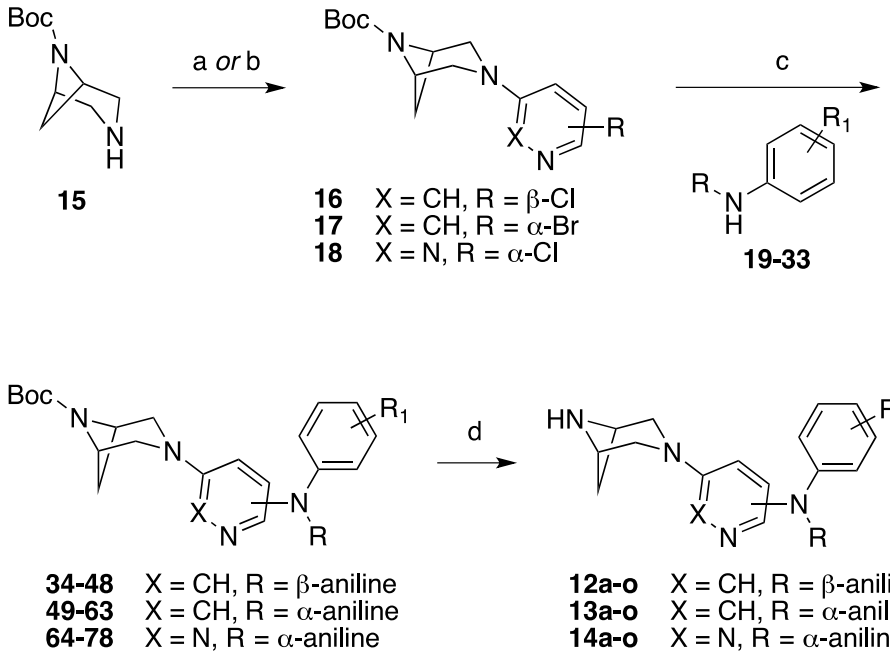
$\alpha_4\beta_2$ K_i = 5.72 pM
α_7 K_i = 176 nM
selectivity ($\alpha_7/\alpha_4\beta_2$) = 30,769



11

$\alpha_4\beta_2$ K_i = 11.17 pM
α_7 K_i = 17 μM
selectivity ($\alpha_7/\alpha_4\beta_2$) = 1,521,934

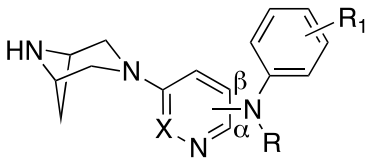
Fig. 3. 3,6-Diazabicyclo[3.3.1]heptanes.



19,**34**,**49**,**64,12-14a** R = H, R_1 = H
20,**35**,**50**,**65,12-14b** R = H, R_1 = 4-Me,
21,**36**,**51**,**66,12-14c** R = H, R_1 = 4-OMe
22,**37**,**52**,**67,12-14d** R = H, R_1 = 4-OEt
23,**38**,**53**,**68,12-14e** R = H, R_1 = 4-Cl
24,**39**,**54**,**69,12-14f** R = H, R_1 = 4-F
25,**40**,**55**,**70,12-14g** R = H, R_1 = 4-NO_2
26,**41**,**56**,**71,12-14h** R = H, R_1 = 4-OH
27,**42**,**57**,**72,12-14i** R = H, R_1 = 4-CF_3
28,**43**,**58**,**73,12-14j** R = H, R_1 = 2-Cl
29,**44**,**59**,**74,12-14k** R = H, R_1 = 3-Cl
30,**45**,**60**,**75,12-14l** R = H, R_1 = 2,4-Cl_2
31,**46**,**61**,**76,12-14m** R = H, R_1 = 3,4-Cl_2
32,**47**,**62**,**77,12-14n** R = H, R_1 = 3,4-OCH_2O-
33,**48**,**63**,**78,12-14o** R = Me, R_1 = H

Scheme 1. Reagents and conditions: a) Dihalopyridine, BINAP, $Pd_2(dba)_3$, Cs_2CO_3, PhMe, rfx, 20 h (for **16** and **17**); b) DCPD, IPr·HCl, $Pd_2(dba)_3$, Cs_2CO_3, dioxane, rfx, 22 h (**18**); c) Xantphos, $Pd_2(dba)_3$, *t*BuOK, PhMe, 130 °C, 0.5-2 h, MW (normal abs); d) HCOOH, rt, overnight.

Table 1. $\alpha_4\beta_2$ and α_7 nAChR binding affinity for compounds 12a-o, 13a-o and -14a-o.



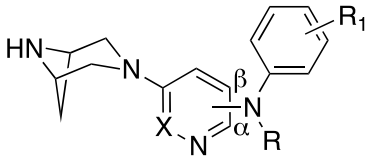
Compound	X	α/β	R	R_1	$\alpha_4\beta_2$ K_i (nM)	CV (%)[a]	α_7 K_i (nM)	CV (%)[a]	Selectivity $\alpha_7/\alpha_4\beta_2$
12a	CH	β	H	H	0.0807	57	26.9	53	333
12b	CH	β	H	4-CH_3	0.148	43	300	116	2,027
12c	CH	β	H	4-OCH_3	0.0598	33	837	66	13,996
12d	CH	β	H	4-OC_2H_5	N.D.[b]		N.D.		-
12e	CH	β	H	4-Cl	0.118	39	116	72	983
12f	CH	β	H	4-F	0.091	45	441	90	4,846
12g	CH	β	H	4-NO_2	0.0225	52	241	51	10,711
12h	CH	β	H	4-OH	0.342	54	2.1 x 10^3	90	6,140
12i	CH	β	H	4-CF_3	0.373	41	186	69	499
12j	CH	β	H	2-Cl	N.D.		N.D.		-
12k	CH	β	H	3-Cl	0.124	41	1.2 x 10^3	90	9,677
12l	CH	β	H	2,4-Cl_2	N.D.		N.D.		-
12m	CH	β	H	3,4-Cl_2	1.25	23	1.9 x 10^3	88	1,520
12n	CH	β	H	3,4-OCH_2O	N.D.		N.D.		-
12o	CH	β	CH_3	H	2.06	29	317	74	154
13a	CH	α	H	H	2.66	30	475	50	179
13b	CH	α	H	4-CH_3	52.1	26	88.8 x 10^3	95	1,704
13c	CH	α	H	4-OCH_3	7.3	74	3.01 x 10^3	80	412

13d	CH	α	H	4-OC_2H_5	N.D.		N.D.		-
13e	CH	α	H	4-Cl	2.49	31	823	58	330
13f	CH	α	H	4-F	2.38	37	1.3×10^3	83	546
13g	CH	α	H	4-NO_2	1.76	33	4.1×10^3	71	2,330
13h	CH	α	H	4-OH	7.1	45	2.2×10^3	50	310
13i	CH	α	H	4-CF_3	0.569	27	235	45	413
13j	CH	α	H	2-Cl	N.D.		N.D.		-
13k	CH	α	H	3-Cl	2.2	37	3.3×10^3	78	1,500
13l	CH	α	H	2,4-Cl_2	N.D.		N.D.		-
13m	CH	α	H	3,4-Cl_2	2.3	40	2.04×10^3	94	887
13n	CH	α	H	3,4-OCH_2O	41	72	6.15×10^3	40	150
13o	CH	α	CH_3	H	N.D.		N.D.		-
14a	N	α	H	H	441	27	10.4×10^3	140	24
14b	N	α	H	4-CH_3	44.3	26	21.7×10^3	84	490
14c	N	α	H	4-OCH_3	578	27	50.2×10^3	79	87
14d	N	α	H	4-OC_2H_5	N.D.		N.D.		-
14e	N	α	H	4-Cl	90.4	24	17.3×10^3	79	191
14f	N	α	H	4-F	868	23	111×10^3	84	128
14g	N	α	H	4-NO_2	86.2	26	6.9×10^3	76	80
14h	N	α	H	4-OH	N.D.		N.D.		-
14i	N	α	H	4-CF_3	6.9	44	2.03×10^3	73	294
14j	N	α	H	2-Cl	N.D.		N.D.		-
14k	N	α	H	3-Cl	1.54×10^3	29	41.8×10^3	72	27
14l	N	α	H	2,4-Cl_2	N.D.		N.D.		-

14m	N	α	H	3,4-Cl_2	516	26	5.1 x 10^3	67	10
14n	N	α	H	3,4-OCH_2O	N.D.		N.D.		-
14o	N	α	CH_3	H	1.43 x 10^3	27	101 x 10^3	91	71
[^{3}H]-Epi[c]					0.050	12	N.D.		
[^{125}I]α-BgTx[d]					N.D.		1.1	40	

[a]Coefficient of variation on three independent experiments. [b]Not Determined. [c][^{3}H]-Epibatidine. [d][^{125}I]α-Bungarotoxine.

Table 2. $\alpha_3\beta_4$ nAChR binding affinity for compounds 12a-c, e-i, k, 13c, f-i, k, m and -14i.



Compound	X	α/β	R	R_1	$\alpha_3\beta_4$ K_t (nM)	CV (%)[a]	Selectivity $\alpha_3\beta_4/\alpha_4\beta_2$
12a	CH	β	H	H	0.799	54	10
12b	CH	β	H	4-CH_3	1.4	65	9
12c	CH	β	H	4-OCH_3	2.2	84	37
12e	CH	β	H	4-Cl	1.2	46	10
12f	CH	β	H	4-F	1.25	54	14
12g	CH	β	H	4-NO_2	0.684	46	30
12h	CH	β	H	4-OH	0.988	46	3
12i	CH	β	H	4-CF_3	1.02	47	3
12k	CH	β	H	3-Cl	293	76	2,363
13c	CH	α	H	4-OCH_3	9.03	83	1
13f	CH	α	H	4-F	13	40	5
13g	CH	α	H	4-NO_2	25	50	14
13h	CH	α	H	4-OH	165	59	23
13i	CH	α	H	4-CF_3	0.818	80	1
13k	CH	α	H	3-Cl	4.8	35	2
13m	CH	α	H	3,4-Cl_2	40.9	53	18
14i	N	α	H	4-CF_3	115	50	17
[^{3}H]-Epi[b]					0.150	30	

[a]Coefficient of variation on three independent experiments. [b][^{3}H]-Epibatidine.

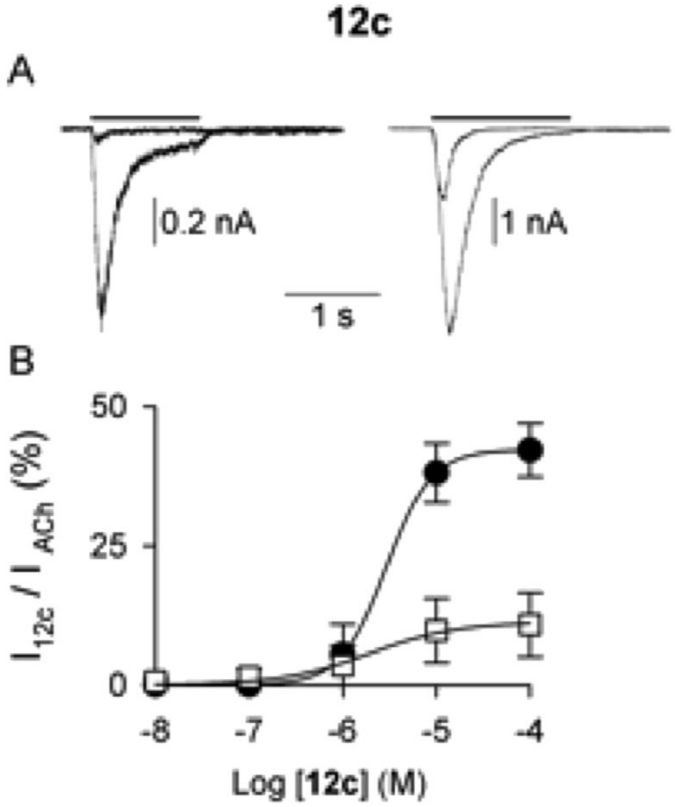


Fig. 4. Partial agonism of compound **12c** on human heteromeric nAChRs.

PANEL A. Typical whole-cell inward currents elicited by ACh (1 mM) and compound **12c** (100 μM) applied on GH4C1 cells transiently transfected with cDNA for human α_4 and β_2 subunits (left) or human α_3 and β_4 subunits (right). Holding potential, -70 mV. Note the lower current amplitude evoked by compound **12c**, behaving as a partial agonist on these receptors. PANEL B. Dose-response curves obtained by best-fitting the normalized current amplitudes, elicited by compound **12c** applied to GH4C1 cells expressing human $\alpha_4\beta_2$ (□) or human $\alpha_3\beta_4$ nAChRs (●), to the following equation: $I = Imax\ [agonist]^{nH}/(EC_{50}{}^{nH} + [agonist]^{nH})$. For $\alpha_4\beta_2$ and $\alpha_3\beta_4$ nAChRs the EC_{50} values were 2.90±0.03 μM and 1.90±0.8 μM, respectively; nH values were 1.79±0.01 μM and 0.90±0.3 μM, respectively.

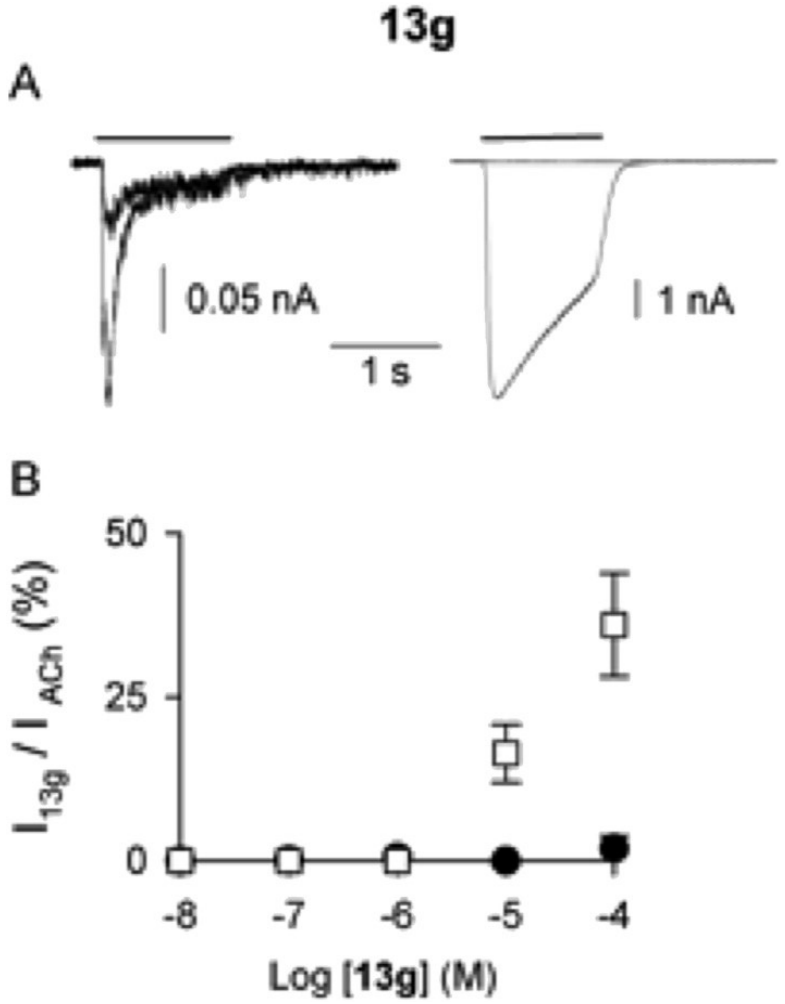


Fig. 5. Partial agonism of compound **13g** on human heteromeric nAChRs.

PANEL A. Typical whole-cell inward currents elicited by ACh (1 mM) and compound **13g** (100 μM) applied on GH4C1 cells transiently transfected with cDNA for human α_4 and β_2 subunits (left) or human α_3 and β_4 subunits (right). Holding potential, -70 mV. Note the absence of response on $\alpha_3\beta_4$ nAChRs. PANEL B. Dose-response relations of the normalized current amplitudes elicited by compound **13g** applied to GH4C1 cells expressing human $\alpha_4\beta_2$ (□) or human $\alpha_3\beta_4$ nAChRs (●). For $\alpha_4\beta_2$ nAChRs the EC_{50} value was > 10 μM.