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Original article

Synthesis and activity of 3-pyridylamine ligands at central nicotinic receptors

Gianfranco Balboni[a], Mauro Marastoni[b], Stefania Merighi[c], Pier Andrea Borea[c], Roberto Tomatis[b]*

[a]Dipartimento di Tossicologia dell'Università di Cagliari, Ferrara, Italy
[b]Dipartimento di Scienze Farmaceutiche e Centro di Biotecnologie dell'Università di Ferrara, Ferrara, Italy
[c]Dipartimento di Medicina Clinica e Sperimentale-Sezione di Farmacologia dell'Università di Ferrara, Ferrara, Italy

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Abstract – A series of thirty 2-(3-pyridylaminomethyl)azetidine, pyrrolidine and piperidine analogues as nicotinic acetylcholine receptor (nAChR) ligands was explored. In general, pyrrolidinyl and many azetidinyl compounds were found to bind with enhanced affinity relative to the piperidines. In the three series, the parallel structural changes (stereochemistry, N-methylation and/or chloro substitution) do not consistently lead to parallel shifts in affinity. The more active compounds (K_i affinity values ranging from 8.9 to 90 nM) were about as analgesic as nicotine in a tail-flick assay in mice after subcutaneous injections. © 2000 Éditions scientifiques et médicales Elsevier SAS

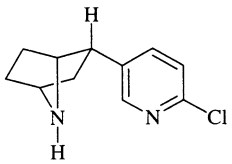
neuronal nicotinic acetylcholine receptor agonists / 3-aminopyridine derivatives / analgesics

1. Introduction

There is considerable evidence suggesting that selective neuronal nicotinic acetylcholine receptor (nAChR) agonists may have therapeutic potential in CNS disorders such as Alzheimer's and Parkinson's diseases, schizophrenia and depression [1–3]. Antinociceptive effects of nicotine have been known for some time but the discovery of the alkaloid epibatidine **1** (*figure 1*) as a potent analgesic that acts via neuronal nAChRs [4] has stimulated renewed interest in targeting nAChRs for analgesia. However, the potential therapeutic actions of epibatidine and older nicotinic agonists are accompanied by evident adverse effects, such as hypertension, neuromuscular paralysis, dependence and seizure. The emerging diversity of nAChR subtypes [5–8] supports the possibility of developing receptor subtype selective therapeutic agents that lack or have substantially attenuated side effects [9–11]. Very recent advances in this expanding area of research include the discovery of 3-pyridyl ethers, which possess high affinity for brain nAChRs [12] and appear to be precursor of suitable radioligands for brain imaging nAChRs with PET [13] and SPECT [14]. In particular, (R)-5-(2-azetidinylmethoxy)-2-chloropyridine **2** [15] has potent antinociceptive properties in different animal models and demonstrates superior selectivity for neuronal nAChRs [16]. Recently, we have prepared a series of 3-aminopyridine derivatives (**3a**) as potential neuronal nAChRs ligands [17]. Amides **3a** displayed negligible or very low affinity, whereas two amines **3b**, achieved by reduction of the corresponding amides **3a**, showed

Abbreviations: Standard abbreviations for amino acid derivatives are according to IUPAC-IUB Biochemical Nomenclature [31]. Other abbreviations are: Azt, 2-azetidincarboxylic acid; Boc, *tert*-butoxycarbonyl; DMF, dimethylformamide; Et_2O, diethyl ether; EtOAc, ethyl acetate; HOBt, hydroxybenzotriazole; HPLC, high-performance liquid chromatography; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; NMM, N-methylmorpholine; Pip, 2-piperidinecarboxylic acid; TFA, trifluoroacetic acid; THF, tetrahydrofuran; WSC, water soluble carbodiimide (1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide).

* Correspondence and reprints: Tel: 0532-291271; fax +39-532-291296
E-mail address: tmt@ifeuniv.unife.it (R. Tomatis).



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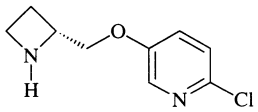

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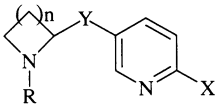

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3a Y = (R) or (S) CONH, $CON(CH_3)$
R = H, CH_3; X = H, Cl; n = 1, 2, 3

3b Y = (R) or (S) CH_2NH, $CH_2N(CH_3)$
R = H, CH_3, X = H, Cl; n = 1, 2, 3

Figure 1. (±) Epibatidine (**1**), (R)-5-(2-azetidinylmethoxy)-2-chloropyridine (**2**) and generic structure of synthesized analogues (**3**).

affinity in the nanomolar range with some dependence on the ring size. Specifically, the piperidinyl compound **33** ($K_i = 623$ nM) binds with half the affinity of its pyrrolidinyl counterpart **12** ($K_i = 305$ nM) (*table I*). These findings prompted us to synthesize further amine analogues **3b** (*figure 1*) in order to attempt a structure–affinity study on these nicotinic ligands. Antinociception effect of some compounds was also evaluated.

2. Chemistry

Aminopyridine derivatives **13**–**40** (*table I*) were prepared as shown in *figures 2 and 3*. Compounds **24**, **25** and **40** were obtained by $LiAlH_4$ reduction of the corresponding amides **3a** (*figure 3*) as previously reported for **12** and **33** [17]. Frequently, attemps to reduce the tertiary amides **3a** ($R' = CH_3$) were fraught with problems resulting in complex mixtures [17], therefore we synthesized most of target derivatives by the approach shown in *figure 2*. The key α-(Boc-amino)-aldehydes **6** were obtained by reduction of the corresponding N-methoxy N-methylamides **5** [18]. Hydroxamates **5** were in turn prepared by reaction of the appropriate Boc-amino acid **4** with O, N-dimethylhydroxylamine in the presence of WSC and HOBt. The protected derivatives **7** were achieved by reductive amination of **6** with the suitable aminopyridine. Methylation of the secondary amine in **7**–**10** was performed with formaldehyde and $NaBH_3CN$. Trifluoroacetic acid treatment of **7** and **10** afforded target compounds **8** and **11**, respectively. Finally, methylation of **8** with excess of HCHO and $NaBH_3CN$ gave target tertiary amine analogues **9**.

For the synthesis of **27** and **31** (*table I*), the precursor (R) Boc-azetidine-2-carboxylic acid was prepared from the free amino acid which was in turn derived from D-Met according to reported protocols [19–21]. Final compounds were isolated as tritrifluoroacetate salts and their structure verification was achieved by ^{1}H-NMR and MS spectrometries.

3. Biological activity

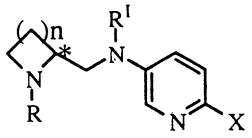
All compounds were tested on rat brain homogenates to evaluate their affinities for the central nicotinic receptors labelled by [^{3}H]-cytisine, which represents primarily $2\alpha_4$ $3\beta_2$ subtype of the high-affinity agonist binding sites in rat brain [10, 22].

Some compounds were also tested as analgesics by a mouse tail-flick assay [23] after subcutaneous injection as described previously [24] (see Section 6).

4. Results and discussion

The binding affinity of new analogues **13**–**40** for the neuronal nAChR is reported in *table I* in comparison with (−) nicotine and compounds **12** and **33** [17]. The binding data of these compounds varied widely, with the K_i values ranging from 9 to $> 12\,000$ nM. The structure–affinity relationship reveals that, generally, the (R) stereochemistry was preferred for nearly all of the pyrrolidinyl derivatives **12**–**23**, with the only exception observed for isomers **24** and **25**. These results are in contrast with the pyrrolidine 3-pyridyl ether analogues [12, 15], where modest or reversed stereoselectivity with respect to binding affinity was observed. On the other hand, the effect of the chloro substituent of the unmethylated compounds (e.g. **14** vs. **12**, **15** vs. **13**) is small, in analogy to results reported for 3-pyridyl ether derivatives [15]. *Table I* shows the effects of pyrrolidine or/and methyleneamino N-methylation (s) on affinity for the [^{3}H]-cytisine binding site. Pyrrolidine N-methylation of **12** and **13** (compounds **24** and **25**) results in an evident attenuation of binding affinity. Such a trend was also observed for the corresponding (R)-pyridyl ether analogue but not for its (S)-enantiomer [12]. A more detrimental effect on receptor binding occurred

Table I. Structure and receptor affinity of compounds.



No.	(n)	R	R^I	X	(*)[a]	HPLC (t_R)	$[\alpha]_D$[b]	MS [M+H$^+$]		K_i (nM)[c]
								Calculated	Found	
12	2	H	H	H	S	–	–	–	–	305[d]
13	2	H	H	H	R	8.07	+23.70	178	178	89.0 ± 3.2
14	2	H	H	Cl	S	10.99	−28.10	212	212	279.2 ± 48
15	2	H	H	Cl	R	10.99	+28.30	212	212	54.3 ± 10.6
16	2	H	CH_3	H	S	8.69	−8.52	191	191	11000 ± 800
17	2	H	CH_3	H	R	8.69	+8.57	191	191	5500 ± 650
18	2	CH_3	CH_3	H	S	9.11	−7.86	205	205	125.0 ± 16
19	2	CH_3	CH_3	H	R	9.11	+7.81	205	205	8.9 ± 2.6
20	2	H	CH_3	Cl	S	12.83	−5.12	225	225	9900 ± 1300
21	2	H	CH_3	Cl	R	12.83	+5.15	225	225	3250 ± 610
22	2	CH_3	CH_3	Cl	S	16.14	−3.57	239	239	2600 ± 500
23	2	CH_3	CH_3	Cl	R	16.14	+3.50	239	239	870 ± 92
24	2	CH_3	H	H	S	8.70	−6.54	191	191	1400 ± 170
25	2	CH_3	H	H	R	8.70	+6.59	191	191	1350 ± 140
26	1	H	H	H	S	9.01	−72.30	163	163	97.2 ± 8.1
27	1	H	H	H	R	9.01	+71.90	163	163	78.1 ± 0.7
28	1	H	H	Cl	S	11.90	−71.40	197	197	88.3 ± 5.1
29	1	H	CH_3	Cl	S	12.80	−69.10	211	211	724 ± 88
30	1	CH_3	CH_3	H	S	10.42	−70.50	191	191	>12000
31	1	CH_3	CH_3	H	R	10.42	+71.00	191	191	9800 ± 1400
32	1	CH_3	CH_3	Cl	S	13.27	−67.30	225	225	10850 ± 950
33	3	H	H	H	S	–	–	–	–	623[c]
34	3	H	H	H	R	10.15	+72.80	191	191	1290 ± 140
35	3	H	CH_3	H	S	11.03	−63.20	205	205	>12000
36	3	H	CH_3	Cl	S	11.84	−68.50	239	239	>12000
37	3	CH_3	CH_3	H	S	11.42	−55.40	219	219	10700 ± 1200
38	3	CH_3	CH_3	H	R	11.42	+55.70	219	219	2200 ± 250
39	3	CH_3	CH_3	Cl	S	12.35	−60.40	253	253	9850 ± 1050
40	3	CH_3	H	H	S	11.05	−71.80	205	205	6210 ± 850
Nicotine										7.91 ± 1.3

[a] (*) Chirality.
[b] c 1.0 methanol; temperature 21 °C.
[c] [^{3}H]-cytisine binding. Values are the average of at least three determinations.
[d] Data from [17].

through methylation at the CH_2NH nitrogen (compounds **16**, **17**, **20**, **21**). The loss in affinity by the methyl group can be attributed to several interrelated factors: e.g. an inability of the $CH_2N(CH_3)$ to form a specific interaction, causes steric impedance, reduces flexibility and stabilizes the compound in a conformation unaceptable to the receptor.

Methylation at both nitrogens differentially affects the affinity of **12** and **13** and their corresponding chloro derivatives **14** and **15**. In fact, dimethylchloro analogues **22** and **23** bind with 10-fold lower affinity than the parents **14** and **15**, while the dimethylated deschloro compounds **18** and **19** were found to bind with higher affinity than the corresponding **12** and **13**, respectively. This is an unexpected trend since the compounds having a chlorine at the 6-position of the pyridyl ring generally demonstrate binding affinity slightly higher than the deschloro counterparts. It is

noteworthy that the individual effects of the single methyl group additions are not cumulative, thus the effect of two methyl functions together is not the sum of the two single methyl group effects. This provides some additional evidence that members of similar series of compounds may not be binding in an identical manner [12, 25], otherwise we must suppose that the electronic and/or hydrophobic effect of the chloro substituent of **23** ($K_i = 870$ nM) may presumably limit the nicotinic binding that pertains to **19** ($K_i = 8.9$ nM).

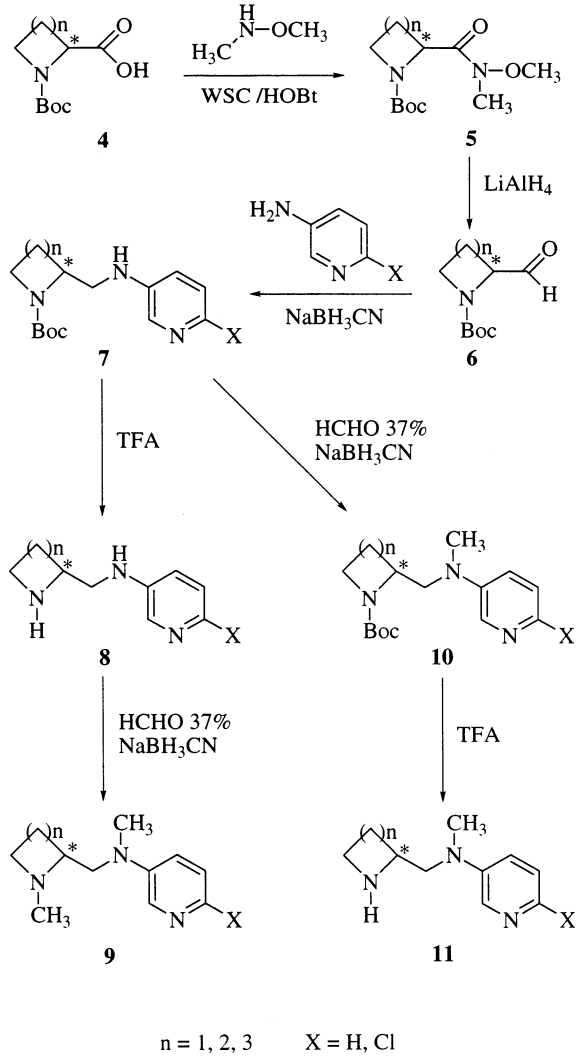


Figure 2. General synthesis of 3-pyridylamine ligands.

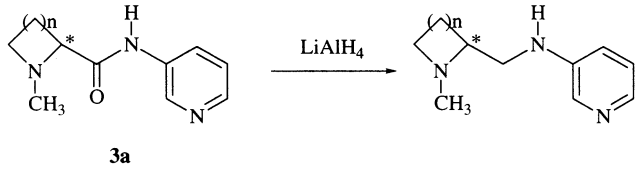


Figure 3. Synthesis of 3-pyridylamine ligands **24**, **25** and **40**.

Table I shows the effect of azacycle ring size on binding affinity (compounds **26**–**40**). The ring reduced and expanded derivatives **26** and **33** ($K_i = 97$ and 623 nM, respectively) displayed a slightly higher or lower affinity than the parent pyrrolidinyl analogue **12** ($K_i = 305$ nM). Like the results with the pyrrolidine series, 6-chloro azetidine **28** ($K_i = 88$ nM) retains the binding property of its deschloro analogue **26**. In the azetidinyl and piperinidyl series azacycle and/or CH_2NH N-methylation results in an attenuation (i.e. **29** and **38**) or more generally in a dramatic loss of affinity (**30**–**32** and **35**–**37**). Interestingly, unlike the corresponding pyrrolidines, azetidines **26** and **27** and piperidines **33** and **34** exhibit negligible or low stereoselectivity. Thus, further azetidine and piperidine analogues having the (R)-stereochemistry were not prepared.

In comparison with pyridyl ethers [12, 15] the present pyridylamines display modest binding properties. For example the deschloro analogue of **2** ($K_i =$ 0.05 nM) (*figure 1*) and its (R)-isomer ($K_i = 0.052$ nM) bind with 1000-fold higher affinity than the corresponding amines **26** ($K_i = 97.2$ nM) and **27** ($K_i = 78.1$ nM). This finding indicates that replacement of the pyridyl ether oxygen atom by nitrogen may perturb important interactions (e.g. hydrogen bond) with the $\alpha_4\beta_2$ sites or may modify critical parameters within the molecule (e.g. slight differences in conformational flexibility or in the putative pharmacophoric elements distance [10], and/or in the electronic effect on the pyridyl ring). In this respect, it is interesting to point out that the substitution of CH_2 or sulfur atom for the oxygen in the pyridyl ethers causes an evident reduction of affinity [26].

Some compounds of *table I* show a significant inhibition of the binding of [^{3}H]-cytisine to $\alpha_4\beta_2$ neuronal nAChRs. Even though the affinity results in this system are not always predictive of analgesic activity [11, 15], $\alpha_4\beta_2$ subunits are crucial for nicotine-eliced antinociception [8]. Therefore, the pyridylamines having the best binding properties were tested as analgesics and the tail-flick results are summarized in *table II*. Although the span of binding affinities for compounds is about 10-fold, there seems to be less variation in their potencies in the in vivo test. In fact,

Table II. Analgesic activity of compounds.

Compound	Tail-flick test ED_{50} (mg/kg)[a]	Rel pot
13	3.45 (2.86–5.50)	0.53
15	4.03 (3.06–6.14)	0.48
19	2.98 (2.02–4.98)	0.65
26	3.88 (2.93–5.68)	0.50
27	2.70 (1.98–4.81)	0.72
28	2.17 (1.63–3.03)	0.85
Nicotine	1.96 (1.47–2.84)	1

[a] ED_{50} values in mg/kg (95% confidence limits) are calculated on compound TFA free. Rel pot = potency relative to nicotine (= 1) = ED_{50} (μmol/kg) nicotine/ED_{50} (μmol/kg) test compound.

pyrrolidines **13**, **15** and **19** and azetidines **26**–**28** are about equipotent and are slightly less potent than nicotine. These apparent discrepancies may be due to different pharmacokinetic properties of compounds, but difference in intrinsic activity at $\alpha_4\beta_2$ sites or in affinity for other neuronal nAChRs cannot be ruled out. Such a trend was already observed for pyridyl ether analogues [15]. The antinociceptive effect of compounds tested was prevented by the noncompetitive neuronal nAChR antagonist mecamylamine (1 mg/kg) but not by hexametonium (2 mg/kg) and the opioid antagonist naloxone (2 mg/kg). These findings support a neuronal nAChR-mediated mechanism of action.

5. Conclusions

Previous efforts in this laboratory resulted in the preparation of the prototype 3-aminopyridine derivatives **12** and **33** showing significant affinity for the neuronal nAChRs labelled by [^{3}H]-cytisine [17]. These findings prompted us to synthesize new analogues in order to attempt a structure affinity study and to evaluate the analgesic potential of these nicotinic ligands.

In general, pyrrolidinyl and many azetinidyl compounds were found to bind with enhanced affinity relative to the piperidines. In the three series, the parallel structural changes (stereochemistry, N-methylation and/or chloro substitution) do not consistently lead to parallel shifts in affinity. Some new analogues were more potent than protype **12** in $\alpha_4\beta_2$ nAChR assay, and compound **19** represents the higher affinity member of the series ($K_i = 8.9$ nM). Finally, the pyridylamines having the higher binding property (K_i affinity values ranging from 8.9 to 90 nM) are about as active as nicotine in a tail-flick assay: they showed significant neuronal nAChRs-mediated analgesic potency in mice after subcutaneous injections. The preparation of conformationally restricted pyridylamine analogues is in progress.

6. Experimental protocols

6.1. General

Melting points were determined by a Kofler apparatus and are uncorrected. Optical rotations were determined with a Perkin Elmer (Vaterstetten, Germany) 141 polarimeter with a 10-cm Water jacketed cell. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) was obtained using the HPG2025A mass spectrometer (Hewlett Packard, Palo Alto, CA, USA). HPLC analysis was performed on a Bruker (Karlsruhe, Germany) liquid chromatograph LC21-C instrument using a Vydac C 18218 TP 5415 column (175 × 4.5 mm, 5 μm particle size) equipped with a Bruker LC 313 UV variable-wavelength detector. Recording and quantification were accomplished with a chromatographic data processor (Epson computer FX80X7). Analytical determinations were carried out by a gradient made up of two solvents: A, 10% (v/v) acetonitrile in water; B: 60% (v:v) acetonitrile in water, both containing 0.1% TFA (trifluoroacetic acid). The gradient program used was as follows: linear gradient from 0 to 100% B in 25 min at a flow of 1 mL/min. Preparative reversed-phase HPLC was carried out with a Waters Delta Prep 3000 (Milford, MA, USA) using a Delta Pack C 18-300 A (30 mm × 30 cm, 15 μm, spherical). The gradient used was identical to that of analytical determinations. Chromatography was performed at a flow rate of 30 mL/min. ^{1}H-NMR spectra were obtained using a Varian Gemini 300 (Palo Alto, CA, USA) instrument. TLC used precoated plates of silica gel F254 (E. Merk, Darmstadt, Germany) in the following solvent systems: (A) 1-butanol/acetic acid/H_2O (3:1:1), (B) EtOAc/pyridine/acetic acid/H_2O (12:4:1.2:2.2), (C) CH_2Cl_2/MeOH/toluene (8.5:1:0.5), (D) $CHCl_3$/MeOH/benzene/H_2O (8:8:8:1). Ninhydrin 1%, fluorescamine and chlorine spray reagents were employed to detect the compounds. Amino acids and amino acid derivatives were purchased from Novabiochem AG. Boc-L-azetidine-2-carboxylic acid was prepared according to literature procedure [27] starting from commercially available free amino acid.

6.2. Chemistry

6.2.1. Preparation of *Boc-L-Pro-N-methoxy-N-methylamide (**5**; n = 2)*

To a stirred solution of Boc-L-Pro-OH (1 g, 4.6 mmol) and N,O-dimethylhydroxylamine hydrochloride (0.55 g, 5.6 mmol) in DMF (10 mL) were added NMM (0.62 mL, 5.6 mmol), HOBt (0.87 g, 5.6 mmol) and WSC (1.09 g, 5.6 mmol) in the above order at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, overnight at room temperature and then diluted with AcOEt (60 mL). The solution was washed with 10% citric acid, 5% $NaHCO_3$, brine, dried over $MgSO_4$ and evaporated to dryness. The resulting oil was reprecipitated from Et_2O–petroleum ether (1.06 g, 88%); Rf (B) 0.82; HPLC t_R 7.90; $[\alpha]_D$ −45.6 (c 1.0, MeOH); MS ($M + H^+$) 259; ^{1}H-NMR (DMSO) δ 1.36–1.46 (d, 9H), 1.83–1.92 (m, 4H), 2.73 (s, 3H), 3.33 (t, 2H), 3.41 (s, 3H), 4.29–4.33 (m, 1H).

The other N-methoxy-N-methyl-α(t-butoxycarbonyl-amino)-carboxamides (**5**) were prepared in an identical manner (80–85%).

*Boc-D-Pro-N-methoxy-N-methylamide (**5**; n = 2)*: MS ($M + H^+$) 259; ^{1}H-NMR (DMSO) δ 1.36–1.46 (d, 9H), 1.83–1.92 (m, 4H), 2.73 (s, 3H), 3.33 (t, 2H), 3.41 (s, 3H), 4.29–4.33 (m, 1H).

*Boc-L-Azt-N-methoxy-N-methylamide (**5**; n = 1)*: MS ($M + H^+$) 245; ^{1}H-NMR (DMSO) δ 1.38–1.46 (d, 9H), 2.41 (m, 2H), 2.80 (s, 3H), 3.36 (t, 2H), 3.48 (s, 3H), 4.25–4.30 (m, 1H).

*Boc-D-Azt -N-methoxy-N-methylamide (**5**; n = 1)*: MS ($M + H^+$) 245; ^{1}H-NMR (DMSO) δ 1.38–1.46 (d, 9H), 2.41 (m, 2H), 2.80 (s, 3H), 3.36 (t, 2H), 3.48 (s, 3H), 4.25–4.30 (m, 1H).

*Boc-L-Pip-N-methoxy-N-methylamide (**5**; n = 3)*: MS ($M + H^+$) 273; ^{1}H-NMR (DMSO) δ 1.40–1.49 (d, 9H), 1.53–1.67 (m, 4H), 1.75–1.79 (m, 2H), 2.66 (s, 3H), 3.19 (t, 2H), 3.38 (s, 3H), 4.19–4.24 (m, 1H).

*Boc-D-Pip-N-methoxy-N-methylamide (**5**; n = 3)*: MS ($M + H^+$) 273; ^{1}H-NMR (DMSO) δ 1.40–1.49 (d, 9H), 1.53–1.67 (m, 4H), 1.75–1.79 (m, 2H), 2.66 (s, 3H), 3.19 (t, 2H), 3.38 (s, 3H), 4.19–4.24 (m, 1H).

6.2.2. *Boc-L-prolinal (**6**; n = 2)*

Lithium aluminum hydride (0.13 g, 3.3 mmol) was added in portions to a stirred solution of **5** ($n = 2$) (0.57 g, 2.2 mmol) in dry Et_2O (10 mL) at 0 °C. Reduction was complete within 25–30 min at room temperature. The mixture was treated with a solution of 0.5N potassium hydrogen sulfate (10 mL) and the product was extracted with Et_2O (3 × 10 mL). The organic phase was washed with 10% citric acid, 5% $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated. The resulting oil was used as such in the following reaction (82%); Rf (B) 0.93; HPLC t_R 9.20; $[\alpha]_D$ −62.3 (c 1.0, MeOH); MS ($M + H^+$) 200; ^{1}H-NMR (DMSO) δ 1.24–1.34 (d, 9H), 1.79–1.85 (m, 2H), 2.14–2.19 (m, 2H), 3.42 (t, 2H), 4.19–4.22 (m, 1H), 9.58 (s, 1H).

The other α(t-butoxycarbonyl)amino-aldehydes (**6**) were prepared by an identical procedure (78–84%).

*Boc-D-prolinal (**6**; n = 2)*: MS ($M + H^+$) 200; ^{1}H-NMR (DMSO) δ 1.24–1.34 (d, 9H), 1.79–1.85 (m, 2H), 2.14–2.19 (m, 2H), 3.42 (t, 2H), 4.19–4.22 (m, 1H), 9.58 (s, 1H).

*Boc-L-azetidine-2-carboxaldehyde (**6**; n = 1)*: MS ($M + H^+$) 186; ^{1}H-NMR (DMSO) δ 1.31–1.37 (d, 9H), 2.33–2.39 (m, 2H), 3.58 (t, 2H), 4.41–4.50 (m, 1H), 9.72 (s, 1H).

*Boc-D-azetidine-2-carboxaldehyde (**6**; n = 1)*: MS ($M + H^+$) 186; ^{1}H-NMR (DMSO) δ 1.31–1.37 (d, 9H), 2.33–2.39 (m, 2H), 3.58 (t, 2H), 4.41–4.50 (m, 1H), 9.72 (s, 1H).

*Boc-L-piperidine-2-carboxaldehyde (**6**; n = 3)*: MS ($M + H^+$) 214; ^{1}H-NMR (DMSO) δ 1.37–1.42 (d, 9H), 1.59–1.71 (m, 4H), 1.93–1.99 (m, 2H), 3.17 (t, 2H), 4.01–4.09 (m, 1H), 9.56 (s, 1H).

*Boc-D-piperidine-2-carboxaldehyde (**6**; n = 3)*: MS ($M + H^+$) 214; ^{1}H-NMR (DMSO) δ 1.37–1.42 (d, 9H), 1.59–1.71 (m, 4H), 1.93–1.99 (m, 2H), 3.17 (t, 2H), 4.01–4.09 (m, 1H), 9.56 (s, 1H).

6.2.3. *(S)-3-(N-1-Boc-pyrrolidin-2-ylmethylamino)-pyridine (**7**, n = 2, X = H)*

To a stirred solution of **6** ($n = 2$) (0.44 g, 2.21 mmol) in MeOH/acetic acid (9:1) (10 mL) were added 3-aminopyridine (0.21 g, 2.21 mmol) and $NaBH_3CN$ (0.28 g, 4.42 mmol) in the above order at room temperature. The mixture was stirred for 30 min, evaporated to dryness and the residue extracted with AcOEt (30 mL). The organic phase was washed with 5% $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated. The crude **7** was purified by preparative HPLC (0.47 g, 77%); Rf (B) 0.75; HPLC t_R 7.02; $[\alpha]_D$ +25.2 (c 1.0, MeOH); MS ($M + H^+$) 278; ^{1}H-NMR ($CDCl_3$) δ 1.49 (s, 9H), 1.61–1.83 (m, 4H), 3.12–3.41 (m, 4H), 3.91–3.98 (m, 1H), 5.33 (bs, 1H), 7.29–7.41 (m, 2H), 8.16–8.21 (m, 1H), 8.52 (s, 1H).

The other analogues (**7**) were synthesized by an identical procedure (75–82%).

(R)-3-(N-1-Boc-pyrrolidin-2-ylmethylamino)pyridine (**7**, $n = 2$, $X = H$): MS ($M + H^+$) 278; ^{1}H-NMR ($CDCl_3$) δ 1.49 (s, 9H), 1.61–1.83 (m, 4H), 3.12–3.41 (m, 4H), 3.91–3.98 (m, 1H), 5.33 (bs, 1H), 7.29–7.41 (m, 2H), 8.16–8.21 (m, 1H), 8.52 (s, 1H).

(S)-2-Chloro-5-(N-1-Boc-pyrrolidin-2-ylmethylamino)-pyridine (**7**, $n = 2$, $X = Cl$): MS ($M + H^+$) 312; ^{1}H-NMR ($CDCl_3$) δ 1.53 (s, 9H), 1.67–1.85 (m, 4H), 3.19–3.42 (m, 4H), 3.95–3.41 (m, 1H), 5.45 (bs, 1H), 7.39 (d, 1H), 7.81 (d, 1H), 8.77 (s, 1H).

(R)-2-Chloro-5-(N-1-Boc-pyrrolidin-2-ylmethyl-amino)pyridine (**7**, $n = 2$, $X = Cl$): MS ($M + H^+$) 312; ^{1}H-NMR ($CDCl_3$) δ 1.53 (s, 9H), 1.67–1.85 (m, 4H), 3.19–3.42 (m, 4H), 3.95–3.41 (m, 1H), 5.45 (bs, 1H), 7.39 (d, 1H), 7.81 (d, 1H), 8.77 (s, 1H).

(S)-3-(N-1-Boc-azetidin-2-ylmethylamino)pyridine (**7**, $n = 1$, $X = H$): MS ($M + H^+$) 264; ^{1}H-NMR ($CDCl_3$) δ 1.50 (s, 9H), 1.84–1.91 (m, 2H), 3.32–3.49 (m, 4H), 4.11–4.21 (m, 1H), 5.28 (bs, 1H), 7.19–7.28 (m, 2H), 8.19–8.24 (m, 1H), 8.57 (s, 1H).

(R)-3-(N-1-Boc-azetidin-2-ylmethylamino)pyridine (**7**, $n = 1$, $X = H$): MS ($M + H^+$) 264; ^{1}H-NMR ($CDCl_3$) δ 1.50 (s, 9H), 1.84–1.91 (m, 2H), 3.32–3.49 (m, 4H), 4.11–4.21 (m, 1H), 5.28 (bs, 1H), 7.19–7.28 (m, 2H), 8.19–8.24 (m, 1H), 8.57 (s, 1H).

(S)-2-Chloro-5-(N-1-Boc-azetidin-2-ylmethylamino)-pyridine (**7**, $n = 1$, $X = Cl$): MS ($M + H^+$) 298; ^{1}H-NMR ($CDCl_3$) δ 1.55 (s, 9H), 1.77–1.89 (m, 2H), 3.30–3.48 (m, 4H), 4.15–4.23 (m, 1H), 5.35 (bs, 1H), 7.42 (d, 1H), 7.77 (d, 1H), 8.72 (s, 1H).

(S)-3-(N-1-Boc-piperidin-2-ylmethylamino)pyridine (**7**, $n = 3$, $X = H$): MS ($M + H^+$) 292; ^{1}H-NMR ($CDCl_3$) δ 1.42 (s, 9H), 1.50–1.71 (m, 6H), 3.08–3.20 (m, 4H), 3.64–3.77 (m, 1H), 5.21 (bs, 1H), 7.20–7.35 (m, 2H), 7.99–8.15 (m, 1H), 8.39 (s, 1H).

(R)-3-(N-1-Boc-piperidin-2-ylmethylamino)pyridine (**7**, $n = 3$, $X = H$): MS ($M + H^+$) 292; ^{1}H-NMR ($CDCl_3$) δ 1.42 (s, 9H), 1.50–1.71 (m, 6H), 3.08–3.20 (m, 4H), 3.64–3.77 (m, 1H), 5.21 (bs, 1H), 7.20–7.35 (m, 2H), 7.99–8.15 (m, 1H), 8.39 (s, 1H).

(S)-2-Chloro-5-(N-1-Boc-piperidin-2-ylmethylamino)-pyridine (**7**, $n = 3$, $X = Cl$): MS ($M + H^+$) 324; ^{1}H-NMR ($CDCl_3$) δ 1.39 (s, 9H), 1.52–1.75 (m, 6H), 3.15–3.43 (m, 4H), 3.97–3.48 (m, 1H), 5.57 (bs, 1H), 7.39 (d, 1H), 7.81 (d, 1H), 8.77 (s, 1H).

6.2.4. (S)-3-(N-1-Boc-pyrrolidin-2-ylmethyl-N-methylamino)pyridine (**10**, $n = 2$, $X = H$)

To a stirred solution of **7** ($n = 2$) (1 mmol) in CH_3CN, 37% formalin (10 mL) and sodium cyanoborohydride (3 mmol) were added, followed after 10 min by acetic acid (0.1 mL). The mixture was stirred for 2 h at room temperature and then acetic acid (0.1 mL) was added. After being stirred for a further 30 min the solution was evaporated, the residue was dissolved in Et_2O (50 mL) washed with 1 N KOH, and brine. The organic phase was dried ($MgSO_4$), evaporated and the crude product was purified by preparative HPLC (0.39 g, 78%); Rf (B) 0.70; HPLC t_R 22.20; $[\alpha]_D$ −8.5 (c 1.0, MeOH); MS ($M + H^+$) 292; ^{1}H-NMR ($CDCl_3$) δ 1.46 (s, 9H), 2.30–2.49 (m, 4H), 3.14 (s, 3H), 3.58–4.12 (m, 5H), 7.20–7.37 (m, 2H), 7.99–8.27 (m, 2H).

The other protected analogues(**10**) were prepared by an identical procedure (71–77%).

(R)-3-(N-1-Boc-pyrrolidin-2-ylmethyl-N-methyl-amino)pyridine (**10**, $n = 2$, $X = H$): MS ($M + H^+$) 292; ^{1}H-NMR ($CDCl_3$) δ 1.46 (s, 9H), 2.30–2.49 (m, 4H), 3.14 (s, 3H), 3.58–4.12 (m, 5H), 7.20–7.37 (m, 2H), 7.99–8.27 (m, 2H).

(S)-2-Chloro-5-(N-1-Boc-pyrrolidin-2-ylmethyl-N-methylamino)pyridine (**10**, $n = 2$, $X = Cl$): MS ($M + H^+$) 326; ^{1}H-NMR ($CDCl_3$) δ 1.43 (s, 9H), 2.35–2.47 (m, 4H), 3.33 (s, 3H), 3.65–4.17 (m, 5H), 7.31–7.53 (m, 2H), 8.25 (m, 1H).

(R)-2-Chloro-5-(N-1-Boc-pyrrolidin-2-ylmethyl-N-methylamino)pyridine (**10**, $n = 2$, $X = Cl$): MS ($M + H^+$) 326; ^{1}H-NMR ($CDCl_3$) δ 1.43 (s, 9H), 2.35–2.47 (m, 4H), 3.33 (s, 3H), 3.65–4.17 (m, 5H), 7.31–7.53 (m, 2H), 8.25 (m, 1H).

(S)-3-(N-1-Boc-azetidin-2-ylmethyl-N-methyl-amino)pyridine (**10**, $n = 1$, $X = H$): MS ($M + H^+$) 278; ^{1}H-NMR ($CDCl_3$) δ 1.51 (s, 9H), 2.40–2.52 (m, 2H), 3.27 (s, 3H), 3.68–4.33 (m, 5H), 7.10–7.31 (m, 2H), 7.89–8.20 (m, 2H).

(S)-2-Chloro-5-(N-1-Boc-azetidin-2-ylmethyl-N-methylamino)pyridine (**10**, $n = 1$, $X = Cl$): MS ($M + H^+$) 312; ^{1}H-NMR ($CDCl_3$) δ 1.48 (s, 9H), 2.45–2.61 (m, 2H), 3.43 (s, 3H), 3.79–4.32 (m, 5H), 7.41–7.90 (m, 2H), 8.31 (m, 1H).

(S)-3-(N-1-Boc-piperidin-2-ylmethyl-N-methyl-amino)pyridine (**10**, $n = 3$, $X = H$): MS ($M + H^+$) 306; ^{1}H-NMR ($CDCl_3$) δ 1.30 (s, 9H), 1.61–1.98 (m, 6H), 3.16 (s, 3H), 3.39–4.13 (m, 5H), 6.87–7.20 (m, 2H), 7.65–7.92 (m, 2H).

(S)-2-Chloro-5-(N-1-Boc-piperidin-2-ylmethyl-N-methylamino)pyridine (**10**, $n = 3$, $X = Cl$): MS ($M + H^+$) 340; ^{1}H-NMR ($CDCl_3$) δ 1.38 (s, 9H), 2.55–2.73 (m, 2H), 3.73 (s, 3H), 3.81–4.22 (m, 5H), 7.74–8.11 (m, 2H), 8.40 (m, 1H).

*6.2.5. (R)-3-(Pyrrolidin-2-ylmethylamino)pyridine (**13**)*

Compound **7** ($n = 2$, X = H) (0.278 g, 1 mmol) was treated with TFA (1 mL) for 30 min at room temperature. The solvent was evaporated in vacuo at 0 °C and the residue was triturated with Et_2O-petroleum ether (1:1); the resulting hygroscopic solid was collected and dried (90%).

^{1}H-NMR ($CDCl_3$) δ 1.59–171 (m, 4H), 3.02–3.37 (m, 5H), 3.90 (bs, 1H), 5.22 (bs, 1H), 7.34–7.59 (m, 2H), 8.22–8.31 (m, 1H), 8.62 (s, 1H).

The analogues of **13** were obtained by the identical procedure (87–93%).**14**: ^{1}H-NMR ($CDCl_3$) δ 1.63–170 (m, 4H), 2.88–3.14 (m, 5H), 3.81 (bs, 1H), 5.20 (bs, 1H), 7.53–7.67 (m, 2H), 8.82 (s, 1H).

15: ^{1}H-NMR ($CDCl_3$) δ 1.63–1.70 (m, 4H), 2.88–3.14 (m, 5H), 3.81 (bs, 1H), 5.20 (bs, 1H), 7.53–7.67 (m, 2H), 8.82 (s, 1H).

26: ^{1}H-NMR ($CDCl_3$) δ 1.49–1.61 (m, 2H), 3.11–3.41 (m, 5H), 3.83 (bs, 1H), 5.29 (bs, 1H), 7.30–7.61 (m, 2H), 8.11–8.30 (m, 1H), 8.71 (s, 1H).

27: ^{1}H-NMR ($CDCl_3$) δ 1.49–1.61 (m, 2H), 3.11–3.41 (m, 5H), 3.83 (bs, 1H), 5.29 (bs, 1H), 7.30–7.61 (m, 2H), 8.11–8.30 (m, 1H), 8.71 (s, 1H).

28: ^{1}H-NMR ($CDCl_3$) δ 1.46–1.65 (m, 2H), 3.03–3.36 (m, 5H), 4.00 (bs, 1H), 5.38 (bs, 1H), 7.17–7.29 (m, 2H), 8.52 (s, 1H).

33: ^{1}H-NMR ($CDCl_3$) δ 1.49–1.75 (m, 6H), 2.81–3.13 (m, 5H), 3.79 (bs, 1H), 4.87 (bs, 1H), 7.41–7.61 (m, 2H), 8.09–8.20 (m, 1H), 8.67 (s, 1H).

34: ^{1}H-NMR ($CDCl_3$) δ 1.49–1.75 (m, 6H), 2.81–3.13 (m, 5H), 3.79 (bs, 1H), 4.87 (bs, 1H), 7.41–7.61 (m, 2H), 8.09–8.20 (m, 1H), 8.67 (s, 1H).

The physicochemical and analytical properties of **13** and related analogues are summarized in *table I*.

*6.2.6. (S)-3-(Pyrrolidin-2-ylmethyl-N-methylamino)-pyridine (**16**)*

Compound **10** ($n = 2$, X = H) (1 mmol) was converted to the title **16** in analogous fashion to the preparation of **13** (89%); ^{1}H-NMR ($CDCl_3$) δ 1.76–2.05 (m, 4H), 2.48–2.80 (m, 5H), 3.07–3.23 (m, 3H), 3.77 (bs, 1H), 7.24–7.37 (m, 2H), 7.91–8.10 (m, 2H). Anal. $C_{17}H_{20}N_3O_6F_9$.

By this procedure the following analogues were obtained (88–91%):

17: ^{1}H-NMR ($CDCl_3$) δ 1.76–2.05 (m, 4H), 2.48–2.80 (m, 5H), 3.07–3.23 (m, 3H), 3.77 (bs, 1H), 7.24–7.37 (m, 2H), 7.91–8.10 (m, 2H).

20: ^{1}H-NMR ($CDCl_3$) δ 1.56–1.78 (m, 4H), 2.42–2.83 (m, 5H), 2.99–3.14 (m, 3H), 3.82 (bs, 1H), 7.71–7.99 (m, 2H), 8.33 (m, 1H).

21: ^{1}H-NMR ($CDCl_3$) δ 1.56–1.78 (m, 4H), 2.42–2.83 (m, 5H), 2.99–3.14 (m, 3H), 3.82 (bs, 1H), 7.71–7.99 (m, 2H), 8.33 (m, 1H).

29: ^{1}H-NMR ($CDCl_3$) δ 1.76–1.88 (m, 4H), 2.40–2.91 (m, 6H), 3.02–3.34 (m, 3H), 7.71–7.99 (m, 2H), 8.33 (m, 1H).

35: ^{1}H-NMR ($CDCl_3$) δ 1.96–2.04 (m, 2H), 2.40–2.91 (m, 6H), 3.02–3.34 (m, 3H), 7.71–7.99 (m, 2H), 8.33 (m, 1H).

36: ^{1}H-NMR ($CDCl_3$) δ 1.41–2.81 (m, 6H), 2.58–2.79 (m, 5H), 3.09–3.28 (m, 3H), 3.42 (bs, 1H), 7.55–7.71 (m, 2H), 8.38–8.48 (m, 1H).

Characterization of these pyridylamines is summarized in *table I*.

*6.2.7. Preparation of (S)-3-(N-1-methylpyrrolidin-2-ylmethyl-N-methylamino)pyridine (**18**)*

Compound **8** ($n = 2$, $X = H$) (1 mmol) was converted to the corresponding N-methyl derivative (**18**) in analogous fashion to the preparation of **10** (76%); Rf (B) 0.50; HPLC t_R 9.10; $[\alpha]_D$ −7.8 (c 1.0, MeOH); MS ($M + H^+$) 205; ^{1}H-NMR ($CDCl_3$) δ 1.50–1.68 (m, 4H), 2.25–2.94 (m, 9H), 3.15–3.23 (m, 2H), 7.34–7.49 (m, 2H), 7.77–8.11 (m, 2H).

The analogues of **18** were prepared by the identical procedure (70–75%).

19: ^{1}H-NMR ($CDCl_3$) δ 1.50–1.68 (m, 4H), 2.25–2.94 (m, 9H), 3.15–3.23 (m, 2H), 7.34–7.49 (m, 2H), 7.77–8.11 (m, 2H). Anal. $C_{18}H_{22}N_3O_6F_9$.

22: ^{1}H-NMR ($CDCl_3$) δ 1.48–1.70 (m, 4H), 2.35–2.97 (m, 9H), 3.30–3.43 (m, 2H), 7.54–7.65 (m, 2H), 8.12–8.33 (m, 1H).

23: ^{1}H-NMR ($CDCl_3$) δ 1.48–1.70 (m, 4H), 2.35–2.97 (m, 9H), 3.30–3.43 (m, 2H), 7.54–7.65 (m, 2H), 8.12–8.33 (m, 1H).

30: ^{1}H-NMR ($CDCl_3$) δ 1.58–1.79 (m, 2H), 2.56–3.10 (m, 9H), 3.04–3.22 (m, 2H), 7.29–7.45 (m, 2H), 8.00–8.20 (m, 2H).

31: ^{1}H-NMR ($CDCl_3$) δ 1.58–1.79 (m, 2H), 2.56–3.10 (m, 9H), 3.04–3.22 (m, 2H), 7.29–7.45 (m, 2H), 8.00–8.20 (m, 2H).

32: ^{1}H-NMR ($CDCl_3$) δ 1.60–1.77 (m, 2H), 2.68–3.19 (m, 9H), 3.11–3.25 (m, 2H), 7.83–7.98 (m, 2H), 8.42–8.53 (m, 1H).

37: ^{1}H-NMR ($CDCl_3$) δ 1.35–1.59 (m, 6H), 2.39–2.73 (m, 9H), 3.01–3.20 (m, 2H), 7.24–7.44 (m, 2H), 7.94–8.03 (m, 2H).

38: ^{1}H-NMR ($CDCl_3$) δ 1.35–1.59 (m, 6H), 2.39–2.73 (m, 9H), 3.01–3.20 (m, 2H), 7.24–7.44 (m, 2H), 7.94–8.03 (m, 2H).

39: ^{1}H-NMR ($CDCl_3$) δ 1.65–1.80 (m, 6H), 2.51–2.87 (m, 9H), 3.09–3.22 (m, 2H), 7.49–7.80 (m, 2H), 8.17–8.23 (m, 1H).

The physicochemical and analytical properties of the title compound and related analogues are reported in *table I*.

*6.2.8. (S)-3-(N-1-methyl-pyrrolidin-2-ylmethyl-amino) pyridine (**24**)*

A solution of N-methyl-L-Pro-N-(pyridyl-3-yl)-carboxyamide [17] (0.20 g, 1 mmol) in dry THF (10 mL) was added, dropwise, to a stirred suspension of $LiAlH_4$ (5 mmol) in THF (10 mL) under an N_2 atmosphere at 0 °C. After 2 h at this temperature, the mixture was stirred overnight at room temperature, cooled at 0 °C and 1 N NaOH (1 mL) was added. The suspension was filtered and the filtrate was concentrated by evaporation and subjected to preparative HPLC. The resulting **24** was crystallized from Et_2O-petroleum ether (38%): ^{1}H-NMR ($CDCl_3$) δ 1.50–161 (m, 4H), 2.44 (s, 3H), 3.13–3.50 (m, 5H), 5.10 (bs, 1H), 7.22–7.57 (m, 2H), 8.02–8.12 (m, 1H), 8.50 (s, 1H).

Compound **25** and the piperidine analogue **40** were prepared by an identical procedure from the corresponding carboxyamide [17].

25 ^{1}H-NMR ($CDCl_3$) δ 1.50–1.61 (m, 4H), 2.44 (s, 3H), 3.13–3.50 (m, 5H), 5.10 (bs, 1H), 7.22–7.57 (m, 2H), 8.02–8.12 (m, 1H), 8.50 (s, 1H).

40 ^{1}H-NMR ($CDCl_3$) δ 1.37–161 (m, 6H), 2.34 (s, 3H), 2.87–3.19 (m, 5H), 5.03 (bs, 1H), 7.20–7.61 (m, 2H), 8.07–8.19 (m, 1H), 8.33 (s, 1H).

Characterization of **24**, **25** and **40** is summarized in *table I*.

6.3. Bioassay

6.3.1. Receptor binding assay

Cerebral cortices of male Wistar rats (150–200 g) were dissected on ice. The tissue was homogenized in 50 mM Tris-HCl buffer (pH 7.4 at 2 °C) containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 2.5 mM $CaCl_2$. The homogenate was centrifuged at 40 000 *g* for 10 min; the pellet was re-suspended in ice-cold buffer, recentrifuged and re-suspended again in buffer. Binding experiments [28] with [^{3}H]-cytisine (New England Nuclear, Boston, MA; 39.7 Ci/mM) were perfomed in 250 μL of buffer which contained 2 nM [^{3}H]-cytisine, membranes from 15 mg (wet weight) of tissue and the compound to be tested. After 75 min of incubation at 2 °C, separation of bound from free radioligand was performed by rapid filtration through Whatman GF/C glass fiber filter, which were washed three times with ice-cold buffer, dried and counted in 5 mL of Aquassure (Packard, Downers Grove, USA). Binding in the presence of 10 mM (−)-nicotine bitartrate was defined unspecific and was, routinely, about 10% of the total binding. K_i values were calculated from the Cheng-Prusoff equation [29] using 1.5 nM as the K_d for [^{3}H]-cytisine, determined by previous saturation experiments.

6.3.2. Antinociception

The analgesic potency of compounds was estimated in Swiss-Webster mice weighing 23–25 g. The tail-flick test was essentially that described by Janssen et al. [23], using water at 55 °C as nociceptive stimulus. Tests were made prior to and at various times after s.c. administration of each compound in saline (4 μL). The average reaction time in control animals was 4 s. Complete analgesia was assumed to be present when no reaction appeared 12 s after application of noxious stimulus. Percent analgesia was calculated according to the formula $[(T - T_0)/(12 - T_0)] \times 100$ (T = reaction time (seconds) after administration of compound; T_0 = 'normal' reaction time before injection of compound; 12 = cutoff time). The specificity of the effects was tested by pretreating the animals with mecamylamine, hexametonium or naloxone (1–2 mg/kg sc). In all cases, the mecamylamine antagonist prevented any analgesic effect. The median antinociceptive dose (ED_{50}) and 95% confidence limits were calculated according to the method of Litchfield and Wilcox [30].

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