Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3847–3851

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Rigidified Acetylcholine Mimics: Conformational Requirements for Binding to Neuronal Nicotinic Receptors

Gérald Villeneuve,[a,*] Danielle Cécyre,[b] Hélène Lejeune,[b] Marc Drouin,[a] Ruoxi Lan[a] and Rémi Quirion[b]

[a]*Département de Chimie, Université de Sherbrooke, 2500 boul. de l'Université, Sherbrooke, Québec, Canada J1K 2R1*
[b]*Centre de Recherche de l'Hôpital Douglas, McGill University, 6857 boul. Lasalle, Verdun, Québec, Canada H4H 1R3*

Received 2 April 2003; revised 8 July 2003; accepted 12 July 2003

Abstract—Rigidified derivatives have been designed and synthesized assuming the $g+t$ conformer of acetylcholine (N–C–C–O = +60°, C–C–O–C = 180°) as active conformation for binding to cytisine sensitive neuronal nicotinic receptors. The SAR of the compounds evaluated, along with those of more flexible analogues, support the $g+t$ conformer hypothesis and highlight the stringent steric limitation of this nicotinic receptor sub-type. Compound **3e** has low μM affinity for cytisine sensitive nicotinic receptor binding sites while being selective with regard to the α-bungarotoxin sensitive subclass. We also report few compounds with μM affinity for the α-bungarotoxin sensitive subclass.

Acetylcholine (**Ach**), the first neurotransmitter to have been characterized, acts on several classes and subclasses of receptors. The nicotinic receptors have been demonstrated to be ligand-gated ion channel while muscarinic receptors belong to the superfamily of G-proteins link receptors. Receptor cloning revealed the existence of five different muscarinic receptors.[1] The case of neuronal nicotinic receptors is far more complex. Most nicotinic receptors are believed to be constituted by the non-covalent association of five proteins subunits. Up to now, eight neuronal α subunits have been cloned (α2–α9) along with three non-α subunits (β2–β4).[2] Although the number of possible combinations is enormous, it seems that some may predominate. The pharmacological receptor responsible for α-bungarotoxin (α-BGT) binding in the brain is probably mainly composed of five α7 subunits.[3] On the other hand, the receptors having high affinity for both cytisine (**Cyt**) and nicotine (**Nic**) mostly involve a combination of α4 and β2.[4]

Nicotinic and muscarinic receptors have been shown to be the most affected in diseases associated with severe memory impairment such as in Alzheimer's disease.[5] An avenue explored was to develop specific acetylcholine esterase inhibitors in order to increase acetylcholine level in the brain. Tacrine and Donepezil are homologated substances for Alzheimer's disease treatment which mainly act via this mechanism.[6] Efforts should also be given to develop selective brain nicotinic agonists that could stimulate for example, presynaptic positive nicotinic autoreceptors.[7] Our approach is related to that goal and to better understand the steric and electronic requirements needed to ensure high affinity and selectivity for neuronal nicotinic receptors. We present herein the rationale design, synthesis and receptor binding affinity of these compounds.

Beer and Reich have proposed that the onnium head and the carbonyl oxygen of **Ach** interact with the nicotinic receptor.[8] **Cyt** can serve as an unambiguous pharmacophore template. Using the amide carbonyl dipole and the secondary amine of **Cyt** to define a three points pharmacophore, a relatively good alignment could be achieved with (+) anatoxin (**Ana**).[9] For **Nic** and (−) epibatidine (**Epi**),[10] the ring center to the pyridine nitrogen was defined as the corresponding dipole. **Ach** can adapt well to that pharmacophore if it adopt the g^+t conformation with τ_2 (N–C–C–O) = +60°, τ_1 (C–C–O–C) = 180° and τ_0 (CH_2–O–C–O) = 0°. The aligned molecules are presented in Figure 1.

Accordingly we attempted to design rigidified analogues of the g^+t conformer of acetylcholine. Our rationale is presented in Figure 2. Firstly, a cyclic ketone will be

*Corresponding author. Tel.: +819-846-1720; fax: +819-821-8070; e-mail: geraldvilleneuve@yahoo.ca

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00728-5

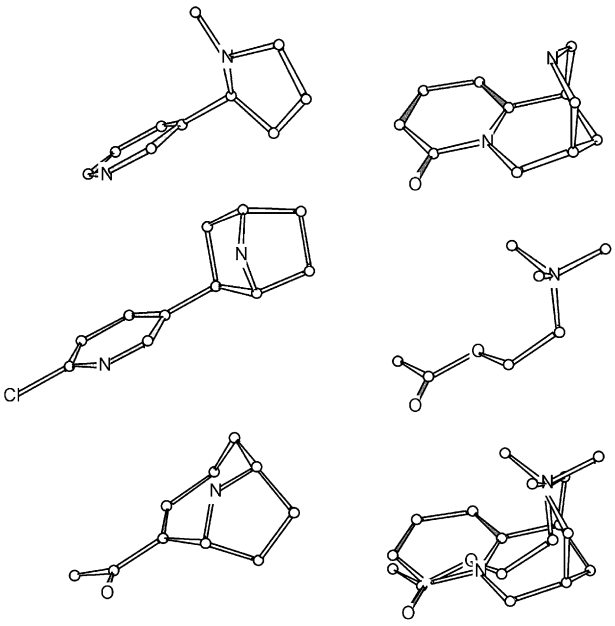


Figure 1. Aligned nicotinic receptor ligands according to Beer and Reich pharmacophore hypothesis. From top, left to right: **Nic**, **Cyt** **Epi**, **Ach** **Ana** and overlay of **Ach** with **Cyt**.

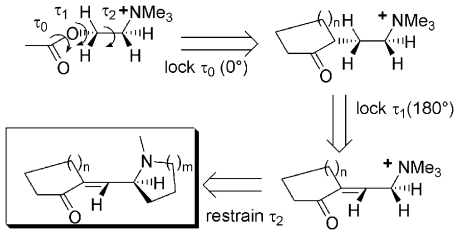
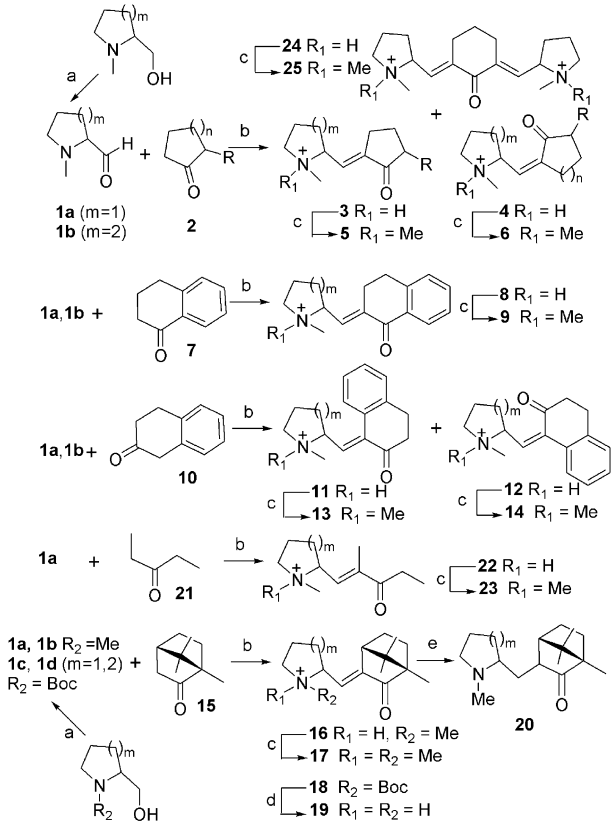


Figure 2. The molecular design of rigidified $g+t$ Ach mimics.

used to mimic the ester bond. Then an *E* double bond will be used to lock τ_1 at 180° (*t*). Finally the incorporation of the amine within a ring would create restrain rotation around τ_2 such that the favored dihedral angle will be close to $+60°$ (g^+) for the S configuration at C2.

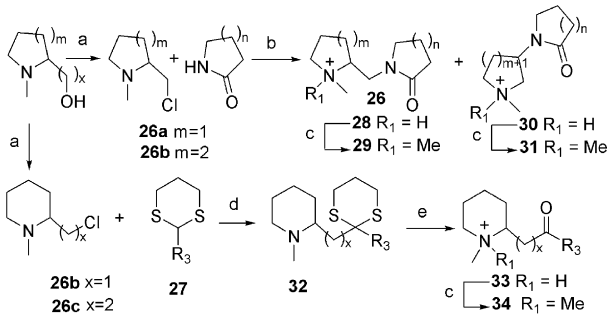
The preparation of rigidified acetylcholine derivatives is shown in Scheme 1. The method relied on the aldol condensation of the lithium enolate of a ketone with *N*-methyl-pyrrolidine carboxaldehyde (**1a**) or *N*-methyl-piperidine carboxaldehyde (**1b**) in THF at −78 °C. The condensation occurred usually with subsequent dehydration to provide mainly the desired *E* enone with few *Z* enone. The *E* configuration was confirmed by X-ray crystallography for compound **9** which by the same token provided the observed τ_2 value of $+117°$.[11] The *Z* enone was always more polar than the *E* enone and purification was possible by flash or layer chromatography. In ^{1}H NMR, the chemical shift of the *E* olefin proton was always more upfield. In one case, the dialkylated ketone **24** was obtained but owing to its much higher polarity, it could be easily separated. Starting material **1a–d** were obtained from the corresponding commercial alcohols using Swern oxidation technique.[12] Measured $[\alpha]_D^{20}$ was consistent to values reported for



Scheme 1. Synthesis of rigidified Ach analogues: (a) $C_2O_2Cl_2$, DMSO, DCM, −50 °C 30 min, then TEA: (b) LDA/THF −78 °C with ketones **2**, **7**, **10**, **15** or **21** 2 h, then **1** (1 equiv) 1–2 h; (c) CH_3I, MeOH, rt, 12 h; (d) PTSA 2 equiv, CH_3CN, 20 h; (e) Na (2 equiv) in liquid NH_3.

related *N*-alkyl-pyrrolidine carboxaldehydes.[13] Furthermore, the aldol condensation did not induce significant epimerization since obtained products had high optical rotations and the diastereomeric ratio for the reaction of **1a** with (+) camphor was 90% (as assessed by GCMS and ^{13}C NMR). Products were Kugelrhor distilled such that GC-MS provided purity and identity of compounds.[14]

More flexible analogues and surrogates were prepared for the sake of comparison and to try to delineate the active portion of (−)lobeline (**Lob**).[15] Their preparations are presented in Scheme 2. Firstly, the corresponding



Scheme 2. Reagents and conditions: (a) PPh_3, CCl_4, CH_3CN, rt to reflux; (b) KF/Al_2O_3, CH_3CN, rt, 24 h; (c) CH_3I, MeOH, rt, 12 h; (d) **27**, 1 equiv *n*BuLi, THF or Et_2O, TMEDA, −78 °C, 30 min, then **26b**,**c**; (e) 1 equiv HCl, $C_6H_5I(CF_3CO_2)_2$, MeOH, H_2O, 1 h.

Table 1. Radioligand binding results at neuronal nicotinic receptors for enone compounds

Compd	*m*	*n*	*R*	Geom.	Ster.	[^{3}H]Cytisine IC_{50} (μM)[a]	[^{125}I]α-BGT IC_{50} (μM)[b]
Epi						0.0008 (±.0001)	0.0037
Ana						0.0045 (±.001)	0.004
Lob						0.087 (±0.026)	18.1
3a	1	1	H	*E*	*S*	0.48 (±0.16)	2.8
3b	1	2	H	*E*	*S*	0.93 (±0.43)	44
3c	2	1	H	*E*	*RS*	11.3 (±2.6)	nd
3d	2	2	H	*E*	*RS*	23.7 (±8.2)	nd
3e	1	2	Me	*E*	*S*	0.46 (±0.16)	31
4d	2	2	H	*Z*	*RS*	207 (±145)	nd
4e	1	2	Me	*Z*	*S*	5.46 (±3.5)	111
5a	1	1	H	*E*	*S*	0.12 (±0.06)	0.16
5b	1	2	H	*E*	*S*	0.84 (±0.10)	0.15
5c	2	1	H	*E*	*RS*	1.5 (±0.24)	134
5d	2	2	H	*E*	*RS*	6.5 (±1.9)	207
5e	1	2	Me	*E*	*S*	0.75 (±0.33)	43
6e	1	2	Me	*Z*	*S*	20.5 (±7.8)	0.21
8a	1			*E*	*S*	12.5 (±4.0)	nd
8b	2			*E*	*RS*	>100	nd
9	2			*E*	*RS*	>100	nd
11	2			*E*	*RS*	>100	37
12	2			*Z*	*RS*	>100	81
13	2			*E*	*RS*	136 (±79)	5.4
14	1			*Z*	*S*	110 (±33)	2.4
16a	1			*E*	*S*	35.9(±16)	nd
16b	2			*E*	*RS*	52.5 (±31)	115
17b	2			*E*	*RS*	12.2 (±3.0)	25.3
19a	1			*E*	*S*	>100	436
19b	2			*E*	*RS*	57.7 (±47)	>100
20	2				*RS*	32.3 (±23)	91
22	2			*E*	*RS*	194 (±104)	nd
23	2			*E*	*RS*	68.6 (±8.4)	nd
24	1	2	—	*E*	*S*	207 (±130)	nd
25	1	2	—	*E*	*S*	89 (±15)	nd

[a]Values are means of two to three independent experiments using triplicate, standard deviation is given in parentheses.
[b]Values from a single triplicate experiment (nd, not determined).

saturated ketones were prepared using a 1–3 dithiane anion[16] which was shown to react well with the activated chloride **26b**,**c**. The resulting alkylated 1–3 dithianes (**32**) were converted to the ketones using the protocol described by Stork and Zhao.[17] On the other hand, isosteric amide compounds were prepared by reacting γ or δ lactams with activated chlorides **26a**,**b** under KF catalyzed conditions.[18] Owing to the formation of aziridinum intermediate, the reaction gave also the ring expanded compounds **30**. Normal products and ring expanded products were separated by silica gel flash or layer chromatography.

All pure compounds were characterized by ^{1}H, ^{13}C NMR and GCMS. Free bases were converted to hydrochloride salts with anhydrous ethereal HCl solution and stored in a moisture free environment. Quaternary salts were generally purified by precipitating them out. All hydrochlorides and quaternary salts were water soluble. The compounds were evaluated for their abilities to compete for specific [^{3}H]cytisine (α4β2 subclass) and [^{125}I]α-bungarotoxin (α7 subclass) binding sites in rat forebrain homogenates.[19]

Binding affinity for the cytisine sensitive neuronal Ach receptors was obtained in the low μM range for compounds within the enone series (Table 1). Tertiary amine compounds **3a**, **3b** and **3e** were the best ligands and parallel affinity was found for corresponding quaternary salts **5a**, **5b**, and **5e**. When the selectivity over the α-BGT-sensitive Ach receptor subclass was considered, compound **3e** cumulates the best affinity/selectivity characteristics. Although compound **5e** has also a good profile, its quaternary salt nature makes it less interesting in consideration of blood–brain barrier penetration. In all cases within the enone series, the pyrrolidine compounds ($m=1$) were 10–20 times more potent than the corresponding piperidines ($m=2$) (**3a** vs **3c**, **3b** vs **3d**,

Table 2. Radioligand binding results at neuronal nicotinic receptors for lactam compounds

Compd	*m*	*n*	R_1	Ster.	[^{3}H] Cytisine IC_{50} (μM)[a]	[^{125}I] α-BGT IC_{50} (μM)[b]
28a	1	1	H	*S*	20.1 (±0.9)	49.5
28b	1	2	H	*S*	>100	>100
28c	2	1	H	*RS*	26.3 (±1.6)	2.72
28d	2	2	H	*RS*	>100	>100
29a	1	1	Me	*S*	93 (±18)	12.2
29b	1	2	Me	*S*	>100	>100
29c	2	1	Me	*RS*	>100	2.87
29d	2	2	Me	*RS*	>100	>100
30	—		H	*RS*	78 (±18)	>100
31	—		Me	*RS*	>100	87

[a]Values are means of two to three independent experiments using triplicate, standard deviation is given in parentheses.
[b]Values from a single triplicate experiment (nd, not determined).

Table 3. Radioligand binding results at neuronal nicotinic receptors for dithiane and ketone compounds

Compd series	x	R_3	[^{3}H]Cytisine IC_{50} (μM) **32**[a]	[^{3}H]Cytisine IC_{50} (μM) **33**[a]	[^{3}H]Cytisine IC_{50} (μM) **34**[a]
a	1	Ph	>100	>100	—[b]
b	1	2-Furyl	>100	5.99 (±1.9)	—[b]
c	1	*p*Cl-Ph	>100	>100	>100
d	1	2-Pyridyl	>100	>100	—[b]
e	1	2-Thienyl	>100	10.4 (±4.6)	—[b]
f	1	3-Pyridyl	53.7 (±23)	—[b]	—[b]
g	2	*p*Cl-Ph	42.6 (±2.1)	>100	62.6 (±29.8)
h	2	2-Thienyl	59.4 (±19.2)	24.6 (±0.2)	29.0 (±6.8)
i	2	Me	>100	3.7 (±1.6) [c]	1.80 (±0.53)[c]
j	2	*p*-F-Ph	59.4 (±31.9)	23.4 (±9.5)	>100

[a]Values are means of two to three independent experiments using triplicate, standard deviation is given in parentheses.
[b]Compound was not prepared.
[c]Binding for [^{125}I]α-BGT were **33i**: 15.0 μM and **34i**: 0.17 μM.

5a vs **5c**, **8a** vs **8b**) which supersed the sole effect of the racemic nature of the piperidine compounds and argue for a ring size effect similar to that observed for nicotine versus anabasine.[20] Having large substituents nearby the ketone function (compounds **8**–**10** and **15**–**20**) or a large substituent within the area that could interact with the receptor carbonyl binding subsite (compounds **11**–**14**) induces significant loss in affinity. This could suggest that results obtained with compounds **22** and **23** are inconsistent (**22** is not larger than **3e** at first glance). However, owing to the non-cyclic nature of **22** and **23**, the enone can assume either the s-*Z* ($\tau_0 = 0°$) or the s-*E* ($\tau_0 = 180°$) conformation. MM2 calculations suggest that the s-*E* conformer is 2 *K*cal/mol more stable than the s-*Z*. The s-*E* conformer will be largely predominant and since the later does not permit to achieve the postulated pharmacophore topology, this may explain the low affinity of **22** and **23**. Finally, when the effect of the stereochemistry of the double bond was studied alone, the *E* compounds were generally 10 times more potent than the corresponding *Z* compounds (**3d** vs **4d**, **3e** vs **4e**, **5e** vs **6e**) which give support to our molecular design. Interestingly, the *Z* compounds **6e** and **14** are fairly potent on α-BGT binding sites with good selectivity over cytisine sensitive sites.

Within the lactam series (Table 2), affinity for the cytisine sensitive subclass was much lower than for the corresponding enone compounds. In principle the lactam series is isosteric to the enone series but the conformation is not as well controlled. In fact, X-ray crystallography of compound **29c**[11] showed that the later adopts a conformation where corresponding τ_2 and τ_1 dihedral angles take the values of $-161°$ and $-112°$, respectively; at departure from the putative pharmacophore. Interestingly, this conformation is apparently more appropriate for α-BGT sites since significant affinity was found for compounds **29c** and related **28c**. The ring size of the lactam ring ($n = 1$) in that series was rather critical.

In the ketone series (Table 3), compounds with X = 1 were made mainly to try to identify the active portion of **Lob** whereas with X = 2 they mimic more **Ach** or carbamylcholine. None of the compound was close to **Lob** affinity (Table 1), although **33b** and **33e** showed some tendency. Compounds bearing an aryl ketone with X = 2 had moderate affinity but were clearly less good ligands than the methyl ketones **33i** and **34i**. Comparison of the affinity of **33i** with that of **3a** supports the beneficial effect of an appropriate locking of the conformation, even if some steric bulk is created under such conditions.

Acknowledgements

This work was supported by a conjoint NSERC Nordic-Merrell-Dow Canadian grant. We would like to thank Dr. André Michel for initiating this project and Pr. Pierre Deslongchamps for taking after. Mr. Gaston Boulay is acknowledged for GC–MS and exact mass.

References and Notes

1. Hulme, E. C.; Birdsall, N. J. M.; Buckley, N. J. *Annu. Rev. Pharmacol. Toxicol.* **1990**, *30*, 633.
2. Sargent, P. *Annu. Rev. Neurosci.* **1993**, *16*, 403.
3. Seguela, P.; Wadiche, J.; Dineley-Miller, K.; Dani, J. A.; Patrick, J. W. *Eur. J. Neurosci.* **1993**, *13*, 596.
4. Flores, C. M.; Rogers, S. W.; Pabreza, L. A.; Wolfe, B. B.; Kellar, K. J. *Mol. Pharmacol.* **1992**, *41*, 31.
5. Coyle, J. T.; Price, D. L.; DeLong, M. *Science* **1983**, *219*, 1184.
6. Snape, M. F.; Misra, A.; Murray, T. K.; DeSouza, R. J.; Williams, J. L.; Cross, A. J.; Green, A. R. *Neuropharmacology* **1999**, *38*, 181.
7. Araujo, D. M.; Lapchak, P. A.; Meaney, M. J.; Collier, B.; Quirion, R. *J. Neurosci.* **1990**, *10*, 3069.
8. Beers, W. H.; Reich, E. *Nature* **1970**, *228*, 917.
9. Thomas, P.; Stephens, M.; Wilkie, G.; Amar, M.; Lunt, G. G.; Whiting, P.; Gallagher, T.; Pereira, E.; Alkondon, M.; Albuquerque, E. X. *J. Neurochem.* **1993**, *60*, 2308.
10. Quian, C.; Li, T.; Shen, T. Y.; Libertin-Garahan, L.; Eckman, J.; Biftu, T.; Ip, S. *Eur. J. Pharm.* **1993**, *250*, R13.
11. Crystallographic data for structures **9** and **29c** have been deposited to the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 205925 and 205926. Copies of the data can be obtained, free of charge, on

application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
12. Mancuso, A. J.; Huang, S. L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480.
13. **1a** $[\alpha]_D^{20} = -130°$ (*c* 7.0, CH_2Cl_2): Linkert, F.; Laschat, S.; Kotila, S.; Fox, T. *Tetrahedron* **1995**, *52*, 955.
14. Selected data: **3d**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.50 (dt, *J* = 9.5, 3.8 Hz, 1H, C=CH), 2.86 (dtd, *J* = 11.2, 3.1, 1.5 Hz, 1H, $NCH_{eq}H_{ax}$), 2.55–2.68 (m, 2H), 2.32–2.50 (m, 3H), 2.11 (s, 3H), 1.93–2.02 (m, 1H), 1.78–1.90 (m, 2H), 1.65–1.78 (m, 3H), 1.56–1.65 (m, 2H), 1.15–1.55 (m, 3H) ; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.7, 140.7 (CH), 136.3, 61.9, 56.0, 44.4, 40.2, 30.8, 27.1, 25.6, 23.5, 23.4, 23.2; *m*/*z* (GC–MS) 207 (M^+), 192 ($M^+ - Me$), 98.
15. Barlow, R. B.; Johnson, O. *Br. J. Pharmacol.* **1989**, *98*, 799.
16. Corey, E. J.; Seebach, D. *Angew. Chem., Int. Ed. Engl* **1965**, *4*, 1075.
17. Stork, G.; Zhao, K. *Tetrahedron Lett.* **1989**, *30*, 287 (addition of HCl prevent formation of amine-oxide).
18. Yamawaki, J.; Ando, T. *Chem. Lett.* **1989**, 755.
19. Membrane-enriched homogenates from rat forebrain were freshly prepared by homogenization in 50 mM Tris buffer, pH 7.4 at 4 °C using a Brinkmann polytron (setting 6, 20 s). The homogenate was centrifuged for 10 min at 49,000*g*, the supernatant was discarded and the pellet washed twice by resuspension in fresh buffer and re-centrifugation. The final pellet was resuspended in incubation buffer, consisting of 50 mM Tris containing 1 mM $MgCl_2$, 120 mM NaCl, 5 mM KCl and 2 mM $CaCl_2$ (pH 7.4), to obtain concentration of 1.0–3.0 mg protein/mL. Aliquots (0.2 mL) were added to assay tubes containing analogue (4–8 concentrations, 10 pM–0.1 mM) and 2 nM [^{3}H]Cytisine or 1.5 nM [^{125}I]α-BGT for a final assay volume of 0.6 mL. Incubation was performed for 90 min at 4 °C for [^{3}H]Cytisine and for 120 min at 22 °C with BSA (1 mg/mL) for [^{125}I]α-BGT. 10 and 100 μM of **Nic** were used respectively to define the non-specific binding. Separation of bound from free radioactive ligand was performed by rapid filtration through #32 glass fiber filters (presoaked in 0.1% polyethyleneimine solution) under reduced pressure using a Brandell Cell Harvester followed by 3×4 mL rapid washes with 50 mM Tris buffer, pH 7.4 at 4 °C. Bound radioactivity was determined via liquid scintillation.
20. Reavill, C.; Jenner, P.; Kumar, R.; Stolerman, I. P. *Neuropharmacology* **1988**, *27*, 235.