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Aporphine metho salts as neuronal nicotinic acetylcholine receptor blockers

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Abstract—(*S*)-Aporphine metho salts with the 1,2,9,10 oxygenation pattern displaced radioligands from recombinant human α7 and α4β2 neuronal nicotinic acetylcholine receptors (nAChR) at low micromolar concentrations. The affinity of the nonphenolic glaucine methiodide (**4**) (vs [^{3}H]cytisine) was the lowest at α4β2 nAChR (K_i = 10 μM), and predicentrine methiodide (**2**) and xanthoplanine iodide (**3**), with free hydroxyl groups at C-2 or C-9, respectively, had the highest affinity at these receptors ($K_i \approx 1$ μM), while the affinity of the diphenolic boldine methiodide (**1**) was intermediate between these values. At homomeric α7 nAChR, xanthoplanine had the highest affinity (K_i = 10 μM) vs [^{125}I]α-bungarotoxin while the other three compounds displaced the radioligand with K_i values between 15 and 21 μM. At 100 μM, all four compounds inhibited the responses of these receptors to EC_{50} concentrations of ACh. The effects of xanthoplanine iodide (**3**) were studied in more detail. Xanthoplanine fully inhibited the EC_{50} ACh responses of both α7 and α4β2 nACh receptors with estimated IC_{50} values of 9 ± 3 μM (α7) and 5 ± 0.8 μM (α4β2).

1. Introduction

One of the earliest generalizations in the structure–activity field was that *N*-methylation of several alkaloids affords compounds with curare-like properties.[1] Almost half a century later a ''nicotinic paralyzing'' action of tetraalkylammonium salts was demonstrated on autonomic ganglia,[2–4] and both neuromuscular and ganglionic blockade were attributed to nicotinic acetylcholine receptors (nAChR). The widespread occurrence and important regulatory roles of nAChR in the central nervous system (CNS) were recognized much more recently, but the diversity of receptor subtypes and the lack of sufficiently selective agonists or blockers have hindered a detailed description of their individual roles.[5] In this sense, the potential of quaternary alkaloids or alkaloid derivatives related to the classical bisbenzylisoquinoline nicotinic receptor blocker tubocurarine (Fig. 1), as blockers of CNS nAChR, has not been sufficiently explored.

The monomeric aporphine alkaloids, regardless of their secondary, tertiary or quaternary ammonium nature (e.g., laurifoline or *N*-methylisoboldinium, Fig. 1), are rigid structural mimics of one of the benzyltetrahydroisoquinoline halves of tubocurarine, which makes them attractive templates for structure–activity studies as nicotinic receptor blockers. Although aporphine alkaloids are widespread in nature and constitute one of the largest alkaloid families, their quaternary ammonium *N*-methyl derivatives or metho salts seem to be relatively rare and little is known of the pharmacology of these quaternary salts.

N-methylboldinium [(6a*S*)-2,9-dihydroxy-1,10-dimethoxyaporphinium] chloride has been described as a constituent of *Cocculus laurifolius* DC (Menispermaceae) together with several other quaternary aporphine alkaloids that are presumably responsible for the neuromuscular blocking and hypotensive activities of the water-soluble alkaloid fraction of that plant.[11] Xanthoplanine or *N*,*N*-dimethyllaurotetaninium [(6a*S*)-9-

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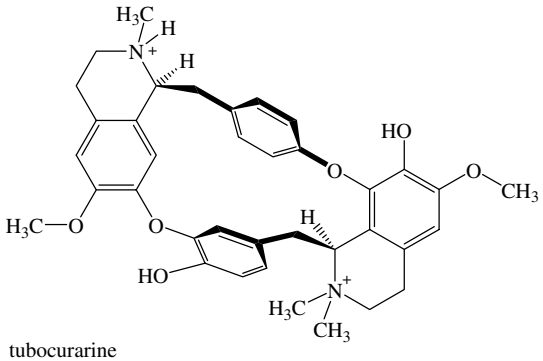
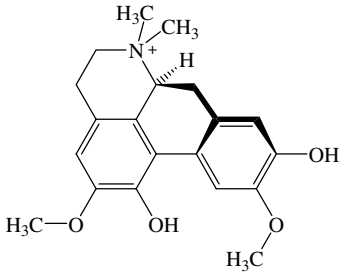
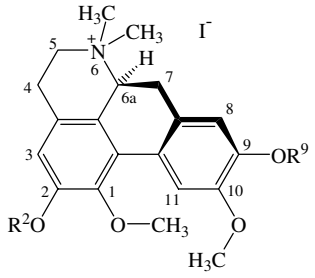


tubocurarine



laurifoline

Figure 1. Structures of tubocurarine, the prototypical nAChR blocker, and laurifoline, a quaternary aporphine alkaloid.



1 $R^2 = R^9 = H$

2 $R^2 = H$; $R^9 = CH_3$

3 $R^2 = CH_3$; $R^9 = H$

4 $R^2 = R^9 = CH_3$

Figure 2. Structures of aporphine metho salts studied in this report.

hydroxy-1,2,10-trimethoxy-*N*-methylaporphinium] has been found on several occasions in plants,[12] while *N*-methylglaucinium [(6a*S*)-1,2,9,10-tetramethoxy-*N*-methylaporphinium] only seems to be known as a synthetic product.[13] To the best of our knowledge, *N*-methylpredicentrinium [(6a*S*)-2-hydroxy-2,9,10-trimethoxy-*N*-methylaporphinium] has neither been found in nature nor prepared synthetically before the present work.

More than 30 years ago the methiodides of both enantiomers of the aporphines corydine (1-hydroxy-2,10,11-trimethoxyaporphine), isocorydine (11-hydroxy-1,2,10-trimethoxyaporphine), glaucine (1,2,9,10-tetramethoxyaporphine), and boldine (2,9-dihydroxy-1,10-dimethoxyaporphine) were prepared and assayed for curaremimetic blockade of the neuromuscular junction. It was concluded that the (6a*S*) isomers, corresponding to the naturally occurring tertiary amines, are slightly more potent than their mirror images, although details are lacking and the different (i.e., 1,2,10,11 and 1,2,9,10) oxygenation patterns are a hindrance to structure–activity analysis.[6] It therefore seemed of interest to reproduce this study in part, analyzing the effects of the metho salts of 1,2,9,10-tetraoxygenated aporphines on the predominant neural subtypes of nAChR (α7 and α4β2), using more modern methodology to look for possible effects of the degree and position of methylation of the free hydroxyl groups of the antioxidant,[7] cytoprotective,[8] and monoaminergic antagonist[9,10] parent alkaloid boldine upon their interactions with these receptors.

2. Results and discussion

The methiodides of boldine (**1**), predicentrine (**2**), *N*-methyllaurotetanine (xanthoplanine iodide, **3**), and glaucine (**4**) (Fig. 2) displaced [^{125}I]α-bungarotoxin (from human α7 nAChR) or [^{3}H]cytisine (from human α4β2 nAChR) with low micromolar K_i values in rather narrow ranges, although glaucine was considerably less potent than its phenolic analogues at hα4β2 nAChR (Table 1, Fig. 3).

Data represent means ± SEM of four experiments, each with triplicate samples. The [^{3}H]cytisine concentration in all α4β2 nACh displacement studies was 1 nM, while the concentration of [^{125}I]α-bungarotoxin (which also binds to the agonist site)[18] in all α7 nACh receptor displacement studies was 0.2 nM. The equilibrium dissociation constants (K_D) used to estimate K_i values were 1 nM for [^{125}I]α-bungarotoxin binding to α7 nACh receptors and 0.43 nM for the binding of [^{3}H]cytisine to hα4β2 nACh receptors.

For the sake of comparison, the nonquaternary boldine (**5**) was assayed against human α7 nAChRs or α4β2

Table 1. Binding affinities of quaternary and tertiary aporphines vs [^{125}I] α-bungarotoxin (α7) and [^{3}H] cytisine (α4β2) at human nAChR expressed in cultured cells

Compound	α7 IC_{50} (μM)	α7 K_i (μM)	α4β2 IC_{50} (μM)	α4β2 K_i (μM)
Boldine MeI (**1**)	18 ± 5	15	8.2 ± 3	2.5
Predicentrine MeI (**2**)	27 ± 8	21	3.2 ± 0.3	0.97
Xanthoplanine iodide (**3**)	12 ± 5	10	3.0 ± 0.7	0.91
Glaucine MeI (**4**)	21 ± 4	18	34 ± 2	10
Boldine (**5**)	81 ± 0.7	67	10 ± 2	3
3-Bromoboldine (**6**)	100 ± 5.9	83	99 ± 4	30
3,8-Dibromoboldine (**7**)	114 ± 8.4	95	101 ± 6	31

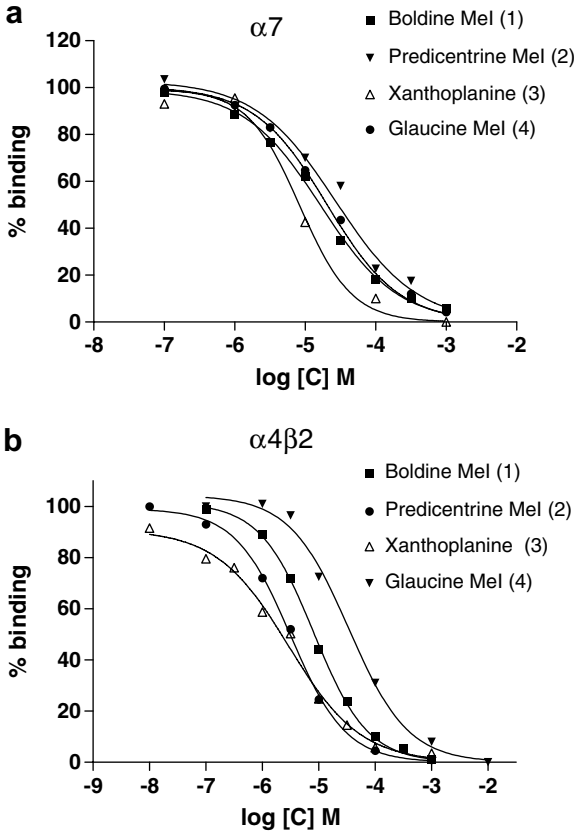


Figure 3. (a) Displacement of [^{125}I]α-bungarotoxin from SH-SY5Y cells stably expressing human α7 receptors,[18] by quaternary aporphine derivatives. (b) Displacement of [^{3}H]cytisine from SH-EP1 cells stably expressing human α4β2 receptors,[19] by quaternary aporphine derivatives.

nAChRs. Boldine completely inhibited 0.2 nM [^{125}I]α-bungarotoxin binding with K_i = 67 μM and thus has at least fourfold weaker affinity for α7 nAChRs than its quaternary salt. Unexpectedly, boldine proved to be a practically equipotent displacer of [^{3}H]cytisine from α4β2 nACh receptors as compared with *N*-methylboldinium (**1**), exhibiting K_i = 3 μM.

The effect of bromination of boldine at C3 (**6**) and at both C3 and C8 (**7**) was also examined. The brominated derivatives were unable to displace 0.2 nM [^{125}I]α-bungarotoxin completely from α7 nAChRs, and their estimated K_i values were slightly worse than for boldine. At this low concentration of toxin, the maximal binding inhibition was 91 ± 5% with 3-bromoboldine (**6**) and 85 ± 3% with 3,8-dibromoboldine (**7**). 3-Bromo- and 3,8-dibromoboldine also inhibited binding of [^{3}H]cytisine to α4β2 nACh receptors with estimated K_i values two to three times better than the K_i's estimated for their effects on α7 nACh receptors.

To examine the effects of boldine and its *O*-methylated derivatives on the function of α7 or α4β2 nACh receptors, we first determined the effect of 100 μM compounds **1**–**4**. As shown in Figure 4, all these compounds inhibited the responses of these receptors to EC_{50} concentrations of ACh. The effects of the most

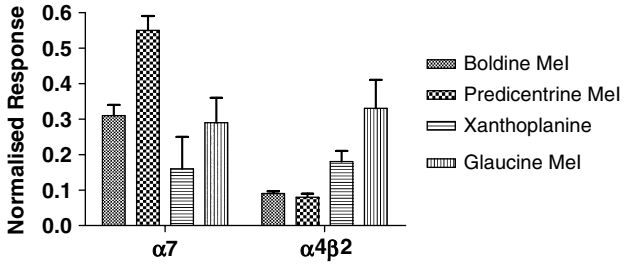


Figure 4. Response of *Xenopus laevis* oocytes transfected with human α7 or α4β4 subunit cDNA to EC_{50} concentrations of acetylcholine in the presence of 100 μM quaternary aporphine derivatives.

potent compound, xanthoplanine (**3**), were studied in more detail. As shown in Figure 5, xanthoplanine fully inhibited the EC_{50} ACh responses of both α7 and α4β2 nACh receptors. The estimated IC_{50} values were: α7, 9 ± 3 μM; α4β2, 5 ± 0.8 μM.

As expected, the affinity of the quaternary *N*-methylboldinium (**1**) was several times greater than that of the nonquaternary boldine (**5**) for human α7 nAChR. The halogenated derivatives of boldine (**6** and **7**) bound more poorly than the parent compound, but the difference was minimal. At α7 nAChR, the 9-*O*-methylated derivative *N*-methylpredicentrinium (**2**) and the 2,9-di-*O*-methylated *N*-methylglaucinium (**4**) did not differ much from *N*-methylboldinium (**1**), while the 2-*O*-methylated xanthoplanine (**3**) had somewhat higher affinity with the rank order **3** ⩾ **1** ≈ **4** ≈ **2**, suggesting a slightly stronger interaction of xanthoplanine with the α7 receptor.

At α4β2 nAChR the affinities of all four quaternary ammonium salts were greater than those at α7 nAChR, judging from their K_i values, with a rank order of affinities of **3** ≈ **2** > **1** > **4**, although at this receptor the nonphenolic glaucine methiodide had an affinity

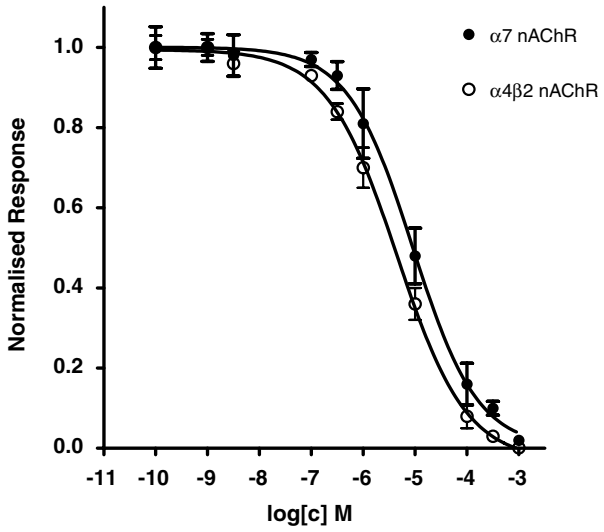


Figure 5. Response of *Xenopus laevis* oocytes transfected with human α7 or α4β4 subunit cDNA to EC_{50} concentrations of acetylcholine in the presence of different concentrations of xanthoplanine iodide.

approaching the same low range as at α7 nAChR. The almost identical micromolar affinities of xanthoplanine (**3**) and predicentrine methiodide (**2**) suggest that α4β2 nAChR receptor blockade by 1,2,9,10-tetraoxygenated quaternary aporphines is insensitive to the position of the free hydroxyl group. The similar affinities of boldine methiodide (**1**) and its parent compound (**5**) for α4β2 nAChR indicate that in this series quaternization is not always a requisite for effective nicotinic receptor blockade and is reminiscent of the effect of tubocurarine, in which one of the benzylisoquinoline moieties is also not quaternary, and of the tertiary *Erythrina* alkaloids and the synthetic secondary amine mecamylamine. However, bromination of boldine at C-3 (**6**) or at both C-3 and C-8 (**7**) resulted in a 10-fold drop in affinity for α4β2 nAChR.

3. Experimental

3.1. General procedures and chemicals

Solvents and reagents were of synthetic quality, purchased from Sigma-Aldrich (St. Louis, MO) or Merck (Darmstadt, Germany). The purity of the aporphine alkaloids used as starting materials (boldine isolated from *Peumus boldus* bark,[14] and *N*-methyllaurotetanine isolated from *Duguetia vallicola* leaves[15]) was assessed by TLC (CH_2Cl_2-MeOH–25% aq NH_3 80:19:1). 3-Bromoboldine (**6**) and 3,8-dibromoboldine (**7**) were prepared as described previously.[16] Melting points are uncorrected. NMR spectra were recorded at 300 MHz (^1H) or 75 MHz (^{13}C) using the solvent peak as an internal reference.

3.1.1. Boldine methiodide (1). Boldine was treated with MeI as described by Bremner and Winzenberg.[13] ^1H NMR (MeOH-d_4) δ 3.15 (3H, s, *N*-CH_3), 3.50 (3H, s, *N*-CH_3), 3.70 (3H, s, 1-*O*-CH_3), 3.93 (3H, s, 10-*O*-CH_3), 4.58 (1H, dd, $J = 14.1$, $J' = 3.4$ Hz, H-6a), 6.78 (1H, s, H-3), 6.93 (1H, s, H-8), 8.03 (1H, s, H-11). ^{13}C NMR (DMSO-d_6) δ 23.49 (C-4), 28.53 (C-7), 43.45 (*N*-CH_3), 53.41 (*N*-CH_3), 56.15 (1-*O*-CH_3), 60.03 (10-*O*-CH_3), 68.44 (C-6a), 112.2 (C-11), 114.7 (C-3), 115.7 (C-8), 118.6 (C-3a), 122.2 (C-7a), 125.5 (C-11a), 126.3 (C-3b), 127.5 (C-11b), 144.2 (C-1), 146.9 (C-9), 147.2 (C-10), 151.4 (C-2).

3.1.2. Predicentrine methiodide (2). Predicentrine (350 mg, 1.03 mmole), prepared by partial methylation of boldine,[14] was treated with methyl iodide in a procedure similar to that used for boldine. The gummy product was crystallized in MeOH-*i*-PrOH to afford 34 mg off-white microcrystalline rods, mp 202–204 °C, ^1H NMR (MeOH-d_4) δ 3.05 (3H, s, *N*-CH_3), 3.40 (3H, s, *N*-CH_3), 3.60 (3H, s, 1-*O*-CH_3), 3.82 (3H, s, 10- or 9-*O*-CH_3), 3.87 (3H, s, 9- or 10-*O*-CH_3), 4.53 (1H, dd, $J = 14.2$, $J' = 3.5$ Hz, H-6a), 6.70 (1H, s, H-3), 7.00 (1H, s, H-8), 7.97 (1H, s, H-11).

3.1.3. Xanthoplanine iodide (*N*-methyllaurotetanine methiodide, 3). *N*-Methyllaurotetanine (256 mg, 0.75 mmole) was dissolved in acetone (2 mL), treated with methyl iodide (1 mL, 2.28 g, 16 mmole), and stirred overnight protected from light. The product was collected by filtration as a white solid (296 mg, 81%), mp 188–190 °C (lit.[17] 190–191 °C), ^1H NMR (D_2O) δ 2.89 (s, 3H, *N*-CH_3), 3.27 (s, 3H, *N*-CH_3), 3.49 (s, 3H, 1-*O*-CH_3), 3.67 (s, 3H, 2-*O*-CH_3), 3.80 (s, 3H, 10-*O*-CH_3), 6.58 (s, 1H, H-3), 6.75 (s, 1H, H-8), 7.60 (s, 1H, H-11).

3.1.4. Glaucine methiodide (4). Glaucine, obtained by complete *O*-methylation of boldine,[14] was treated with methyl iodide as described by Bremner and Winzenberg.[13] ^1H NMR (MeOH-d_4) δ 3.08 (3H, s, *N*-CH_3), 3.43 (3H, s, *N*-CH_3), 3.68 (3H, s, 1-*O*-CH_3), 3.84 (3H, s, 2- or 9-*O*-CH_3), 3.88 (3H, s, 9- or 2-*O*-CH_3), 3.89 (3H, s, 10-*O*-CH_3), 4.63 (1H, dd, $J = 14.2$, $J' = 3.2$ Hz, H-6a), 6.89 (1H, s, H-3), 7.21 (1H, s, H-8), 7.98 (1H, s, H-11).

3.2. Pharmacology

3.2.1. Receptor binding. All seven compounds were assayed for binding to human α7 and α4β2 nAChR stably expressed in clonal cell lines,[18,19] using [^{125}I]α-bungarotoxin (α7) or [^{3}H]cytisine (α4β2) as radioligands,[20] The binding saline used in [^{125}I]α-bungarotoxin studies consisted of (in mM) 140 NaCl, 1 EDTA, and 50 Tris–HCl at pH 7.4, while for [^{3}H]cytisine binding the saline contained (in mM) 120 NaCl, 5 KCl, 1 $MgCl_2$, 2.5 $CaCl_2$, and 50 Tris, pH 7.0. [^{125}I]α-bungarotoxin binding and [^{3}H]cytisine binding studies were carried out in membrane homogenates prepared from α7- or α4β2-clonal cell lines, respectively, as described previously.[21,22] Non-specific binding was defined using 10 μM nicotine.

3.2.2. Functional studies. *Xenopus* oocytes were prepared and injected with human α7 or combinations of α4 + β2 nicotinic acetylcholine receptor subunit cDNAs as previously described.[20] Whole-cell currents were measured by two-electrode voltage clamp (GeneClamp 500, Axon Instruments, USA) using 0.5–1.5 MΩ agarose-cushioned electrodes containing 3 M KCl. Oocytes were continually supplied with fresh Ringer solution (mM: 115 NaCl; 2.5 KCl; 1.8 $BaCl_2$; 10 HEPES, pH 7.2) in a 60 μL bath, using a gravity-driven perfusion system at a rate of 10 ml/min. Acetylcholine and the aporphine derivatives were applied dissolved in the same Ringer medium by gravity perfusion using a manually activated valve. Acetylcholine was applied for a period sufficient (approximately 10–20 s) to obtain a stable plateau response (at low concentrations) or until the beginning of a sag after the peak response (at higher concentrations). To construct antagonist dose–response curves, the responses elicited by co-application of the agonist and increasing concentrations of antagonist were normalized to the responses elicited by EC_{50} concentrations of acetylcholine alone. Constant responses to acetylcholine were obtained before the co-application of agonist and antagonist. In these studies oocytes were preincubated with antagonist for two min prior to the co-application procedure to ensure equilibration between receptors and antagonists. To maintain ongoing measurements of the control response to agonist throughout the experiment, each co-application was bracketed by an application of

EC_{50} acetylcholine alone. Between successive drug applications, the cell was perfused with Ringer solution for 4 min to allow drug clearance and prevent receptor desensitization.

3.3. Data analyses

Concentration-response functional or radioligand binding data for antagonists were fit by non-linear regression (Prism 4.01, GraphPad, USA) to the equations:

$$i = i_{max}/[1 + (IC_{50}/x)^{nHill}],$$

wherein i_{max}, maximal normalized current response (in the absence of antagonist for inhibitory currents); x, antagonist concentration, IC_{50}, antagonist concentration eliciting half-maximal inhibition, and nHill, Hill coefficient.

For radioligand binding data, the K_i value of the test compounds was determined using the equation of Cheng and Prusoff, $K_i = EC_{50}/1 + [X]/K_d$.

Results are presented as means ± standard error of the mean (SEM) of at least four separate experiments from at least two different batches of oocytes or membrane preparations. Where appropriate, One-way ANOVA or Student's t test for unpaired data was used, and values of $P \leqslant 0.05$ were regarded as significant.

Acknowledgments

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References and notes

1. Brown, A. C.; Fraser, T. R. *Trans. R. Soc. Edinburgh* **1869**, *25*, 151–203.
2. Marshall, C. R. *Trans. R. Soc. Edinburgh* **1913**, 17–40.
3. Dale, H. H. *J. Pharmacol. Exp. Ther.* **1914**, *6*, 147–190.
4. Burn, J. H.; Dale, H. H. *J. Pharmacol. Exp. Ther.* **1915**, *6*, 417–438.
5. Cassels, B. K.; Bermúdez, I.; Dajas, F.; Abin-Carriquiry, J. A.; Wonnacott, S. *Drug Discov. Today* **2005**, *10*, 1657–1665.
6. Erhardt, P. A.; Soine, T. O. *J. Pharm. Sci.* **1975**, *64*, 53–62.
7. Cassels, B. K.; Asencio, M.; Conget, P.; Speisky, H.; Videla, L. A.; Lissi, E. A. *Pharmacol. Res.* **1995**, *31*, 103–107.
8. Bannach, R.; Valenzuela, A.; Cassels, B. K.; Núñez-Vergara, L. J.; Speisky, H. *Cell Biol. Toxicol.* **1996**, *12*, 89–100.
9. Madrero, Y.; Elorriaga, M.; Martínez, S.; Noguera, M. A.; Cassels, B. K.; D'Ocon, M. P.; Ivorra, M. D. *Br. J. Pharmacol.* **1996**, *119*, 1563–1568.
10. Asencio, M.; Delaquerrière, B.; Cassels, B. K.; Speisky, H.; Comoy, E.; Protais, P. *Pharmacol. Biochem. Behav.* **1999**, *62*, 7–13.
11. Saxena, N. K.; Bhakuni, D. S. *J. Indian Chem. Soc.* **1979**, *56*, 1020–1023.
12. Guinaudeau, H.; Lebœuf, M.; Cavé, A. *J. Nat. Prod.* **1994**, *57*, 1033–1135, and work cited therein.
13. Bremner, J. B.; Winzenberg, K. N. *Aust. J. Chem.* **1978**, *31*, 313–320.
14. Asencio, M.; Cassels, B. K.; Speisky, H. *Bol. Soc. Chil. Quim.* **1993**, *38*, 331–334.
15. Arango, O.; Pérez, E.; Granados, H.; Rojano, B.; Sáez, J. *Actual. Biol.* **2004**, *26*, 11–16.
16. Sobarzo-Sánchez, E. M.; Arbaoui, J.; Protais, P.; Cassels, B. K. *J. Nat. Prod.* **2001**, *63*, 480–484.
17. Ishii, H. *Yakugaku Zasshi* **1961**, *81*, 243–248.
18. Peng, J. H.; Lukas, R. J. *Soc. Neurosci. Abstr.* **1998**, *24*, 831.
19. Pacheco, M. A.; Pastoor, T. E.; Lukas, R. J.; Wecker, L. *Neurochem. Res.* **2001**, *26*, 686–693.
20. Exley, R.; Iturriaga-Vásquez, P.; Lukas, R. J.; Sher, E.; Cassels, B. K.; Bermudez, I. *Br. J. Pharmacol.* **2004**, *146*, 15–24.
21. Houlihan, L. M.; Slater, Y.; Beadle, D.; Lukas, R. J.; Bermúdez, I. *Neuropharmacology* **2000**, *39*, 2533–2542.
22. Houlihan, L. M.; Slater, Y.; Guerra, D. L.; Peng, J.-H.; Kuo, J.-P.; Lukas, R.; Cassels, B. K.; Bermúdez, I. *J. Neurochem.* **2001**, *78*, 1029–1043.