Halogenated Boldine Derivatives with Enhanced Monoamine Receptor Selectivity

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Received September 3, 1999

(*S*)-(+)-Boldine (**1**) was brominated, chlorinated, and iodinated using molecular bromine in acetic acid or *N*-halosuccinimides in trifluoroacetic acid. Initial halogenation occurs at C-3, followed (in the cases of chlorine and bromine) by the less reactive C-8, to afford 3-haloboldines- and 3,8-dihaloboldines (**2**−**5**). Using a 2:1 ratio of *N*-iodosuccinimide to boldine, however, only the 3-iodo derivative **6** was obtained. Radioligand binding studies of these products showed that halogenation of boldine at C-3 favors affinity for D_1- (vs D_2-) dopaminergic receptors, attaining a low nanomolar IC_{50} value in the case of 3-iodoboldine (**6**).

(*S*)-(+)-Boldine (**1**), the major alkaloid present in the leaves and bark of the Chilean boldo tree (*Peumus boldus* Molina, Monimiaceae), has been characterized in the past few years as an antioxidant that effectively protects different systems against free-radical-induced lipid peroxidation or enzyme inactivation.[1] This activity presumably underlies the hepatoprotective and cytoprotective effects recently demonstrated for this alkaloid[1,2] and may also be related to its antipyretic and antiinflammatory behavior[3] and to its protective effect in experimental colitis.[4] Independently of these properties, boldine is a slightly selective α_{1A}-adrenergic antagonist in vascular tissue[5] and a nonselective D_1- and D_2-dopaminergic antagonist in the central nervous system.[6] After its oral ingestion, boldine has a plasma half-life of only a few minutes and is rapidly glucuronidated in the liver,[7] a behavior that may explain its relatively weak and short-lived systemic actions and certainly limits its potential clinical usefulness. It therefore seemed interesting to study whether, by introducing substituents on the readily available boldine molecule, more favorable pharmacokinetics could be obtained while maintaining or improving some of its useful pharmacological activities.

Structure−antioxidative activity studies on boldine and related compounds suggest that the phenol groups bonded to the aporphine ring system and that the basic benzylic amine function both contribute to these properties.[8] Such structural features should be retained in boldine derivatives of interest as potential antioxidants. On the other hand, there is presently no way of predicting the effects of derivatization of boldine upon its scantily known monoaminergic properties. Therefore, it seemed reasonable that variations upon the boldine motif should aim for increased lipophilicity without greatly affecting the key phenolic and amine functional groups. One way of achieving this would be to replace aromatic ring hydrogens with not very bulky substituents expected to make a positive contribution to the overall lipophilicity.

Among the substituents meeting this requirement, the most easily accessible are chlorine, bromine, and iodine. These atoms may be expected to contribute to the lipophilicity of the corresponding haloboldines, in terms of log P (where P = the octanol/aqueous buffer partition constant), by values of about 0.62, 0.75, and 1.12, respectively,[9] to be added to the lipophilicity of boldine itself, which we estimate to be considerably less than unity (glaucine, the *O,O*-dimethyl ether of boldine, is reported to have log P = 1.22).[9] The bromination of boldine with Br_2 in CCl_4 to afford a mixture of 3-bromoboldine (**2**) and 3,8-dibromoboldine (**3**) was reported more than 20 years ago.[10] Nevertheless, aporphines are readily dehydrogenated with I_2 to the corresponding 6a,7-didehydro derivatives,[11] presumably via hydrogen abstraction from the benzylic 6a position, to afford a relatively stable free radical[8] and aromatization of ring C.

Results and Discussion

We first reexamined the reaction of boldine with Br_2 in acetic acid instead of CCl_4,[10] assuming that protonation of the nitrogen might hinder the dehydrogenation side reaction, and found that a considerable excess of the halogen was required to achieve high conversions of the alkaloid. Under these conditions, 3,8-dibromoboldine (**3**) was obtained in 64% isolated yield, and 3-bromoboldine (**2**) in only 9% yield, with chromatographic evidence of the formation of several other products.[11] Bromination of other aporphines has been carried out quite effectively using *N*-bromosuccinimide (NBS),[12] so we then studied the reaction of boldine with NBS in trifluoroacetic acid in order to ensure complete protonation of the basic nitrogen atom. By varying the NBS−boldine ratio we were able to control the extent of halogenation, obtaining reasonable yields of either 3-bromoboldine (**2**) or the 3,8-dibromo derivative (**3**).

We then studied the chlorination of boldine with *N*-chlorosuccinimide (NCS). Using a 1:1 molar ratio of the reagents, only 44% of 3-chloroboldine (**4**) could be isolated, with no evidence of dichloro products and much unreacted boldine remaining. The yield of 3-chloroboldine rose to 48% with a 2:1 NCS−boldine ratio, and 19% 3,8-dichloroboldine (**5**) could also be isolated. The steric and electronic environments of C-3 and C-8 of boldine appear to be very similar, and consequently iodination with *N*-iodosuccinimide (NIS) might be expected to afford both 3-iodoboldine and 3,8-diiodoboldine. Our results showed that, even using a 2:1 ratio of NIS to boldine, only the 3-iodo derivative (**6**) could be isolated in moderate yield.

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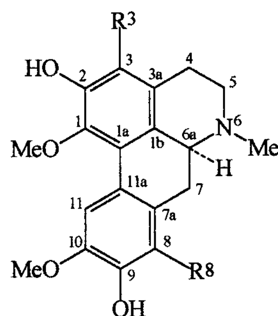
10.1021/np990433j CCC: $19.00 © 2000 American Chemical Society and American Society of Pharmacognosy
Published on Web 03/15/2000



Identification of the halogenated positions was quite straightforward from the ^{1}H NMR spectra of the products, as in every case the downfield (δ 7.83−7.88) H-11 resonance was preserved, and when an additional aromatic proton signal was present (compounds **2**, **4**, and **6**), this appeared in the δ 6.74−6.82 range, in close agreement with the H-8 resonance of boldine (δ 6.81) and considerably downfield from the boldine H-3 resonance (δ 6.61). These assignments were confirmed by interpretation of the 2D HMBC/HMQC spectra of each of our products.

Although the ^{13}C NMR spectra could also be interpreted tentatively on the basis of the well-substantiated boldine spectra in $CDCl_3$ and in DMSO-d_6, whose signals differ by, at the most, 1.7 ppm on going from one solvent to the other,[13] the analysis of 2D HMBC/HMQC spectra made unambiguous assignments possible for all our compounds. In the case of boldine, the ^{13}C resonances are in agreement with the published assignments, with the exception of the reversal of the quaternary C-1a and C-7a signals.[13] In particular, the anomalous ^{13}C methoxyl resonance (δ 60.7) attributable to the out-of-plane C-1 methoxyl group shows a long-range correlation with the signal at δ 144.1 (which may, therefore, be assigned to C-1), and this, in turn, correlates to the C-2 OH resonance at δ 9.00 and the H-3 signal at δ 6.50. Conversely, this OH signal also correlates to the ^{13}C resonance at δ 115.6 (which should, thus, correspond to C-3). In the one-bond C/H correlation spectrum this signal correlates with the δ 6.50 peak, confirming our conclusion. The latter resonance shows other long-range correlations, most significantly to C-4 (δ 23.0), and also to C-1b (δ 127.0) and C-2 (δ 150.5). Long-range cross-correlations were found between H-11 (δ 7.85) and C-8 (δ 116.7), on one hand, and H-8 (δ 6.72) and C-11 (δ 113.4), on the other, allowing a similar rationale to be applied to the assignment of the δ 2.30 apparent triplet to H-7β. This latter signal is also clearly correlated to the C-11a (δ 124−26), C-1b (δ 127.0), and C-1a (δ 131.0) resonances, as well as the *N*-methyl carbon peak. The 2D spectra of the halogenated derivatives were analyzed in the same way.

As expected from tabulated ^{13}C substituent shifts,[14] the chlorinated carbon atom resonances are shifted downfield by 4.1−4.9 ppm, the brominated ones are shifted upfield by 3.0−3.9 ppm, and the iodinated carbon signal lies 24.1 ppm upfield from the corresponding signal in the boldine spectrum. The only other noteworthy features associated with halogenation in the ^{13}C NMR spectra of boldine derivatives are a fairly strong (4.8 ppm) deshielding of C-4 in 3-iodoboldine (**6**) and a weaker (3.8 ppm) shielding of C-7 in 3,8-dichloroboldine (**5**) with regard to the corresponding resonances in the boldine spectra. In comparison, the C-4 signals in 3-chloroboldine- and 3,8-dichloroboldine lie only 1.2−1.5 ppm upfield, and in 3-bromoboldine- and 3,8-dibromoboldine, they are shifted downfield by 1.5−2.0 ppm from the corresponding boldine signals.

The introduction of a halogen atom at C-3 leads to changes in the chemical shifts of the C-4 protons and the *N*-methyl carbon, increasing with the volume of the substituent. In the boldine ^{1}H NMR spectrum (in DMSO-d_6), H-4α and H-4β resonate at δ 2.50 and 2.90, respectively. In all five halogenated boldine derivatives, the corresponding proton signals become indistinguishable at 300 MHz: both appear near δ 2.77 in 3,8-dichloroboldine, near 2.70 in 3-chloroboldine and 3,8-dibromoboldine, near 2.69 in 3-bromoboldine, and near 2.62 in 3-iodoboldine. These results suggest that in the time-averaged conformations of these compounds both protons are placed symmetrically on either side of the ring A plane and that both are increasingly shielded on going from the smaller to the larger, electron-rich halogen substituents. This assumed displacement of the conformational equilibria, mainly affecting ring B in the monohalogenated compounds, might also be related to the upfield shift of the *N*-methyl carbon resonance from δ 45.2 in boldine to 43.9, 43.7, and 43.3 in the 3-chloro, 3-bromo, and 3-iodo derivatives, respectively.

In these electrophilic aromatic substitution reactions of boldine, the greater stabilization of the intermediate 3-substituted σ complex (which may be explained by conjugation throughout the biphenyl system, unavailable to the alternative 8-substituted complex) seems to be the dominant factor in the orientation observed. In the reactions with NBS and NCS, only the 3-haloboldines- and 3,8-dihaloboldines were isolated, suggesting that substitution at C-3 is more rapid than at C-8, although the latter may still occur on the monohalogenated substrate. In the case of NIS, only 3-iodoboldine was obtained. If NIS is an appreciably weaker donor of electrophilic halogen than NBS or NCS, it is reasonable that selectivity should increase in the order NCS $\leq$ NBS $<$ NIS, thus explaining the apparent absence of 8-iodo substitution.

Radioligand displacement studies in rat-brain membranes show that, in the cases of the chloro and bromo derivatives, halogenation at C-3 leads to slight increases in affinity and selectivity for cortical α_{1A}- (vs. α_{1B}-) adrenergic receptors.[15] Thus, boldine exhibits pK_i values of 8.31 and 6.50 vs. [^{3}H]-prazosin (a subtype-nonselective α_1-adrenergic receptor ligand) for the high and low affinity sites interpreted as α_{1A} and α_{1B} receptors, respectively (with a selectivity ratio—K_{low}/K_{high}—of 70); in the case of 3-bromoboldine, the corresponding pK_i values are 8.93 and 6.87 (with a selectivity ratio of 120), and for 3,8-dibromoboldine the figures are 8.87 and 6.92 (90). The introduction of a chlorine atom at C-3 has a somewhat lesser effect on affinity—pK_i values of 8.65 and 6.57—but raises selectivity to the same extent (122) as bromine.[15] These results are paralleled by experiments with cloned human α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptors. In the latter study, 3,8-dichloroboldine and 3-iodoboldine were included and found to have slightly lower affinities than 3-chloroboldine or 3-bromoboldine for α_{1A}- and α_{1B}-receptors, but were not significantly different at the α_{1D} subtype.[16]

Binding studies in rat-striatal membranes, using [^{3}H]-SCH 23390 (a selective D_1-dopaminergic receptor ligand) or [^{3}H]-raclopride (a selective D_2-dopaminergic ligand) (Figure 1 and Table 1), have now shown that chlorination or bromination of boldine at C-3 leads to a significant increase of affinity for D_1, but not D_2, receptors, while 3,8-dibromoboldine displays only a slight increase of affinity for D_1 and a decrease for D_2 receptors. In all cases, halogenation leads to some selectivity for D_1 receptors.

Within this small series, 3-bromoboldine surpasses 3-chloroboldine in both affinity and selectivity at α_1-adrenergic and D_1 dopaminergic receptors, both compounds binding more tightly and selectively than unsubstituted

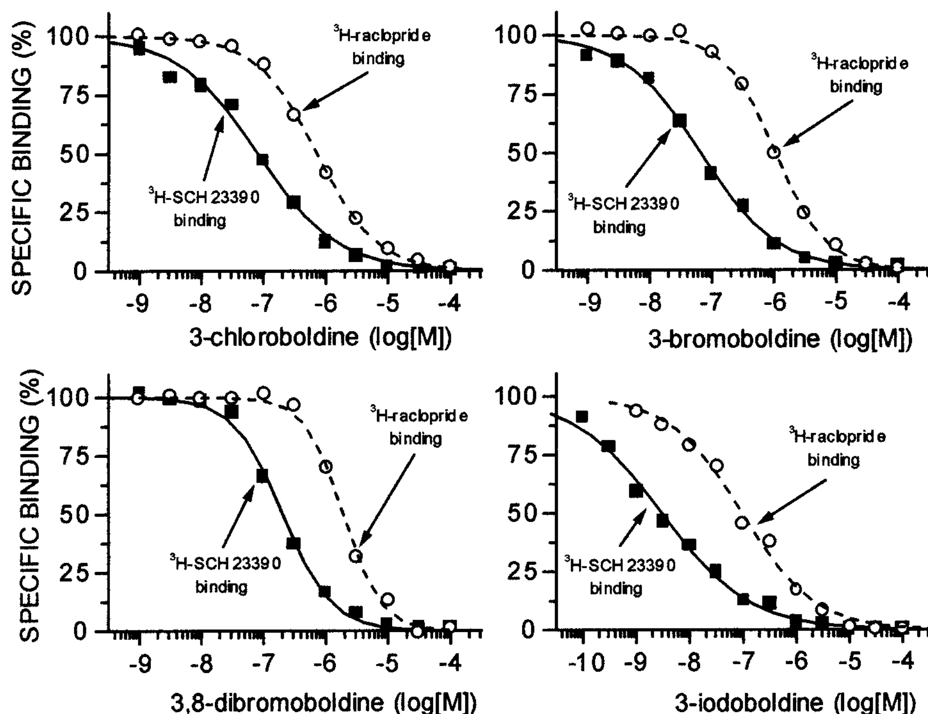


Figure 1. Displacement of [³H]-SCH 23390 or [³H]-raclopride (D_1- and D_2-dopaminergic radioligands, respectively) by 3-chloroboldine; 3-bromoboldine; 3,8-dibromoboldine; and 3-iodoboldine.

Table 1. Displacement of [³H]-SCH 23390 or [³H]-Raclopride (D_1 and D_2 Dopaminergic Radioligands, Respectively) by Boldine,[6] 3-Chloroboldine; 3-Bromoboldine; 3,8-Dibromoboldine; and 3-Iodoboldine[a]

compounds	IC_{50} (μM) on specific binding of [³H]-SCH 23390	[³H]-raclopride	ratio D_2/D_1
boldine	0.40 ± 0.03	0.52 ± 0.11	1.3
3-chloroboldine	0.081 (0.020−0.380)	0.72 (0.15−3.52)	8.9
3-bromoboldine	0.066 (0.018−0.242)	1.05 (0.25−4.39)	15.9
3,8-dibromoboldine	0.206 (0.054−0.670)	1.91 (0.69−5.28)	9.3
3-iodoboldine	0.003 (0.0004−0.025)	0.096 (0.018−0.512)	32.0

[a] IC_{50} and their 95% confidence limits were calculated by the method of Litchfield and Wilcoxon,[17] from dose−response curves with 8−12 determinations at each concentration.

boldine, while introduction of a second halogen atom at C-8 seems to defeat this trend. As molecular models show the bromine atom rather deeply embedded between the C-2 hydroxyl and the C-4 methylene groups, a hydrophobic interaction of the halogen with a lipophilic site in the α_{1A}-adrenergic or D_1-dopaminergic receptor molecule seems unlikely. On the other hand, the relatively low electronegativity of bromine might allow (and the greater electronegativity of chlorine, hinder) an interaction of the aporphine A ring with aromatic residues believed to form a cluster in the receptor binding site.[18] The parallel between the behavior of the 3-haloboldines at α_1-adrenergic and dopaminergic receptors breaks down, however, when 3-iodoboldine is taken into account. In the case of α_{1A}-adrenoceptors, the introduction of iodine at C-3 leads to a loss of affinity and selectivity, while in the case of D_1-dopaminergic receptors there is a remarkable increase of both parameters despite an also quite significant increase of affinity for D_2 receptors. In fact, 3-iodoboldine stands at the top of the range of affinities for dopamine receptors in the aporphine field,[19] and its selectivity for D_1-dopaminergic receptors is also one of the best. As iodine is even less electronegative than bromine, it might be expected to favor aromatic ring interactions; as it is larger, it may be more likely to interact with a lipophilic site; finally, its effect on the conformation of ring B, as suggested by the NMR spectra, might also contribute to its rather high affinity for dopaminergic receptors.

Although only reliable docking and molecular dynamics studies with appropriate receptor models may allow these issues to be resolved, our binding results illuminate a promising approach to subtype-selective natural-product-based monoaminergic drugs. Studies on the antioxidative activity of these halogenated boldine derivatives are also in progress.

Experimental Section

General Experimental Procedures. Melting points were determined on a Reichert-Jung Galen III Kofler hot stage. Optical rotations were determined with a Schmidt-Haensch Polartronic electronic polarimeter. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 using a Bruker AMX 300 instrument, operating at 300.13 MHz (¹H) or 75.48 MHz (¹³C).

Bromination of Boldine. Boldine (**1**), isolated from *P. boldus* bark and crystallized in $CHCl_3$ as the 1:1 complex with this solvent, was brominated by two different methods: with Br_2 in HOAc, or with NBS in TFA.

Bromination with Br_2. A solution of **1**−$CHCl_3$ (1.17 g, 2.62 mmol) dissolved in HOAc (50 mL) was treated dropwise at 20 °C with Br_2 (4 M in HOAc, 4 mL) with constant stirring. A light violet precipitate, which was separated by filtration, formed overnight in the reddish solution. The precipitate was made weakly basic with dilute NH_3, the suspension (pH 8−9) was extracted with $CHCl_3$, and the organic extracts were combined, dried over Na_2SO_4, and concentrated to afford a residue of 3,8-dibromoboldine (**3**) (676 mg, 1.68 mmol, 64%). The filtrate of the reaction mixture was similarly made basic

with NH_3 and worked up giving a brown solid that was purified by Si gel flash chromatography (EtOAc) to give 3-bromoboldine (**2**) (118 mg, 0.24 mmol, 9%).

Bromination with NBS. A solution of **1**−$CHCl_3$ (502 mg, 1.12 mmol) in TFA (15 mL) was treated with NBS (200 mg, 1.12 mmol) at room temperature (20 °C). After 1 h stirring, the mixture was poured into cold H_2O (50 mL), and the aqueous solution was adjusted to pH 8−9 with concentrated NH_3, extracted with $CHCl_3$, worked up, and chromatographed as before to give **2** (365 mg, 0.90 mmol, 80%) and **3** (6 mg, 0.11 mmol, 1%). Changing the NBS−boldine ratio, **1**−$CHCl_3$ (507 mg, 1.14 mmol) in TFA was treated with NBS (405 mg, 2.28 mmol) under similar conditions to yield **2** (60 mg, 0.15 mmol, 13%) and **3** (299 mg, 0.62 mmol, 54%).

3-Bromoboldine (2): light tan needles from C_6H_6; mp 192−194 °C (lit. 208−209 °C);[10] $[\alpha]^{18}_{D}$ +88° (*c* 0.11, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.81 (1H, s, H-11), 6.75 (1H, s, H-8), 3.78 (3H, s, *O*-10-CH_3), 3.50 (3H, s, *O*-1-CH_3), 3.03 (1H, dd, *J* = 11.5 Hz, *J'* = 4.3 Hz, H-5α), 2.97 (1H, dd, *J* = 13.4 Hz, *J'* = 3.9 Hz, H-7α), 2.83 (1H, dd, *J* = 13.4 Hz, *J'* ≈ 3 Hz, H-6a), 2.69 (2H, m, H-4α and H-4β), 2.39 (3H, s, *N*-CH_3), 2.30 (1H, m, H-5β), 2.26 (1H, dd, *J* = *J'* = 13.4 Hz, H-7β); ^{13}C NMR (DMSO-d_6) δ 146.7 (C-2), 146.4 (C-10), 146.3 (C-9), 143.2 (C-1), 129.6 (C-7a), 129.0 (C-3a), 127.3 (C-1a), 125.5 (C-1b), 121.9 (C-11a), 115.3 (C-8), 111.6 (C-11), 110.3 (C-3), 62.4 (C-6a), 60.0 (*O*-1-CH_3), 55.8 (*O*-10-CH_3), 52.7 (C-5), 43.6 (*N*-CH_3), 33.4 (C-7), 30.5 (C-4); *anal.* C 56.34%, H 4.98%, N 3.46%, calcd for $C_{19}H_{20}BrNO_4$, C 56.17%, H 4.96%, N 3.46%.

3,8-Dibromoboldine (3): pale violet needles from C_6H_6; mp 196−198 °C (lit. 199−200 °C);[10] $[\alpha]^{24}_{D}$ +158° (*c* 0.11, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.86 (1H, s, H-11), 3.86 (3H, s, *O*-10-CH_3), 3.48 (3H, s, *O*-1-CH_3), 3.08 (1H, m, H-7α), 3.02 (1H, m, H-5α), 2.78 (1H, dd, *J* = 14.1 Hz, *J'* ≈ 3 Hz, H-6a), 2.70 (2H, m, H-4α and H-4β), 2.44 (3H, s, *N*-CH_3), 2.32 (1H, m, H-5β), 2.14 (1H, dd, *J* = *J'* = 14.1 Hz, H-7β); ^{13}C NMR (DMSO-d_6) δ 146.8 (C-2), 146.7 (C-10), 143.6 (C-9), 143.4 (C-1), 128.9 (C-1a), 128.7 (C-1b), 127.2 (C-3a), 125.0 (C-7a), 123.0 (C-11a), 111.4 (C-8), 111.1 (C-3), 110.2 (C-11), 61.9 (C-6a), 60.1 (*O*-1-CH_3), 56.2 (*O*-10-CH_3), 52.5 (C-5), 43.5 (*N*-CH_3), 33.0 (C-7), 30.0 (C-4); *anal.* C 47.25%, H 3.97%, N 2.90%, calcd for $C_{19}H_{19}Br_2NO_4$, C 47.03%, H 3.95%, N 2.90%.

Chlorination of Boldine. A solution of **1**−$CHCl_3$ (927 mg, 2.08 mmol) in TFA (20 mL) was treated with NCS (283 mg, 2.12 mmol) at room temperature (22 °C). After stirring for 1 h, the mixture was poured into 70 mL of cold H_2O and worked up as described above to give a brown residue. Flash chromatography (Si gel and EtOAc) afforded 3-chloroboldine (**4**) (334 mg, 0.92 mmol, 44%). No dichloro derivative was observed by TLC of the crude reaction mixture. Increasing the NCS−boldine ratio, **1**−$CHCl_3$ (926 mg, 2.08 mmol) in TFA (20 mL) was treated similarly with NCS (572 mg, 4.28 mmol), giving **4** (360 mg, 1.0 mmol, 48%) and 3,8-dichloroboldine (**5**) (153 mg, 0.40 mmol, 19%).

3-Chloroboldine (4): light tan needles (C_6H_6); mp 183−184 °C; $[\alpha]^{17}_{D}$ +102° (*c* 0.11, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.81 (1H, s, H-11), 6.75 (1H, s, H-8), 3.79 (3H, s, *O*-10-CH_3), 3.52 (3H, s, *O*-1-CH_3), 3.02 (1H, m, H-5α), 2.97 (1H, dd, *J* = 13.3 Hz, *J'* = 3.7 Hz, H-7α), 2.78 (1H, dd, *J* = 13.3 Hz, *J'* = 3.6 Hz, H-6a), 2.70 (2H, m, H-4α and H-4β), 2.46 (3H, s, *N*-CH_3), 2.32 (1H, m, H-5β), 2.25 (1H, dd, *J* = *J'* = 13.4 Hz, H-7β); ^{13}C NMR (DMSO-d_6) δ 146.3 (C-10), 146.2 (C-9), 145.6 (C-2), 143.5 (C-1), 129.6 (C-7a), 127.4 (C-3a), 126.9 (C-1a), 124.9 (C-1b), 121.9 (C-11a), 118.2 (C-3), 115.3 (C-8), 111.6 (C-11), 62.3 (C-6a), 59.8 (*O*-1-CH_3), 55.8 (*O*-10-CH_3), 52.4 (C-5), 43.6 (*N*-CH_3), 33.4 (C-7), 27.3 (C-4); *anal.* C 63.65%, H 5.67%, N 3.98%, calcd for $C_{19}H_{20}ClNO_4$, C 63.07%, H 5.57%, N 3.89%.

3,8-Dichloroboldine (5): tan needles ($CHCl_3$−light petroleum); mp 131.5−133.5 °C; $[\alpha]^{25}_{D}$ +177° (*c* 0.03, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.83 (1H, s, H-11), 3.86 (3H, s, *O*-10-CH_3), 3.51 (3H, s, *O*-1-CH_3), 3.02 (2H, m, H-5α and H-7α), 2.78 (1H, dd, *J* = 13.4 Hz, *J'* ≈ 5 Hz, H-6a), 2.73 (2H, m, H-4α and H-4β), 2.44 (3H, s, *N*-CH_3), 2.32 (1H, m, H-5β), 2.12 (1H, dd, *J* = *J'* = 13.4 Hz, H-7β); ^{13}C NMR (DMSO-d_6) δ 146.8 (C-10), 145.7 (C-2), 143.8 (C-1), 142.6 (C-9), 127.4 (C-1a), 127.0 (C-1b), 126.6 (C-3a), 124.3 (C-7a), 122.6 (C-11a), 119.2 (C-8), 119.0 (C-3), 109.7 (C-11), 61.7 (C-6a), 60.0 (*O*-1-CH_3), 56.2 (*O*-10-CH_3), 52.2 (C-5), 43.5 (*N*-CH_3), 30.0 (C-7), 27.0 (C-4); **5** (100 mg, 0.25 mmol) was converted into the hydrochloride, which was crystallized in *i*-PrOH−Et_2O (**5·**HCl, 50 mg, 46%): light tan amorphous powder; mp 171−173 °C; $[\alpha]^{25}_{D}$ + 123° (*c* 0.18, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.87 (1H, s, H-11), 4.26 (1H, m), 3.87 (3H, s, *O*-10-CH_3), 3.76 (1H, m), 3.58 (1H, m), 3.55 (3H, s, *O*-1-CH_3), 3.40 (1H, m), 3.19 (1H, m), 3.04 (3H, s, *N*-CH_3), 3.02 (1H, m), 2.80 (1H, dd, *J* = *J'* = 14.5 Hz.); ^{13}C NMR (DMSO-d_6) δ 147.2 (C-2), 147.1 (C-10), 144.4 (C-9), 143.1 (C-1), 125.1 (C-7a), 124.5 (C-3a), 124.4 (C1b), 121.9 (C-1a), 120.6 (C-11a), 119.3 (C-8), 119.0 (C-3), 109.7 (C-11), 60.2 (C-6a), 60.0 (*O*-1-CH_3), 56.1 (*O*-10-CH_3), 50.8 (C-5), 40.8 (N−CH_3), 26.8 (C-7), 24.1 (C-4); *anal.* C 52.43%, H 4.63%, N 3.22%, calcd for $C_{19}H_{19}Cl_2NO_4$−HCl, C 52.73%, H 4.66%, N 3.25%.

Iodination of Boldine. A solution of **1**−$CHCl_3$ (1.51 g, 3.38 mmol) dissolved in TFA (30 mL) was treated with NIS (910 mg, 4.05 mmol) at room temperature (20 °C). After stirring for 2 h, the mixture was poured into cold H_2O (75 mL) and worked up as usual to afford a dark red, semisolid residue. Flash chromatography (Si gel and EtOAc) gave an amorphous product identified as 3-iodoboldine (**6**) by its NMR spectra (646 mg, 1.42 mmol, 42%), which did not crystallize in C_6H_6 or $CHCl_3$. Increasing the NIS−boldine ratio, **1**−$CHCl_3$ (2.47 g, 5.53 mmol) was treated with NIS (2.53 g, 11.3 mmol) in TFA at room temperature (20 °C) giving only **6** (1.2 g, 2.6 mmol, 47%).

3-Iodoboldine (6): amorphous brown solid; $[\alpha]^{24}_{D}$ +65° (*c* 0.2, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.84 (1H, s, H-11), 6.78 (1H, s, H-8), 3.81 (3H, s, *O*-10-CH_3), 3.51 (3H, s, *O*-1-CH_3), 2.98 (2H, m, H-5α and H-7α), 2.83 (1H, dd, *J* = 13.3 Hz, *J'* ≈ 3 Hz, H-6a), 2.62 (2H, m, H-4α and H-4β), 2.42 (3H, s, *N*-CH_3), 2.29 (1H, dd, *J* = *J'* = 13.3 Hz, H-7β), 2.27 (1H, m, H-5β); ^{13}C NMR (DMSO-d_6) δ 149.0 (C-2), 146.3 (C-10), 146.2 (C-9), 141.9 (C-1), 131.9 (C-7a), 129.6 (C-3a), 127.6 (C-1a), 126.1 (C-1b), 121.9 (C-11a), 115.2 (C-8), 111.6 (C-11), 90.0 (C-3), 62.4 (C-6a), 59.7 (*O*-1-CH_3), 55.8 (*O*-10-CH_3), 53.2 (C-5), 43.3 (*N*-CH_3), 35.5 (C-7), 33.3 (C-4). Compound **6** (90 mg, 0.20 mmol) was converted into its crystalline hydrobromide monohydrate (**6**-HBr·H_2O, 35 mg, 30%): light tan microcrystalline solid (*i*-PrOH−Et_2O); mp > 220 °C dec; $[\alpha]^{24}_{D}$ +54° (*c* 0.2, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.83 (1H, s, H-11), 6.84 (1H, s, H-8), 4.25 (1H, m), 3.80 (3H, s, *O*-10-CH_3), 3.78 (1H, m), 3.51 (3H, s, *O*-1-CH_3), 3.33 (3H, m), 3.09 (3H, s, *N*-CH_3), 2.96 (1H, m), 2.71 (1H, dd, *J* = *J'* = 13.8 Hz); ^{13}C NMR (DMSO-d_6) δ 150.8 (C-2), 146.4 (C-10), 146.8 (C-9), 142.4 (C-1), 129.3 (C-7a), 126.7 (C-3a), 126.4 (C-1b), 121.4 (C-1a), 121.2 (C-11a), 115.4 (C-8), 111.4 (C-11), 89.8 (C-3), 61.2 (C-6a), 60.0 (*O*-1-CH_3), 55.7 (*O*-10-CH_3), 51.8 (C-5), 40.9 (*N*-CH_3), 32.7 (C-7), 29.9 (C-4); *anal* C 41.60%, H 4.22%, N 2.63%, calcd for $C_{19}H_{20}INO_4$·HBr·H_2O, C 41.47%, H 4.21%, N 2.56%.

Binding Experiments. Binding experiments were performed on Wistar rat striatal membranes. Each striatum was homogenized in 2 mL of ice-cold Tris−HCl buffer (50 mM, pH = 7.4 at 22 °C) with a Polytron (4 s, maximal scale) and immediately diluted with Tris buffer. The homogenate was centrifuged either twice ([^{3}H]-SCH 23390 binding experiments) or four times ([^{3}H]-raclopride binding experiments) at 20 000 *g* for 10 min at 4 °C, with resuspension in the same volume of Tris buffer between centrifugations. For [^{3}H]-SCH 23390 binding experiments, the final pellet was resuspended in Tris buffer containing 5 mM $MgSO_4$, 0.5 mM EDTA, and 0.02% ascorbic acid (Tris−Mg buffer), and the suspension was briefly sonicated and diluted to a protein concentration of 1 mg/mL. A 100-μL aliquot of freshly prepared membrane suspension (100 μg of striatal protein) was incubated for 1 h at 25 °C with 100 μL of Tris buffer containing [^{3}H]-SCH 23390 (0.25 nM final concentration) and 800 μL of Tris−Mg buffer containing the required drugs. Nonspecific binding was determined in the presence of 30 μM SK&F 38393 and represented around 2−3% of total binding. For [^{3}H]-raclopride binding experiments, the final pellet was resuspended in Tris buffer containing 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, and 0.1% ascorbic acid (Tris−

ions buffer), and the suspension was treated as described above. A 200-μL aliquot of freshly prepared membrane suspension (200 μg of striatal protein) was incubated for 1 h at 25 °C with 200 μL of Tris−ions buffer containing [^{3}H]-raclopride (0.5 nM final concentration) and 400 μL of Tris−ions buffer containing the drug being investigated. Nonspecific binding was determined in the presence of 50 μM of apomorphine and represented ca. 5−7% of total binding. In both cases, incubations were stopped by addition of 3 mL of ice-cold buffer (Tris−Mg buffer or Tris−ions buffer, as appropriate) followed by rapid filtration through Whatman GF/B filters. Tubes were rinsed with 3 mL of ice-cold buffer. After the filters had been dried, radioactivity was counted in 4 mL of BCS scintillation liquid at an efficiency of 45%. Filter blanks corresponded to approximately 0.5% of total binding and were not modified by drugs.

Acknowledgment. This work was funded in part by the Presidential Chair in Science (B.K.C., Chile, 1996). The principal author acknowledges a generous gift of equipment from the Alexander von Humboldt Foundation (Germany). An exchange program between France and Chile (ECOS/CONICYT) made valuable face-to-face discussions possible. E.M.S. is the recipient of a FONDECYT scholarship.

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NP990433J