Halogenated Boldine Derivatives with Enhanced Monoamine Receptor Selectivity

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(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 D1‐ (vs D2) dopaminergic receptors, attaining a low nanomolar IC50 value in the case of 3‐iodoboldine (6).

Results and Discussion

We first reexamined the reaction of boldine with Br2 in acetic acid instead of CCl4, 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),13 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% of 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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Identification of the halogenated positions was quite straightforward from the $^1$H NMR spectra of the products, as in every case the downfield ($\delta$ 7.83–8.88) H-11 resonance was preserved, and when an additional aromatic proton signal was present (compounds 2, 4, and 6), this appeared in the $\delta$ 6.74–6.82 range, in close agreement with the H-8 resonance of boldine ($\delta$ 6.81) and considerably downfield from the boldine H-3 resonance ($\delta$ 6.61). These assignments were confirmed by interpretation of the 2D HMBC/HSQC 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, the analysis of 2D HMBC/HSQC 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. In particular, the anomalous $^{13}$C methoxyl resonance ($\delta$ 60.7) attributable to the out-of-plane C-1 methyl group shows a long-range correlation with the signal at $\delta$ 144.1 (which may, therefore, be assigned to C-1), and this, in turn, correlates to the C-2 OH resonance at $\delta$ 9.00 and the H-3 signal at $\delta$ 6.50. Conversely, this OH signal also correlates to the $^{13}$C resonance at $\delta$ 115.6 (which should, thus, correspond to C-3). In the one-bond C/H correlation spectrum this signal correlates with the $\delta$ 6.50 peak, confirming our conclusion. The latter resonance shows other long-range correlations, most significantly to C-4 ($\delta$ 23.0), and also to C-1b ($\delta$ 127.0) and C-2 ($\delta$ 150.5). Long-range cross-correlations were found between H-11 ($\delta$ 7.85) and C-8 ($\delta$ 116.7), on one hand, and H-8 ($\delta$ 6.72) and C-11 ($\delta$ 113.4), on the other, allowing a similar rationale to be applied to the assignment of the $\delta$ 2.30 apparent triplet to H-7/$\beta$. This latter signal is also clearly correlated to the C-11a ($\delta$ 124–26), C-1b ($\delta$ 127.0), and C-1a ($\delta$ 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, 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. Only the 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 $\delta$ 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-4a and H-4 further resonate at $\delta$ 2.50 and 2.90, respectively. In all five halogenated boldine derivatives, the corresponding proton signals become indistinguishable at 300 MHz: both appear near $\delta$ 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 $\delta$ 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 $\alpha$ 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 $\approx$ 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 $\alpha_{1A}$- (vs. $\alpha_{1B}$-) adrenergic receptors.$^{15}$ Thus, boldine exhibits $pK_i$ values of 8.31 and 6.50 vs. [H]$^+$-prazosin (a subtype-nonselective $\alpha_1$-adrenergic receptor ligand) for the high and low affinity sites interpreted as $\alpha_{1A}$ and $\alpha_{1B}$ receptors, respectively (with a selectivity ratio $K_{iO/A}/K_{iO/B}$ 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 $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$-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 $\alpha_{1A}$- and $\alpha_{1B}$-Receptors, but were not significantly different at the $\alpha_{1D}$ subtype.$^{16}$

Binding studies in rat-striatal membranes, using [H]$^+$- SCH 23390 (a selective $D_1$-dopaminergic receptor ligand) or [H]$^+$-radopride (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 $\alpha_{1A}$-adrenergic and $D_1$ dopaminergic receptors, both compounds binding more tightly and selectively than unsubstituted
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 R<sub>1A</sub>-adrenergic or D<sub>1</sub>-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. The parallel between the behavior of the 3-haloboldines at R<sub>1A</sub>-adrenergic and dopaminergic receptors breaks down, however, when 3-iodoboldine is taken into account. In the case of R<sub>1A</sub>-adrenoceptors, the introduction of iodine at C-3 leads to a loss of affinity and selectivity, while in the case of D<sub>1</sub>-dopaminergic receptors there is a remarkable increase of both parameters despite an also quite significant increase of affinity for D<sub>2</sub> receptors. In fact, 3-iodoboldine stands at the top of the range of affinities for dopamine receptors in the aporphine field, and its selectivity for D<sub>1</sub>-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<sub>3</sub> or DMSO-d<sub>6</sub> 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<sub>3</sub> as the 1:1 complex with this solvent, was brominated by two different methods: with Br<sub>2</sub> in HOAc, or with NBS in TFA.

**Bromination with Br<sub>2</sub>.** A solution of 1–CHCl<sub>3</sub> (1.17 g, 2.62 mmol) dissolved in HOAc (50 mL) was treated dropwise at 20 °C with Br<sub>2</sub> (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<sub>4</sub>OH, the suspension (pH 8–9) was extracted with CHCl<sub>3</sub>, and the organic extracts were combined, dried over Na<sub>2</sub>SO<sub>4</sub>, 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.

**Table 1.** Displacement of [3H]-SCH 23390 or [3H]-Raclopride (D<sub>1</sub> and D<sub>2</sub> Dopaminergic Radioligands, Respectively) by Boldine,<sup>6</sup> 3-Chloroboldine, 3-Bromoboldine, 3,8-Dibromoboldine, and 3-Iodoboldine<sup>a</sup>

<table>
<thead>
<tr>
<th>compounds</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (uM) on specific binding of [3H]-SCH 23390</th>
<th>[3H]-Raclopride</th>
<th>ratio D&lt;sub&gt;2&lt;/sub&gt;/D&lt;sub&gt;1&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>boldine</td>
<td>0.40 ± 0.03</td>
<td>0.52 ± 0.11</td>
<td>1.3</td>
</tr>
<tr>
<td>3-chloroboldine</td>
<td>0.081 (0.020–0.380)</td>
<td>0.72 (0.15–3.52)</td>
<td>8.9</td>
</tr>
<tr>
<td>3-bromoboldine</td>
<td>0.066 (0.018–0.242)</td>
<td>1.05 (0.25–4.39)</td>
<td>15.9</td>
</tr>
<tr>
<td>3,8-dibromoboldine</td>
<td>0.206 (0.054–0.670)</td>
<td>1.91 (0.69–5.28)</td>
<td>9.3</td>
</tr>
<tr>
<td>3-iodoboldine</td>
<td>0.003 (0.0004–0.025)</td>
<td>0.096 (0.018–0.512)</td>
<td>32.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>IC<sub>50</sub> and their 95% confidence limits were calculated by the method of Litchfield and Wilcoxon,<sup>17</sup> from dose–response curves with 8–12 determinations at each concentration.

![Figure 1. Displacement of [3H]-SCH 23390 or [3H]-Raclopride (D<sub>1</sub>- and D<sub>2</sub>-dopaminergic radioligands, respectively) by 3-chloroboldine; 3-bromoboldine; 3,8-dibromoboldine; and 3-iodoboldine.](image-url)
with NH₃ 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-CHCl₃ (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 H₂O (50 mL), and the aqeous solution was adjusted to pH 8–9 with concentrated NH₄OH, extracted with CHCl₃, worked up, and chromatographed as described to give 2 (365 mg, 80%) and 3 (90 mg, 0.11 mmol, 1%). Changing the NBS–bolidine ratio, 1–CHCl₃ (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 C₆H₁₅OH; mp 192–194 °C (lit. 208–209 °C), [α]D₀²⁰ = +88° (c 0.11, MeOH); ¹H NMR (DMSO-d₆) δ 7.81 (1H, s, H-11), 6.75 (1H, s, H-8), 3.78 (3H, s, O-10-CH₃), 3.50 (3H, s, O-1-Ch), 3.03 (1H, dd, J = 11.5 Hz, J' = 7.4 Hz, H-7a), 2.97 (1H, dd, J = 13.4 Hz, J' = 3.9 Hz, H-6a), 2.69 (2H, m, H-4x and H-4'), 2.39 (3H, s, N-Ch₂), 2.30 (1H, m, H-5x), 2.26 (1H, dd, J = J' = 13.4 Hz, H-7); ¹³C NMR (DMSO-d₆) δ 146.2 (C-2), 146.2 (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-CH₃), 55.8 (O-10-CH₃), 52.7 (C-5), 43.6 (N-Ch₃), 33.4 (C-7), 30.5 (C-4); anal. C 56.34%, H 4.98%, N 3.46%, calcd for C₁₉H₁₈BrN₂O₄ C 56.17%, H 4.96%, N 3.46%.

3,8-Dibromoboldine (3): pale yellow needles from C₆H₁₅OH; mp 196–198 °C (lit. 199–200 °C), [α]D₀²⁰ = +158° (c 0.11, MeOH); ¹H NMR (DMSO-d₆) δ 7.86 (1H, s, H-11), 3.86 (3H, s, O-10-CH₃), 3.48 (3H, s, O-1-Ch), 3.08 (1H, m, H-7a), 3.02 (1H, m, H-5x), 2.78 (1H, dd, J = 141 Hz, J' = 3.0 Hz, H-6a), 2.70 (2H, m, H-4x and H-4'), 2.44 (3H, s, N-Ch₂), 2.32 (1H, m, H-5x), 2.14 (1H, dd, J = J' = 141 Hz, H-7); ¹³C NMR (DMSO-d₆) δ 146.8 (C-2), 146.7 (C-10), 143.6 (C-9), 143.4 (C-1), 129.8 (C-3a), 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-CH₃), 56.2 (O-10-CH₃), 52.5 (C-5), 43.5 (N-Ch₃), 33.0 (C-7), 30.4 (C-4); anal. C 47.25%, H 3.97%, N 2.90%, calcd for C₁₉H₁₉Br₂N₂O₄ C 47.65%, H 3.93%, N 2.90%.

Chlorination of Boldine. A solution of 1-CHCl₃ (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 H₂O 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 dicloro derivative was observed by TLC. The crude product was sonicated and diluted to a protein concentration of 1 mg/mL. The solution was freed of traces of TFA by passage through a coarse-pore cellulose column. The suspension was briefly frozen and then sonicated a second time. The resulting suspension was centrifuged and the final pellet was resuspended in Tris buffer containing 120 mM MgCl₂, 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 1 µM ⁵¹Cr-SCH 23930 (0.25 mM final concentration) and 800 nL of [H]-raclopride buffer containing the required drugs. Non-specific binding was determined in the presence of 30 nM SK&F 38393 and represented around 2–3% of total binding. For [H]-raclopride binding experiments, the final pellet was resuspended in Tris buffer containing 120 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 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 [3H]-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.

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References and Notes


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