# Displacement Activity of Bisbenzylisoquinoline Alkaloids at Striatal H-SCH 23390 and H-Raclopride Binding Sites

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# DISPLACEMENT ACTIVITY OF BISBENZYLISOQUINOLINE ALKALOIDS AT STRIATAL $^3$H-SCH 23390 AND $^3$H-RACLOPRIDE BINDING SITES

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ABSTRACT.—Fifteen bisbenzylisoquinoline alkaloids (BBIQ) with one ether bridge (thaligrisine [**1**], berbamunine [**2**], dimethylgrisabine [**3**], pampulhamine [**4**], and methyldauricine [**5**]), with two ether bridges (homoaromoline, isotetrandrine, and obaberine), with one ether bridge and one biphenyl bridge (oxandrine, dimethylpseudoxandrine, pseudoxandrine, and antioquine) or secoderivatives (secoobaberine, secoantioquine, and secolucidine), were tested for their ability to displace $^3$H-raclopride or $^3$H-SCH 23390 from their specific dopaminergic binding sites to rat striatal membranes. The most active compounds were found in the group of BBIQs with one ether bridge. Inactive or weakly active compounds were found in this group of BBIQs with one ether bridge and in the other groups. Analysis of tridimensional representations indicates that the different activities among the BBIQs with one ether bridge could be related to strong differences between the spatial occupancy of these compounds according to their stereochemistry.

The bisbenzylisoquinolines (BBIQs) constitute one of the most important groups of isoquinoline alkaloids. More than 400 different compounds have been isolated, particularly from species of the Berberidaceae, Menispermaceae, Monimiaceae, and Ranunculaceae families (1–4), and recently from some species of the Annonaceae family, especially from *Pseudoxandra esclerocarpa* Maas. (5). BBIQs are synthesized by dimerization of trioxygenated benzyltetrahydroisoquinolines in which aryl ether bonds are formed by phenol oxidative coupling reactions (6). Recently, we have studied the smooth muscle relaxing properties of antioquine on isolated rat uterus (7). Antioquine, isolated from the stem bark of *Ps. esclerocarpa* (8), is a BBIQ which presents one diaryl ether bridge and one biphenyl bridge. The relaxant effects produced by (*S,R*)-antioquine may be due to the blockade of calcium movements across the cell membrane (7). Very recently, we have observed the BBIQs with different stereochemistry (*R,R* or *R,S*) present another mechanism of relaxation.

In this paper, we describe the displacing activity at central dopaminergic binding sites labeled with $^3$H-SCH 23390, a selective ligand at D-1 dopamine receptors (9), and with $^3$H-raclopride, a selective ligand at D-2 dopamine receptors (10), of five BBIQs with a diaryl ether bridge coupled "tail-to-tail," (+)-(*R,S*)-thaligrisine [**1**], (+)-(*R,S*)-berbamunine [**2**] (5), (−)-(*S,R*)-dimethylgrisabine [**3**] (11), (−)-(*R,R*)-pampulhamine [**4**] (12), and (−)-(*R,R*)-methyldauricine [**5**] (13), and of ten BBIQs with two bridges (both diaryl-ether or one diaryl-ether and one biphenyl) isolated from *Ps. esclerocarpa* (5,8,14,15) (Table 1). We have investigated the structure-activity relationships of the BBIQ (*R,S*), (*S,R*), and (*R,R*) series.

## EXPERIMENTAL

PLANT MATERIAL.—Thaligrisine [**1**] and berbamunine [**2**] were isolated from the bark of *Ps. esclerocarpa* (Annonaceae) (5), dimethylgrisabine [**3**] from the leaves of *Phaecanthus vietnamensis* Ban. (An-

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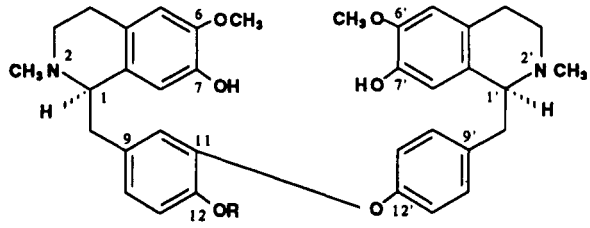
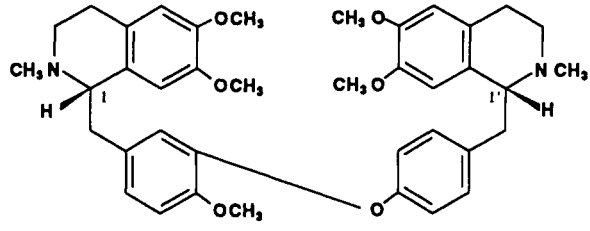
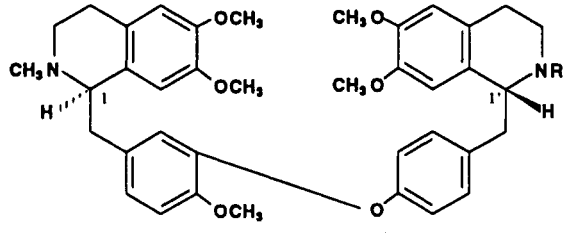


**1** R=Me
**2** R=H



**3**



**4** R=H
**5** R=Me

nonaceae) (11), pampulhamine [**4**] from the leaves of *Aristolochia gigantea* Mart. (Aristolochiaceae) (12), and methyldauricine [**5**] from the bark of *Popowia pisocarpa* (Bl.) Endl. (Annonaceae) (13). The other products were isolated from the bark of *Ps. esclerocarpa* (5). Voucher specimens are deposited as follows: *Phaecanthus vietnamensis* at the Institute of Medical Chemistry, Palacky University, Czechoslovakia; *Aristolochia gigantea* at the Faculty of Pharmacy of the U.F.M.G., Belo Horizonte, Brasil; *Popowia pisocarpa* at the Museum d'Histoire Naturelle, Paris, France; *Pseudoxandra esclerocarpa* at the University of Antioquia, Medellin, Colombia and at the Museum d'Histoire Naturelle, Paris, France.

EXTRACTION AND ISOLATION.—The alkaloids were extracted with $CH_2Cl_2$ after alkalinization with $NH_4OH$. The organic extract was purified, and the alkaloids were isolated by Si gel chromatography. The structures of the BBIQ alkaloids were elucidated by spectroscopic methods (5, 8, 11–15). The absolute configurations of the chiral centers of BBIQ alkaloids with a diaryl ether bridge coupled "tail-to-tail" were determined by the study of CD spectra (2).

*(1R,1'S)-Thaligrisine* [**1**].—$C_{37}H_{42}N_2O_6$: [α]D +27 (*c*=0.3, MeOH); cims ($NH_3$) *m/z* $[MH]^+$ 611; CD (MeOH) Δε (nm) +24 (219), +11.8 (290), 0 (358); see Schiff (3) and Cortes *et al.* (5).

*(1R,1'S)-Berbamunine* [**2**].—$C_{36}H_{40}N_2O_6$: [α]D +40 (*c*=0.13, $CHCl_3$); cims ($NH_3$) *m/z* $[MH]^+$ 597; CD (MeOH) Δε (nm) +9 (208), +6 (244), +4 (283), 0 (358); see Schiff (4) and Cortes *et al.* (5).

*(1S,1'R)-Dimethylgrisabine* [**3**].—$C_{39}H_{46}N_2O_6$: [α]D −26 (*c*=0.19, $CHCl_3$); cims ($NH_3$) *m/z* $[MH]^+$ 639; CD (MeOH) Δε (nm) −10.7 (216), −6.2 (246), −2.6 (282); see Sedmera *et al.* (11).

TABLE 1. Comparative $IC_{50}$s of Various Bisbenzylisoquinoline Derivatives on Specific $^3$H-SCH 23390 and $^3$H-raclopride Binding to Rat Striatal Membranes.[a]

| Product | $IC_{50}$ (μM) on binding of | | ratio D-1/D-2[b] |
|---|---|---|---|
| | $^3$H-SCH 23390 | $^3$H-raclopride | |
| Derivatives with one ether bridge (11-12′) | | | |
| (*R,S*)-thaligrisine [**1**] | 5.6 (1.2–26.9) | 0.027 (0.007–0.107) | 207 |
| (*R,S*)-berbamunine [**2**] | 1.04 (0.08–13.42) | 0.30 (0.03–2.68) | 3.5 |
| (*S,R*)-*O,O*-dimethygrisabine [**3**] | 5.64 (0.48–66.21) | 1.45 (0.22–9.69) | 3.9 |
| (*R,R*)-pampulhamine [**4**] | >100 | >100 | N.C.[c] |
| (*R,R*)-*O*-methyldauricine [**5**] | >100 | >100 | N.C. |
| Derivatives with two ether bridge (7-8′ or 8-7′ and 11-12′) | | | |
| (*R,S*)-homoaromoline | 15.4 (2.2–109.4) | 66.4 (33.2–132.6) | 0.2 |
| (*S,R*)-isotetrandrine | 33.3 (2.3–486.0) | 0.67 (0.06–7.66) | 49.6 |
| (*R,S*)-obaberine | 39.1 (3.4–454.8) | 28.4 (7.9–101.7) | 1.4 |
| Derivatives with one ether bridge and one biphenyl bridge (8-7′ and 11-11′) | | | |
| (*S,S*)-oxandrine | 11.1 (1.61–76.5) | 2.76 (0.31–24.6) | 4.0 |
| (*S,R*)-dimethylpseudoxandrine | 21.9 (2.8–170.7) | 3.8 (0.32–44.6) | 5.8 |
| (*S,R*)-pseudoxandrine | 90.2 (5.0–1626) | 16.2 (1.8–147.7) | 5.6 |
| (*S,R*)-antioquine | >100 | 3.18 (0.63–16.05) | >31.4 |
| Seco derivatives | | | |
| (*S*)-secoobaberine | >30 | >30 | N.C. |
| (*S*)-secoantioquine | >30 | 10.0 (1.18–85) | >3 |
| (*S*)-secolucidine | >100 | 81.6 (7.48–889.3) | >1.2 |

[a]$IC_{50}$s were calculated by the method of Lichtfield and Wilcoxon (18) from concentration-effect curves with 6 to 11 concentrations and 4 to 8 determinations for each concentration.

[b]Ratio of $IC_{50}$ on $^3$H-SCH 23390 binding to $IC_{50}$ on $^3$H-raclopride binding.

[c]Not calculable.

*(1R,1′R)-Pampulhamine* [**4**].—$C_{36}H_{40}N_2O_6$; $[\alpha]_D$ −58 ($c$ = 0.18, MeOH); cims ($NH_3$) *m/z* $[MH]^+$ 597; CD (MeOH) Δε (nm) −11.6 (226), 0 (248), +0.2 (250), 0 (256), −3.1 (289), 0 (310); see Cortes *et al.* (12).

*(1R,1′R)-Methyldauricine* [**5**].—$C_{39}H_{46}N_2O_6$; $[\alpha]_D$ −142 ($c$ = 0.13, $CHCl_3$); cims ($NH_3$) *m/z* $[MH]^+$ 639; see Ghua *et al.* (1) and Jossang *et al.* (13).

PREPARATION OF RAT STRIATAL MEMBRANES.—Binding experiments were performed on striatal membranes from male Wistar rats (200–250 g, IFFA-CREDO, L'Arbresle, France). Each striatum was homogenized in 2 ml ice-cold Tris-HCl buffer (50 mM, pH = 7.4 at 22°) with a polytron (4 sec, 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° (Sigma 2K15, Bioblock, Strasbourg, France) with resuspension in the same volume of Tris buffer between each centrifugation.

BINDING EXPERIMENTS.—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 (16). A 100-μl aliquot of freshly prepared membrane suspension (100 μg of striatal protein) was incubated for 1 h at 25° with 100 μl Tris-Mg buffer containing $^3$H-SCH 23390 (71.3 Ci/mmol, NEN, Paris, France, 0.25 nM final concentration) and 800 μl of Tris-Mg buffer containing the required drugs. Non-specific binding was determined in the presence of 30 μM SK&F 38393 (RBI, Natick, USA) (17) and represented around 2 to 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 $CaCl_2$, 1 mM $MgCl_2$, 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° with 200 μl of Tris-ions buffer containing $^3$H-raclopride (71.9 Ci/mmol, NEN, Paris, France, 0.5 nM final concentration) and 400 μl of Tris-ions buffer contain-

ing the drug being investigated. Non-specific binding was determined in the presence of 50 μM apomorphine (17) and represented around 5 to 7% of total binding.

In both cases, incubations were stopped by the addition of 3 ml 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 ice-cold buffer, and filters were washed with 3 × 3 ml ice-cold buffer. After the filters had been dried, radioactivity was counted in 4 ml BCS scintillation liquid (Amersham, Paris, France) at an efficiency of 45%. Filter blanks corresponded to approximately 0.5% of total binding and were not modified by drugs.

All the compounds, used as hydrochloride salts, were tested comparatively at 6 or 11 concentrations from $10^{-9}$ M to $10^{-4}$ M, depending upon the available amounts of the compounds being investigated. An $IC_{50}$ and 95% confidence limits were calculated by the method of Lichtfield and Wilcoxon (18).

## RESULTS AND DISCUSSION

Most of the tested compounds displayed a low displacing activity on binding of $^3$H-SCH 23390, a selective ligand at D-1 dopamine receptors (9) and on binding of $^3$H-raclopride, a selective ligand at D-2 dopamine receptors (10). This was especially the case for seco-derivatives and for BBIQs with two bridges (either two diaryl ether bridges or one diaryl ether bridge and one biphenyl bridge) (Table 1). Among these derivatives, the more active compounds were oxandrine, dimethylpseudoxandrine, and isotetrandrine. Some of them (isotetrandrine and antioquine) appeared more effective for displacing $^3$H-raclopride binding than $^3$H-SCH 23390 binding to rat striatal membranes, whereas the reverse was true for homoaromoline which appeared more effective on $^3$H-SCH 23390 although at high concentrations (Table 1).

The most active compounds were found among BBIQs with one ether bridge. Thaligrisine [**1**] was active at nanomolar concentrations and was more effective on $^3$H-raclopride binding than on $^3$H-SCH 23390 binding, berbamunine [**2**] and dimethylgrisabine [**3**] were active at micromolar concentrations, and pampulhamine [**4**] and methyldauricine [**5**] were ineffective on both $^3$H-SCH 23390 and $^3$H-raclopride binding (Figure 1, Table 1). From their structures related to known dopamine receptor agonists and from the biphasic displacement of the $^3$H-ligands observed with increasing concentrations of thaligrisine [**1**] (Figure 1), it is likely that these BBIQs could act as agonists at dopamine receptors. This could be further demonstrated when higher amounts of the products are available.

CD studies indicate that dimethylgrisabine [**3**], with the *S,R* configuration, is characterized by negative Cotton effects for 282, 246, and 216 nm wave lengths (11), that thaligrisine [**1**] and berbamunine [**2**] with *R,S* configuration possess positive Cotton effects for the same wave lengths (283, 244, and 208 nm) (5), whereas pampulhamine [**4**] and methyldauricine [**5**] alkaloids with the *R,R* configuration show two negative Cotton effects at 289 and 226 nm wave lengths and one positive Cotton effect for the 250 nm wave length (12).

The great differences observed in the displacing activity of thaligrisine [**1**], dimethylgrisabine [**3**], and methyldauricine [**5**] may be explained by the presence or absence of methoxy groups but mainly by the influence of the configurations of the chiral centers. Epimerization at C-1 and/or C-1′ is probably responsible for a large effect on the conformations of the molecules and therefore on the spatial occupancies of the amino and hydroxyl groups. Interactions between these functional groups and the dopaminergic binding sites are probably involved in the displacing activity. Then (*R,S*)-thaligrisine, the most active compound of this series, must have hydroxyl and amino groups disengaged from intramolecular interactions, allowing interactions with the binding sites of the receptors. On the other hand, inversion of the configuration of the chiral center at C-1′ as for (*R,R*)-methydauricine [**5**] leads to modifications of the conformation of the corresponding molecule, with disappearence of biological activity, since (*R,R*)-methyldauricine [**5**] displaced neither $^3$H-SCH 23390 nor $^3$H-raclopride

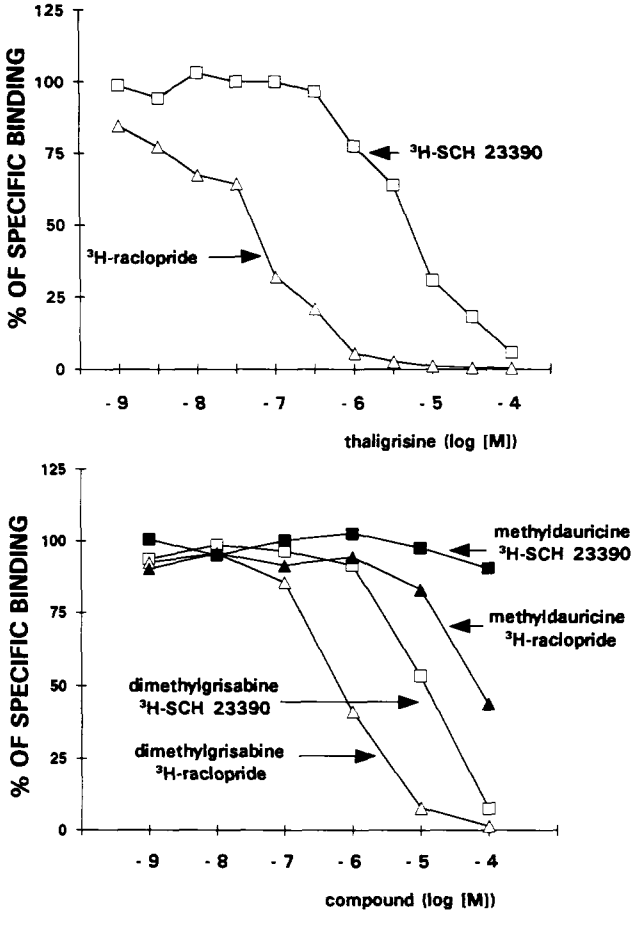



FIGURE 1. Inhibition of specific $^3$H-SCH 23390 and $^3$H-raclopride binding to rat striatal membranes by increasing concentrations of thaligrisine [**1**] (upper panel) and by dimethylgrisabine [**3**] and methyldauricine [**5**] (lower panel). Displacement curves correspond to 4–8 values at each concentration.

from their specific binding sites. In addition, inversion of both chiral centers at C-1 and C-1′ as for (*S,R*)-dimethylgrisabine [**3**] shows a slight decrease of the activity by comparison with (*R,S*)-thaligrisine [**1**]. Preliminary computational studies of the three-dimensional structures of the three compounds support this hypothesis based on the conformational effects, but further studies will be necessary to fully understand and explain the mechanism of action of such compounds.

Finally, in contrast to natural cularine derivatives which appeared to display a similar displacing activity at $^3$H-SCH 23390 and $^3$H-raclopride binding sites (19), thaligrisine [**1**] has an interesting selectivity for $^3$H-raclopride binding sites. Therefore, it can be envisaged to isolate or synthetize BBIQs with one ether bridge displaying a high affinity and a great selectivity for D-2 dopamine receptors.

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