# *In Vitro* and *in Vivo* Studies of Benzisoquinoline Ligands for the Brain Synaptic Vesicle Monoamine Transporter

Lihsueh C. Lee, Thierry Vander Borght, Phillip S. Sherman, Kirk A. Frey, and Michael R. Kilbourn*

*Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan 48109*

*Received February 13, 1995*[⊗]

Tetrabenazine is a high-affinity inhibitor of the vesicular monoamine transporter in mammalian brain. As part of a program to develop *in vivo* imaging agents for these transporters in human brain, a series of 2-alkylated dihydrotetrabenazine ligands was synthesized and evaluated *in vitro* and *in vivo* for binding to the brain vesicular monoamine transporter. Additions of organometallic reagents to tetrabenazine produced 2-methyl, 2-ethyl, 2-*n*-propyl, 2-isopropyl, and 2-isobutyl derivatives of dihydrotetrabenazine. The stereochemistry and conformation of the addition products were thoroughly verified by two-dimensional NMR techniques. All of these alkyl derivatives displayed *in vitro* affinity for the vesicular monoamine transporter binding site in rat brain using competitive assays with the radioligand [$^3$H]methoxytetrabenazine. Except for the isopropyl derivative, all compounds when tested at 10 mg/kg iv showed an ability to inhibit *in vivo* accumulation of the radioligand [$^{11}$C]methoxytetrabenazine in the mouse brain striatum. Derivatives with small alkyl groups (methyl, ethyl) were more effective than those with large groups (propyl, isobutyl). These studies suggest that large groups in the 2-position of the benzisoquinoline structure will significantly diminish both *in vitro* and *in vivo* binding of these compounds to the vesicular monoamine transporter.

## Introduction

The brain vesicular monoamine transporter (VMAT2) is an integral component of monoaminergic neurons and functions to transport newly synthesized or recovered monoamine neurotransmitters from the cytosol into the lumen of the synaptic storage vesicles.[1] The synaptic vesicular transporter of human brain has been recently cloned and sequenced and its chromosomal location identified.[2,3] The ontogeny, pharmacology, and regional brain distribution of VMAT2 in mammalian brain including humans have been extensively studied using the tritiated radioligand α-[$^3$H]dihydrotetrabenazine (2α-hydroxy-3-isobutyl-1,2,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine, **1a**).[4] Determination of VMAT2 binding sites has been proposed as a measure of monoaminergic neuronal density, based on demonstrated reductions in VMAT2 sites in animal models of neurodegenerative diseases and in postmortem samples of patients with Parkinson's disease.[5] Recently, we proposed the use of radiolabeled benzisoquinolines related to tetrabenazine (2-oxo-3-isobutyl-1,2,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine, **2**) as potential *in vivo* imaging agents for quantification of VMAT2 sites in living human brain using positron emission tomography (PET).[6] Using the benzisoquinolines **1a**, **2** and methoxytetrabenazine (2α-methoxy-3-isobutyl-1,2,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine, **3**) (Figure 1) isotopically labeled with carbon-11 ($\beta^+$, $t_{1/2}$ = 20.4 min), studies in monkeys and humans have demonstrated the feasibility of imaging and quantifying this transporter using PET.[7−10]

Tetrabenazine (**2**) has a long history of clinical use, and its *in vivo* effects on monoamine concentrations in the brain have been extensively studied.[11] *In vivo*, **2** is rapidly and extensively metabolized to **1a**, which is likely the active pharmacological agent; *in vitro*, **1a** and **2** have similar binding affinities for VMAT2.[12] As part of our development of radioligands for *in vivo* emission tomographic imaging and quantification of VMAT2 in the human brain, we have become interested in the structure−activity relationship of benzisoquinoline binding to the VMAT2. Our goal is the preparation of benzisoquinolines labeled with positron- and single-photon-emitting radionuclides for *in vivo* imaging using PET and SPECT (single-photon emission-computed tomography). For development of SPECT radioligands in particular, molecular modifications to accommodate an iodine atom or a chelating group for a metal radionuclide (e.g., $^{99m}$Tc) are necessary; such substituents add considerable steric bulk and lipophilicity to an existing chemical structure. Previous attempts to modify the structure of **1a** to include a iodine-bearing group have produced a radioligand suitable for *in vitro* studies of the VMAT2 site but have not led to successful *in vivo* radioligands.[13] Development of new imaging agents for the vesicular monoamine transporter will require a better knowledge of the structural requirements and limitations for high-affinity binding to the VMAT2. A

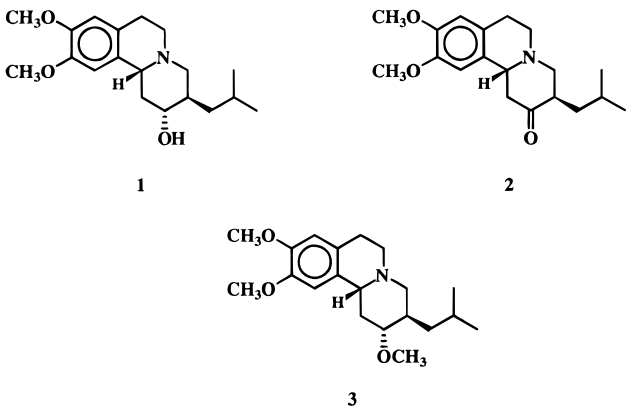
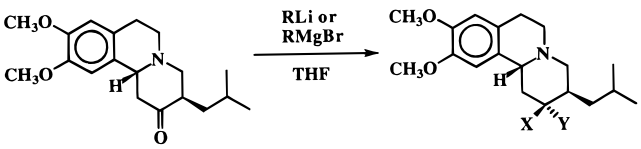



**Figure 1.**

⊗ Abstract published in *Advance ACS Abstracts*, November 1, 1995.

0022-2623/96/1839-0191$12.00/0 

**Scheme 1.** Synthesis of 2-Alkyldihydrotetrabenazine Derivatives




| | X | Y |
|---|---|---|
| 4a | $CH_3$ | OH |
| 4b | OH | $CH_3$ |
| 5 | OH | $CH_2CH_3$ |
| 6 | OH | $CH_2CH_2CH_3$ |
| 7 | OH | $CH(CH_3)_3$ |
| 8 | OH | $CH_2CH(CH_3)_2$ |

number of benzisoquinolines have been prepared over the years, including (a) analogs of **2** with different 3-alkyl substituents,[11] (b) derivatives of **1a** with alkyl substituents at the 2-position,[11,13] and (c) benzisoquinoline analogs with amino, amido, and ester functional groups at the 2-position.[12,14] There has, however, never been a systematic study of the *in vitro* or *in vivo* structure−activity relationship of benzisoquinoline binding to the VMAT2 site (or, for that matter, any systematic study of the binding site requirement for VMAT2). Alterations of the structure of **2** do have profound effects on its biological activity; some modifications increase pharmacologic activity,[11] while others produce differential depletions of certain monoamines.[15] As an initial investigation of the effects of adding substituents to the benzisoquinoline structure, we report here the synthesis, structural characterization, *in vitro* binding affinities, and *in vivo* biological activities of a series of alkylated derivatives of dihydrotetrabenazine.

## Results and Discussion

**Syntheses of 2-Alkyldihydrotetrabenazines**. The 2-alkyldihydrotetrabenazines **4**−**8** (Scheme 1) were prepared by the addition of Grignard or organolithium reagents (RMgX or RLi; R = Me, Et, *n*-Pr, *i*-Pr, *i*-Bu; X = Cl, Br) to the ketone group of **2**. Crude products were purified by flash column chromatography. As confirmed by spectroscopic data (see following sections), these derivatives retained the same relative configurations at carbons C-3 and C-11b and the *trans* B/C ring junction as found in the parent compounds **1** and **2**. In the addition of an organometallic reagent to the ketone of **2**, two isomers can be formed, with the incoming alkyl group assuming either the axial or equatorial position. These isomers are designated the $\beta$- and $\alpha$-isomers, respectively. For such additions, the major product of the reactions would be expected to have the R group in the sterically less hindered, equatorial ($\alpha$) position. For addition of $CH_3Li$, both the $\alpha$- and the $\beta$-methyl isomers were obtained with the major product being **3a** in a ratio of about 3:1. Increasing the chain length (ethyl, *n*-propyl, *i*-propyl, *i*-butyl, and *i*-pentyl) resulted in the isolation of only the $\alpha$-alkyl-$\beta$-hydroxy isomers (only one isomer apparent from TLC and NMR analyses); whether the other isomers were formed in minor amounts during the addition reactions was not determined. With increasing size of the alkyl group, additional side products were obtained which were identified as a mixture of the two isomers of **1**, the result of simple reduction of the ketone of **2**. This was not surprising, as reduction to the alcohol can be observed during Grignard reagent additions to ketones.[16]

The reduction of **2** using $NaBH_4$ at 0 °C produced $\alpha$-dihydrotetrabenazine (**1a**) and $\beta$-dihydrotetrabenazine (**1b**), in an 4:1 ratio. Two recrystallizations provided **1a** in 99% purity as determined by HPLC. Flash chromatography was used to isolate the **1b** isomer from the residue.

**Structural Assignments and Conformation by NMR.** A variety of NMR spectroscopic tools were employed to unequivocally determine the relative configurations and ring conformations for compounds **1**−**8**. These methods included acquisition of spectra using the experimental techniques of HETCOR ($^1H-^{13}C$ heteronuclear shift-correlated spectroscopy), GATEDEC (gated decoupled *J*-spectroscopy), JMODXH (heteronuclear *J*-modulation spectroscopy), COSY ($^1H-^1H$ chemical shift-correlated spectroscopy), and NOESY (nuclear Overhauser enhancement chemical shift-correlated spectroscopy). It was usually possible to assign the complete $^1H$ NMR spectrum of these compounds based on HETCOR experiments only, as most of the peaks were well separated on the $^{13}C$ NMR spectrum. In most cases, however, $^{13}C$ NMR JMODXH experiments were also performed to eliminate problems related to the $^{13}C$ NMR signals that were too close to each other. COSY spectra were then obtained to confirm spectral assignments. Finally, NOESY experiments allowed the complete assignment of the relative configurations and confirmed the assigned conformation for the ring structure. Our NMR studies were in agreement with previous investigations of such benzisoquinolines and related structures.[17−24]

The combined NMR experiments allowed assignment of the 2-ethyl, *n*-propyl, isopropyl, and isobutyl groups as being *trans* to the isobutyl group at the 3-position and a B,C ring conformation unchanged from that of the parent compounds **1** and **2**. For the sterically smaller methyl groups, both isomers are formed in the addition reaction, again with no alterations in the conformation of the ring system. For all compounds synthesized, these NMR analyses indicated that the relative configurations of substituents on the carbon atoms at positions C-3 and C-11b were maintained during the Grignard addition reactions.

We have recently shown that $\alpha$-dihydrotetrabenazine, synthesized by reduction of commercially available tetrabenazine, is a mixture of two enantiomers. Only one of the two isomers is biologically active in either *in vitro* or *in vivo* assays of radioligand binding to the vesicular monoamine transporter of mouse or human brain.[25,26] All the benzisoquinolines we have synthesized here from tetrabenazine are thus racemic mixtures.

***In Vitro* Studies**. The *in vitro* $K_i$ values for inhibition of [$^3H$]methoxytetrabenazine binding to the VMAT2 of rat striatum[27] are shown in Table 1 for **2** and the six alkylated derivatives **4**−**8** prepared in this study. The highest affinity was shown by the $\beta$-methyl compound **3a** with a general decrease in binding affinity upon either lengthening or branching of the carbon chain. Stereoselectivity of binding was shown between the two isomers **4a**,**b**, with a nearly 5-fold higher affinity found for **4a.** For comparison, literature $IC_{50}$ values for inhibition of [$^3H$]dihydrotetrabenazine binding in rat

**Table 1.** *In Vitro* $K_i$ Values of Benzisoquinoline Ligands for the Binding of [$^3$H]Methoxytetrabenazine to Rat Brain Vesicular Monoamine Transporter[a]

| ligand | $K_i$ (nM) |
|---|---|
| **2** | 8.1 ± 0.4 |
| **4a** | 2.6 ± 0.2 |
| **4b** | 12 ± 1 |
| **5** | 42 ± 5 |
| **6** | 84 ± 14 |
| **7** | 136 ± 12 |
| **8** | 33 ± 2 |

[a] Data are shown as mean ± SD ($n$ = 3).

**Table 2.** Competition of Unlabeled Benzisoquinolines for the *in Vivo* Localization of [$^{11}$C]Methoxytetrabenazine in Mouse Striatum and Hypothalamus[a]

| competing ligand | striatum | hypothalamus (%ID/g) |
|---|---|---|
| none (controls, $N$ = 64) | 6.38 ± 1.37 | 3.87 ± 0.63 |
| **2** | 2.26 ± 0.42[b] | 1.71 ± 0.25[b] |
| **4a** | 2.09 ± 0.27[b] | 1.96 ± 0.39[b] |
| **4b** | 2.35 ± 0.32[b] | 1.79 ± 0.27[b] |
| **5** | 3.24 ± 0.58[b] | 2.00 ± 0.27[b] |
| **6** | 4.26 ± 0.37[b] | 1.96 ± 0.35[b] |
| **7** | 6.21 ± 1.4 | 3.06 ± 0.7[b] |
| **8** | 5.33 ± 0.86[b] | 2.93 ± 0.51[b] |

[a] CD-1 mice were coinjected with 10 mg/kg blocking agent and 100−200 $\mu$Ci of [$^{11}$C]methoxytetrabenazine and regional brain distributions determined at 15 min after injection. Control animals were injected with radiotracer in saline but with no added competing agent. Data are shown as mean ± SD of percent injected dose/g (%ID/g). Statistical analyses were done using an unpaired Student's $t$-test. [b] $p < 0.005$ as compared to controls.

brain[12] by **1a**,**b** are 6 and 20 nM, respectively, and we have in separate studies determined a $K_i$ of 0.97 nM for (+)-α-dihydrotetrabenazine.[25]

***In Vivo* Studies.** In the development of new organ- or tissue-specific imaging radiopharmaceuticals, a difficult step is the selection of a candidate compound for subsequent radiolabeling and *in vivo* evaluation. For practical or economic reasons, it is not usually feasible to radiolabel all compounds in a series (such as all of the alkylated benzisoquinolines prepared here), and thus there is a need for methods to reduce such lists to a more manageable number of candidate compounds.

The *in vivo* biological activities of the new compounds prepared here were assayed using their ability to compete for the accumulation of [$^{11}$C]methoxytetrabenazine ([$^{11}$C]-**3**) in mouse striatum and hypothalamus after peripheral administration. We have previously demonstrated that the retention of tritiated and carbon-11-labeled forms of **1**−**3** in rodent brain tissues *in vivo* is due to binding to the VMAT2 site[6,28] and that the *in vivo* regional brain distributions of these radioligands determined 10−15 min after iv injection are highly correlated with *in vitro* measures of [$^3$H]dihydrotetrabenazine binding sites ($r$ = 0.99) and regional *in vitro* monoamine concentrations ($r$ = 0.98; correlations not shown).[29] Using this simple assay, we have demonstrated (Table 2) that all of the new benzisoquinolines with the exception of the isopropyl derivative are capable of inhibition of radiotracer binding to the vesicular monoamine transporter, although with different efficiencies: Derivatives with smaller alkyl groups (methyl, ethyl) are more potent than those incorporating the larger groups (propyl, isopropyl, isobutyl).

***In Vivo*−*in Vitro* Correlations.** In the design of new drugs and radiopharmaceuticals, it is of interest to determine if measures of *in vitro* properties, such as binding affinities, lipophilicities, or serum protein binding, necessarily predict *in vivo* biological activities. In the area of radiopharmaceutical design, Katzenellenbogen and co-workers have demonstrated the usefulness of an *in vitro* measure, termed the binding selectivity index (defined as the ratio of the measured *in vitro* binding affinity to estimated nonspecific binding), as an indicator of the *in vivo* behavior of steroid radiotracers.[30,31] Similarly, Eckelman et al.[32] have shown a good correlation between *in vitro* binding affinities and *in vivo* radioligand-binding inhibition for a series of iodinated derivatives of 3-quiniclidinyl benzilate, a muscarinic cholinergic receptor antagonist.

We have obtained both *in vitro* and *in vivo* data for a series of benzisoquinoline ligands for the VMAT2 and can examine whether the *in vitro* data would have predicted the *in vivo* results. Although *in vivo* competition studies performed here do not control for confounding factors of pharmacokinetics, plasma protein binding, nonspecific distribution, and metabolism, it is interesting that the *in vitro* binding affinities and *in vivo* data are indeed correlated. Thus, the greatest *in vivo* competition is demonstrated by the highest *in vitro* affinity compounds, bearing either no (**2**) or small (**4a**,**b** and **5**) alkyl groups. Addition of the larger alkyl groups (propyl (**6**), isopropyl (**7**), and isobutyl (**8**)) reduces both *in vitro* binding affinity as well as *in vivo* competition for radioligand binding, although the *in vivo* results certainly reflect the effects of increased lipophilicity and possibly less drug delivery to the tissue. If the isobutyl derivative **8** is excluded (see below), the correlation between *in vitro* binding affinities and *in vivo* radioligand competition is remarkably good ($r^2$ = 0.994; correlation not shown).

The isobutyl compound **8** proves very interesting. Despite a good binding affinity ($K_i$ = 33 nM), this derivative shows relatively poor ability *in vivo* to compete for radioligand binding to the vesicular monoamine transporter. This is likely due to greater protein binding and partitioning into membranes due to the higher lipophilicity. The binding affinity observed for **8** may, however, indicate an important option in the future development of *in vivo* imaging agents. The increase in binding affinity when moving from the isopropyl to the isobutyl derivative may be due to new, favorable interactions of the longer 2-alkyl group with a hydrophobic site near the binding site for these benzisoquinolines. This would explain the very high binding affinity observed *in vitro* for one of the two possible 2-iodovinyl derivatives of dihydrotetrabenazine reported by Kung and co-workers;[13] although the stereochemistry of the high-affinity isomer remains undetermined, if it proves to be the $\beta$-iodovinyl-α-hydroxy derivative, then it would possibly present a large lipophilic substituent into approximately the same space as reached by the 2$\beta$-isobutyl derivative **8** tested here. If such substituents contribute to a high binding affinity, a successful *in vivo* radiotracer will then require modifications of other structural features of the benzisoquinolines to reduce overall lipophilicity of the molecule.

## Conclusions

Tetrabenazine and related benzisoquinolines are high-affinity ligands for the VMAT2 in mammalian brain. A

methyl group in either the α- or *β*-configuration at the 2-position of dihydrotetrabenazine is well tolerated, and the highest *in vitro* affinity for the VMAT2 site of rat brain was found for the 2*β*-methyl-α-hydroxy isomer **4a**. Addition of alkyl groups larger than a methyl group in the 2α-position of **1b** reduces both *in vitro* binding affinity as well as the ability to occupy VMAT2 sites *in vivo*.

## Experimental Section

**General.** Chemicals were purchased from Aldrich Chemical Co. (St. Louis, MO) and are reagent grade unless otherwise noted. Kieselgel (silica gel 60, 6−300 *μ*m, 70−230 mesh) and thin layer chromatographic plastic sheets (silica gel 60 $F_{254}$, layer thickness 0.2 mm) were purchased from Merck Co. Melting points were determinated in a capillary tube on a Mel-Temp apparatus and are uncorrected. $^1$H and $^{13}$C NMR spectra were obtained on a Bruker AC-360 spectrometer and recorded in $CDCl_3$ (7.24 ppm for $^1$H NMR and 77.00 ppm for $^{13}$C NMR as a reference). Mass spectra were measured with a VG 70-250s spectrometer. High-resolution mass spectra were measured with a Fisons magnetic sector mass spectrometer. Elemental analyses were performed by the University of Michigan CHN/AA Laboraotry (Ann Arbor, MI) and are within 0.4% of the calculated values, unless otherwise noted.

[$^3$H]Methoxytetrabenazine (82 Ci/mmol) was prepared by custom tritiation (Amersham Corp.). [$^{11}$C]Methoxytetrabenazine (>500 Ci/mmol) was prepared as previously described.[33]

**Syntheses of 2-Alkyldihydrotetrabenazine Derivatives. 2α- and 2*β*-Hydroxy-2-methyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine (4a,b).** Syntheses of alkylated dihydrotetrabenazines **4**−**8** were done using the general procedure described here for the synthesis of compound **4**. To a stirred solution of **2** (1.621 g, 5.106 mmol) in dry tetrahydrofuran (30 mL) at 0 °C was added methyllithium (4.0 mL, 1.4 M solution in THF) dropwise over 10 min. The reaction mixture was stirred at 0 °C for another 2.5 h and then allowed to warm to room temperature. After 12 h, the reaction was quenched by addition of water (2 mL). The organic layer was separated, and the aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried over sodium sulfate and filtered. Removal of the solvent from the filtrate gave an oily solid. Column chromatographic purification using a methanol in dichloromethane gradient yielded two compounds, **4a** (189 mg, 0.567 mmol) and **4b** (533 mg, 1.60 mmol), in 11% and 31% yields, respectively (total 42%).

**4a**: mp 76−78 °C; $^1$H NMR ($CDCl_3$) *δ* 6.56 (1H, s, H-8), 6.62 (1H, s, H-11), 3.83 (3H, s, $CH_3$-9), 3.82 (3H, s, $CH_3$-10), 3.17 (1H, d, *J* = 12 Hz, H-11b), 3.08 (1H, m, H-7), 3.00 (1H, m, H-6), 2.93 (1H, dd, *J* = 11.9, 4.1 Hz, H-4), 2.06 (1H, dd, *J* = 11.6, 11.6 Hz, H-4), 2.62 (1H, m, H-7), 2.47 (1H, ddd, *J* = 10.9, 10.9, 4.7 Hz, H-6), 2.29 (1H, dd, *J* = 12.4, 4.2 Hz, H-1), 1.82 (1H, m, H-3), 1.59 (2H, m, H-1,2′), 1.38 (1H, ddd, *J* = 13, 10.2, 2.5 Hz, H-1′), 1.22 (3H, s, $CH_3$-1″), 0.99 (1H, ddd, *J* = 13, 9.8, 3.2 Hz, H-1′), 0.93 (3H, d, *J* = 6.3 Hz, $CH_3$-3′), 0.90 (3H, d, *J* = 6.3 Hz, $CH_3$-3′); $^{13}$C NMR ($CDCl_3$) *δ* 147.45 (C-10), 147.13 (C-9), 129.49 (C-11a), 126.44 (C-7a), 111.52 (C-8), 107.86 (C-11), 72.36 (C-2), 59.99 (C-11b), 59.05 (C-4), 55.96 and 55.81 (2 × $OCH_3$), 51.78 (C-6), 44.50 (C-3), 47.46 (C-1), 36.21 (C-1′), 29.11 (C-7), 25.74 (C-2′), 24.24 (C-3′), 21.79 (C-3′), 20.64 (C-1″); HRMS calcd 333.2304, found 333.2292. Anal. ($C_{20}H_{31}NO_3$) C,H,N.

**4b:** mp 108−111 °C; $^1$H NMR ($CDCl_3$) *δ* 6.64 (1H, s, H-8), 6.54 (1H, s, H-11), 3.82 (3H, s, $CH_3$-9), 3.81 (3H, s, $CH_3$-10), 3.39 (1H, d, *J* = 12 Hz, H-11b), 3.08 (1H, m, H-7), 2.95 (1H, m, H-6), 2.80 (1H, dd, *J* = 11.6, 4.3 Hz, H-4), 2.27 (1H, dd, *J* = 11.6, 11.6 Hz, H-4), 2.61 (1H, m, H-7), 2.50 (1H, ddd, *J* = 11.4, 11.4, 4 Hz, H-6), 2.22 (1H, dd, *J* = 14.1, 2.6 Hz, H-1), 1.82 (1H, m, H-3), 1.57 (2H, m, H-1,2′), 1.30 (1H, ddd, *J* = 13.4, 11, 2.4 Hz, H-1′), 1.06 (1H, ddd, *J* = 13.4, 9.7, 4 Hz, H-1′), 1.27 (3H, s, $CH_3$-1″), 0.92 (3H, d, *J* = 6.6 Hz, $CH_3$-3′), 0.88 (3H, d, *J* = 6.6 Hz, $CH_3$-3′); $^{13}$C NMR ($CDCl_3$) *δ* 147.34 (C-10), 147.10 (C-9), 129.82 (C-11a), 126.64 (C-7a), 111.46 (C-8), 107.90 (C-11), 71.07 (C-2), 57.89 (C-11b), 57.48 (C-4), 55.96 and 55.79 (2 × $OCH_3$), 52.15 (C-6), 41.93 (C-3), 45.56 (C-1), 36.03 (C-1′), 29.05 (C-7), 25.90 (C-2′), 24.12 (C-3′), 21.69 (C-3′), 28.43 (C-1″). Anal. ($C_{20}H_{31}NO_3$) C,H,N.

**2*β*-Ethyl-2α-hydroxy-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine (5):** yield 620 mg (38%); mp 122−123 °C; $^1$H NMR ($CDCl_3$) *δ* 6.70 (1H, s, H-8), 6.61 (1H, s, H-11), 3.82 (3H, s, $CH_3$-9), 3.87 (3H, s, $CH_3$-10), 3.48 (1H, d, *J* = 12 Hz, H-11b), 3.00 (1H, m, H-7), 3.01 (1H, m, H-6), 2.88 (1H, dd, *J* = 11.6, 4.3 Hz, H-4), 2.37 (1H, dd, *J* = 11.6, 11.6 Hz, H-4), 2.67 (1H, m, H-7), 2.56 (1H, ddd, *J* = 11.4, 11.4, 4 Hz, H-6), 2.16 (1H, dd, *J* = 14.1, 2.6 Hz, H-1), 1.88 (1H, m, H-3), 1.67 (1H, m, H-1), 1.65 (2H, m, H-2, 1″), 1.29 (1H, ddd, *J* = 12.9, 10.5, 2.5 Hz, H-1′), 1.12 (1H, ddd, *J* = 13.9, 9.8, 4 Hz, H-1′), 0.96 (3H, t, *J* = 8.4 Hz, H-2″), 0.98 (3H, d, *J* = 6.4 Hz, $CH_3$-3′), 0.95 (3H, d, *J* = 6.4 Hz, $CH_3$-3′); $^{13}$C NMR ($CDCl_3$) *δ* 147.31 (C-10), 147.04 (C-9), 130.09 (C-11a), 126.75 (C-7a), 111.48 (C-8), 108.04 (C-11), 73.25 (C-2), 57.64 (C-11b), 57.10 (C-4), 55.99 and 55.76 (2 × $OCH_3$), 52.10 (C-6), 38.93 (C-3), 41.45 (C-1), 35.51 (C-1′), 29.08 (C-7), 25.57 (C-2′), 24.22(C-3′), 21.67 (C-3′), 33.03 (C-1″), 8.03 (C-2″); HRMS calcd 347.2460, found 347.2468. Anal. ($C_{21}H_{33}NO_3$) C,H,N.

**2*β*-*n*-Propyl-2α-hydroxy-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine (6):** yield 227 mg (33%); mp 147−148 °C; $^1$H NMR ($CDCl_3$) *δ* 6.62 (1H, s, H-8), 6.53 (1H, s, H-11), 3.80 (3H, s, $CH_3$-9), 3.81 (3H, s, $CH_3$-10), 3.42 (1H, d, *J* = 11.2 Hz, H-11b), 3.08 (1H, m, H-7), 2.93 (1H, m, H-6), 2.80 (1H, dd, *J* = 11.6, 4.3 Hz, H-4), 2.31 (1H, dd, *J* = 11.6, 11.6 Hz, H-4), 2.60 (1H, m, H-7), 2.49 (1H, ddd, *J* = 11.4, 11.4, 4 Hz, H-6), 2.12 (1H, dd, *J* = 13.5, 2.6 Hz, H-1), 1.80 (1H, m, H-3), 1.58 (2H, m, H-1,2′), 1.52 (2H, m, H-1″,2″), 1.34 (2H, m, H-1″,2″), 1.22 (1H, ddd, *J* = 12.9, 10.5, 2.5 Hz, H-1′), 1.05 (1H, ddd, *J* = 16, 11.8, 4.4 Hz, H-1′), 0.90 (3H, t, *J* = 7.1 Hz, H-3″), 0.90 (3H, d, *J* = 6.4 Hz, $CH_3$-3′), 0.87 (3H, d, *J* = 6.4 Hz, $CH_3$-3′); $^{13}$C NMR ($CDCl_3$) *δ* 147.02 (C-9), 147.30 (C-10), 129.95 (C-11a), 126.65 (C-7a), 111.45 (C-8), 108.00 (C-11), 72.95 (C-2), 57.64 (C-11b), 57.05 (C-4), 55.98 and 55.73 (2 × $OCH_3$), 52.05 (C-6), 39.41 (C-3), 42.07 (C-1), 35.53 (C-1′), 28.99 (C-7), 25.56 (C-2′), 24.21 (C-3′), 21.65 (C-3′), 43.13 (C-1″), 16.81 (C-2″), 14.67 (C-3″); HRMS calcd 361.2617, found 361.2607. Anal. ($C_{22}H_{35}NO_3$) C, H, N.

**2*β*-Isopropyl-2α-hydroxy-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine (7):** yield 340 mg (26%); mp 167−168; $^1$H NMR ($CDCl_3$) *δ* 6.54 (1H, s, H-8), 6.63 (1H, s, H-11), 3.81 (3H, s, $CH_3$-9), 3.80 (3H, s, $CH_3$-10), 3.41 (1H, d, *J* = 12 Hz, H-11b), 3.08 (1H, m, H-7), 2.96 (1H, m, H-6), 2.83 (1H, dd, *J* = 11.6, 4.3 Hz, H-4), 2.33 (1H, dd, *J* = 11.6, 11.6 Hz, H-4), 2.61 (1H, m, H-7), 2.59 (1H, ddd, *J* = 11.4, 11.4, 4 Hz, H-6), 2.06 (1H, dd, *J* = 13.6, 2.8 Hz, H-1), 2.02 (3H, m, H-3,1″), 1.44 (1H, dd, *J* = 13.4, 12 Hz, H-1), 1.60 (1H, m, H-2′), 1.22 (1H, ddd, *J* = 13.2, 10.5, 2.6 Hz, H-1′), 1.03 (1H, ddd, *J* = 15.2, 10.8, 4 Hz, H-1′), 1.01 (3H, d , *J* = 6.9 Hz, H-2″), 0.84 (3H, d, *J* = 6.9 Hz, H-2″), 0.90 (3H, d, *J* = 6.6 Hz, $CH_3$-3′), 0.88 (3H, d, *J* = 6.6 Hz, $CH_3$-3′); $^{13}$C NMR ($CDCl_3$) *δ* 146.86 (C-9), 147.20 (C-10), 130.18 (C-11a), 126.68 (C-7a), 111.36 (C-8), 108.02 (C-11), 74.96 (C-2), 57.28 (C-11b), 56.73 (C-4), 55.92 and 55.61 (2 × $OCH_3$), 51.87 (C-6), 37.41 (C-3), 35.58 (C-1′), 35.08 (C-1), 28.92 (C-7), 25.07 (C-2′), 24.22 (C-3′), 21.39 (C-3′), 33.94 (C-1″), 17.26 (C-2″), 16.23 (C-2″); HRMS calcd 361.2617, found 361.2607. Anal. ($C_{22}H_{35}NO_3$) C,H,N.

**2*β*-Isobutyl-2α-hydroxy-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine (8):** yield 750 mg (39%); mp 175−177 °C; $^1$H NMR ($CDCl_3$) *δ* 6.55 (1H, s, H-8), 6.51 (1H, s, H-11), 3.81 (3H, s, $CH_3$-9), 3.82 (3H, s, $CH_3$-10), 3.40 (1H, d, *J* = 11.4 Hz, H-11b), 2.94 (1H, m, H-7), 3.04 (1H, m, H-6), 2.81 (1H, dd, *J* = 11.6, 4.3 Hz, H-4), 2.28 (1H, dd, *J* = 11.6, 11.6 Hz, H-4), 2.60 (1H, m, H-7), 2.49 (1H, ddd, *J* = 11.4, 11.4, 4 Hz, H-6), 2.24 (1H, m, H-1), 1.87 (1H, m, H-2″), 1.66 (1H, m, H-3), 1.59 (1H, m, H-2′), 1.56 (1H, m, H-1), 1.50 (1H, m, H-1″), 1.38 (1H, m, H-1″), 1.25 (1H, ddd, *J* = 12.9, 10.5, 2.5 Hz, H-1′), 1.02 (1H, ddd, *J* = 14.2, 10.4, 4.7 Hz, H-1′), 1.01 (3H, d, *J* = 6.7 Hz, H-3″), 0.94 (3H, d, *J* = 6.7 Hz, H-3″), 0.92 (3H, d, *J* = 6.4 Hz, $CH_3$-3′), 0.89 (3H, d, *J* = 6.4 Hz, $CH_3$-3′); $^{13}$C NMR ($CDCl_3$) *δ* 146.89 (C-9), 147.21 (C-10), 129.91 (C-11a), 126.64 (C-7a), 111.37 (C-8), 108.07 (C-11),

73.24 (C-2), 57.50 (C-11b), 57.02 (C-4), 55.90, 55.58 (2 × $OCH_3$), 51.81 (C-6), 40.75 (C-3), 42.24 (C-1), 35.54 (C-1′), 28.89 (C-7), 23.24 (C-2′), 25.10 (C-3′), 21.45 (C-3′), 49.13 (C-1″), 25.49 (C-2″), 24.56 (C-3″); HRMS calcd 375.2773, found 375.2773. Anal. ($C_{23}H_{37}NO_3$) C,H,N.

**2α- and 2β-Hydroxy-3-isobutyl-1,2,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine (1a,b).** The two isomers of dihydrotetrabenazine were isolated both as side products in the synthesis of the alkylated derivatives, above, and from $NaBH_4$ reduction of the ketone in ethanol solution.

**1a:** $^1$H NMR ($CDCl_3$) $\delta$ 6.62 (1H, s, H-8), 6.71 (1H, s, H-11), 3.88 (6H, s, $OCH_3$-9,10), 3.42 (1H, m, H-2), 3.16 (1H, d, $J$ = 12 Hz, H-11b), 3.09 (2H, m, H-4,7), 3.03 (1H, m, H-6), 2.69 (1H, m, H-7), 2.63 (1H, m, H-1), 2.49 (1H, ddd, $J$ = 10.9, 10.9, 4.7 Hz, H-6), 2.01 (1H, dd, $J$ = 11.6, 11.6 Hz, H-4), 1.75 (2H, m, H-3, 2′), 1.62 (1H, ddd, $J$ =13, 10.2, 2.5 Hz, H-1′), 1.53 (1H, dd, $J$ = 13.6, 12 Hz, H-1), 1.09 (1H, ddd, $J$ = 13, 9.8, 3.2 Hz, H-1′), 0.98 (3H, d, $J$ = 6.6 Hz, $CH_3$-3′), 0.96 (3H, d, $J$ = 6.6 Hz, $CH_3$-3′); $^{13}$C NMR ($CDCl_3$) $\delta$ 147.24 (C-9), 147.52 (C-10), 129.39 (C-11a), 126.44 (C-7a), 111.49 (C-8), 107.96 (C-11), 74.60 (C-2), 60.96 (C-11b), 60.11 (C-4), 55.99 and 55.89 (2 × $OCH_3$), 51.96 (C-6), 41.63 (C-3), 40.60 (C-1), 39.74 (C-1′), 29.22 (C-7), 25.38 (C-2′), 24.24 (C-3′), 21.82 (C-3′).

**1b:** $^1$H NMR ($CDCl_3$) $\delta$ 6.56 (1H, s, H-8), 6.65 (1H, s, H-11), 4.07 (1H, bs, H-2), 3.82 (6H, s, $OCH_3$-9,10), 3.51 (1H, d, $J$ = 12 Hz, H-11b), 3.08 (1H, m, H-7), 2.95 (1H, m, H-6), 2.67 (3H, m, H-4,6,7), 2.40 (2H, m, H-1,4), 2.00 (1H, m, H-3), 1.71 (2H, m, H-1,2′), 1.26 (1H, m, H-1′), 1.19 (1H, m, H-1′), 0.95 (3H, d, $J$ = 6.2 Hz, $CH_3$-3′), 0.93 (3H, d, $J$ = 6.2 Hz, $CH_3$-3′); $^{13}$C NMR ($CDCl_3$) $\delta$ 146.97 (C-9), 147.25 (C-10), 129.92 (C-11a), 126.74 (C-7a), 111.39 (C-8), 107.95 (C-11), 67.73 (C-2), 56.27 (C-11b), 56.18 (C-4), 55.86 and 55.69 (2 × $OCH_3$), 52.31 (C-6), 37.59 (C-3), 39.06 (C-1), 38.74 (C-1′), 29.02 (C-7), 38.74 (C-2′), 22.87 (C-3′).

***In Vitro* Binding Assays: [$^3$H]Methoxytetrabenazine Binding.** Determinations of the *in vitro* $K_i$ values of unlabeled benzisoquinolines **2** and **4**−**8** for [$^3$H]methoxytetrabenazine binding to the rat brain VMAT2 were done using an autoradiographic method.[27] Studies were done in triplicate using intact brain cryostat sections of male Sprague−Dawley rats (190−240 g; Charles River Laboratories, Portage, MI). The binding of [$^3$H]methoxytetrabenazine was assayed under minor modifications of the conditions utilized for [$^3$H]dihydrotetrabenazine.[34] Adjacent coronal sections (20 m) at the level of the caudate-putamen were mounted on subbed microscope slides and prewashed at 25 °C in 300 mM sucrose, 50 mM Tris, and 1 mM EDTA, pH 8.0 (sucrose buffer), to remove endogenous competing substances. Sections were then incubated for 3 h in the presence of 10 nM [$^3$H]methoxytetrabenazine and varying concentration of test drug ranging from 1 nM to 2 M. Following incubation, slices were washed 3 × 3 min in fresh sucrose buffer, briefly dipped in distillated water at 4 °C to remove excess buffer, and allow to air-dry before autoradiography. Sections were then apposed to tritium-sensitive X-ray film (Hyperfilm-$^3$H; Amersham Corp., U.K.) and exposed for 2 weeks. Autoradiographs were analyzed by computer-assisted video densitometry (MCID; Imaging Research, St. Catherines, Ontario, Canada). Calibrated plastic radioactive standards were included with each exposure to convert the optical density to radioligand tissue concentrations as described previously.[35] The binding isotherms, resulting from striatal binding at various competitive ligand concentrations, were analyzed by nonlinear curve fitting using the Ligand program[36] (Elsevier-Biosoft, Cambridge, U.K) with both one- and two-site model configurations. The model of least complexity was assumed correct unless significant ($p < 0.05$) improvement in residual error was noted. The apparent affinity constants were calculated using a $K_d$ of 3.9 nM for [$^3$H]methoxytetrabenazine binding in rat striatum.[27]

***In Vivo* Radioligand Competition Studies.** Female CD-1 mice (20−25 g; obtained from Charles River) were anesthetized with diethyl ether, injected via the tail vein with a mixture of [$^{11}$C]methoxytetrabenazine (100−300 $\mu$Ci) and 10 mg/kg test compound (**2**, **4**−**8**) in 10% ethanol in saline, and then allowed to recover. After 15 min, the animals were anesthetized (ether) and decapitated and the brains quickly removed and dissected. Samples of striatum (left and right combined) and hypothalamus (whole) were quickly counted for carbon-11 (automatic $\gamma$-counter) and then weighed. Data were calculated as percent injected dose/g (%ID/g) for each tissue.

**Acknowledgment.** This work was supported by National Institutes of Health Grant NS 15655, MH 47611, AG 08671, and T-32-CA09015 (to L.C.L.) and the Department of Energy Grant DE-FG021-87ER60561. We thank Mr. Frank Parker, Dr. Christopher Kojiro, and Dr. Peter Toogood (Department of Chemistry, University of Michigan) for their assistance in obtaining and interpreting the NMR spectra.

## References

(1) Shuldiner, S. A molecular glimpse of vesicular monoamine transporters. *J. Neurochem*. **1994**, *62*, 2067−2078.
(2) Erickson, J. D.; Eiden, L. E. Functional identification and molecular cloning of a human brain vesicle monoamine transporter. *J. Neurochem*. **1993,** *61*, 2314−2317.
(3) Surratt, C.; Persico, A. M.; Yang, X.-D.; Edgar, S. R.; Bird, G. S.; Hawkins, A. L.; Griffin, C. A.; Li, X.; Jabs, E. W.; Uhl, G. R. A human synaptic vesicle monoamine transporter cDNA predicts posttranslational modifications, reveals chromosome 10 gene localization and identifies TaqI RFLPs. *FEBS Lett*. **1993**, *318*, 325−330.
(4) Henry J.-P.; Scherman, D. Radioligands of the vesicular monoamine transporter and their use as markers of monoamine storage vesicles. *Biochem. Pharmacol.* **1989**, *38*, 2395−2404.
(5) Scherman, D.; Desnos, C.; Darchen, F.; Pollak, P.; Javoy-Agid, F.; Agid, Y. Striatal dopamine deficiency in Parkinson's disease: role of aging. *Ann. Neurol.* **1989**, *26*, 551−557.
(6) DaSilva, J. N.; Kilbourn, M. R. *In vivo* binding of [$^{11}$C]tetrabenazine to vesicular monoamine transporters in mouse brain. *Life Sci.* **1992**, *51*, 593−600.
(7) DaSilva, J. N.; Kilbourn, M. R.; Domino, E. F. *In vivo* imaging of monoaminergic nerve terminals in normal and MPTP-lesioned primate brain using positron emission tomography (PET) and [$^{11}$C]tetrabenazine. *Synapse* **1993**, *14*, 128−131.
(8) Kilbourn, M. R.; DaSilva, J. N.; Frey, K. A.; Koeppe, R. A.; Kuhl, D. E. *In vivo* imaging of vesicular monoamine transporters in human brain using [$^{11}$C]tetrabenazine and positron emission tomography. *J. Neurochem*. **1993**, *60*, 2315−2318.
(9) Koeppe, R. A.; Frey, K. A.; Vander Borght, T. M.; Kilbourn, M. R.; Jewett, D. M.; Lee, L. C.; Kuhl, D. E. Kinetic evaluation of [C-11]dihydrotetrabenazine (DTBZ) by dynamic PET: a marker for the vesicular monoamine transporter. *J. Cerebral Blood Flow Metab.* **1995,** *15*, S651.
(10) Vander Borght, T. M.; Kilbourn, M. R.; Koeppe, R. A.; DaSilva, J. N.; Carey, J. E.; Kuhl, D. E.; Frey, K. A. In vivo imaging of the brain vesicular monoamine transporter. *J. Nucl. Med*. **1995**, in press.
(11) Pletscher, A.; Brossi, A.; Gey, K. F. Benzoquinolizine derivatives: a new class of monoamine decreasing drugs with psychotropic action. *Int. Rev. Neurobiol.* **1962**, *4*, 275−306.
(12) Scherman, D.; Gasnier, B.; Jaudon, P.; Henry, J.-P. Hydrophobicity of the tetrabenazine-binding site of the chromaffin granule monoamine transporter. *Mol. Pharmacol.* **1988**, *33*, 72−77.
(13) Kung, M.-P.; Canney, D. J.; Frederick, D.; Zhuang, Z.; Billings, J. J.; Kung, H. F. Binding of $^{125}$I-iodovinyltetrabenazine to CNS vesicular monoamine transport sites. *Synapse* **1994**, *18*, 225−232.
(14) Aranda, G.; Beaucourt, J. P.; Ponchant, M.; Isambert, M. F.; Henry, J. P. Synthesis and biological activity of iodinated and photosensitive derivatives of tetrabenazine. *Eur. J. Med. Chem*. **1990**, *25*, 369−374.
(15) Pletscher, A.; Besendorf, H.; Gey, K. F. Depression of norepinephrine and 5-hydroxytryptamine in the brain by benzisoquinoline derivatives. *Science* **1959**, *129*, 844.
(16) Cowan, D. O.; Mosher, H. S. Comparison of the reactions of Grignard reagents and dialkylmagnesium compounds in addition, reduction and emolization reactions. *J. Org. Chem*. **1962**, *27*, 1−5.
(17) Sugiura, M.; Takao, N.; Iwasa, K.; Sasaki, Y. Stereochemistry of quinolizidines. IV. Conformation of benzo[a]-quinolizidines and their $^{13}$C chemical shifts. *Chem. Pharm. Bull.* **1978**, *26*, 1901−1907.
(18) Sugiura, M.; Takao, N.; Iwasa, K.; Sasaki, Y. Stereochemistry of quinolizidines. VI. Conformation of benzo[a]-quinolizidines and their $^{13}$C chemical shifts. (2). *Chem. Pharm. Bull.* **1979**, *27*, 3144−3149.
(19) Sugiura, M.; Takao, N.; Iwasa, K.; Sasaki, Y. Stereochemistry of quinolizidines. III. Carbon-13 magnetic resonance spectra of benzo[a]quinolizidines. *Chem. Pharm. Bull.* **1978**, *26*, 1168−1176.

(20) Menendez, J. C.; Sollhuber, M. M. Stereochemistry of diastereomeric 9,10-dimethoxy-1,2,4,6,7,11b-hexahydrospiro[benzo[a]-quinizolidine]-2′,4′-diones. *Heterocycles* **1987**, *26*, 3203−3209.
(21) Uskokovic, M.; Bruderer, H.; von Planta, C.; Williams, T.; Brossi, A. The nuclear magnetic resonance spectra of the angular proton in benzo[a]- and indolo[a]quinolizidines. *J. Am. Chem. Soc.* **1964**, *86*, 3364−3367.
(22) Jennings, W. B.; Boyd, D. R.; Watson, C. G.; Becker, E. D.; Bradley, R. B.; Jerina, D. M. The stereochemical dependence of $^{15}$NCH and $^{13}$CH coupling constants in oxaziridines. *J. Am. Chem. Soc.* **1972**, *94*, 8501−8504.
(23) Van Binst, G.; Tourwe, D. Benzo- and Indolequinolizine compounds. IV. The use of $^{13}$C-H coupling constants in the stereochemical study of nitrogen bridgehead compounds. *Heterocycles* **1973**, *1*, 257−265.
(24) Tourwe, D.; Van Binst, G.; Kametani, T. Conformational analysis of the dibenzo[a,g]quinolizidines by spectroscopic means. II. The use of high resolution 270 MHz proton magnetic resonance. *Org. Magn. Reson.* **1977**, *9*, 341−346.
(25) Kilbourn, M. R.; Lee, L.; Vander Borght, T.; Jewett, D.; Frey, K. Binding of α-dihydrotetrabenazine to the vesicular monoamine transporter is stereospecific. *Eur. J. Pharmacol.* **1995**, *278*, 249−252.
(26) Kilbourn, M. R.; Lee, L. C.; Jewett, D. M.; Vander Borght, T. M.; Koeppe, R. A.; Frey, K. A. In vitro and in vivo binding of α-dihydrotetrabenazine to the vesicular monoamine transporter is stereospecific. *J. Cerebral Blood Flow Metab.* **1995**, *15*, S650.
(27) Vander Borght, T. M.; Sima, A. A. F.; Kilbourn, M. R.; Desmond, T. J.; Frey, K. A. [$^3$H]Methoxytetrabenazine: a high specific activity ligand for estimating monoaminergic neuronal integrity. *Neuroscience* **1995**, *68*, 955−962.
(28) DaSilva, J. N.; Carey, J. E.; Sherman, P. S.; Pisani, T. J.; Kilbourn, M. R. Characterization of [$^{11}$C]tetrabenazine as an *in vivo* radioligand for the vesicular monoamine transporter. *Nucl. Med. Biol.* **1994**, *21*, 151−156.
(29) Kilbourn, M. R. PET radioligands for vesicular neurotransmitter transporters. *Med. Chem. Res.* **1994**, *5*, 113−126.
(30) Katzenellenbogen, J. A. The Pharmacology of Steroid Radiopharmaceuticals: Specific and nonspecific Binding and Uptake Selectivity. In *Radiopharmaceuticals: Chemistry and Pharmacology*; Nunn, A. D., Ed.; Marcel Dekker: New York, 1992; pp 297−331.
(31) Buckman, B. O.; Bonasera, T. A.; Kirschbaum, K. S.; Welch, M. J.; Katzenellenbogen, J. A. Fluorine-18-labeled progestin 16a,17a-dioxalanes: development of high-affinity ligands for the progesterone receptor with high in vivo target site selectivity. *J. Med. Chem.* **1995**, *38*, 328−337.
(32) Eckelman, W. C.; Grissom, M.; Conklin, J.; Rzeszotarski, W. J.; Gibson, R. E.; Francis, B. E.; Jagoda, E. M.; Eng, R.; Reba, R. C. In vivo competition studies with analogues of 3-quiniclidinyl benzilate. *J. Pharm. Sci.* **1984**, *73*, 529−534.
(33) DaSilva, J. N.; Kilbourn, M. R.; Mangner, T. J. Synthesis of a [$^{11}$C]methoxy derivative of α-dihydrotetrabenazine: a radioligand for studying the vesicular monoamine transporter. *Appl. Radiat. Isot.* **1993**, *44*, 1487−1489.
(34) Scherman, D.; Raisman, R.; Ploska, A.; Agid, Y. [$^3$H]Dihydrotetrabenazine, a new *in vitro* monoaminergic probe for human brain. *J. Neurochem.* **1988**, *50*, 1131−1136.
(35) Pan, H. S.; Frey, K. A.; Young, A. B.; Penney, J. B. Change in [$^3$H]muscimol binding in substantia nigra, entopeduncular nucleus, globus pallidus, and thalamus after striatal lesions as demonstrated by quantitative receptor autoradiography. *J. Neurosci.* **1983**, *3*, 1189−1198.
(36) Munson, P. J.; Rodbard, D. Ligand: a versatile computerized approach for characterization of ligand-binding system. *Anal. Biochem.* **1980**, *107*, 220−239.

JM950117B