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Preparation and Characterization of Tetrabenazine Enantiomers against Vesicular Monoamine Transporter 2

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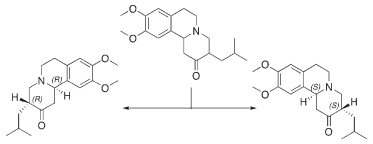
ABSTRACT As a clinical medication for the treatment of hyperkinetic movement disorders, in conditions such as Huntington's disease, tetrabenazine (TBZ) has always been used in its racemic form. To establish whether or not its beneficial therapeutic actions are enantiospecific, a practical total synthetic route was developed to yield each enantiomeric form to allow their chemical and pharmacological characterization. We briefly summarize the total synthesis of TBZ and report a detailed procedure for resolution of TBZ into its enantiomers, (+)-TBZ and (−)-TBZ. This allowed determination of the optical rotation and absolute configurations of each TBZ enantiomer, based on X-ray crystallographic analysis, together with characterization of their inhibitory action at the vesicular monoamine transporter 2, where (+)-TBZ proved 3-fold more active than (−)-TBZ.

KEYWORDS Tetrabenazine enantiomers, vesicular monoamine transporter 2 (VMAT2), Huntington's chorea, hyperkinesias



Tetrabenazine (TBZ) (**8**), also known as Ro 1-9569,[1] Nitoman (Hoffmann-La Roche, Nutley, NJ), and Xenazine (Lundbeck, Deerfield, IL), is a benzoquinolizine derivative with the chemical name 1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-3-(2-methylpropyl)-2*H*-benzo[*a*]quinolizin-2-one. Originally developed as an antipsychotic agent in the 1950s,[2] it has proved more valuable for other indications, as in the treatment of disorders characterized by excessive involuntary movement.[3,4] These hyperkinesias have a largely unknown pathophysiology, appear to involve neurotransmitter dysfunction, and include tremor, dystonia, ballism, tics, akathisia, stereotypies, chorea, myoclonus, and athetosis. TBZ depletes brain monoamine levels, including serotonin, dopamine, and norepinephrine. It achieves this by reversibly binding to the vesicular monoamine transporter 2 (VMAT2)[5,6] to inhibit monoamine uptake into granular vesicles of presynaptic neurons and, thereby, augment their degradation by monoamine oxidases within the cytoplasm. Despite five decades of medical history and approval for use in Britain in 1971, TBZ was only recently approved (August 15, 2008) by the FDA in the United States as the first drug to treat chorea associated with Huntington's disease.[7]

Whereas TBZ has been shown to be efficacious for the treatment of a variety of disabling hyperkinetic movement disorders, it has potential side effects, such as dysphasia, depression, sedation, parkinsonism, and others that are common to neuroleptic and psychotic medications.[8,9] One approach to reducing the risk of potential side effects has been to combine TBZ with other neuroleptic medications.[3] The structure of TBZ utilized as a medication, with the exception of some of its derivatives, is a racemic form.[3,7−9] The medicinally active enantiomeric form of TBZ remains to be determined, and synthesis and characterization of each may allow more effective use of this increasingly used drug.

An approach to obtain optically active TBZ can be initiated by catalytic asymmetric condensation of dihydro-isoquinoline (**2**) and desired malonate to give an enantiomeric excessive intermediate as described in the literature.[10] Because our objective was to characterize both enantiomers, the classical resolution of racemic TBZ seemed more efficient for our study. Moreover, Openshaw and Whittaker[11] have described the racemization of benzo[α]quinolizine, the primary ring structure of TBZ enantiomers **12** or **13**, via isoquinolinium structure **9**. Hence, asymmetric synthesis may encounter racemization midway within the synthetic pathway and may provide a final product that lacks optical purity.

There are two known primary methods for the synthesis of TBZ: the cyclization of tetrahydro-isoquinoline derivatives[1] or the condensation of a 3,4-dihydro-isoquinoline

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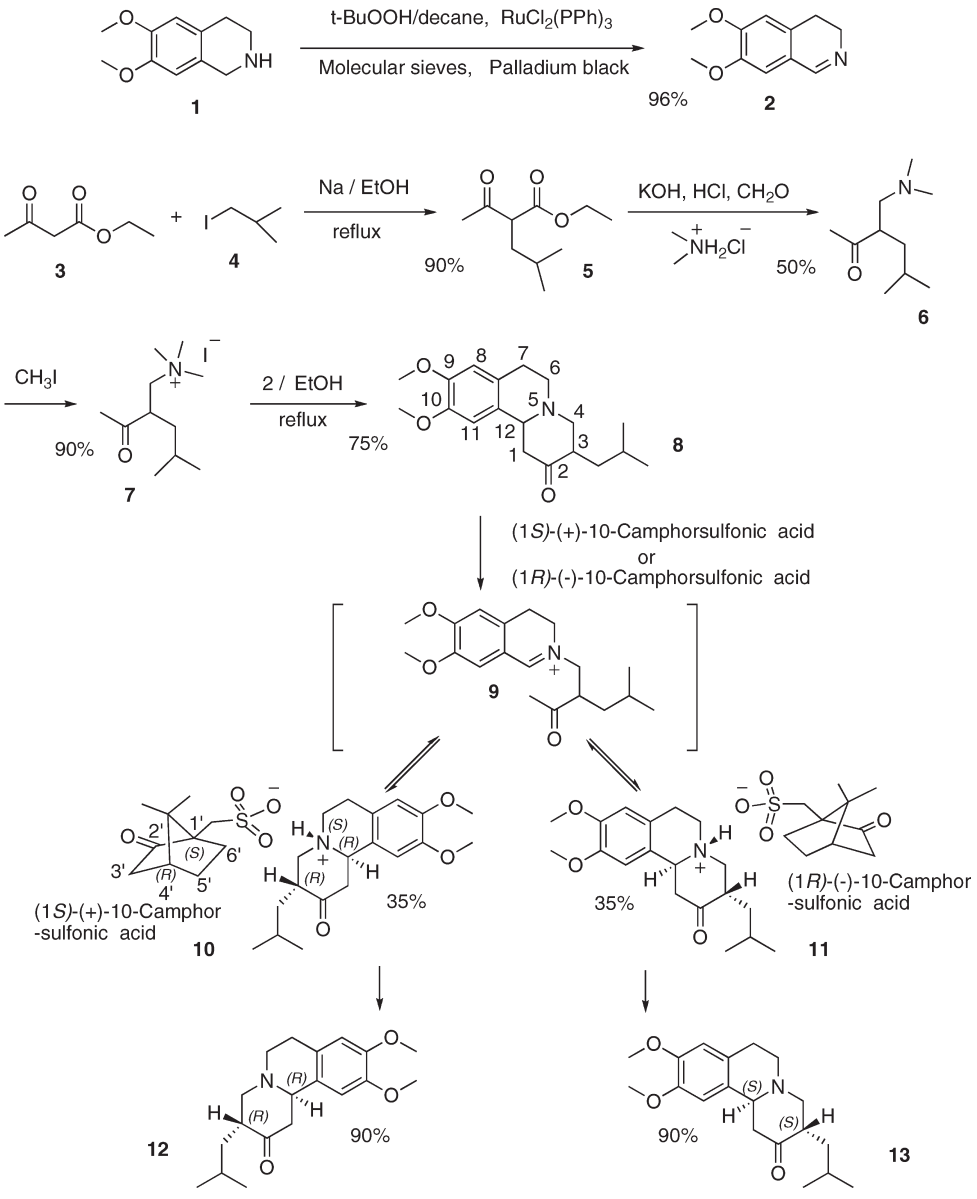
Scheme 1. Synthesis of TBZ Enantiomers



derivative with β-amino ketone (**7**).[12] A brief description of the synthesis of TBZ based on the second condensation method is illustrated in Scheme 1.

6,7-Dimethoxy-3,4-dihydro-quinoline (**2**) was obtained by catalytic oxidization of 6,7-dimethoxy-1,2,3,4-tertahydro-quinoline (**1**) in almost quantitative yield, according to a known procedure in the literature.[13] Compound **2** was separated from the reaction mixture by forming a solid fumarate[14] to avoid evaporation of the solvent, decane, which has a high boiling point.

Ethyl α-isobutyl-acetoacetate (**5**) was generated by condensation of ethyl acetoacetate (**3**) with 1-iodo-2-methylpropane (**4**) in a solution of Na/EtOH. The specific conditions utilized were adapted from the literature,[15] following their use in the synthesis of a compound similar to **5**. After work up, final distillation of the reaction mixture by a short column provided relatively pure compound **5**[16] (97 °C/15 mg). Following the procedure for the synthesis of 3-dialkylaminomethyl-alkane-2-ones and their methiodides,[12] compound **5** was reacted with formaldehyde and dimethylamine hydrochloride; thereafter, decarbonylation gave compound **6**[17] that then was reacted with iodomethane to provide compound **7**.[18] In accord with the literature,[12] compounds **2** and **7** (1:1 mol) were refluxed in EtOH overnight to generate a crude product that was then chromatographed and crystallized to provide pure racemic TBZ (**8**).[19] Under such a condition, only thermodynamically stable enantiomers (**8**) were formed, as shown in the ^{1}H NMR of compound **8**. No other diastereomers, such as epimers, were found to be present. Finally, adaptation of the methodology developed to successfully resolve racemic 2-oxo-3-ethyl-benzo[α]qinolizine[11] allowed the generation of (+)-TBZ (**12**) and (−)-TBZ (**13**), as detailed below.

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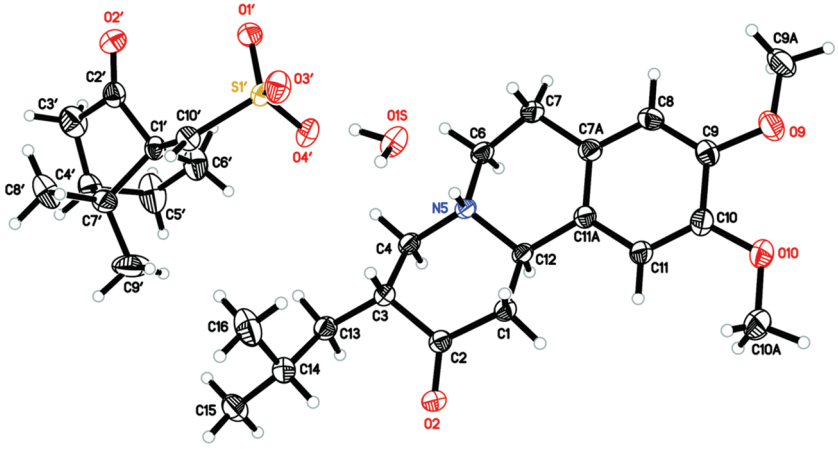

Figure 1. Structure and absolute configuration of (1*S*)-(+)-10-camphorsulfonate of (+)-TBZ (**10**) provided by X-ray crystallography: The absolute configuration was determined unambiguously from the anomalous data [Flack = −0.02(7)]. The absolute configuration as reported by PLATON is C3 = R, N5 = S, C12 = R, C1′ = S (from acid), and C4′ = R (also from acid). For additional details, see the Supporting Information.

A solution of TBZ (317.4 mg, 1 mmol) and (1*S*)-(+)-10-camphorsulfonic acid (232.3 mg, 1 mmol) in ethyl acetate (10 mL), together with a small magnetic stirring bar, was placed in a sealed tube. This was heated while stirring in an oil bath at 80 °C for 28 h, and the resulting solution was set aside at room temperature overnight. Filtration provided crystalline (1*S*)-(+)-10-camphorsulfonate (400 mg, 0.7 mmol, 70%), $[\alpha]_D^{23}$ +20.0 (*c* 0.35, EtOH), which was then dissolved in acetone (10 mL) and kept at room temperature for 2 days. Filtration gave the crystalline (1*S*)-(+)-10-camphorsulfonate of (+)-TBZ (**10**) (200 mg, 0.35 mmol, 35%): prism, mp 132−133 °C, $[\alpha]_D^{23}$ +36.00 (*c* 0.36, EtOH). Anal. ($C_{29}H_{43}NO_7S.H_2O$) C, H, N. These transparent prism crystals were recrystallized two times from acetone, and the [α] value was determined to be constant. X-ray crystallography analysis of the optically pure compound gave an unambiguous absolute configuration of **10**, which is shown in Figure 1. The salt, **10**, was transferred to base, **12**, by a routine procedure: dissolved in water, basified by aqueous Na_2CO_3, extracted by ethyl ether, and dried over Na_2SO_4. The resulting ether solution was evaporated to remove ether and provide the crystalline base of (+)-TBZ (**12**): prism, mp 108−110 °C (mp 126 °C, lit.[10]), $[\alpha]_D^{23}$ +71.02 (*c* 0.5, EtOH), $[\alpha]_D^{23}$ +67.60 (*c* 0.4, CH_2Cl_2) {$[\alpha]_D^{23}$ +37.2 (*c* 0.4, CH_2Cl_2), lit.[10]}. ^{1}H NMR and MS (CI) are identical with that of compound **8**. Anal. ($C_{19}H_{27}NO_3$) C, H, N.

Using (1*R*)-(−)-10-camphorsulfonic acid, instead of (1*S*)-(+)-10-camphorsulfonic acid, the same procedures gave the (1*R*)-(−)-10-camphorsulfonate of (−)-TBZ (**11**): prism, mp 132−133 °C, $[\alpha]_D^{23}$ −36.01 (*c* 0.35, EtOH). Anal. ($C_{29}H_{43}NO_7S.H_2O$) C, H, N. The base of (−)-TBZ (**13**): prism, mp 108−110 °C, $[\alpha]_D^{23}$ −71.12 (*c* 0.5, EtOH), $[\alpha]_D^{23}$ −67.75 (*c* 0.3, CH_2Cl_2). ^{1}H NMR and MS (CI) are identical with that of compound **8**. Anal. ($C_{19}H_{27}NO_3$) C, H, N.

Assessment of VMAT2 binding affinity was undertaken by quantifying displacement of [^{3}H]dihydrotetrabenazine ([^{3}H]DHTBZ) from rat striatum by compounds (Supporting Information) and is shown as a dissociation constant (K_i) value in Table 1. Whereas both enantiomeric forms of TBZ possessed high affinities for VMAT2 that were in the pharmacologically relevant nanomolar range, the (+)-form (**10**) proved to be 3-fold more potent than the (−)-enantiomer (**11**) ($p < 0.05$, Bonferonni *t* test). As expected, the K_i value of the racemic form, **8**, was midway between those of the pure enantiomers and was in accord with the literature.[5,6]

Table 1. VMAT2 Binding Affinity of TBZ Enantiomeric Forms

	compound	K_i value ± SEM (nM)[a]
11	(1*R*)-(−)-10-camphorsulfonate of (−)-TBZ	11.20 ± 1.03
10	(1*S*)-(+)-10-camphorsulfonate of (+)-TBZ	4.61 ± 0.31
8	(±)-TBZ	8.07 ± 0.20

[a] Mean ± standard error of the mean ($N = 3$); each is statistically different from the others ($p < 0.05$, Bonferonni *t* test).

The efficacy of TBZ in a variety of hyperkinetic movement disorders demonstrates that the pharmacological regulation of VMAT2 function in humans can have clinical benefits and can be well-tolerated.[3,4,7−9] Additionally, studies of VMAT2 heterozygote knockout mice in stimulant place conditioning (e.g., reward) are attenuated, suggesting a role of VMAT2 in mediating the behavioral effects of abused psychostimulant drugs[20−23] and furthering interest in VMAT2 as a drug target.

In vivo, TBZ is rapidly and extensively hepatically metabolized to its reduced form, DHTBZ, in both humans and rodents.[24,25] DHTBZ, like TBZ, has a high affinity for VMAT2,[5,6,22] and its concentrations over time following TBZ administration to humans have been reported to be 148-fold greater than that of TBZ.[24] As the affinity of DHTBZ is similar to that of (±)-TBZ (**8**), the actions of TBZ are likely mediated primarily by its metabolite(s). Consequent to the chiral centers within clinically available (±)-TBZ (**8**), at C-3 and C-11b, several stereoisomers can be generated in vivo, each of which may then give rise to further metabolites.[24,25] Synthesis, separation, and characterization of several of these have determined that for α-DHTBZ, VMAT2 affinity is highly enantioselective, with the (+)-isomer possessing high affinity (0.97 ± 0.48 nM)[26,27] and the (−)-isomer low affinity

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(2.2 ± 0.3 μM).[26,27] The VMAT2 affinity of (+)-α-DHTBZ is both in the range of (+)-TBZ, reported herein, and shows similar chiral preference with a far greater (2000-fold) selectivity. The use of enantiomerically pure (+)-TBZ in the clinic may reduce the reported high individual variability in the pharmacokinetics and metabolism associated with (±)-TBZ in humans[24,25] and optimize its benefits in hyperkinetic movement disorders as well as other conditions in which inhibition of VMAT2 is efficacious.

SUPPORTING INFORMATION AVAILABLE

Elemental analysis, X-ray crystallography of **10**, and VMAT2 binding. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) Brossi, A.; Lindlar, H.; Walter, M.; Schnider, O. Syntheseversuche in der Emetin Reihe 1. Mitteilung 2-Oxo-hydrobenzo-[a]chinolizine. *Helv. Chim. Acta* 1958, *41*, 119−139; U.S. 2830993 (1958 to Hoffmann-La Roche).

(2) 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.

(3) Kenney, C.; Jankovic, J. Tetrabenazine in the treatment of hyperkinetic movement disorders. *Expert Rev. Neurother.* **2006,** *6*, 7–17.

(4) Fasano, A.; Bentivoglio, A. R. Tetrabenazine. *Expert Opin. Pharmacother.* **2009,** *10* (17), 2883–2896.

(5) Scherman, D.; Jaudon, P.; Henry, J. Characterization of the monoamine carrier of chromaffin granule membrane by binding of [2−3H]dihydrotetrabenazine. *Proc. Natl. Acad. Sci. U.S.A.* **1983,** *80*, 584–588.

(6) Thiriot, D. S.; Ruoho, E. A. Mutagenesis and Derivatization of Human Vesicle Monoamine Transporter 2 (VMAT2) Cysteines Identifies Transporter Domains Involved in Tetrabenazine Binding and Substrate Transport. *J. Biol. Chem.* **2001,** *276*, 27304–27315.

(7) Mestre, T.; Ferreira, J.; Coelho, M. M.; Rosa, M.; Sampaio, C. Therapeutic interventions for symptomatic treatment in Huntington's disease. *Cochrane Database Syst. Rev.* 2009, *3*, CD006456.

(8) Kenney, C.; Hunter, C.; Jankovic, J. Long-term tolerability of tetrabenazine in the treatment of hyperkinetic movement disorders. *Mov. Disord.* **2007,** *22*, 193–197.

(9) Fasano, A.; Cadeddu, F.; Guidubaldi, A.; Piano, C.; Soleti, F.; Zinzi, P.; Bentivoglio, A. R. The long-term effect of tetrabenazine in the management of Huntington disease. *Clin. Neuropharmacol.* **2008,** *31*, 313–318.

(10) Rishel, M. J.; Amarasinghe, K. K. D.; Dinn, S. R.; Johnson, B. F. Asymmetric synthesis of tetrabenazine and dihydrotetrabenazine. *J. Org. Chem.* **2009,** *74*, 4001–4004.

(11) Openshaw, H. T.; Whittaker, N. The synthesis of emetine and related compounds. Part V. A stereochemically favourable synthesis of emetine. *J. Chem. Soc.* **1963,** 1461–1471.

(12) Openshaw, H. T.; Whittaker, N. The synthesis of emetine and related compounds. Part IV. A new synthesis of 3-substituted 1,2,3,4,6,7-hexahydro-9,10-dimethoxy-2-oxo-11bH-benzo[a]-quinolizines. *J. Chem. Soc.* **1963,** 1449–1460.

(13) Murahashi, S.; Naota, T.; Taki, H. Ruthenium-catalysed oxidation of secondary amines to imines using *t*-butyl hydroperoxide. *J. Chem. Soc. Chem. Commun.* **1985,** 613.

(14) Fumarate of compound **2**: ^{1}H NMR (300 MHz, DMSO): δ = 2.55 (t, 2H, −CH_2−), 2.65 (t, 2H, −CH_2−N═) 3.82 (s, 3H, −OCH_3), 3.85 (s,3H, −OCH_3), 6.60 (s, 1H, −CH═N), 6.85 (s, 1H, Ar−H), 7.05 (s, 1H, Ar−H), 8.20 (s, 1H, NH^+). Base of compound **2**: MS (CI) *m/z* 192 $(M + 1)^+$.

(15) *Organic Syntheses*; Wiley & Sons: New York, 1941; Collect. Vol. I, p 248.

(16) Compound **5**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.90 (d, 6H, 2-CH_3), 1.30 (t, 3H, −CH_3), 1.50−1.80 (m, 3H, −CH_2−CH<), 2.20 (s, 3H, −CO−CH_3), 3.45 (t, 1H, −CO−CH−CO−), 4.16 (q, 2H, −OCH_2−). MS (CI) *m/z* 187 $(M + 1)^+$.

(17) Compound **6**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.89 (d, 3H, −CH_3), 0.92 (d, 3H, −CH_3), 1.12−1.55 (m, 3H, −CH_2−CH<), 2.13 (s, 3H, −CO−CH_3), 2.16 [s, 6H, −N$(CH_3)_2$], 2.12−2.75 (m, 3H, −CO−CH−CH_2−N<). MS (CI) *m/z* 172 $(M + 1)^+$.

(18) Compound **7**: mp 170−171 °C. ^{1}H NMR (300 MHz, DMSO): δ = 0.92 (d, 3H, −CH_3), 0.98 (d, 3H, −CH_3), 1.10−1.70 (m, 3H, −CH_2−CH<), 2.28 (s, 3H, −CO−CH_3), 3.02 [s, 9H, −$N^+(CH_3)_3I^-$], 2.12−2.75 (m, 3H, −CO−CH−CH_2−N).

(19) Compound **8**: mp 128 °C. ^{1}H NMR (300 MHz, DMSO): δ = 0.90 (d, *J* = 5.0 Hz, 3H, −CH_3), 0.92 (d, *J* = 5.0 Hz, 3H, −CH_3), 1.05 (m, 1H, −CH<), 1.65 (m, 1H, −CH−), 1.80 (m, 1H, −CH−), 2.35 (dd, *J* = 12.0, 12.0 Hz, 1H, −CH−), 2.54 (m, 1H, −CH−), 2.58 (m, 1H, −CH−), 2.71 (m, 1H, −CH−), 2.74 (m, 1H, −CH−), 2.90 (dd, *J* = 15.0, 3.0 Hz, 1H, −CH−), 3.09 (m, 1H, −CH−), 3.14 (m, 1H, −CH−), 3.28 (dd, *J* = 12.0, 6.0 Hz, 1H, −CH−), 3.50 (d, *J* = 12.0 Hz, 1H, −CH−), 3.82 (s, 3H, −CO−CH_3), 3.88 (s, 3H, −CO−CH_3), 6.54 (s, 1H, Ar−H), 6.61 (s, 1H, Ar−H). MS (CI) *m/z* 318 $(M + 1)^+$.

(20) Takahashi, N.; Miner, L. L.; Sora, I.; Ujike, H.; Revay, R. S.; Kostic, V.; Jackson-Lewis, V.; Prezedborski, S.; Uhl, G. R. VMAT2 knockout mice: Heterozygotes display reduced amphetamine conditioned reward, enhanced amphetamine locomotion and enhanced MPTP toxicity. *Proc. Natl. Acad. Sci. U.S.A.* **1997,** *94*, 9938–9943.

(21) Riddle, E. L.; Fleckenstein, A. E.; Hanson, G. R. Role of monoamine transporters in mediating psychostimulant effects. *AAPS J.* **2005,** *7*, E847–E851.

(22) Zheng, G.; Dwoskin, L. P.; Crooks, P. A. Vesicular monoamine transporter 2: Role as a novel target for drug development. *AAPS J.* **2006,** *8*, E682–E692.

(23) Nickell, J. R.; Krishnamurthy, S.; Norrholm, S.; Deaciuc, G.; Siripurapu, K. B.; Zheng, G.; Crooks, P. A.; Dwoskin, L. P. Lobelane Inhibits Methamphetamine-Evoked Dopamine Release via Inhibition of the Vesicular Monoamine Transporter-2. *J. Pharmacol. Exp. Ther.* **2010,** *332*, 612–621.

(24) Mehvar, R.; Jamali, F.; Watson, M. W.; Skelton, D. Pharmacokinetics of tetrabenazine and its major metabolite in man and rat. Bioavailability and dose dependency studies. *Drug Metab. Dispos.* **1987,** *15*, 250–255.

(25) Roberts, M. S.; McLean, S.; Millingen, K. S.; Galloway, H. M. The Pharmacokinetics of tetrabenazine and its hydroxy metabolite in patients treated for involuntary movement disorders. *Eur. J. Clin. Pharmacol.* **1986,** *29*, 703–708.

DOI: 10.1021/ml1000189 | *ACS Med. Chem. Lett.* **2010,** *1*, 105–109

(26) Kilbourn, M. R.; Lee, L.; Vander Borght, T.; Jewett, D.; Frey, K. Binding of alpha-dihydrotetrabenazine to the vesicular monoamine transporter is stereospecific. *Eur. J. Pharmacol.* **1995,** *278*, 249–252.
(27) Kilbourn, M. R.; Lee, L.; Jewett, D. M.; Vander Borght, T.; Koeppe, R. A.; Frey, K. A. In vitro and in vivo binding of α-dihydrotetrabenazine to the vesicular monoamine transporter is stereospecific. *J. Cereb. Blood Flow Metab.* **1995,** *15*, S650.

DOI: 10.1021/ml1000189 | *ACS Med. Chem. Lett.* **2010,** *1,* 105–109