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Synthesis and biological evaluation of 3-alkyl-dihydrotetrabenazine derivatives as vesicular monoamine transporter-2 (VMAT2) ligands

Pinguan Zheng [a], Brian P. Lieberman [a], Seok Rye Choi [b], Karl Plöessl [a], Hank F. Kung [a,c,*]

[a] Department of Radiology, University of Pennsylvania, Philadelphia, PA 19104, USA
[b] Avid Radiopharmaceuticals, Philadelphia, PA 19104, USA
[c] Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104, USA

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ABSTRACT

In the search of new probes for in vivo brain imaging of vesicular monoamine transporter type 2 (VMAT2), we have developed an efficient synthesis of a novel series of 3-alkyl-dihydrotetrabenazine (DTBZ) derivatives. The affinity of VMAT2 was evaluated by an in vitro inhibitory binding assay using [^{125}I]-iodovinyl-TBZ or [^{18}F](+)-FP-DTBZ as radioligands in rat striatal tissue homogenates. New DTBZ derivatives exhibited moderate to good binding affinity to VMAT2. Among these new ligands, compound **4b** showed the best affinity for VMAT2 (K_i = 5.98 nM) and may be a useful lead compound for future structure–activity studies.

Vesicular monoamine transporter (VMAT) is responsible for the movement of monoamine neurotransmitters from the synaptic cleft into the presynaptic vesicules.[1] Two subtypes of VMAT (type 1 and type 2) have been cloned.[2–4] Interestingly, the central nervous system (CNS) of rodents and human predominantly expresses VMAT2. In the brain, VMAT2 is relatively nonspecific and transports dopamine, serotonin and norepinephrine. Therefore, imaging VMAT2 provides a measurement reflecting the total number of all three monoamine neurons in the brain, which is important in the understanding of neurological and psychiatric disorders such as Parkinson's disease.

Tetrabenazine (TBZ, xenazine, **1**), first introduced in 1956 by Brossi et al.,[5] was approved by FDA to treat hyperkinetic movement disorders.[6] Both TBZ and its metabolites dihydrotetrabenazine (DTBZ, **2**) exhibit high inhibitory activity of VMAT2. Compared with its parent compound TBZ, DTBZ exhibits higher biological stability and less sensitivity to drugs affecting dopamine levels in the brain. DTBZ has been successfully labeled with radionuclide ^{11}C and applied in the diagnosis of Parkinson's disease.[7–9] In vivo studies showed that ^{11}C-DTBZ is an excellent positron emission tomography (PET) tracer for measuring VMAT2 sites in the brain.

However, the nature of short half-life of ^{11}C radionuclide ($t_{1/2}$: 20 min) impeded the large-scale application of this tracer in the clinical studies. We have therefore started a program to look for new VMAT2 ligands, which is suitable for radiolabeling with a longer half-life radionuclide such as ^{18}F ($t_{1/2}$: 110 min). The new F-18 labeled VMAT2 radiotracer would have broad clinical application given the established national supply network of [^{18}F]-FDG. Several series of DTBZ derivatives have been developed in our laboratory and tested in vitro.[10–12] Of these compounds, the optically active (+)-9-FP-DTBZ (AV-133)(**3**) exhibits the highest binding affinity of VMAT2 (K_i: 0.10 ± 0.01 nM). Further in vivo studies showed that [^{18}F]-9-FP-DTBZ is an excellent brain imaging agent with a striatum (target) to cerebellum (background) ratio of 4.51 at 30 min postinjection.[10c] As a continuing interest in this area, we herein reported the modular synthesis and biological evaluation of a new series of 3-alkyl-DTBZ as potential PET imaging radiotracers (Fig. 1, compound **4**, **10**). In addition to be useful as an imaging agent for mapping the VMAT2 receptors in the brain, (+)-9-FP-DTBZ (AV-133)(**3**) also demonstrated potential for imaging VMAT2 on the beta cells in the pancreas.[10e–g] By substitution of the isobutyl group with cycloalkyl (four-, five-, six-membered) methyl group, we aimed to further explore the lipophilic binding site of VMAT2 and we expect to modulate the in vivo kinetics of VMAT2 in favor of binding to the VMAT2 binding sites of beta cells in the pancreas.

A modified synthesis[13] of **4a** and **4b** is shown in Scheme 1. Alkylation of 2,4-pentadione afforded **6a** and **6b** in good yields.[14] According to Amri's protocol,[15] allylated methyl vinyl ketone **7a** and **7b** were obtained in multi-gram scale via addition of formaldehyde followed by in situ decarboxylation. Heating the ethanolic mixture of methyl vinyl ketone and dihydroisoquinoline at 80 °C

* Corresponding author. Tel.: +1 215 6623096; fax: +1 215 3495035.
E-mail address: kunghf@sunmac.spect.upenn.edu (H.F. Kung).

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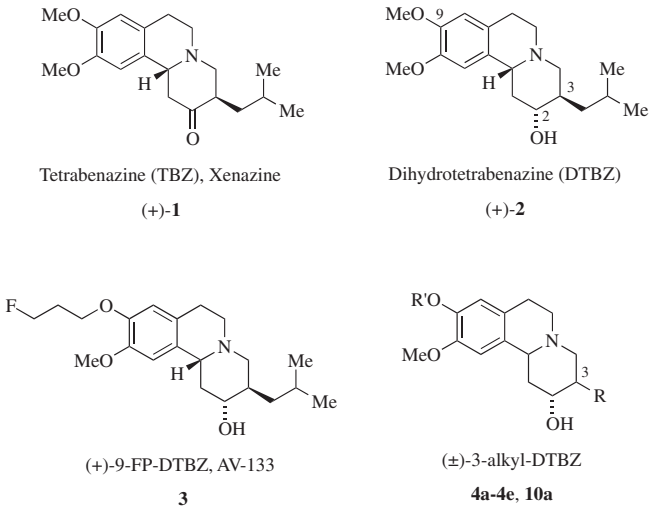


Figure 1. Structure of TBZ (**1**), DTBZ (**2**), FP-DTBZ (**3**) and 3-alkyl-DTBZ (**4a–4e**, **10a**).

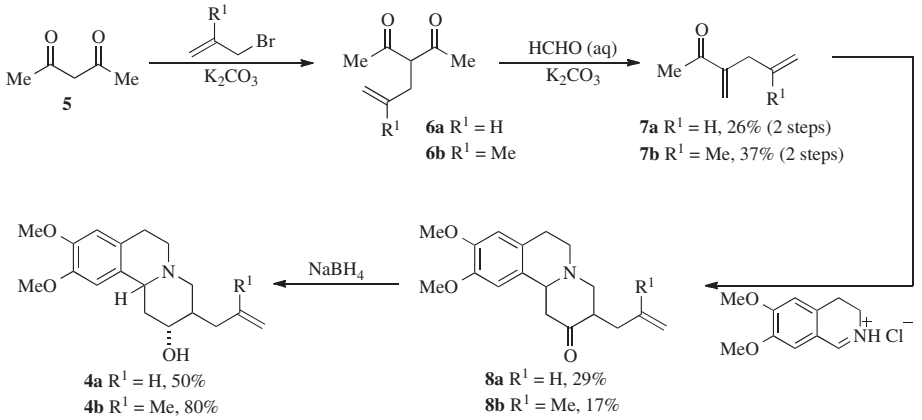
for 2 days provided the desired TBZ derivatives in moderate yields. TBZs were further converted to DTBZs (**4a** and **4b**).[16,17]

Desmethylation[18] at C-9 position of **4a** was complicated with base-induced isomerization of terminal olefin. Instead, **9a** was obtained in 47% yield with major product of *E* configuration as indicated by coupling constant (J = 15.2 Hz). Alkylation delivered the FP-DTBZ derivative **10a** (Scheme 2).[16,19]

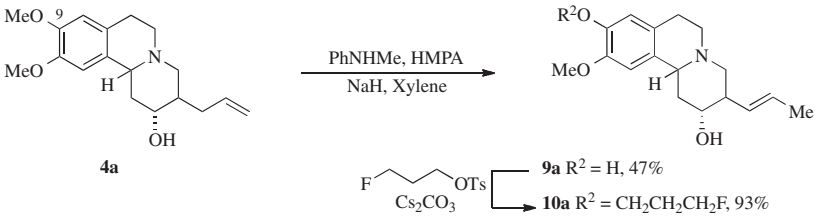
Attempts on the synthesis of cycloalkyl methyl DTBZ derivatives (**4c**, **4d** and **4e**) with the method as depicted in Scheme 1 turned out to be unsuccessful. Alkylation of 2,4-pentadione **5** with different cycloalkyl methyl halides failed to provide any desired products.[20] Therefore, an alternative synthetic route was designed and executed (Scheme 3).[16,21] Tetrahydroisoquinoline **14** was synthesized according to Battersby's protocol.[22] Commercially available 3,4-dimethoxy-phenethylamine **11** was converted to amide **12**. Compound **12** underwent P_2O_5-mediated Bischler–Napieralski reaction to deliver dihydroisoquinoline **13** in 73% yield. Hydrogenation in the presence of Adams' catalyst provided the tetrahydroisoquinoline **14**. Diester **15** was obtained via Michael addition to ethyl acrylate.[23] Intramolecular Dieckmann condensation occurred smoothly to deliver the key intermediate β-keto ester **16**.[5] Alkylation of resulting β-keto ester with various cycloalkyl methyl halides successfully afforded **17** in 15–71% yield (unoptimized). Finally, decarboxylation[24] followed by reduction delivered the desired 3-alkyl-DTBZ (**4c–4e**). The relative stereochemistry of **4c** was established by X-ray crystallography (Fig. 2).[25] As reported by Kilbourn,[18b] product **4c** is in the thermodynamically stable chair conformation. Both hydroxy group at C-2 position and cyclobutyl methyl group at C-3 position are in the equatorial position, whereas C-11b hydrogen is in the axial positions. All DTBZ ligands used for in vitro studies are racemic, which contain the active isomer (2*R*,3*R*,11b*R*) and inactive isomer (2*S*,3*S*,11b*S*).[10c]

In vitro studies were carried out by using [^{125}I]-iodovinyl-TBZ or [^{18}F](+)-FP-DTBZ as radioligands in rat striatal tissue homogenates and the results are summarized in Table 1.

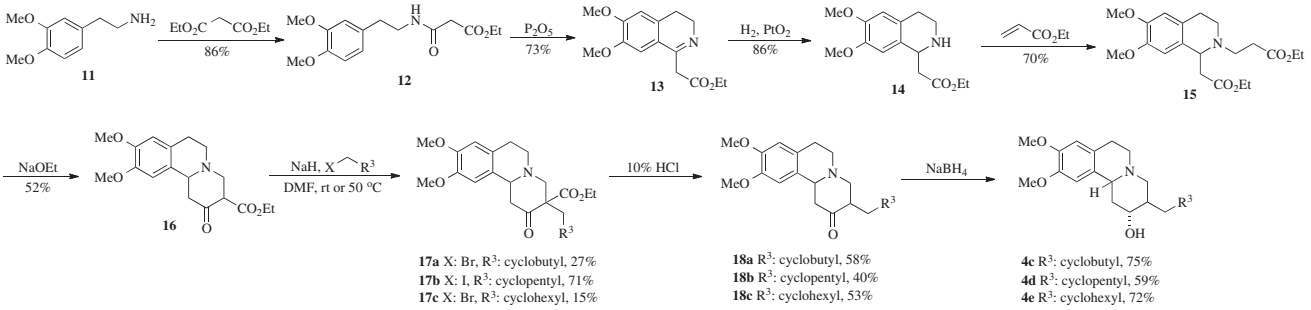
Our results further reinforced the vital role of isobutyl group at C-3 position in the VMAT2 binding, which is consistent with previous structure–activity relationship studies.[26] When replacing isobutyl chain with smaller allyl group, **4a** displayed a loss of 245-fold binding affinity to VMAT2 (Table 1, entries 1 and 3). An increase of the size of substituent at C-3 position restored the amine-depleting activity to the level of DTBZ (entries 1 and 4). Surprisingly, prop-1-en-1-yl FP-DTBZ **10a** showed moderate binding affinity of 22.4 nM, which is 11-fold more potent than **4a**. Considering the comparable size of allyl and prop-1-en-1-yl side chain, the difference in binding affinity could be attributed to the presence of 9-fluoropropoxy on the phenyl ring, which might have the secondary interaction with VMAT2.[10a] A further study on this secondary effect is currently ongoing in our laboratory.



Scheme 1. Synthesis of 3-alkyl-DTBZ derivative (**4a**, **4b**).



Scheme 2. Synthesis of FP-DTBZ derivative (**10a**).



Scheme 3. The synthesis of 3-alkyl-DTBZ derivatives (**4c**–**4e**).

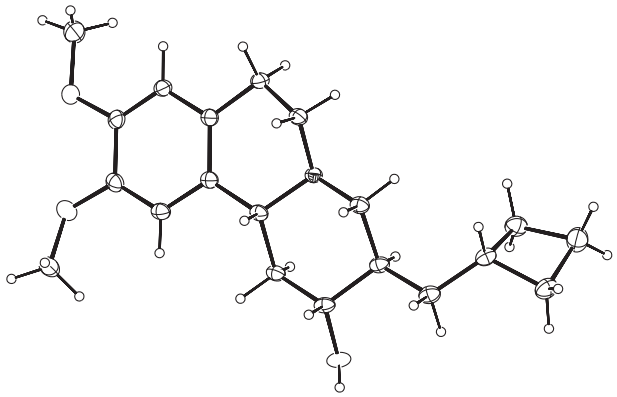


Figure 2. X-ray structure of 3-cyclobutyl methyl DTBZ (**4c**).

Table 1
Inhibition constants of new ligands on [^{125}I]-iodovinyl-TBZ or [^{18}F](+)-FP-DTBZ binding to VMAT2 in rat striatal homogenates

Entry	Compound	K_i[a] (nM)
1	(+)-DTBZ, **2**	1.03 ± 0.11
2	(+)-FP-DTBZ, **3**	0.10 ± 0.01[10c]
3	**4a**	253 ± 6.0[b]
4	**4b**	5.98 ± 0.23
5	**4c**	26.9 ± 3.43
6	**4d**	227 ± 19.0
7	**4e**	137 ± 35.0
8	**10a**	22.4 ± 1.25[b]

[a] K_d value of 8.2 nM for [^{125}I]-iodovinyl-TBZ was used for the calculation based on the reported K_i value (see Ref. 12b). The results are the mean ± S.E.M. of three independent measurements done in duplicates. [^{125}I]-iodovinyl-TBZ was used as radioligand, unless otherwise stated.

[b] [^{18}F](+)-FP-DTBZ was used.

When the isobutyl side chain was replaced with cycloalkyl methyl group, the binding affinity generally decreased (Table 1, entries 5–7). Cyclobutyl substrate **4c** showed better affinity than cyclopentyl **4d** and cyclohexyl **4e**. Interestingly, cyclohexyl **4e** exhibited slightly better affinity than cyclopentyl derivative **4d**.

In summary, we have synthesized a new series of 3-alkyl DTBZ derivatives. These new ligands were tested in vitro for their potency to inhibit the binding of [^{125}I]-iodovinyl-TBZ or [^{18}F](+)-FP-DTBZ in rat striatal membrane homogenates. These compounds exhibited moderate to good binding affinity to VMAT2 binding sites in the brain. We have demonstrated that the hydrophobic binding pocket of vesicular monoamine transporter 2 (VMAT2) is highly sensitive to steric size of the side chain at the C-3 position. Further structure–activity relationship studies of DTBZ derivatives are currently being pursued in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data (Experimental procedures, characterization data, as well as biological studies. This material is available free of charge via the Internet) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.113.

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16. See the Supplementary data for the details.
17. *Typical procedure for the synthesis of* **8b**: A solution of dihydroisoquinoline hydrochloride salt (9.3 g, 40.9 mmol) and methyl vinyl ketone **7b** (6.1 g, 49.1 mmol) in absolute EtOH (41 mL) was stirred at 90 °C for 48 h. The solvent

and the excess of reagent were removed and the residue was chromatographed (SiO_2, 20% EtOAc/Hexanes to 50% EtOAc/Hexanes). Compound **8b** was obtained as pale yellow solid (2.2 g, 17% yield).
Typical procedure for the synthesis of **4b**: To a solution of **8b** (2.2 g, 6.97 mmol) in 70 mL absolute EtOH at 0 °C, was portionwise added $NaBH_4$ (791 mg, 20.9 mmol). After 2 h, the reaction mixture was concentrated in vacuo and taken up in DCM. Wash with conc. K_2CO_3 solution and dry over Na_2SO_4. The solvent was removed in vacuo. Crude product was triturated with acetone to provide 0.98 g of **4b** as single diastereomer and 1.0 g of **4b** as diastereomeric mixture (*d.r.*: 4:1), 90% combined yield.

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19. *Typical procedure for the synthesis of* **9a**: N-methylaniline (789 mg, 7.4 mmol) was added dropwise to a stirred suspension of NaH (444 mg, 11.1 mmol) and HMPA (1.28 mL, 7.4 mmol) in dry xylene (2.0 mL) at 65 °C. 15 min later, a suspension of **4a** in 1.0 mL xylene was added dropwise. The stirring was continued for 48 h at 65 °C. After the suspension was cooled to rt, it was quenched by 5% HCl (5.0 mL) and extracted with Et_2O (5 times) to remove the unreacted **4a** and HMPA. The aqueous layer was neutralized with conc. HCl solution and **9a** was separated as brown oil. Triturate with acetone to provide **9a** was obtained as brown solid (511 mg, 41% yield).
Typical procedure for the synthesis of **10a**: A solution of **9a** (100 mg, 0.88 mmol), tosylate (266 mg, 1.14 mmol) and Cs_2CO_3 (371 mg, 1.14 mmol) in 3.5 mL acetone was refluxed for 16 h. After the suspension was cooled to rt, it was filtered through a pad of celite and the filtrate was concentrated in vacuo. The crude product was purified by chromatography (SiO_2, 2% MeOH/DCM to 5% MeOH/DCM) to provide **10a** as foam (113 mg, 93% yield).
20. Several conditions were employed in the alkylation of 2,4-pentadione. (a) Ref. 14. (b) Shih, C. C. Y.; Shi, Q.; Wang, H.-K.; Su, C.-Y. PCT Int. Appl. WO 2008085984 A1, 2008; Scifinder Scholar AN 2008: 857452.
21. *Typical procedure for the synthesis of* **17a**: To the solution of **16** (250 mg, 0.75 mmol) in DMF (5.0 mL) at 0 °C, was added NaH (33 mg, 0.82 mmol). 15 min later, cyclobutyl methyl bromide (168 mg, 1.12 mmol) was added dropwise. The resulting mixture was heated at 50 °C for 4 h. Cool to rt and quench with H_2O. The reaction mixture was concentrated in vacuo and the residue was taken up in DCM. Wash with H_2O, dry over Na_2SO_4 and concentrate in vacuo. The crude product was chromatographed (SiO_2, 20% EtOAc/Hexanes to 30% EtOAc/Hex) to provide **17a** as yellow oil (81 mg, 27% yield).
Typical procedure for the synthesis of **18a**: **17a** was dissolved in 10% HCl (3.0 mL) and refluxed (115 °C) for 17 h. Cool to rt and neutralize with concd. K_2CO_3 solution. Extract with DCM, dry over Na_2SO_4 and concentrate in vacuo. Crude product was chromatographed (SiO_2, 20% EtOAc/Hexanes to 30% EtOAc/Hexanes) to provide **18a** as white solid (38.4 mg, 58% yield). Compound **18a** was converted to **4c** according to Ref. 17.
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25. CCDC 799803 contains the supplementary crystallographic data for this Letter. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
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