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Derivatization of (±) dihydrotetrabenazine for copper-64 labeling towards long-lived radiotracers for PET imaging of the vesicular monoamine transporter 2

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ABSTRACT

Dihydrotetrabenazine (DTBZ) derivatized from (+) Tetrabenazine (TBZ) has been used for imaging the expression of VMAT2 when labeled with ^{11}C ($t_{1/2}$ = 20.3 min) or ^{18}F ($t_{1/2}$ = 110 min) in neurodegenerative diseases or pancreatic beta-cell. Because ^{11}C or ^{18}F radiolabels are only available in the proximity of a biomedical cyclotron facility, here we report our work of derivatizing (+) and (−) DTBZ using a ^{64}Cu-specific bifunctional chelator scaffold (^{64}Cu: $t_{1/2}$ = 12.7 h) for the preparation of long-lived VMAT2 targeted radiotracers, ^{64}Cu-CB-TE2A-(+)-DTBZ and ^{64}Cu-CB-TE2A-(−)-DTBZ. The specific VMAT2 binding affinity of ^{64}Cu-CB-TE2A-(+)-DTBZ measured using rat brain homogenate or porcine islets was not compromised by our chemical modifications while that of its (−) counterpart remained low as in ^{11}C or ^{18}F labeled (±) DTBZ.

Vesicular monoamine transporter (VMAT2) is a membrane protein responsible for transporting monoamines (dopamine, serotonin, norepinephrine, epinephrine and histamine) from within the neuron into the storage granules (vesicles). It has been linked to several of neurological and psychiatric disorders such as Parkinson's disease. The level of VMAT2 expression provides an understanding of neurological and psychiatric diseases. To date, positron emission tomography (PET) imaging of VMAT2 density in the basal ganglia area of the brain using [^{11}C] (+)-dihydrotetrabenazine ([^{11}C] (+)-DTBZ) has been successfully applied to the clinical diagnosis of Parkinson's disease and neurodegenerative diseases.[1,2]

Recently it has been demonstrated that VMAT2 binding sites are expressed predominantly on the beta cells in the islets of Langerhans.[3,4] Therefore, VMAT2 based ligands have been used for imaging beta cell mass (BCM). Of them, [^{11}C] (+)-DTBZ has been used for PET imaging of VMAT2 binding sites in the pancreas of rodents, primates, and humans.[4,5]

Currently, ^{11}C and ^{18}F are the only PET nuclides reported for the development of DTBZ targeted imaging probes (Fig. 1). However, the short half-lives of these two radioisotopes (^{11}C $t_{1/2}$ = 20.3 m, ^{18}F $t_{1/2}$ = 110 m) limit their application as the chemical procedures to incorporate these isotopes must be carried out in the proximity of a biomedical cyclotron. Among nonstandard PET nuclides, ^{64}Cu ($t_{1/2}$ = 12.7 h; β^+ 0.653 MeV, 17.4%) has drawn considerable interest in PET research owing to its short positron range, commercial availability, and reasonably long decay half-life. In this work, we derivatized DTBZ for radiolabeling with ^{64}Cu in order to construct a relatively long-lived PET imaging probe for noninvasive assessment of VMAT2 expression, preferably in the pancreatic beta cell. This was achieved by conjugating VMAT2 targeting (+)-DTBZ to a bifunctional chelator scaffold[6] derived from 2,2′-(1,4,8,11-tetraazabicyclo[6.6.2]hexadecane-4,11-diyl)diacetic acid (CB-TE2A), an ideal Cu(II) chelator for PET imaging. In vitro assays of the resulted ^{64}Cu radiotracers were performed on rat brain homogenates and porcelain islets.

The synthesis of CB-TE2A-DTBZ conjugate was accomplished in two steps (Supplementary information). The first encompassed the synthesis and resolution of DTBZ while the second dealt with the synthesis of CB-TE2A conjugate and its subsequent deprotection. There are two known primary methods for the synthesis of DTBZ: the cyclization of tetrahydro-isoquinoline derivatives[7] or the condensation of a 3,4-dihydro-isoquinoline derivative with β-amino ketone.[8] We followed the later method and synthesized DTBZ by condensation of 6,7-dimethoxy-3,4-dihydro-quinoline[9] and β-amino ketone[8] according to the published literature.[10] Resolution of (±) DTBZ was carried out based on a published procedure.[10,11] The separation was based on the existence of an interconversion between benzo[α]quinolizine and isoquinolinium upon exposure to an acid. Amino functional group was then

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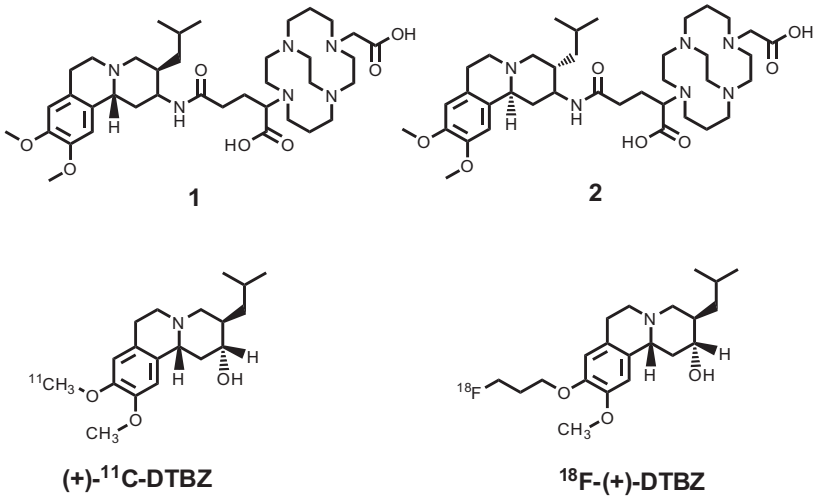
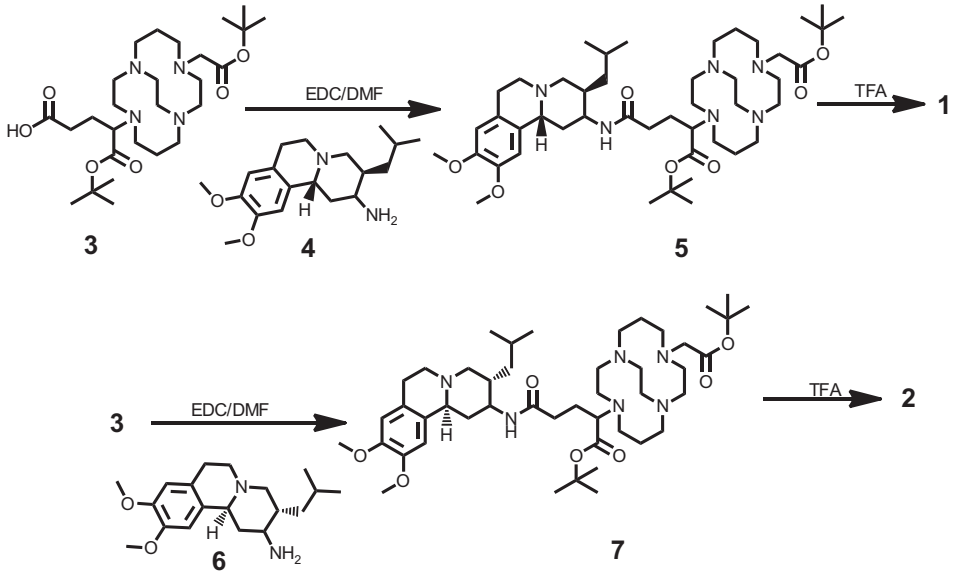


Figure 1. Structures of CB-TE2A conjugate of (+) DTBZ (**1**) and (−) DTBZ (**2**). Also depicted are the structures of ^{11}C and ^{18}F labeled (+) DTBZ.

introduced on the DTBZ molecule by following a published procedure.[12] The CB-TE2A-DTBZ conjugate was then synthesized by acid-amine conjugation via carbodiimide chemistry. The CB-TE2A scaffold containing γ-carboxylic acid was reacted with amine terminated DTBZ derivatives (**4** and **6**) in DMF as solvent in the presence of ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as coupling agent and triethylamine as proton scavenger to give protected DTBZ conjugates **5** and **7** (Scheme 1). Finally, the *t*-butyl carboxylate group was removed using trifluoroacetic acid and dichloromethane mixture (1:1) to provide **1** and **2**, each containing two free carboxylic acids to ensure the biological stability of the ^{64}Cu label.

The ^{64}Cu labeling was performed using ammonium acetate buffer (0.4 M) under mild acidic condition (pH = 6.5)[6] for all the conjugates. Reaction was carried out at 85 °C for 30 min. Both conjugates were radiolabeled with ^{64}Cu in >60% yields. The ^{64}Cu-labeled conjugates were purified in one-step using a preactivated C-18 Sep-Pak light cartridge. The radiochemical purity of the ^{64}Cu-labeled conjugates after cartridge purification was >99% as determined by radio-TLC and radio-HPLC. The specific activity of ^{64}Cu-CB-TE2A-(+)-DTBZ and ^{64}Cu-CB-TE2A-(−)-DTBZ was in the range 33–44 MBq/nmol. Both ^{64}Cu-labeled conjugates were eluted from HPLC about 1 min earlier than their respective cold counterparts.

The in vitro binding affinities of CB-TE2A-(+)-DTBZ and CB-TE2A-(−)-DTBZ were determined by a competitive binding assay using rat brain homogenates with ^{64}Cu-CB-TE2A-(+)-DTBZ as the VMAT2 radio-ligand. The rat brain homogenates were obtained from the striatum regions of rat brains. While both CB-TE2A-(+)-DTBZ and CB-TE2A-(−)-DTBZ inhibited the radioligand binding in a dose-dependent manner, the former had a much more pronounced effect than the latter (Fig. 2a). The calculated half maximal inhibitory concentration (IC_{50}) values of CB-TE2A-(+)-DTBZ and CB-TE2A-(−)-DTBZ expressed by the 50% inhibitory concentration to the binding of ^{64}Cu-CB-TE2A-(+)-DTBZ to the homogenates were 16.8 ± 6.9 nM and 253.2 ± 107.8 nM, respectively. In comparison, the reported IC_{50} values of (+)-DTBZ and (−)-DTBZ measured using the rat brain striatum were 0.97 ± 0.48 nM and 2.2 ± 0.3 μM, respectively.[13] The competitive binding assay result clearly demonstrates the anticipated higher VMAT2 binding affinity of



Scheme 1. Synthetic routes to amino derivative of CB-TE2A-DTBZ conjugate.

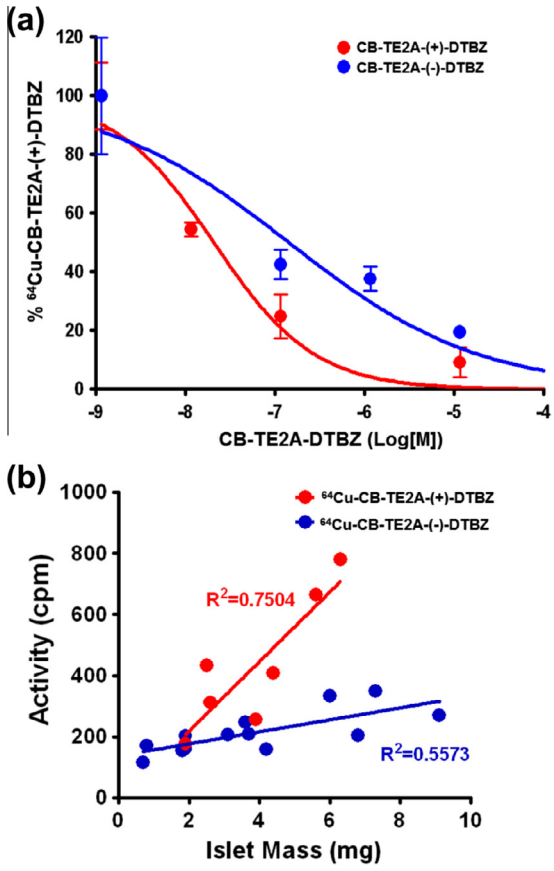


Figure 2. (a) Competitive binding assay using rat brain homogenates with ^{64}Cu-CB-TE2A-(+)-DTBZ as the VMAT2 radioligand. (b) Ex vivo binding assay of ^{64}Cu-CB-TE2A-(+)-DTBZ and ^{64}Cu-CB-TE2A-(−)-DTBZ using freshly isolated porcine islets.

CB-TE2A-(+)-DTBZ as compared to its (−) counterpart. In addition, it shows that the conjugation of (+) DTBZ to CB-TE2A does not substantially alter the binding affinity of the parent compound.

To test the feasibility of using ^{64}Cu-CB-TE2A-(+)-DTBZ for pancreatic beta cell imaging, we performed an ex vivo binding assay of ^{64}Cu-CB-TE2A-(+)-DTBZ and ^{64}Cu-CB-TE2A-(−)-DTBZ using freshly isolated porcine islets. For the assay, a fixed amount of radioactivity of ^{64}Cu-CB-TE2A-(+)-DTBZ and ^{64}Cu-CB-TE2A-(−)-DTBZ was added to varying numbers of islets. After incubation and washing, the activity trapped in the islets was measured. The results of the assay are shown in Figure 2b. The amount of radioactivity of ^{64}Cu-CB-TE2A-(+)-DTBZ bound to the islets was much higher than that of ^{64}Cu-CB-TE2A-(−)-DTBZ, indicating the preferential islet uptake of ^{64}Cu-CB-TE2A-(+)-DTBZ, a determinant for BCM imaging probe development.

Taken together, we successfully derivatized (±) DTBZ for ^{64}Cu labeling without significantly compromising their VMAT2 binding affinity. Between the two conjugates, CB-TE2A-(+)-DTBZ exhibited the desired preferential accumulation in freshly isolated porcine islets. This may potentiate its use as a VMAT2-targeted imaging agent for noninvasive assessment of pancreatic beta-cell imaging or other VMAT2 expressing non-brain organs.

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Supplementary data

Supplementary data (synthesis and evaluation) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.10.070.

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