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Communications to the Editor

A New Single-Photon Emission Computed Tomography Imaging Agent for Serotonin Transporters: [^{123}I]IDAM, 5-Iodo-2-((2-((dimethylamino)methyl)-phenyl)thio)benzyl Alcohol

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The serotonin reuptake or transporter sites (SERT) are located specifically on the terminals and cell bodies of the serotonergic neurotransmission system in the brain. They are the primary binding sites for selective serotonin reuptake inhibitors (SSRI)—common antidepressant drugs such as fluoxetine, sertraline, and paroxetine.[1,2] Imaging of SERT in humans would provide a useful tool to understand how alterations of this system are related to depressive illness and other psychiatric disorders. In addition, the saturation of SERT and its clinical consequences can be evaluated by a direct measurement of SERT binding in patients undergoing various drug treatments targeting these sites. In the past few years, development of specific tracers for in vivo PET (positron emission tomography) or SPECT (single-photon emission computed tomography) imaging of SERT has only met with a limited success. The most successful PET ligand for imaging SERT is [^{11}C](+)McN5652 (*trans*-1,2,3,5,6,10β-hexahydro-6-[4-(methylthio)phenyl]pyrrolo[2,1-α]isoquinolone),[3−5] which showed excellent inhibition of 5-HT reuptake in rat brain synaptosomes (K_i = 0.40 nM) and moderate selectivity toward other monoamine transporters (DAT (dopamine) and NET (norepinephrine) transporters: K_i = 23.5 and 1.82 nM, respectively). Since SPECT is more widely available than PET, there is a strong impetus to develop clinically useful SPECT ligands for imaging SERT. Several radioiodinated tracers designed for SPECT imaging have been tested unsuccessfully, including 4-iodotomoxetine reported previously from this laboratory.[6] Only [^{123}I]5-iodo-6-nitroquipazine[7−9] showed promising properties as an in vivo tracer for imaging SERT sites in monkey's brain. However, no human study of this agent using SPECT imaging has been reported. It has been widely suggested that [^{123}I]β-CIT (2β-carbomethoxy-3β-(4-iodophenyl)-tropane), a SPECT imaging agent that binds to both DAT and SERT, will be able to clarify pathological changes in both dopaminergic and serotonergic systems. However, differential kinetics of [^{123}I]β-CIT binding to DAT and SERT were observed.[10−12] The effect of a selective serotonin uptake inhibitor in human brain in vivo has been directly measured by [^{123}I]β-CIT/SPECT imaging of SERT sites in depressed patients undergoing treatment with citalopram.[13] A more selective series of compounds, nor-β-CIT (N-demethylated analogue of β-CIT)[14] and its related derivatives,[15] has recently been reported as improved SPECT imaging agents for SERT. It is suggested that [^{123}I]nor-β-CIT might be a suitable alternative tracer for visualization of SERT sites in the human brain with SPECT.[14,16] However, [^{123}I]nor-β-CIT binds to both DAT and SERT, and the selectivity is not sufficient to distinguish between these two monoamine transporter sites. There is a strong impetus to find more selective tracers for imaging SERT in the brain.

A chlorinated compound, 5-chloro-2-((2-((dimethylamino)methyl)phenyl)thio)benzyl alcohol (403U76), was reported as an inhibitor of serotonin uptake and norepinephrine uptake in rat brain synaptosomes (K_i = 2.1 and 55 nM, respectively).[17−20] To develop a new SERT selective imaging agent, a radioiodinated analogue, 5-iodo-2-((2-((dimethylamino)methyl)phenyl)thio)-benzyl alcohol (IDAM), has been synthesized in our laboratory. The synthesis of IDAM (**6**) and its bromo derivative **4** was achieved by a reaction sequence outlined in Scheme 1. The direct coupling of 2,5-dibromo-

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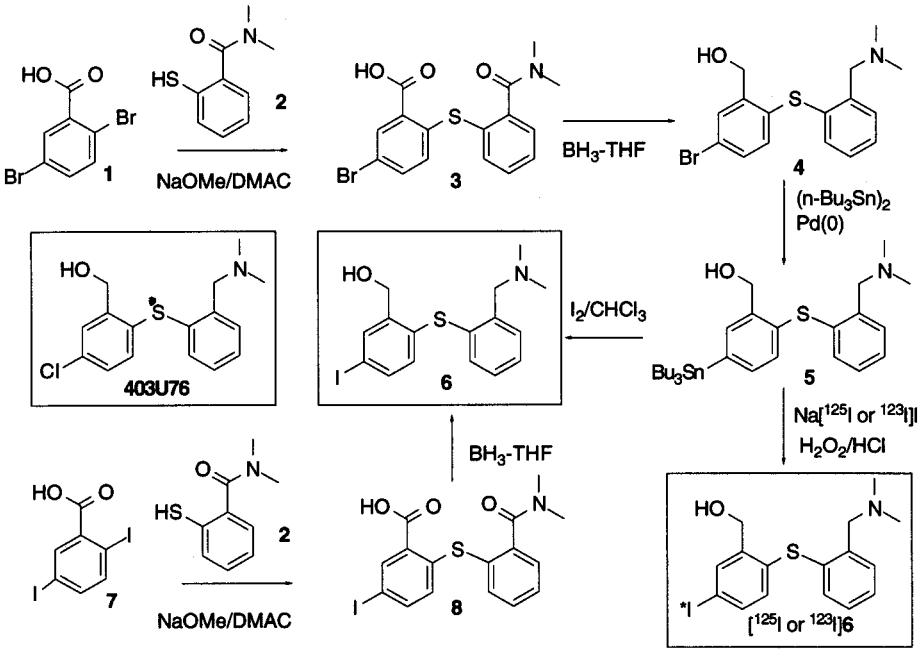
Scheme 1



benzoic acid **(1)** or 2,5-diiodobenzoic acid **(7)** with 2-thio-*N*,*N*-dimethylbenzamide **(2)** was carried out in *N*,*N*-dimethylacetamide (DMAC) with sodium methoxide to give the desired compounds in good yield (59% and 44% for **3** and **8**, respectively). Only when 2-thio-*N*,*N*-dimethylbenzamide **(2)** was freshly prepared was a good coupling yield achieved. This may due to the fact that the free thiol **2** was not stable upon prolonged standing at room temperature. Compound **3** was reduced successfully with BH_3−THF. The bromo compound **4** was converted to the corresponding tri-*n*-butyltin derivative **5** by a tetrakis(triphenylphosphine)palladium(0)-catalyzed reaction with good yield (66%). The tin derivative **5** was successfully converted to IDAM (**6**) with excellent yield (97%), or alternatively, 2-((4-iodo-2-carboxyphenyl)thio)-*N*,*N*-dimethylbenzamide (**8**) was reduced to IDAM (**6**) with 66% yield.[21] Radioiodination was carried out by an iododestannylation reaction; **5** was reacted with radioactive sodium iodide (I-125 or I-123) in the presence of hydrogen peroxide. The final no-carrier-added [^{125}I or ^{123}I]IDAM (**6**) was purified using an HPLC method (purity > 99%, t_R = 11.38 min, PRP-1 column eluted, 1 mL/min, with a 80:20 mixture of acetonitrile and 3,3-dimethylglutaric acid buffer, pH 7.4) as reported previously.[6]

In vitro binding studies were performed using membrane preparation from LLC-PK_1 cells individually expressing monoamine transporters (kindly provided by Dr. Rudnick, Yale University[22]). IDAM (**6**) displayed a high binding affinity to SERT (K_i = 0.097 nM). The binding affinity to the other two monoamine transporters is much lower (K_i = >10 000 and 234 nM for DAT and NET, respectively). The bromo derivative **4** displayed a lower binding affinity for SERT and lower SERT/NET selectivity (K_i = 0.52, >10 000, and 35 nM, for SERT, DAT, and NET, respectively, while (+)-McN5652 showed K_i = 0.01, 112, and 11.3 nM under the same conditions). It appears that IDAM (**6**) displayed a superior binding affinity for SERT and higher SERT/DAT and SERT/NET selectivity than **4** and (+)-McN5652. It is interesting to note that by changing the substitution group from the chloro of 403U76 to the iodo group as in IDAM (**6**) the selectivity for SERT binding is dramatically improved: K_i(NET)/K_i(SERT) > 1000. Initial biodistribution study in rats (iv injection) showed a rapid brain uptake and washout (2.78%, 1.17%, 0.50%, and 0.17% dose/organ at 2, 30, 60, and 120 min, respectively). More importantly the hypothalamus region where the serotonin neurons are located exhibited a high specific uptake. Hypothalamus/cerebellum ratio based on %dose/g of these two regions showed a value of 1.29, 2.21, 2.76, and 2.68 at 2, 30, 60, and 120 min, post iv injection, respectively. Preliminary imaging study of [^{123}I]IDAM ([^{123}I]**6**) in the brain of a baboon by

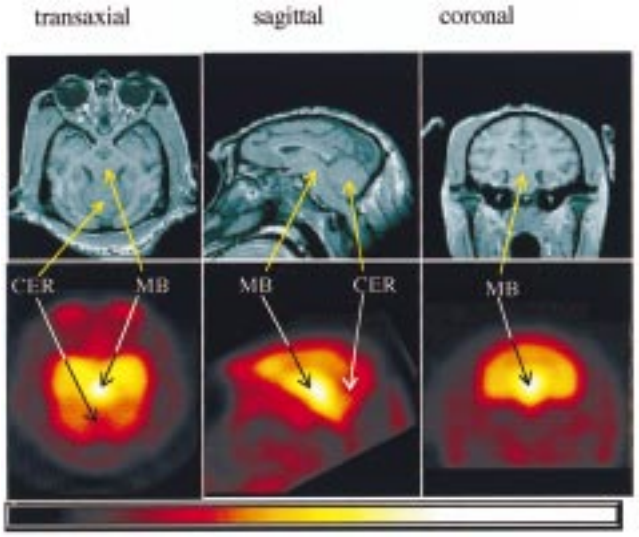


Figure 1. Comparison of MRI images (anatomy) and SPECT images[23] of [^{123}I]IDAM (**6**) (SERT localization) presented in transaxial, sagittal, and coronal views (between 60 and 120 min post iv injection). [^{123}I]IDAM ([^{123}I]**6**) localized with high concentration in midbrain (MB) (raphe nucleus, substantia nigra, hypothalamus), where the SERT concentration is high; it displayed no specific uptake in cerebellum (CER) (area lacking SERT). Ratio of MB/CER at 120 min was 2.4.

SPECT[23] at 60−120 min post iv injection showed an excellent contrast in midbrain area where SERT are found with a high density (Figure 1). The specific uptake in the midbrain region in the brain of baboons can be displaced by a chasing dose of (+)McN5652, a SERT ligand, but not with nisoxetine, a selective NET ligand (data not shown). The biological properties of [^{123}I]IDAM (**6**) show promise to be a superior SPECT imaging agent for in vivo evaluation of this important serotonin binding site in the brain.

In conclusion, a new iodinated SERT ligand, 5-iodo-2-((2-((dimethylamino)methyl)phenyl)thio)benzyl alcohol (IDAM) (**6**), was developed. This novel ligand showed excellent binding affinity and selectivity, and it may be useful as an in vitro and in vivo tool for studying the SERT binding sites.

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Supporting Information Available: Experimental procedures and data for compounds reported in this paper. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) 5-Tri-*n*-butyltin-2-((2-((dimethylamino)methyl)phenyl)thio)-benzyl alcohol (**4**). IR (cm^{-1}, neat) 3414, 2958, 2927, 1459, 1378, 1066, 752; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.2−7.5 (m, 4H), 6.9−7.1 (m, 3H), 4.50 (s, 2H), 3.55 (s, 2H), 2.30 (s, 3H), 1.2−1.8 (m, 12H), 1.1 (m, 6H), 0.91 (t, J = 7.2 Hz, 9H). Anal. Calcd for $C_{28}H_{45}NOSSn\cdot H_2O$: C, 57.94; H, 8.16; N, 2.41. Found: C, 57.48; H, 7.88; N, 2.63. 5-Iodo-2-((2-((dimethylamino)methyl)phenyl)thio)-benzyl alcohol (**6**): IR (cm^{-1}, neat) 3391, 2940, 1586, 1459, 1249, 1037, 840, 730; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.74 (d, J = 1.8 Hz, 1H), 7.58 (dd, J = 8.1, 1.8 Hz, 1H), 7.1−7.3 (m, 4H), 7.00 (m, 1H), 4.41 (s, 2H), 3.48 (s, 2H), 2.29 (s, 6H). Anal. Calcd for $C_{16}H_{18}INOS$: C, 48.13; H, 4.54; N, 3.51. Found: C, 48.17; H, 4.89; N, 3.25.
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(23) The SPECT images were obtained by a triple-head gamma camera equipped with ultra-high-resolution fan-beam collimators (Picker Prism 3000). The acquisition parameters comprised a rotational radius of 14 cm, a 15% energy window centered on 159 keV, 120 projection angles over 360°, and a 128 × 128 matrix with a pixel width of 2.11 mm in the projection domain. Data collection started immediately after iv injection of 555 MBq (15 mCi) of [^{123}I]IDAM. Forty-eight 5-min scans were carried out over a total time period of 240 min. The projection images were reconstructed by filtered-back projection. Then, a 3D low-pass Butterworth filter was applied. For uniform attenuation correction, Chang's first-order method was used. The MRI scans were acquired on a 1.5-T instrument (GE Medical Systems, Milwaukee, WI) with a spoiled GRAS sequence that produces 0.97- × 0.97- × 1-mm voxels. The MRI scans were resized and resliced in planes parallel to the one containing the anterior and posterior commissures.

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