SYNTHESIS OF [2-{(4-CHLOROPHENYL)(4-[^{125}I]IODOPHENYL)} METHOXYETHYL]-1-PIPERIDINE-3-CARBOXYLIC ACID, [^{125}I]CIPCA: A POTENTIAL RADIOTRACER FOR GABA UPTAKE SITES

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SUMMARY

The synthesis of racemic [2-{(4-Chlorophenyl)(4-iodophenyl)} methoxyethyl]-1-piperidine-3-carboxylic acid, (CIPCA) and its radioiodinated analog [^{125}I]CIPCA is described. CIPCA was synthesized from 4-iodobenzoyl chloride in five steps in 16% overall yield. Ammonium sulfate catalyzed solid-state isotopic exchange of CIPCA with Na^{125}I provided [^{125}I]CIPCA in 34% isolated radiochemical yield at a specific activity of 118 Ci/mmol. [^{125}I]CIPCA demonstrated moderate brain extraction and good in vivo radiostability in preliminary biodistribution studies conducted in CD-1 mice. [^{125}I]CIPCA is a potentially useful radiotracer for study of the GABA uptake system.

Key Words: GABA uptake inhibitor, Iodine-125, radiotracer, mouse brain uptake, SPECT.

INTRODUCTION

The neutral amino acid, γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system (1,2). Dysfunctions of the GABA neuronal system have been implicated in the development of neurological disorders such as Huntington's chorea (3), Parkinson's disease (4) and epilepsy (5). In particular, reduced synaptic concentrations of GABA are reported to be a contributory factor in certain seizure disorders in humans (6). Consequently, attention has been directed towards the development of GABA uptake inhibitors as an approach to increase synaptic concentrations of GABA (7).

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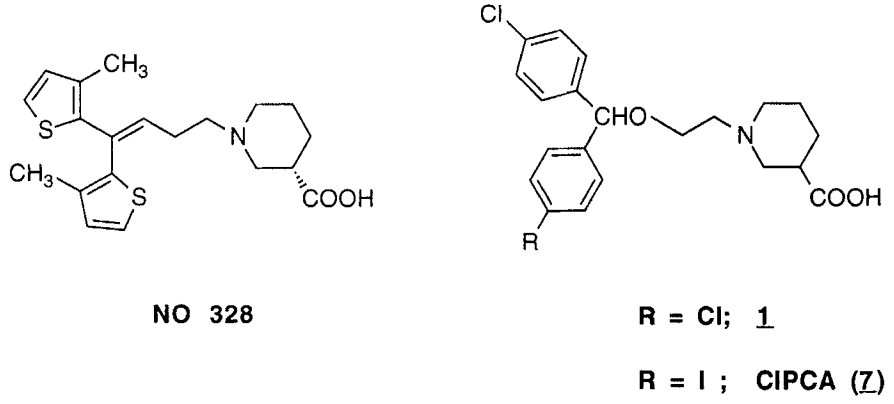
Structure-activity-relationship studies on conformationally restricted GABA analogs demonstrate that the R stereoisomer of nipecotic acid, (R-(-)-3-piperidine-carboxylic acid) is an inhibitor of GABA uptake (IC_{50} = 1.7 μM) with no detectable affinity for GABA receptors (8). The hydrophilic nature of nipecotic acid prevents significant permeation of the blood-brain-barrier following peripheral administration. However, enhancement of brain permeability has been achieved by the introduction of lipophilic groups such as a diphenylmethoxyethyl or a diphenylbutenyl group at the nitrogen atom of nipecotic acid (9). These derivatives often display similar selectivity and, in some instances, increased inhibitor potency for the GABA uptake site (9). Two notable examples are the R stereoisomers of the bis(3-methyl-2-thienyl) analog, (NO 328) and bis(4-chlorophenyl) analog, 1 (Figure 1), with in vitro IC_{50} values for GABA uptake inhibition of 0.067 and 0.18 μM respectively (10,11).



NO 328

R = Cl; 1

R = I ; CIPCA (7)

FIGURE 1. Structures of GABA uptake inhibitors

The development of selective, high affinity radioligands for specific neurotransmitter binding sites has played a major role in the rapid growth of neuropharmacology. However, few radioligands are currently available for study of the GABA neuronal system, and development of new ligands in this area has been limited mostly to the benzodiazepine subset of the GABA-benzodiazepine receptor complex. The synthesis of a ^{18}F-labeled GABA uptake inhibitor has been recently reported for positron emission tomography (PET) studies (12). Currently, ^{3}H-labeled

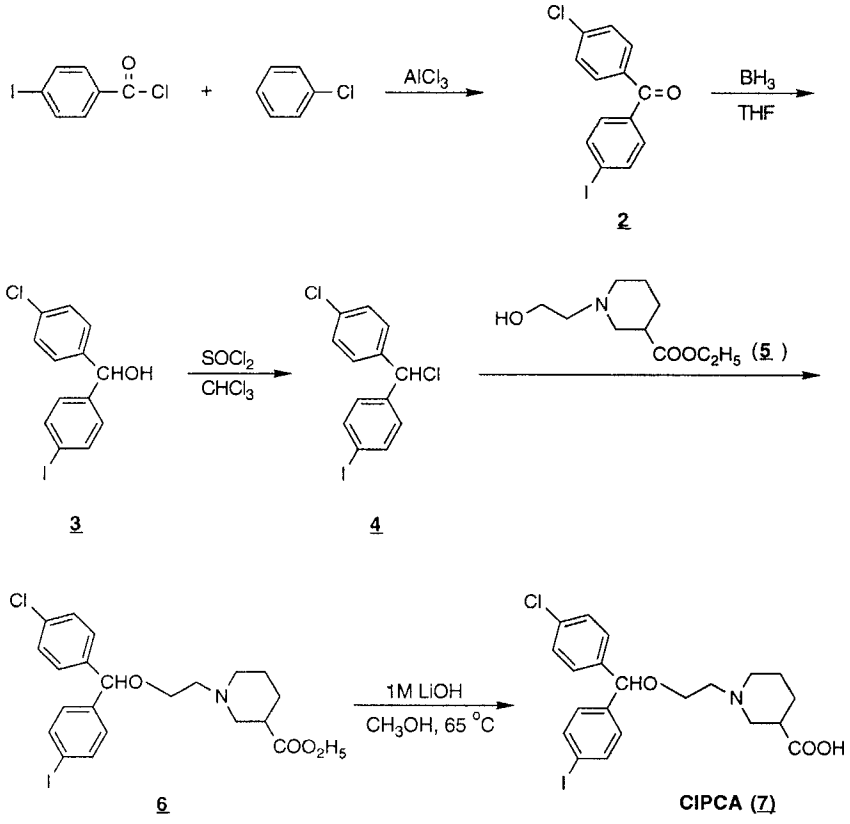
(R,S)-nipecotic acid (Kd = 0.62 μM) is the only available radioligand for study of GABA uptake sites (13). This tracer has been used to map GABA uptake sites in rat brain as well as in postmortem brain tissues of normal and Alzheimers disease subjects (14,15). A radioiodinated ligand would offer many advantages over a tritium labeled ligand, including a higher specific activity and, therefore, a higher sensitivity for labeling very low receptor densities. An added advantage would be the possibility of conducting in vivo imaging studies with the corresponding ^{123}I-labeled analog using single photon emission computed tomography (SPECT). The objective of this study was to develop an ^{125}I radioiodinated analog of the prototype GABA uptake inhibitor **1**, and to conduct preliminary in vivo biodistribution studies to ascertain its suitability for study of the GABA uptake system.

RESULTS AND DISCUSSION

[2-{(4-Chlorophenyl)(4-[^{125}I]iodophenyl)}methoxyethyl]-1-piperidine-3-carboxylic acid ([^{125}I]CIPCA, Figure 1), wherein a single chlorine atom in **1** is replaced with iodine-125, was chosen for evaluation (16). CIPCA was prepared via a synthetic route starting from 4-iodobenzoyl chloride as outlined in Scheme I. Acylation of chlorobenzene with 4-iodobenzoyl chloride under Friedel-Crafts conditions afforded **2** in 87% yield. Diborane reduction of the ketone provided the alcohol, **3** which was subsequently converted to the corresponding chloro analog **4** with $SOCl_2$ under reflux conditions. Ethyl N-(2-hydroxyethyl)nipecotate (**5**) was synthesized by refluxing ethyl nipecotate, 2-chloroethanol and anhydrous K_2CO_3 in toluene in the presence of tetrabutylammonium iodide as catalyst. Condensation of the benzhydryl chloride (**4**) with **5** in the absence of solvent at 160-170 °C afforded the ester **6** as an oil in 55% yield following purification by chromatography. Attempts at deprotection of the ester to the corresponding carboxylic acid **7** under acid hydrolysis conditions resulted in cleavage of the benzhydryl ether functionality (17). Hydrolysis of **6** with aqueous LiOH according to the published procedure (11), however, provided the desired carboxylic acid (CIPCA, **7**) in 73% yield.

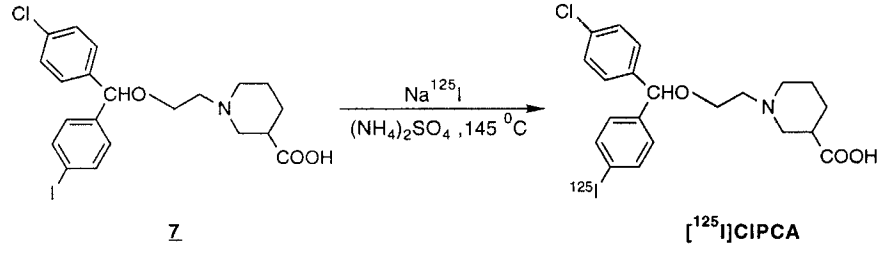
The radiosynthesis of [^{125}I]CIPCA was achieved via the $(NH_4)_2SO_4$ catalyzed solid state exchange labeling technique as shown in Scheme 2 (18,19). The initial

SCHEME 1. Synthesis of ClPCA (7)



radiopurity of [^{125}I]ClPCA was 60% (as determined by radio-TLC analysis) the rest of the radioactivity representing mostly free [^{125}I]iodide. Attempts at removal of the unreacted radioiodide by either Anion exchange chromatography (Amberlite IRA-400) or Cation exchange chromatography (Dowex 50W-X8) resulted in substantial

SCHEME 2. Synthesis of [^{125}I]ClPCA



losses of product. This was probably due to a strong interaction of the lipophilic amino side chain with the polymeric matrix of the resin. Similarly, attempts at purification of [^{125}I]CIPCA using a silica Sep Pak under normal phase conditions were unsuccessful due to tailing of the labeled compound. Subsequently, use of a cyano Sep Pak under reverse-phase conditions provided [^{125}I]CIPCA in 95% radiochemical purity with good product recovery.

[^{125}I]CIPCA displayed moderately good brain extraction (0.82 % of the injected dose at 30 min post injection) in preliminary biodistribution studies conducted in female CD-1 mice. Thyroid radioactivity concentrations at 4 h post injection indicated <1% in vivo deiodination. A slight regional heterogeneity of uptake in brain was observed with a cortex to striatum ratio of 1.2 at 30 min. Braestrup and coworkers reported a cortex to striatum ratio of 1.53 for the in vitro specific binding (rat brain synaptosomes) of the R stereoisomer of [^{3}H]NO 328 (10). Our studies with [^{125}I]CIPCA a) utilized a racemic mixture and b) were not corrected for the non-specific binding component, which could account, in part, for the observed lower cortex to striatum ratio. Further studies with the R stereoisomer of [^{125}I]CIPCA (ie. R configuration at the C-3 carbon of the piperidine ring), in conjunction with pharmacological blocking studies, should provide insight into the specificity of the in vivo binding of this radiotracer in mouse brain.

In conclusion, we report here the synthesis of an ^{125}I-labeled radiotracer for GABA uptake sites which crosses the blood-brain-barrier and has good in vitro and in vivo stability. The moderately high specific activity (118 Ci/mmol) of [^{125}I]CIPCA is adequate for in vivo studies in small animals. However, for clinical application a no-carrier-added synthesis of the corresponding ^{123}I-labeled analog is preferable in order to minimize possible pharmacological or toxicity effects (11). [^{123}I]CIPCA is a potentially useful radiotracer for scintigraphic visualization of GABA uptake sites in brain by single photon emission computed tomography (SPECT).

EXPERIMENTAL

Melting points were obtained with a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 727B spectrometer. ^{1}H-NMR spectra were obtained on a Bruker WM-360 (360 MHz)

instrument with tetramethylsilane as internal standard. Mass spectra were obtained on a Finnigan 4021 GCMS/DS (low resolution) or a UG70-250-S (high resolution) instrument. Na[^{125}I]iodide was obtained from Nordion Ltd, Ontario, Canada as a no-carrier-added solution in 0.1N NaOH (pH = 10 - 12). All chemical reagents were purchased from Aldrich Chemical Company, Milwaukee, WI. Flash chromatography was performed by the method of Still (20). Elemental analyses were performed by Spang Microanalytical Laboratories, Eagle Harbor, MI.

Thin-layer chromatography of the radioactive products were performed on Whatman K6F silica gel glass-backed plates (20 cm, 250 μ). TLC chromatograms were scanned for radioactivity using a Berthold Model LB 2832 TLC - linear analyzer equipped with a Model LB 500 data acquisition system.

HPLC was carried out on a Beckman Instrument Model 344 liquid chromatograph equipped with a Beckman Model 155-00 UV detector. Radioactivity was monitored with a Radiomatic Instruments Model DR/IC Flo-one radioactive flow detector with a Model CU data acquisition system upgrade containing a 340 μL solid scintillant cell.

The radiochemical purity of [^{125}I]CIPCA was determined using a μBondapak Cyano column, 10 μ, (3.9 x 300 mm) in series with a Cyano guard cartridge (Brownlee) with 0.1 M ammonium acetate (pH=6.8):CH_3CN (7:3, v/v) as the mobile phase at a flow rate of 1.5 mL/min. UV absorbance was monitored at 214 nm. The retention time for [^{125}I]CIPCA under these conditions was 8.7 min. Specific activity was determined from a standard curve relating mass to UV absorbance peak area (19).

4-Chloro-4'-iodobenzophenone (2)

Aluminum chloride (2.25 g, 16.9 mmol) was added in one portion to a suspension of 4-iodobenzoyl chloride (2.25 g, 8.4 mmol) in dry chlorobenzene (15 mL) at room temperature under an argon atmosphere. The pale yellow solution was then heated at 70 ^{o}C (oil-bath) for 3 h and the warm reaction mixture poured over a mixture of crushed ice and concentrated HCl (10 mL). The aqueous suspension was extracted with $CHCl_3$ (3 x 50 mL) and the combined organic layers washed successively with aqueous 10% Na_2CO_3 (50 mL), H_2O (50 mL) and dried

(Na_2SO_4). Removal of the solvent under reduced pressure and recrystallization of the residue from EtOH:$CHCl_3$ (4:1) afforded 2.5 g (87%) of **2** as white shiny flakes. mp 169-170 °C [lit(21) 171.2-171.7 °C]. IR (KBr) 1640 cm^{-1} (carbonyl); ^1H NMR ($CDCl_3$): δ 7.87 (d, 2H, J = 8.5 Hz), 7.73 (d, 2H, J = 8.5 Hz) 7.51-7.46 (pair of doublets, 4H). Anal. Calcd. for $C_{13}H_8ClIO$: C, 45.58; H, 2.35. Found: C, 45.61; H, 2.48.

4-Chloro-4'-iodobenzhydrol (3)

A solution of **2** (2.3 g, 6.71 mmol) in anhydrous THF (25 mL) was treated dropwise with stirring at 5 °C (ice-water bath) with a 1.0 M solution of BH_3 in THF (13.4 mL, 13.4 mmol). The solution was warmed to room temperature, stirred for 3 h and quenched by the slow addition at 0 °C of a solution of 50 mL of THF:H_2O (1:1). The reaction mixture was poured into saturated aqueous K_2CO_3 (100 mL), extracted with Et_2O (2 x 100 mL) and dried ($MgSO_4$). Flash chromatography [silica-gel, hexanes:EtOAc (4:1)] of the crude product gave 2.04 g (88%) of **3** as a white solid which was crystallized from hexanes: mp 98-99 °C. IR (KBr) 3340 cm^{-1} (OH). ^1H NMR ($CDCl_3$): δ 7.65 (d, 2H, J = 8.4 Hz), 7.31-7.24 (pair of doublets, 4H), 7.08 (d, 2H, J = 8.5 Hz), 5.72 (d, 1H, J = 3.2 Hz), 2.37 (d, 1H, J = 3.4 Hz). Anal. Calcd. for $C_{13}H_{10}ClIO$: C, 45.31; H, 2.92. Found: C, 45.11; H, 2.97.

4-Chloro-4'-iodobenzhydryl chloride (4)

A solution of **3** (2.0 g, 5.8 mmol) in dry $CHCl_3$ (20 mL) was treated in one portion under argon with excess $SOCl_2$ (20 mL) and refluxed for 18 h. Following removal of volatiles under reduced pressure the residue was partitioned between $CHCl_3$ (100 mL) and H_2O (50 mL). The aqueous layer was further extracted with $CHCl_3$ (100 mL), and the combined organic layers dried (Na_2SO_4), filtered, and evaporated under reduced pressure. Flash chromatography of the crude product [silica-gel; hexanes:EtOAc (19:1)] gave a white crystalline solid: 1.47 g (70%) which was recrystallized from hexane: mp 63-65 °C. ^1H NMR ($CDCl_3$): δ 7.66 (d, 2H, J = 8.5 Hz), 7.36-7.22 (m, 4H), 7.10 (d, 2H, J = 8.5 Hz), 6.00 (s, 1H). Anal. Calcd. for $C_{13}H_9Cl_2I$: C, 43.01; H, 2.50. Found: C, 43.13; H, 2.63.

Ethyl N-(2-Hydroxyethyl)nipecotate (5)

A refluxing, stirred, slurry of ethyl nipecotate (1.0 g, 6.4 mmol), anhydrous K_2CO_3 (1.8 g, 12.7 mmol), and tetrabutylammonium iodide (0.23 g, 0.64 mmol) in dry toluene (10 mL) was treated dropwise with a solution of 2-chloroethanol (0.51 g, 6.4 mmol) in dry toluene (10 mL) and refluxed for a further 4 h. The mixture was filtered while hot, the residue washed with hot toluene (30 mL) and the combined organic extracts dried ($MgSO_4$). Removal of solvent under reduced pressure and flash chromatography of the residue [silica-gel; $CHCl_3$:CH_3OH:NH_4OH (19:1:0.1)] gave 0.93 g (73%) of a clear colorless oil. ^1H NMR ($CDCl_3$): δ 4.15 (q, 2H), 3.61 (m, 2H), 2.88 (d, 1H), 2.71 (d, 1H), 2.57 (m, 3H), 2.41 (t, 1H), 2.21 (t, 1H), 1.90 (m, 1H), 1.76 (m, 1H), 1.58 (t, 2H), 1.26 (t, 3H).

Ethyl-1-[2-{(4-chlorophenyl)(4-iodophenyl)}methoxyethyl]piperidine-3-carboxylate (6)

The nipecotate ester derivative 5 (0.55 g, 2.70 mmol) and 4 (1.0 g, 2.7 mmol) were combined in a 5 mL Pierce Reacti-vial, the vial sealed with a teflon-lined cap and heated in an oil bath at 160-170 °C for 45 min. The warm reaction mixture was solubilized in hot THF (5 mL) and poured into a mixture of 100 mL of ether: saturated brine (1:1). The organic layer was removed, the aqueous layer further extracted with ether (2 x 50 mL), and the combined organic layers dried (Na_2SO_4). Removal of solvent under reduced pressure and flash chromatography [silica-gel; $CHCl_3$:CH_3OH (197:3)] of the residue afforded 0.80 g (55%) of a pale yellow oil.
IR (Nujol) 1728 cm^{-1} (carbonyl). ^1H NMR ($CDCl_3$): δ 7.64 (d, 2H, J = 8.4 Hz), 7.26 (dd, 4H, J = 8.6 Hz) 7.06 (d, 2H, J = 8.5 Hz), 5.28 (s, 1H), 4.11 (q, 2H, J = 7.1 Hz), 3.55 (t, 2H, J = 5.9 Hz), 3.01 (d, 1H), J = 8.0 Hz), 2.76 (d, 1H, J = 11.3 Hz), 2.65 (t, 2H, J = 5.9 Hz), 2.54 (m, 1H), 2.24 (t, 1H, J = 10.6 Hz), 2.08 (dt, 1H), 1.94 (dd, 1H, J = 11.4 Hz), 1.69 (m, 1H), 1.62-1.39 (m, 2H), 1.23 (t, 3H, J = 7.1 Hz). HRMS (CI with CH_4) MH+ at 528.0786 (Calcd 528.0802). Anal. Calcd. for $C_{23}H_{27}ClINO_3$: C, 52.33; H, 5.16; N, 2.65. Found: C, 52.34; H, 5.25; N, 2.64.

[2-{(4-Chlorophenyl)(4-iodophenyl)}methoxyethyl]-1-piperidine-3-carboxylic acid (7)

A mixture of 6 (0.15 g, 0.3 mmol) and a 1.0 M solution of aqueous LiOH (0.5 mL, 0.5 mmol) in CH_3OH (5 mL) was refluxed with stirring at 65 °C until

homogenous (1 h). The cooled mixture was poured into H_2O (20 mL), extracted with EtOAc (3 x 50 mL), and the organic layer dried (Na_2SO_4). Removal of solvent under reduced pressure and flash chromatography of the residue [silica-gel; $CHCl_3$: CH_3OH: NH_4OH (7:3:0.1)] gave 0.11 g (73%) of an amorphous solid. IR (KBr) 1710 cm-^{1} (carbonyl); ^{1}H NMR (CD_3COCD_3): δ 7.71 (d, 2H, J = 7.9 Hz); 7.43 (d, 2H, J = 8.4 Hz), 7.36 (d, 2H, J = 8.2 Hz) 7.24 (d, 2H, J = 7.9 Hz), 5.51 (s, 1H), 3.62 (t, 2H), 2.87-2.42 (br m, 7H), 1.85-1.51 (br m, 4H). HRMS (CI with CH_4) MH^+ at 500.0477 (Calcd 500.0489). Anal. Calcd. for $C_{21}H_{23}ClINO_3$: C, 50.47; H, 4.64; N, 2.80. Found: C, 50.29; H, 4.67; N, 2.82.

[2-[(4-Chlorophenyl)(4-[^{125}I]iodophenyl)]methoxyethyl]-1-piperidine-3-carboxylic acid ([^{125}I]CIPCA; [^{125}I]Z)

[^{125}I]CIPCA was synthesized by $(NH_4)_2SO_4$ catalyzed isotopic exchange as follows: A solution of $(NH_4)_2SO_4$ (6 mg in 20 μL of deionized H_2O), Z (20 μg in 40 μL of acetone), Na^{125}I (7.8 mCi in 16μl of 0.1N NaOH), and three layers of 3 mm borosilicate glass beads were combined in a 3 mL multi-dose vial, and the mixture was heated in an oil bath to 145 ^{o}C over a 25 min period. At this point 20 cc of air was slowly introduced into the reaction vial by syringe over a 2 min period, and the dry reaction mixture was maintained at 145 ^{o}C for an additional 25 min. The vial was cooled and radio-TLC analysis performed [silica; $CHCl_3$: CH_3OH: NH_4OH (7:3:0.1)]. The R_f of [^{125}I]Z and Na^{125}I were 0.23 and 0.55 respectively in this TLC system, and the radiochemical purity of the product was 60%. Further purification was achieved by chromatography on a cyano Sep Pak (Waters). The crude product (7.1 mCi) in EtOH (0.50 mL) was diluted with H_2O (10 mL) and transferred to an activated cyano Sep Pak [prewashed with CH_3OH (5 mL) followed by H_2O (5 mL)]. The Sep Pak was rinsed sequentially with 0.01% aqueous NaI (5 mL) and H_2O (5 mL) to remove residual Na^{125}I. Following sequential rinsing of the Sep Pak with CH_3OH:H_2O (1:9, v/v; 5 mL) and CH_3OH:H_2O (1:4, v/v; 5 mL), elution with 100% CH_3OH (2 x 1 mL) afforded 2.65 mCi of [^{125}I]CIPCA with a radiochemical purity of 95%. Isolated radiochemical yield was 34% and the specific activity was determined to be 118 Ci/mmol. The solvent was removed using an argon stream, the product redissolved in absolute EtOH to a concentration of 1 mCi/mL and stored at 4 ^{0}C until

further use. Under these conditions, the labeled product showed less than 5% radiolytic decomposition in 48 h by radio-TLC and radio-HPLC analysis. The radiotracer was formulated in 0.150 M sodium acetate (pH = 4.5):EtOH (95:5, v/v) for animal evaluation.

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16. Replacement of a single chlorine atom in 1 with iodine does generate an additional chiral center at the diphenylmethane carbon. Studies comparing the influence of chirality at this particular site on GABA uptake inhibitor activity have, to our knowledge, never been reported for these analogs. It is reported, however, that removal of one of the 4-chloro substituents of 1 leads to a 10-fold lowering of activity (ref 11).

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