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Iodine-125 and Fluorine-18 Labeled Aryl-1,4-dialkylpiperazines: Potential Radiopharmaceuticals for *In Vivo* Study of the Dopamine Uptake System

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A series of fluorine-18 and iodine-125 labeled aryl-1,4-dialkylpiperazine analogs, derivatives of GBR 12935, were synthesized as radiotracers for positron emission tomography or single photon emission computerized tomography imaging of the brain based on their affinity for the presynaptic dopamine reuptake system High specific activity fluorine-18 tracers were prepared by nucleophilic aromatic substitution reactions, iodine-125 tracers were prepared by isotopic exchange reactions *In vitro* competitive binding studies demonstrated that iodine substitution is tolerated in the 4-position of the phenyl ring of the phenalkylpiperazine group *In vivo* regional brain biodistribution studies in mice indicated no selectivity of the radioiodinated ligands for the dopamine reuptake site, with striatum/cerebellum concentration ratios of 1. Similar negative results with the new fluorine-18 derivatives demonstrated that *in vivo* selectivity for the dopamine reuptake site appears to be critically dependent on the carbon chain length between the piperazine ring and the solitary aromatic ring These studies suggest that development of new radiopharmaceuticals based on the GBR 12935 structure cannot be based solely on considerations of *in vitro* binding affinities

Introduction

Potent, highly selective dopamine reuptake inhibitors in the aryl-1,4-dialk(en)ylpiperazine series (Scheme 1) have been recently reported (van der Zee *et al*, 1980) An interesting feature of this class of compounds is their higher selectivity for the dopaminergic uptake system than for the noradrenergic or serotoninergic uptake systems compared to previously reported dopamine reuptake inhibitors such as nomifensine, mazindol and cocaine. The latter compounds also have the disadvantage of being potent releasers of dopamine, a property not shared by the aryl-1,4-dialk(en)ylpiperazines (Hoffman *et al.*, 1986). Studies conducted with a phenylpropenyl derivative, [^{3}H]GBR 12783 (Chagraoui *et al*, 1987; Leroux-Nicollet and Costentin, 1988), and the phenylpropyl derivatives [^{18}F]GBR 13119 (Kilbourn, 1988) and [^{18}F]GBR 12909 (Haka *et al.*, 1989), have demonstrated that these compounds retain their high affinity and specificity for the dopamine reuptake system *in vivo* [^{18}F]GBR 12909 has been recently used to image the presynaptic dopamine reuptake system in the human brain using positron emission tomography (PET) (Koeppe *et al*, 1990) An I-125 labeled azido analog in this series was also recently reported to be a potential photoaffinity label for the dopamine uptake carrier protein (Grigoriadis *et al*, 1989) An important observation was the high *in vitro* potency (K_i = 10–20 nM) displayed by this particular analog despite the incorporation of an iodine atom and an azido group in the 3 and 4 positions, respectively, of the solitary aromatic ring. Encouraged by these results, we have pursued the development of new radiotracers based on this series of compounds, and in particular radioiodinated analogs which might find more widespread clinical use due to the greater availability of single photon emission computed tomography (SPECT) instrumentation.

Examination of the single limited SAR study of the aryl-1,4-dialkylpiperazines revealed that analogs

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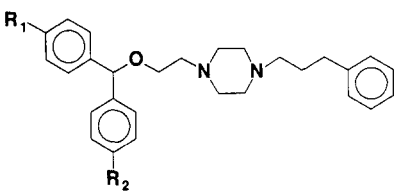


GBR 12935 $R_1 = R_2 = H$

GBR 13119 $R_1 = H, R_2 = F$

GBR 12909 $R_1 = R_2 = F$

Scheme 1

bearing small-volume halogen substituents on the aromatic rings led to highly potent inhibitors (van der Zee *et al.*, 1980) No significant reduction in dopamine reuptake inhibition resulted from replacement of the phenylpropenyl portion of GBR 12783 with a phenylpropyl group (GBR 12935), and furthermore, phenethyl or phenylbutyl groups caused only slight decreases in potency (van der Zee *et al*, 1980)

Our goal in this work was twofold

(1) to explore the effect of shortening the carbon chain length between the solitary aromatic ring and the piperazine ring on *in vivo* specificity for the dopamine reuptake system; and
(2) to determine if analogs labeled with iodine-125 on an aromatic ring would retain the high affinity and specificity displayed by the parent compounds.

The radiofluorinated derivatives [^{18}F]**3a** and [^{18}F]**3b** and the radioiodinated analogs [^{125}I]**8a**, [^{125}I]**8b**, [^{125}I]**8c** and [^{125}I]**12** were synthesized and their regional *in vivo* brain distribution in CD-1 mice were compared to [^{18}F]GBR 13119 and [^{18}F]GBR 12909, the [^{18}F]fluorinated ligands currently being evaluated in PET studies of the dopamine reuptake system

Experimental

Melting points were obtained with a Fisher–Johns 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 3-Iodophenylacetic acid was purchased from Sapon Laboratories, Aurora, OH All other chemical reagents were purchased from Aldrich Chemical Company, Milwaukee, WI Flash chromatography was performed by the method of Still *et al* (1978) Elemental analyses were performed by Spang, Microanalytical Laboratories, Eagle Harbor, MI

Thin-layer chromatography of the radioactive products was performed on either Whatman K6F silica gel glass-backed plates (20 cm, 250 μm) or Whatman KC18F reversed phase glass-backed plates (20 cm, 200 μm) 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 Beckmann Instrument Model 344 liquid chromatograph equipped with a Beckman Model 155-00 u.v detector Radioactivity was monitored with either a Beckman Model 170 radioisotope detector or 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 For the purification of the I-125 labeled tracers a Phenomenex Ultremex C-18, 5 μm (4 6 × 150 mm) column was used with 0 1 M ammonium acetate, pH = 6 8 (A) and 95% ethanol (B) mixtures under the following conditions For [^{125}I]**8a** A·B 1:4; flow rate 1 mL min^{-1}, retention time 10 6 min; For [^{125}I]**8b** A B 1 4, flow rate 1 5 mL min^{-1}; retention time 11 0 min For [^{125}I]**8c** A B 13 87, flow rate 1.2 mL min^{-1}, retention time 7 0 min For [^{125}I]**12** A B 15 85, flow rate 1 mL min^{-1}, retention time 9 0 min. Ultraviolet absorbance was monitored at 233 nm in the above cases Specific activity determinations for the radioiodinated tracers [^{125}I]**8a**, [^{125}I]**8b** and [^{125}I]**8c** were estimated from a standard curve relating mass to u v absorbance peak area as described previously (Van Dort *et al.*, 1988) utilizing the HPLC conditions described above For [^{125}I]**12** a μ Bondapak cyano column, 10 μm, (3 9 × 300 mm) was used with 0 1 M ammonium acetate (pH = 6 8)· acetonitrile (1 1) at a flow rate of 2 mL min^{-1} and u.v detection at 237 nm The retention time of [^{125}I]**12** was 7.9 min under these conditions

High specific activity, no-carrier-added [^{18}F]-fluoride ion was prepared by irradiation of a [^{18}O]-water target, as previously described (Mulholland *et al*, 1989) Na[^{125}I] iodide was obtained from Nordion Ltd, Ontario, Canada as a no-carrier-added solution in 0 1 N NaOH (pH = 10–12)

*Preparation of 1-(2-hydroxyethyl)-4-(benzyl)-piperazine (**2a**) and 1-(2-hydroxyethyl)-4-(2-phenylethyl)-piperazine (**2b**)*

Synthesis of these intermediate alcohols was based on methods previously reported (van der Zee *et al*, 1980) 1-(2-Hydroxyethyl)piperazine (1 equiv.) in ethanol was condensed with the appropriate halide [benzyl chloride or 2-bromoethylbenzene (1 equiv)] under reflux conditions The crude mixture was evaporated and the product isolated by repeated liquid–liquid extractions (chloroform. 2 N HCl, followed by partitioning between 6 N NaOH chloroform) The final organic layer was washed with brine and dried (Na_2SO_4). The isolated products were suitable for use in the subsequent condensation reactions

*1-(2-Hydroxyethyl)-4-(benzyl)piperazine **2(a)***

^{1}H-NMR (free base, $CDCl_3$) δ 7.24–7 32 (m, 5H, Ar), 3 62 (t, 2H, —CH_2—OH), 3 57 (s, 2H,

benzylic), 2.51–2.66 (m, 10H, —NCH_2) EIMS (70 eV), *m/e* (rel. int.): 220 (M+, 2.2) 202 (9.8), 190 (24.8), 189 (47.8), 146 (11.4), 134 (6.2), 119 (8.4), 98 (7.3), 91 (100).

*1-(2-Hydroxyethyl)-4-(2-phenylethyl)piperazine (**2b**)*

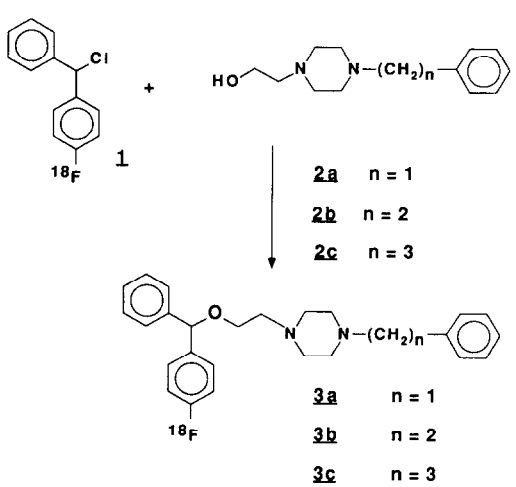
^{1}H-NMR (free base, $CDCl_3$) δ: 7.17–7.32 (m, 5H, Ar), 3.61 (t, 2H, —CH_2—OH), 2.78–2.84 (dd, 2H, benzylic), 2.48–2.63 (m, 12H, —NCH_2). EIMS (70 eV), *m/e* (rel int.): 234 (M+, 0.68), 189 (1), 160 (2.24), 143 (100).

*Synthesis of 1-[2-{(4′-[^{18}F]fluorophenyl)(phenyl) methoxy}ethyl]-4-(benzyl)piperazine (**3a**) and 1-[2-{(4′-[^{18}F]fluorophenyl)(phenyl)methoxy}ethyl]-4-(2-phenylethyl)piperazine (**3b**)*

These fluorine-18 labeled derivatives were prepared in a manner identical to that recently described for [^{18}F]GBR 12909 and [^{18}F]GBR 13119 (Kilbourn and Haka, 1988). In brief, 4-[^{18}F]fluorobenzhydryl chloride (**1**) was prepared in no-carrier-added form through a three step reaction: nucleophilic substitution of the nitro group in 4-nitrobenzophenone with F-18, reduction of the ketone to the alcohol followed by chlorination with $SOCl_2$. **1** was then condensed with the appropriately substituted 2-hydroxyethylpiperazine derivative to give **3a–c** (Scheme 2) The products were isolated by silica gel Sep-Pak chromatography, and radiochemical purities and specific activities determined by TLC and HPLC Isolated products were prepared for *in vivo* studies by evaporation of organic solvent and dissolution in 0.9% bacteriostatic saline containing up to 10% ethanol

*2-(3-Iodophenyl)ethanol (**5a**)*

A solution of 3-iodophenylacetic acid **4a** (1.0 g, 3.82 mmol) in dry THF (5 mL) was treated dropwise with stirring at 0°C under argon with a 1.0 M solution of BH_3 in THF (4.96 mL, 4.96 mmol). The solution was stirred at 0° to 5°C for 1 h and the excess BH_3 was destroyed by treatment dropwise at 0°C with a solution of 3 mL of THF H_2O (1:1). The reaction mixture was poured into saturated aqueous K_2CO_3 (25 mL), extracted with Et_2O (3 × 25 mL) and the organic layers dried ($MgSO_4$), filtered and evaporated. Purification by flash chromatography (silica gel, $CHCl_3$:CH_3OH, 97:3) yielded 0.92 g (97%) of pure **5a** as a colorless oil: ^{1}H-NMR ($CDCl_3$) δ: 7.60 (s, 1H), 7.57 (d, 1H, J = 7.8 Hz), 7.20 (d, 1H, J = 7.6 Hz), 7.05 (t, 1H, J = 7.7 Hz), 3.85 (t, 2H, J = 6.5 Hz), 2.81 (t, 2H, J = 6.5 Hz), 1.45 (br s, 1H) HRMS *m/e* 247.9685 (C_8H_9IO requires 247.9698). EIMS (70 eV) *m/e* (relative intensity) 248 (M+, base) 218(56), 217(58), 91(69), 90(52), 89(29), 86(18), 84(26), 77(15), 65(15), 63(17), 49(30), 39(15).



Scheme 2

*3-(3-Iodophenyl)propanol (**5b**)*

Similarly, diborane reduction of 3-(3-iodophenyl)propionic acid **4b** gave **5b** in 95% yield. i.r. (Nujol) 3330 cm^{-1} (OH); ^{1}H-NMR ($CDCl_3$) δ 7.57 (s, 1H), 7.53 (d, 1H, J = 7.9 Hz), 7.16 (d, 1H, J = 7.9 Hz), 7.02 (t, 1H, J = 7.7 Hz), 3.67 (t, 2H, J = 6.4 Hz), 2.66 (t, 2H, J = 7.7 Hz), 1.91–1.83 (m, 2H), 1.34 (br s, 1H). HRMS *m/e* 261.9851 ($C_9H_{11}IO$ requires 261.9855). EIMS (70 eV) *m/e* (relative intensity) 264(13), 262(98), 244(61), 218(56), 217(36), 117(84), 115(34), 104(41), 103(27), 91(base), 90(42), 89(29), 78(28), 77(34), 51(23), 43(29).

*3-(4-Iodophenyl)propanol (**5c**)*

By a similar approach diborane reduction of 3-(4-iodophenyl)propionic acid **4c** gave **5c** in 95% yield. ^{1}H-NMR ($CDCl_3$) δ: 7.59 (d, 2H, J = 8.3 Hz), 6.94 (d, 2H, J = 8.3 Hz), 3.64 (t, 2H, J = 6.40 Hz), 2.65 (t, 2H, J = 7.41 Hz), 1.87–1.83 (m, 2H), 1.58 (br s, 1H). HRMS *m/e* 261.9847 ($C_9H_{11}IO$ requires 261.9855) EIMS (70 eV) *m/e* (relative intensity) 262(12), 244(10), 217(10), 117(10), 91(9), 74(23), 73(24), 71(9), 61(14), 59(36), 45(base), 43(43), 42(18), 41(20)

*2-(3-Iodophenyl)ethyl tosylate (**6a**)*

A stirred solution of the alcohol **5a** (0.74 g, 2.98 mmol) and pyridine (0.56 g, 7.15 mmol) in dry CH_2Cl_2 (5 mL) was treated under argon with *p*-toluenesulfonyl chloride (0.68 g, 3.58 mmol) in one portion at 0°C The reaction was allowed to warm to room temperature and stirred a further 3 h. Volatiles were removed under high vacuum and the residue partitioned between saturated aqeuous $NaHCO_3$ (25 mL) and EtOAc (25 mL). The aqueous layer was further extracted with EtOAc (2 × 25 mL), the combined organic layers washed with H_2O (25 mL) and dried (Na_2SO_4) The solvent was evaporated and the residue flash chromatographed (silica-gel, hexanes·EtOAc, 3.1) to afford 1.05 g (87%) of pure **6a** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 7.66 (d, 2H, J = 8.3 Hz), 7.54 (d, 1H, J = 7.8 Hz), 7.41 (s, 1H), 7.29 (d, 2H, J = 8.3 Hz), 7.09 (d, 1H, J = 7.6 Hz), 6.98 (t, 1H, J = 7.7 Hz), 4.19 (t, 2H, J = 6.7 Hz), 2.88 (t,

2H, J = 6.7 Hz), 2 44 (s, 3H) HRMS *m/e* 401.9779 ($C_{15}H_{15}ISO_3$ requires 401.9787) EIMS (70 eV) *m/e* (relative intensity) 405(2), 231(11), 230(base), 217(18), 155(11), 103(16), 91(40), 90(17), 65(13)

*3-(3-Iodophenyl)propyl tosylate (**6b**)*

Similarly tosylation of alcohol **5b** gave **6b** in 75% yield ^{1}H-NMR ($CDCl_3$) δ 7 79 (d, 2H, J = 8 1 Hz), 7.51 (d, 1H, J = 7 7 Hz), 7.44 (s, 1H), 7.35 (d, 2H, J = 8 3 Hz) 7 04 (d, 1H, J = 7 6 Hz), 6 97 (t, 1H, J = 7 7 Hz), 4 02 (t, 2H, J = 6 1 Hz), 2.59 (t, 2H, J = 7.6 Hz), 2 46 (s, 3H), 1 97–1 89 (m, 2H) HRMS *m/e* 415 9932 ($C_{16}H_{17}ISO_3$ requires 415 9943). EIMS (70 eV) *m/e* (relative intensity) 416(20), 245(12), 244(base), 217(10), 118(15), 117(46), 115(13), 104(10), 91(33), 65(10)

*3-(4-Iodophenyl)propyl tosylate (**6a**)*

Similarly tosylation of alcohol **5c** gave **6c** in 86% yield ^{1}H-NMR ($CDCl_3$) δ 7 77 (d, 2H, J = 8 2 Hz), 7 54 (d, 2H, J = 8 2 Hz), 7 35 (d, 2H, J = 8.4 Hz), 6 82 (d, 2H, J = 8 1 Hz), 4 00 (t, 2H, J = 6 1 Hz), 2 60 (t, 2H, J = 7 4 Hz), 2 47 (s, 3H), 1 92 (pentet, 2H, J = 7.7 Hz) HRMS *m/e* HRMS *m/e* 415 9934 ($C_{16}H_{17}ISO_3$ requires 415 9943) EIMS (70 eV) *m/e* (relative intensity) 416(7), 245(13), 244(base), 217(13), 118(9), 117(43), 116(9), 115(17), 104(11), 103(7), 91(25), 90(12), 89(8), 78(7), 77(8), 65(11)

*1-[2-{Diphenylmethoxy}ethyl]piperazine (**7**)*

A well stirred mixture of piperazine (3 14 g, 36.45 mmol) and K_2CO_3 (3 36 g, 24 31 mmol) in dry toluene (10 mL) was treated dropwise under reflux with a solution of benzhydryl β-chloroethyl ether (van der Zee *et al*, 1980) (3 0 g, 12.16 mmol) in toluene (10 mL) Following reflux for 5 h the cooled mixture was diluted with toluene (30 mL) extracted with H_2O (2 × 50 mL) and the organic layers dried (Na_2SO_4) Flash chromatography (silica gel, $CHCl_3$ CH_3OH NH_4OH, 8 2 0 1) of the crude product gave 2 18 g (60%) of pure **7** as a colorless oil ^{1}H-NMR ($CDCl_3$) δ 7 36–7 21 (m, 10H), 5 37 (s, 1H), 3 60 (t, 2H, J = 6 1 Hz), 2 89(t, 4H, J = 4 8 Hz), 2 67 (t, 2H, J = 6 1 Hz), 2 50 (br, m, 4H), 2 11 (s, 1H) HRMS *m/e* 297.1968 ($C_{19}H_{25}N_2O$ requires 297 1967). CIMS (methane), *m/e* (relative intensity), 297(78), 295(19), 195(14), 168(13), 167(base), 113(18), 99(14), 86(12)

*1-{2-(Diphenylmethoxy)ethyl}-4-(2-(3-iodophenyl) ethyl}piperazine (**8a**)*

The tosylate derivative **6a** (0 27 g, 0.67 mmol) in dry toluene (3 mL) was added dropwise under argon to a warm (75°C) stirred suspension of the piperazine derivative **7** (0 20 g, 0 67 mmol) and anhydrous K_2CO_3 (0.19 g, 1 35 mmol) in dry toluene (3 mL) The reaction mixture was refluxed for 24 h The cooled mixture was then poured into H_2O (25 mL) extracted with toluene (2 × 25 mL) and the combined organic layers washed with H_2O (25 mL) and dried (Na_2SO_4) Flash chromatography (silica gel, $CHCl_3$:CH_3OH, 98:2) of the crude product afforded 0.32 g (90%) of pure **8a** as an oil A portion of this material was converted to the dihydrochloride salt by treatment of an ethanol solution with ethereal HCl. Recrystallization from isopropanol afforded white crystals m p 204–206°C (d). ^{1}H-NMR (free base, $CDCl_3$) δ. 7 56 (s, 1H), 7 52 (d, 1H, J = 7 7 Hz), 7 35–7 20 (m, 10H), 7.15 (d, 1H, J = 7.7 Hz), 7 00 (t, 1H, J = 7.7 Hz), 5 37 (s, 1H), 3 60 (t, 2H, J = 6 1 Hz), 2 75–2 41 (m, 14H). Anal calcd for ($C_{27}H_{31}N_2IO$ 2HCl) C, 54.10, H, 5 55, N, 4 67 Found: C, 54 18; H, 5 61, N, 4 61

*1-{2-(Diphenylmethoxy)ethyl}-4-{3-(3-iodophenyl) propyl}piperazine (**8b**)*

Similarly treatment of the piperazine derivative 7 with 3-(3-iodophenyl)propyl tosylate (**6b**) afforded **8b** after workup in 60% yield, m.p for the dihydrochloride salt 185–186°C (d) (isopropanol) ^{1}H-NMR (free base, $CDCl_3$) δ 7 54 (s, 1H), 7 49 (d, 1H, J = 7 7 Hz), 7 34–7 20 (m, 10H), 7.12 (d, 1H, J = 7.7 Hz), 6 98 (t, 1H, J = 7 7 Hz), 5.37 (s, 1H), 3 59 (t, 2H, J = 6 1 Hz), 2 67 (t, 2H, J = 6.0 Hz) 2 58–2.30 (m, 12H) 1 76 (m, 2H) Anal calcd for ($C_{28}H_{33}N_2IO \cdot 2HCl$). C, 54 82, H, 5 75, N. 4 57 Found C, 54 88; H 5 66; N, 4 53

*1-{2-(Diphenylmethoxy)ethyl}-4-{3-(4-iodophenyl) propyl}piperazine (**8c**)*

Similarly treatment of the piperazine derivative **7** with 3-(4-iodophenyl)propyl tosylate (**6c**) afforded **8c** after workup in 70% yield, m p for the dihydrochloride salt 190–192°C (d) (ethanol) ^{1}H-NMR (free base, $CDCl_3$) δ 7 58 (d, 2H, J = 8 3 Hz), 7 35–7 21 (m, 10H), 6.93 (d, 2H, J = 8.3 Hz), 5 37 (s, 1H), 3 60 (t, 2H, J = 6 1 Hz), 2 68 (t, 2H, J = 5 9 Hz), 2 58–2 31 (m, 12H), 1 77 (pentet, 2H). Anal calcd for ($C_{28}H_{33}N_2IO$ 2HCl) C, 54 82, H, 5 75, N, 4 57. Found C, 54 81, H, 5 75, N, 4 45

*4-Fluoro-4'-iodobenzophenone (**9**)*

A well stirred suspension of 4-iodobenzoyl chloride (1 00 g, 3 75 mmol) in dry fluorobenzene (5 mL) under argon was treated with $AlCl_3$ (1 00 g, 7 5 mmol) in one portion at room temperature Following 2 h of reflux the clear warm solution was poured over a mixture of crushed ice and concentrated HCl The product was extracted into $CHCl_3$ (3 × 25 mL) and the combined organic layers washed with aqueous 10% Na_2CO_3 (75 mL), H_2O (75 mL), and dried (Na_2SO_4). Following removal of solvent the residue was recrystallized from EtOH to give 1 10 g (90%) of white shiny flakes, m.p 129–130°C. ir (KBr) 1640 cm^{-1}, ^{1}H-NMR($CDCl_3$)δ 7 87–7 80(m, 4H),7 49(d, 2H, J = 8 5 Hz), 7 17 (m, 2H) Anal calcd for ($C_{13}H_8FIO$) C, 47 88; H, 2 47 Found C, 47 74, H, 2 61

*4-Fluoro-4'-iodobenzhydrol (**10**)*

A solution of **9** (1 0 g, 3 1 mmol) in dry THF (10 mL) was treated dropwise with stirring at 0–5°C with a

1 0 M solution of BH_3 in THF (4.0 mL, 4.0 mmol). The solution was allowed to warm to room temperature and stirred a further 3 h. The excess BH_3 was destroyed by treatment dropwise at 0°C with a solution of 25 mL of THF.H_2O (1:1). The reaction mixture was poured into saturated aqueous K_2CO_3 (50 mL), extracted with Et_2O (3 × 50 mL) and the organic layers dried ($MgSO_4$), filtered and evaporated. Purification by flash chromatography (silica gel, hexanes:EtOAc; 4.1) afforded 0.96 g (94%) of pure **10** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ 7.65 (d, 2H, J = 8 4 Hz), 7.32–7.29 (m, 2H), 7.10 (d, 2H, J = 8.1 Hz), 7 02–6.99 (m, 2H), 5.75 (d, 1H, J = 3 2 Hz), 2.29 (d, 1H, J = 3.4 Hz). Anal. calcd for ($C_{13}H_{10}FIO$) C, 47 58; H, 32.07. Found C, 47.74, H, 3 16

4-Fluoro-4'-iodobenzhydryl chloride (11)

A solution of **10** (0.73 g, 2 22 mmol) in dry $CHCl_3$ (5 mL) was treated in one portion at room temperature under argon with excess $SOCl_2$ (5 mL,). The reaction mixture was refluxed for 2 h and volatiles were removed under reduced pressure The residue was partitioned between $CHCl_3$ (25 mL) and H_2O (25 mL) The aqueous layer was further extracted with $CHCl_3$ and the combined organic layers dried (Na_2SO_4), filtered and evaporated under reduced pressure Flash chromatography (silica gel, hexanes:EtOAc 19 1) of the crude product gave 0 73 g (95%) of **11** as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ. 7 68 (d, 2H, J = 8 5 Hz), 7.36–7 32 (m, 2H), 7.13 (d, 2H, J = 8.5 Hz), 7 05–7 01 (m, 2H), 6 04 (s, 1H) HRMS *m/e* 345 9413 ($C_{13}H_9ClFI$ requires 345 9421) EIMS (70 eV) *m/e* (relative intensity) 346(12), 312(16), 311(base), 185(21), 184(43), 183(92), 182(12), 92(20), 91(50), 86(34), 84(49), 82(20), 81(16), 63(17), 51(14), 50(16), 49(25), 47(24), 39(14), 35(15).

1-[2-{4-Fluorophenyl)(4-iodophenyl)methoxy}ethyl]-4-(3-phenylpropyl)piperazine(12)

1-(2-hydroxyethyl)-4-(3-phenylpropyl)piperazine (van der Zee *et al*, 1980) (0.36 g, 1.44 mmol) and **11** (0 50 g, 1.44 mmol) were combined in a 1 mL glass V-vial (Pierce) sealed with a Teflon liner and cap and heated at 165°C for 45 min. The dark brown residue was dissolved in the minimum volume of hot THF and added to a 100 mL mixture of Et_2O saturated brine (1 1). The Et_2O layer was removed, the aqueous layer extracted with Et_2O (1 × 50 mL) and the combined organic layers dried (Na_2SO_4), filtered and evaporated under reduced pressure Flash chromatography of the residue (silica gel, $CHCl_3$.CH_3OH; 98.2) afforded 0.53 g (66%) of pure **12** as a pale yellow oil. A portion was converted to the dihydrochloride salt and recrystallized from isopropanol:Et_2O, m.p. 211–213°C (d). ^{1}H-NMR (free base, $CDCl_3$) δ· 7.64 (d, 2H, J = 8.4 Hz), 7.30–7.23 (m, 5H), 7 18 (d, 2H, J = 7.8 Hz), 7.06 (d, 2H, J = 8.4 Hz), 7.01–6.96 (m, 2H), 5 29 (s, 1H), 3 55 (t, 2H, J = 6.0 Hz), 2.67–2.34 (m, 14H), 1.81 (m, 2H). Anal. calcd for ($C_{28}H_{32}N_2FIO$·2HCl). C, 53.09; H, 5.41; N, 4.42. Found: C, 53.12; H, 5.58, N, 4.60.

Radioiodination

In a typical procedure, a solution of $(NH_4)_2SO_4$ (5.0 mg in 15 μL of deionized H_2O), **8a** (20 μg of dihydrochloride salt in 40 μL in EtOH), and 9.1 mCi of $Na^{125}I$ were combined and the mixture was heated to dryness at 145°C in an oil bath. Air was then slowly passed through the reaction vial for 2 min and the dry reaction mixture maintained at 145°C for 25 min. After cooling, the reaction mixture was dissolved in EtOH (250 μL) and subjected to radio-TLC analysis (silica; $CHCl_3$:CH_3OH; 19.1) The solution was passed through an anion-exchange column (Amberlite IRA 400, OH form, strongly basic) to remove residual [^{125}I]iodide. Further elution with EtOH (250 μL) provided 5.41 mCi (59%) of crude radioactive product of *ca* 90% radiochemical purity. Further purification was accomplished by reversed phase HPLC as described which provided the tracers in 98% chemical and radiochemical purity as determined by analytical HPLC. The HPLC purified radiotracers were diluted with 0 9% bacteriostatic saline to a final ethanol concentration of 10% for *in vivo* biological evaluations.

The specific activity values (Ci mmol^{-1}) and isolated radiochemical yields (shown in parentheses) for the radioiodinated tracers [^{125}I]**8a**, [^{125}I]**8b**, [^{125}I]**8c** and [^{125}I]**12** were 200 (37%), 249 (50%), 239 (57%) and 266 (63%) respectively.

Tissue distribution studies

CD-1 mice (20–25 g, Charles River, Wilmington, MA) were injected via the tail vein under light ether anesthesia with either the fluorine-18 labeled ligands (7–10 μCi) or the iodine-125 labeled ligands (3–10 μCi). The animals were sacrificed by decapitation at designated time intervals and the brain rapidly removed and dissected into regions of interest (Glowinski and Iverson, 1966) (striatum, cerebral cortex, cerebellum and remainder of brain tissue). Blood samples were also obtained. All samples were assayed for radioactivity in an automatic γ counter and then weighed. Data were calculated as % ID/g for all tissues.

In vitro binding studies

In vitro competition studies for the binding of [^{3}H]GBR 12935 were performed as previously described (Richfield, 1991). Brains from adult male Sprague–Dawley rats (180–200 g) were obtained after rapid decapitation and frozen by immersion in isopentane at −30°C. Brains were mounted on tissue pedestals and warmed to −18°C, coronal sections 8–15 μm were cut on a cryostat microtome and thaw mounted onto gelatin coated slides. Slides were dehydrated at 20°C for several minutes and then frozen at −25°C until used in assays. The final [^{3}H]GBR 12935 binding buffer was a 50 mM sodium phosphate buffer

(pH 7 5), adjusted to a final sodium concentration of 120 mM with sodium chloride, 0 001% ascorbic acid, 0.025% fatty acid free bovine serum albumin and 0 75 μM *trans*-flupentixol Competition studies were performed at a [^{3}H]GBR 12935 concentration of 0.25 nM and included various concentrations of unlabeled competitors Equilibrium occurred at 30 h. Specific binding was determined by subtracting the amount bound in the presence of 25 μM mazindol (nonspecific binding) from the amount bound in the absence of mazindol (total binding). Dried slides were placed in an x-ray cassette with carbon-14 plastic standards, previously calibrated with tritiated brain paste sections, and apposed to LKB ultrofilm ^{3}H at 25°C for 21 days The LKB ultrofilm ^{3}H was developed in Kodak D19 for 4 min at room temperature and fixed in Kodak rapid fix for 3 5 min All binding data were determined directly from film densities in regions of interest using a video based densitometer (MCID BRS, Imaging Research, Inc). Linear and nonlinear methods were used to determine inhibitory constants (K_I) One and two site nonlinear analyses were performed using computer assisted modeling program (London Software, Inc , Cleveland, OH) Competition models were constrained by having the K_D for [^{3}H]GBR 12935 fixed to a single value determined by independent saturation experiments

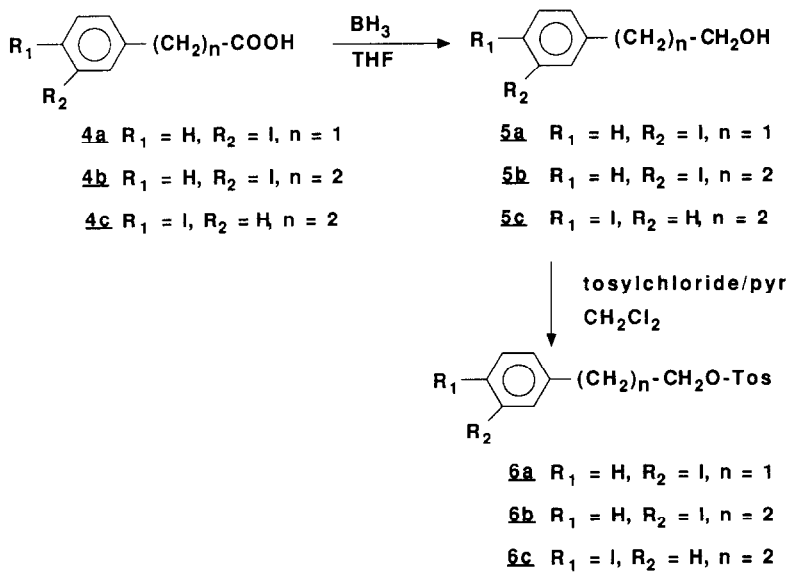
Results

Chemistry and radiochemistry

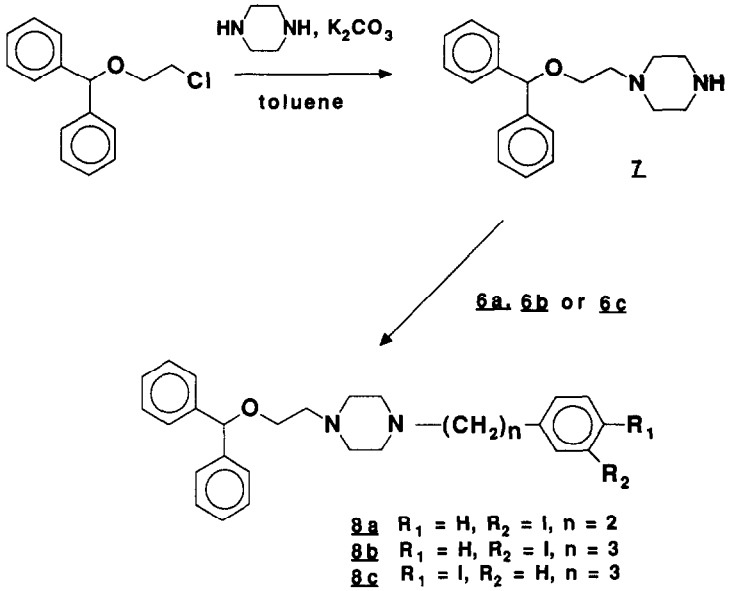
Preparation of the fluorine-18 analogs with benzyl or phenethyl chains was straightforward, and followed the synthetic route (Scheme 2) that we have previously described for the preparation of the phenylpropyl compound [^{18}F]GBR 13119 (Haka *et al*, 1989, Kilbourn and Haka, 1988) The required 1-phenylalkyl-4-(2-hydroxyethyl)piperazines (**2a**, **2b** or **2c**) were prepared according to the procedure of van der Zee *et al.* (1980) and reacted with 4-[^{18}F]fluorobenzhydryl chloride (Haka *et al.*, 1989). The final products, isolated by silica gel chromatography, were obtained in high radiochemical purities (>95%) and high specific activities (>1000 Ci/mmol)

The synthesis of the iodinated analogs was based on the methodology reported initially by van der Zee and coworkers (van der Zee *et al*, 1980). The synthetic route outlined for the 3-iodophenylalkyl homologs **8a**, **8b** and **8c** required the tosylate analogs **6a**, **6b** and **6c**, respectively, which were prepared as shown in Scheme 3. Diborane reduction of the appropriate 3-iodophenylalkanoic acid proceeded smoothly to give the alcohol which was converted to the tosylate by treatment with *p*-toluenesulfonyl chloride in pyridine/CH_2Cl_2 Condensation of the tosylates **6a**, **6b** or **6c** with **7** in the presence of K_2CO_3 in refluxing toluene afforded **8a**, **8b** or **8c** in 60–90% isolated yields (Scheme 4)

The synthesis of the analog bearing iodine in the benzyhydryl portion of the molecule (**12**) utilized a different synthetic approach (Scheme 5) similar to that used in the preparation of the fluorine-18 labeled compounds. Friedel–Crafts acylation of fluorobenzene with 4-iodobenzoyl chloride gave 4-iodo-4′-fluorobenzophenone **9** which was reduced with diborane to the benzhydryl alcohol analog **10** Treatment of **10** with $SOCl_2$ in refluxing $CHCl_3$ yielded the benzhydryl chloride **11**, which was condensed with 1-(3-phenylpropyl)-4-(2-hydroxyethyl)piperazine at 165°C to afford **12** in 66% yield. Radioiodination was accomplished by the ammonium sulfate catalyzed solid state isotope exchange technique previously reported (Mangner *et al*, 1982) Unreacted $Na^{125}I$



Scheme 3



Scheme 4

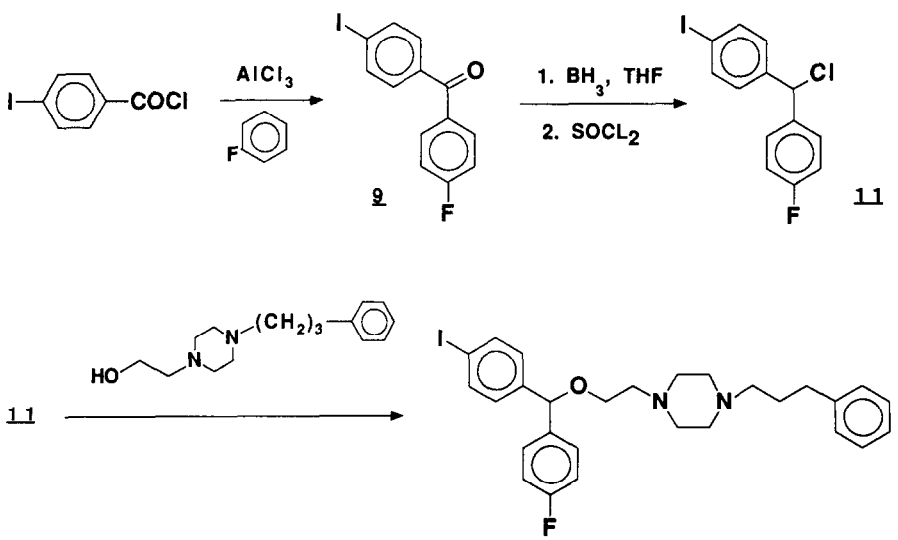
was removed by passage of an ethanol solution of the crude labeled product through an anion exchange column Further purification by reversed phase HPLC provided the radioligands in 37–63% isolated radiochemical yield and 98% chemical and radiochemical purity. Specific activities were determined to be greater than 200 Ci mmol^{-1}. All radioiodinated ligands showed less than 3% radiolytic decomposition when stored at 5°C in the HPLC elution solvent (0 1 M ammonium acetate, pH = 6.8:EtOH; 1:4) for up to 1 week The radiotracers were formulated immediately prior to i v administration by dilution with 0.9% bacteriostatic saline.

In vitro biological results

The abilities of the new iodinated derivatives **8a–c**, **12** and GBR 12909 to compete for [^{3}H]GBR 12935 binding were determined (Table 1). For compounds **8a–b** and **12**, substitution with iodine produced a significant (4- to 13-fold) loss in affinity. In the case of iodine substitution in the 4-position of the single aromatic ring (**8c**), however, the *in vitro* affinity was not significantly different from that of GBR 12909 (13 nM).

In vivo biological results

The regional mouse brain distributions of the radiolabeled analogs are reported in Tables 2 and 3.



Scheme 5

Table 1 Inhibition of [^{3}H]GBR 12935 binding by various GBR analogs

Compound	K_i(nM)
8a	176 ± 14 1
8b	53 0 ± 14 1
8c	21 4 ± 12 3
12	56 2 ± 8 3
GBR 12909	13 ± 9 0
GBR 12935	1–2[a]

[a] K_d value from van der Zee *et al* (1980)

For the fluorine-18 labeled analogs (Table 2), no specific uptake and retention of the radiotracer in the striatum was evident, with striatum-to-cerebellum concentration ratios near unity for both the benzyl (**3a**) and phenethyl (**3b**) compounds. Uptake and retention of radiotracer in nontarget tissue (cerebellum) and blood levels of radioactivity were not significantly different from that we have previously reported with [^{18}F]GBR 13119 or [^{18}F]GBR 12909 (Kilbourn, 1988, Haka *et al*, 1989)

For all of the radioiodinated ligands (Table 3), the striatal accumulations at 60 min post-injection were consistently less than 50% of that observed with [^{18}F]GBR 13119, and the derivatives labeled in the solitary aromatic ring all showed a much higher uptake of radioactivity in nontarget tissues such as the cerebellum and cortex With the iodine in the benzhydryl portion of the molecule, as in **12**, levels of radioactivity in the nontarget tissues, cerebellum and cortex, were very similar to those found with [^{18}F]GBR 13119 For all four iodinated radiotracers the striatum-to-cerebellum concentration ratio never increased to greater than 1 The relative regional distributions of radioactivity did not change at later times, and with the exception of **8c**, radioactivity levels slowly decreased with time. For the radioiodinated ligands, blood levels of radioactivity at 1 and 5 h were also comparable to those seen with [^{18}F]GBR 13119, except for **12** which displayed much higher levels The identity of the radioactivity in blood was not determined for any of the radioiodinated compounds

Discussion

The aryl-1,4-dialkylpiperazines, exemplified by GBR 12935 (Scheme 1, $R_1 = R_2 = H$), are the first reported dopamine reuptake inhibitors having both high affinity and selectivity. In this study structural modifications were conducted on GBR 12935 to ascertain the effect on *in vivo* selectivity of

(1) chain length between the solitary phenyl ring and the piperazine ring;
(2) location of the iodine atom at various positions on the aromatic rings

The data shown in Table 2 forms a limited *in vivo* structure–distribution relationship study of fluorine-18 labeled diarylalkylpiperazines Dopaminergic terminals are known to be highly concentrated in striatal tissue and virtually absent in the cerebellum (Grigoriadis *et al*, 1989) The striatum to cerebellum concentration ratio (STR/CER) therefore serves as a useful index of dopaminergic specificity The goal of this study was to reduce the lipophilicity of [^{18}F]GBR 12909 and hopefully improve the target-to-nontarget (STR/CER) ratio Unexpectedly, the shortening of the phenylpropyl chain of [^{18}F]GBR 13119 by two (**3a**) or one (**3b**) methylene groups caused a complete loss of selective binding of the radiotracer in mouse brain This was surprising, since the original SAR study of this class of compounds (van der Zee *et al*, 1980) reported only a 3-fold decrease in *in vitro* binding affinity in going from a phenylpropyl (GBR 12935, $IC_{50} = 2$ nM) to the phenethyl analog ($IC_{50} = 6\ 7$ nM), although a larger decrease was reported for the benzyl analog ($IC_{50} = 34$ nM). The loss of specific *in vivo* binding with such small changes in *in vitro* IC_{50} values is highly unusual For both of the chain-shortened analogs the striatal uptake of fluorine-18 was reduced to that of the cerebellum (giving STR/CER values near unity), and those levels were very comparable to the cerebellar levels found with [^{18}F]GBR 13119, the parent phenylpropyl analog Thus, the low STR/CER ratios can be ascribed to loss of specific binding rather than increased nonspecific binding As all of these molecules are labeled in the same position, metabolic differences should be minimized All of the mono-fluoro compounds, GBR 13119, **3a** and **3b**, are chiral but were studied as racemic mixtures, the influence of this chirality on binding to the DA uptake site is unknown

In Table 2 it can also be found for comparison the previously published regional brain distributions of [^{18}F]GBR 12909 and a thiophene ring substituted analog, *thienyl*-[^{18}F]GBR 13119, (1-[(4-[^{18}F]fluorophenyl)(2-thienyl)methoxy)ethyl]-4-(3-phenylpropyl)

Table 2 *In vivo* regional brain distribution of [^{18}F]GBR analogs 60 min after i v administration to CD-1 mice[a]

Compound	Striatum	Cortex	Cerebellum	Blood	Striatum / Cerebellum	% ID/Brain
[^{18}F]**3a**	0 67 ± 0 03[b]	0 73 ± 0 03	0 72 ± 0 03	0 60 ± 0 05	0 94 ± 0 06	0 36 ± 0 02
[^{18}F]**3b**	0 50 ± 0 09	0 38 ± 0 03	0 41 ± 0 04	0 73 ± 0 06	1 17 ± 0 14	0 26 ± 0 03
[^{18}F]**3c** (GBR 13119)	2 80 ± 0 11	0 98 ± 0 08	0 66 ± 0 06	0 89 ± 0 16	4 24 ± 0 48	0 44 ± 0 04
[^{18}F]GBR 12909	1 82 ± 0 59	0 91 ± 0 16	0 70 ± 0 12	1 28 ± 0 25	2 70 ± 0 28	0 55 ± 0 17
Thienyl-[^{18}F]GBR 13119	2 50 ± 0 35	1 0 ± 0 21	0 63 ± 0 16	0 78 ± 0 06	4 14 ± 0 48	0 47 ± 0 05

[a] 3–28 animals per data point
[b] Data reported as % ID/g (mean ± SD)

Table 3 *In vivo* regional brain distribution of [^{125}I]GBR Analogs after i v administration to CD-1 mice[a]

Compound	Time (h)	Striatum	Cortex	Cerebellum	Blood	Striatum / Cerebellum	% ID/Brain
[^{125}I]**8a**	1 0	1 06 ± 0 13[b]	1 21 ± 0 19	1 09 ± 0 17	0 74 ± 0 07	0 97	0 55 ± 0 10
	5 0	0 13 ± 0 03	0 16 ± 0 04	0 12 ± 0 03	0 28 ± 0 02	1 08	0 07 ± 0 01
[^{125}I]**8b**	1 0	1 23 ± 0 23	1 27 ± 0 19	1 26 ± 0 19	1 13 ± 0 10	0 98	0 59 ± 0 08
	5 0	0 42 ± 0 11	0 41 ± 0 09	0 45 ± 0 11	0 87 ± 0 05	0 93	0 20 ± 0 05
[^{125}I]**8c**	1 0	1 16 ± 0 45	1 30 ± 0 48	1 23 ± 0 45	0 56 ± 0 15	0 94	0 60 ± 0 22
	5 0	1 77 ± 0 49	2 22 ± 0 61	1 76 ± 0 48	0 41 ± 0 08	1 00	0 95 ± 0 26
[^{125}I]**12**	1 0	0 79 ± 0 19	0 83 ± 0 19	0 85 ± 0 20	3 22 ± 0 58	0 93	0 39 ± 0 08
	5 0	0 40 ± 0 05	0 43 ± 0 05	0 43 ± 0 07	2 48 ± 0 30	0 93	0 22 ± 0 02

[a]3–5 animals per data point
[b]Data reported as % ID/g (Mean ± SD)

piperazine) (Kilbourn, 1989). We have consistently observed a lower striatal uptake and lower STR/CER ratio for [^{18}F]GBR 12909 when compared with [^{18}F]GBR 13119, even though these two compounds differ by only a single fluorine atom Different *in vivo* behavior for such a small structural change was unexpected Fluorine substitutions on aromatic rings can have large effects on *in vivo* pharmacology where the strongly electron-withdrawing fluorine atom affects the ionization of other ring substituents (hydroxyls, amines) or participates in through-space interactions with groups on other portions of the molecule However, this should not be the case with GBR 12909, as such groups are absent A clear explanation of the lower selectivity of the bis-fluoro analog is thus lacking Finally, the substitution of one of the phenyl rings with a thiophene ring provides a derivative with essentially the same *in vivo* distribution as [^{18}F]GBR 13119, indicating that some modifications of the structure are tolerated

Substitution of GBR 12935 and analogs with iodine proved uniformly unsuccessful, a complete loss of selective *in vivo* accumulation in the striatum was observed (Table 3) despite an *in vitro* binding affinity for compound **8c** which was essentially identical to GBR 12909 The sensitivity of the dopamine uptake carrier binding site to the length of the phenylalkyl chain, seen for the fluorine-18 compounds, was reinforced by the *in vitro* binding results for the 3-iodo analogs **8a** (IC_{50} = 176 nM) and **8b** (IC_{50} = 53 nM). The nonspecific binding of compounds **8a–c**, as evidenced by radiotracer accumulation in cerebellum and cortex, is generally higher than that seen with the fluorinated derivatives and this would be consistent with an expected increase in the lipophilicity upon iodine substitution Surprisingly, substitution of an iodine for a fluorine, as in the conversion of GBR 12909 to compound **12**, did not increase the apparent nonspecific binding, as radiotracer levels in cerebellum are quite similar Thus, although cerebellum radiotracer levels are a first approximation of nonspecific binding, caution should be exercised in comparing different radiotracers due to possible differences in radiotracer metabolism and delivery. This is perhaps supported by the very different blood level of radioactivity found for the sole analog with the iodine in the benzhydryl ether half of the molecule.

The *in vivo* biodistribution results obtained with the iodinated ligands are most easily explained on the basis of a combination of lower affinity for the dopamine uptake site and high nonspecific binding of these lipophilic compounds to hydrophobic cellular constituents. Recently, high affinity binding of this class of drugs to a second site, identified as a cytochrome P450IID1 enzyme, has been reported during *in vitro* studies with [^{3}H] GBR 12935 (Niznik *et al.*, 1990). Why the iodination of GBR 12935 should increase binding to this site is unclear. It should be noted, however, that significantly different *in vivo* biodistributions were observed for compounds **8b** and **8c**, which differ only in the position of the iodine on the aryl ring The 4-iodophenylpropyl derivative **8c** continues to accumulate in all brain tissues at 5 h, and even at 24 h does not return to the low levels observed with the isomeric compound **8b** (data not shown). Clearly these two compounds, which should have nearly identical physiochemical characteristics, show markedly different *in vivo* behavior, whether this is due to binding to a second site, or result from differences in metabolism and clearance, remains unresolved. We do know, based on thyroid radioactivity levels (data not shown), that the *in vivo* deiodination of these analogs 1 h after i.v. injection is low (< 5%) and thus does not likely contribute greatly to differences in the biodistributions of **8b** and **8c**

These *in vivo* studies have demonstrated some important requirements of the binding site of the dopamine uptake carrier as they relate to design of new radiopharmaceuticals. First, a three carbon-chain spacer between the piperazine ring nitrogen and the single phenyl ring appears optimal for an *in vivo* radiotracer Second, large substituents such as iodine can be tolerated with minimal losses of *in vitro* binding affinity, but such iodinated derivatives exhibit uniformly poor *in vivo* behavior Small substituents such as fluorine at the 4′-positions of the diphenylmethoxy group (GBR 13119, GBR 12909) are well tolerated, but large groups such as iodine have a negative effect These results complement the original SAR study of van der Zee *et al.* (1980) which showed decreases in *in vitro* binding affinities upon substitutions with methyl, methoxy and chloro groups.

In conclusion, the fluorine-18 labeled analogs prepared here do not appear to offer any advantage over

currently available radiotracers such as [^{18}F]GBR 13119 and [^{18}F]GBR 12909 for *in vivo* study of the dopamine reuptake system The radioiodinated derivatives we have prepared also do not show promise as ligands for SPECT imaging of the dopamine reuptake system

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