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Carbon-11 labeled indolylpropylamine analog as a new potential PET agent for imaging of the serotonin transporter

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ABSTRACT

The synthesis and structure–activity relationship of a new class of indole derivatives with low-nanomolar affinity for the SERT and high selectivity versus the 5-HT1A receptor were recently reported. Based on their chemical structure, four new indolylpropylamine derivatives which contain atoms to afford future labeling with PET isotopes, were synthesized and evaluated as SERT ligands. The chemistry of these novel derivatives, their biological evaluation, the general method of preparing the precursor indole for labeling, and the C-11 labeling of the most promising indole derivative, are described herein.

1. Introduction

Serotonergic neurotransmission is known to modulate numerous physiological and behavioral functions in the central nervous system (CNS).[1] It is involved in 'integrating' emotion, cognition, motor function, and pain as well as circadian rhythm and neuroendocrine functions. The serotonin (5-hydroxytryptamine, 5-HT) transporter (SERT; 5-HTT), located on presynaptic nerve endings, terminates the synaptic actions of serotonin and recycles it into the presynaptic neurotransmitter pool. Deficits in serotonergic transmission have been implicated in several neurological and psychiatric disorders such as depression, drug addiction, eating disorders, schizophrenia and obsessive compulsive disorder (OCD).[2,3] Depression affects about 100 million people worldwide, and has been ranked by the World Health Organization (WHO) as the leading cause of disability and premature death, which results in high utilization of health services and decreased work productivity.[4] Based on the implication of SERT in the pathogenesis and therapy of neuropsychiatric diseases and the possibility to use SERT as a marker of the integrity of the serotonergic system, considerable efforts have been invested in recent years in the design, synthesis, radiolabeling, and biological and pharmacological characterization of radiolabeled biomarkers (radiotracers) for non-invasive quantitative molecular imaging of SERT using nuclear medicine modalities such as Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET).[5,1] In vivo mapping of human brain SERT by PET could be very valuable for understanding alterations of the serotonergic system and might prove useful for the monitoring of antidepressant therapy.[6] Several putative SPECT and PET radiotracers for SERT have been suggested and reported, but none proved to be the ultimate agent for the imaging of the transporter. The first generation of SERT radiotracers, such as C-11 labeled cyanoimipramine, citalopram, and sertraline was characterized by various shortcomings, such as poor signal-to-noise ratios,[7–9] and lack of selectivity for the SERT over the dopamine transporter (DAT)[10] or the norepinephrine transporter (NET).[11] Until recently, the most widely used PET radiotracer for the in vivo investigation of highly expressed midbrain and thalamic SERT has been [^{11}C]McN5652.[12–14] It has been used for quantification of SERT in the normal human brain,[15–18] and in patients intoxicated with methylenedioxymethamphetamine (MDMA),[19,20] with social phobia,[21] major depression and bipolar disorder.[22] However, its rather high nonspecific binding in the brain and the resulting weak signal-to-noise ratio precludes imaging of SERT in regions of moderate or low densities such as the limbic and neocortical regions. Introducing F-18 into McN5652 could only partially improve its imaging properties.[23–25] In the series of arylnortropane compounds, radiolabeled β-CIT derivatives have been developed and used in SPECT and PET.[26–28] These SERT imaging agents have been tested in normal subjects[29–31] and in subjects with neurologic[32] and psychiatric disorders[33–35] however, they have not proven to be effective as expected in part because of their considerable and undesirable affinity for the DAT. More recently, a new series of PET radiotracers, based on the substituted diarylsulfide class of compounds, such as [^{11}C]DASB, [^{11}C]MADAM, and

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[^{11}C]AFM have emerged.[36–48] All of these compounds have been shown to be selective SERT radiotracers with varying specific-to-nonspecific binding ratios in vivo and diverse brain kinetics. The path toward the development of a useful radiotracer for in vivo imaging of SERT continues its course. The search for fluorine-18 labeled radiotracers in order to reliably determine to what extent the serotonergic system is affected in neuropsychiatric disorders remains a challenge.

Given the growing importance of the above-mentioned brain diseases for public health and the closely connected socio-economic impact of neuropsychiatric disorders, there is indeed a pressing need to develop a more suitable PET radiotracer for SERT in terms of low nonspecific binding, absence of radiolabeled metabolites that cross the blood brain barrier (BBB), measurable free fraction in plasma, and optimal affinity and selectivity. Based on a new class of indole derivatives which hold low-nanomolar affinity for the SERT and high selectivity versus the 5-HT1A receptor,[49] we synthesized four new indolylpropylamine derivatives which contain atoms to afford future labeling with PET isotopes. The chemistry of these novel derivatives, their biological evaluation, the general method of preparing the precursor indole for labeling, and the C-11 labeling of the most promising indole derivative are described herein.

2. Materials and methods

2.1. General methods

All chemicals were purchased from Sigma–Aldrich, Fisher Scientific, Merck, or J.T. Baker. Chemicals were used as supplied, excluding THF, which was refluxed over sodium and benzophenone and freshly distilled prior to use. Mass spectra were obtained on a LCQ DUO, Thermo Quest Finnigan spectrometer, equipped with ESI probe and GC Mass spectroscopy was performed in EI mode on a Thermo Quest, Finnigan Trace MS spectrometer at the Hadassah-Hebrew University Mass Spectroscopy Facility. HRMS results were obtained on MALDI-TOF and ESI mass spectrometers at the Schulich Faculty of Chemistry, Technion - Israel Institute of Technology. ^{1}H NMR spectra were obtained on a Bruker AMX 300 MHz instrument. Radiosyntheses were carried out with a [^{11}C]-CH_3I module (GE, Munster, Germany). Specific radioactivity was determined by HPLC, using cold mass calibration curves. [^{11}C]-CO_2 was produced by the ^{14}N(p, α)^{11}C nuclear reaction on nitrogen containing 1% oxygen, using an 18/9 IBA-cyclotron. Bombardment was carried out for 15–30 min with a 26 μA beam of 16 MeV protons. At the end of bombardment, the target gas was delivered and trapped by a cryogenic trap in the [^{11}C]-CH_3I module.

2.2. Chemistry

2.2.1. 3-(5-Fluoro-1H-indole-3-yl) propanol (1a)[49]

To a solution of 4-fluoro phenylhydrazine (6.92 mmol) in aqueous H_2SO_4 (4%, 10 mL), dihydropyran (630 μL) was added dropwise. The mixture was refluxed for 2 h. After cooling it was extracted with ethyl acetate (2 × 100 mL). The organic phase was washed with water and evaporated. Further purification on silica gel (1% MeOH: 99% CH_2Cl_2) yielded 300 mg (43%) of **1a**. ^{1}H NMR (DMSO-d_6) δ 10.843 (s, NH), 7.312 (m, 1H), 7.282 (d, J = 4.5 Hz, 1H), 7.205 (s, 2H), 4.474 (s, OH), 3.393 (t, J = 5.1 Hz, 2H), 2.67 (m, 2H), 1.765 (t, J = 1.2 Hz, 2H). GCMS, m/z 179.

2.2.2. 3-(5-Bromo-1H-indole-3-yl) propanol (1b)

^{1}H NMR (DMSO-d_6) δ 10.962 (s, NH), 7.679 (s, 1H), 7.32 (d, J = 3 Hz, 1H), 7.205 (s, 2H), 4.501 (s, OH), 3.497 (m, 2H), 2.7157 (m, 2H), 1.822 (t, J = 8.1 Hz, 2H). GCMS, m/z 239. 44% yield.

2.2.3. 3-(5-Iodo-1H-indole-3-yl) propanol (1c)

^{1}H NMR (DMSO-d_6) δ 10.934 (s, NH), 7.822 (s, 1H), 7.191 (m, 1H), 7.162 (s, 2H), 4.427 (s, OH), 3.429 (m, 2H), 2.669 (m, 2H), 1.774 (t, J = 6.3 Hz, 2H). GCMS, m/z 287, 36% yield.

2.2.4. 3-(3-Bromo-propyl)-5-fluoro-1H-indole (2a)[49]

To a solution of triphenylphosphine (0.025 mol) in 80 mL of dry acetonitrile, a solution of **1a** (0.02 mol) in 75 mL acetonitrile was added dropwise. Then a solution of carbon tetrabromide (0.027 mol) in 25 mL acetonitrile was added dropwise. The reaction mixture is allowed to stir at rt for 3 h. Evaporation and purification on silica-gel column (50% hexanes:50% CH_2Cl_2) yielded 4.5 g (80%) of **2a**. ^{1}H NMR (DMSO-d_6) δ 11.02 (s 1H), 7.55 (s,1H), 7.23 (d, J = 1.2 Hz, 1H), 7.08 (m, 2H), 3.42 (t, J = 1.4 Hz, 2H), 2.89 (t, J = 7.1 Hz, 2H), 2.112 (m, 2H). GCMS, m/z 241.

2.2.5. 3-(3-Bromo-propyl)-5-bromo-1H-indole (2b)

^{1}H NMR (DMSO-d_6) δ 11.036 (s, NH), 7.684 (s, 1H), 7.309 (d, J = 0.6 Hz, 1H), 7.197 (s, 1H), 7.158 (d, J = 6.6 Hz, 1H), 3.536 (t, J = 0.9 Hz, 2H), 2.794 (t, J = 7.2 Hz, 2H), 2.125 (m, 2H). GCMS, m/z 203.

2.2.6. 3-(3-Iodo-propyl)-5-bromo-1H-indole (2c)

^{1}H NMR (DMSO-d_6) δ 11.006 (s, NH), 7.856 (s,1H), 7.308 (d, J = 8.1 Hz, 1H), 7.201 (m, 2H), 3.539 (t, J = 3.9 Hz, 2H), 2.783 (t, J = 6.6 Hz, 2H), 2.119 (m, 2H). MS, m/z 349.

2.2.7. 6-Methoxy-1,2,3,4-tetrahydroisoquinoline (3a)[50]

3-2-(3-Methoxy-phenyl)-ethylamine(0.013 mol) was dissolved in 1 N HCl to obtain pH = 0.4. Then, aqueous formaldehyde (39%, 1.88 mL, 25 mmol) was added. The mixture was stirred at room temperature for 24 h. The solution was basified with sodium hydrogen carbonate, extracted with ethyl acetate (3 × 50 mL) and evaporated. Purification of the two isomers; 6-methoxy- and 8-methoxy-1,2,3,4-tetrahydroisoquinoline with silica-gel column yielded 0.7 g (35%) of **3a**. ^{1}H NMR (DMSO-d_6) δ 9.05 (s, NH), 7.11 (d, J = 2.7 Hz, 1H), 6.92 (s, 2H) 4.08 (s, 2H), 3.88 (s, 3H), 3.28 (s, 2H), 3.05 (t, J = 5.0 Hz, 2H). MS (m/z) 163 (MH^+).

2.2.8. 5-Methoxy-1,2,3,4-tetrahydroisoquinoline (3b)[50]

2-(2-Methoxy-phenyl)-ethylamine (0.013 mmol) was dissolved in 1N HCl to obtain pH = 0.4. Then, aqueous formaldehyde (1.88 mL, 39%, 25 mmol) was added. The mixture was stirred at room temperature for 24 h. The solution was basified with sodium hydrogen carbonate, extracted with ethyl acetate (3 × 50 mL) and evaporated. Purification with silica-gel column yielded 0.8 g (38%) of **3b**. ^{1}H NMR (DMSO-d_6) δ 9.12 (s, NH), 7.10 (d, J = 2.7 Hz, 1H), 6.81 (s, 2H) 4.2 (s, 2H), 3.85 (s, 3H), 3.35(s, 2H), 3.1 (t, J = 5.0 Hz, 2H). MS (m/z) 163 (MH^+).

2.2.9. 2-[3-(5-Fluoro-1H-indol-3-yl)-propyl]-6-methoxy-1,2,3,4- tetrahydro-isoquinoline (4)[50]

A mixture of 3-(3-bromo-propyl)-5-fluoro-1H-indole (0.0039 mol) and 6-methoxy-1,2,3,4-tetrahydro-isoquinoline (0.0039 mol) and triethylamine (0.8 mL, 0.0058 mol) in DMSO (10 mL) was stirred at 100 °C for 24 h. The reaction mixture was cooled and extracted with ethyl acetate (3× 50 mL). The organic phase was washed with water (200 mL), dried with magnesium sulfate, and evaporated. Purification on silica gel (2% MeOH: 98% CH_2Cl_2) yielded 0.26 g (20%) of **4**. ^{1}H NMR (DMSO-d_6) δ 10.858 (s, NH), 7.315 (m, 3H), 6.949 (m, 2H), 6.679 (m, 2H), 3.677 (s, 3H), 3.439 (s, 2H), 2.762 (t, J = 5.1 Hz, 2H), 2.664 (t, J = 6.6 Hz, 2H), 2.598 (t, J = 5.4 Hz, 2H) 2.476 (m, 2H), 1.859 (t, J = 7.2 Hz, 2H). MS (m/z) 338 (MH^+), HRMS ($C_{21}H_{24}N_2OF$): 339.1870.

2.2.10. 2-[3-(5-Fluoro-1H-indol-3-yl)-propyl]-5-methoxy-1,2,3,4-tetrahydro-isoquinoline (5)[49]

^{1}H NMR (DMSO-d_6) δ 10.858 (s, NH), 7.315 (m, 3H), 6.949 (m, 2H), 6.679 (m, 2H), 3.677 (s, 3H), 3.439 (s, 2H), 2.762 (t, J = 5.1 Hz, 2H), 2.664 (t, J = 6.6 Hz, 2H), 2.598 (t, J = 5.4 Hz, 2H) 2.476 (m, 2H), 1.859 (t, J = 7.2 Hz, 2H). MS (m/z) 338 (MH^+). HRMS ($C_{21}H_{24}N_2OF$): 339.1873.

2.2.11. 2-[3-(5-Bromo-1H-indol-3-yl)-propyl]-6-methoxy-1,2,3,4-tetrahydro-isoquinoline (6)

^{1}H NMR (DMSO-d_6) δ 10.992 (s, NH), 7.666 (s, 1H), 7.298 (d, J = 1.8 Hz, 1H), 7.184 (m, 2H), 6.952 (d, J = 8.4 Hz, 1H), 6.677 (m, 2H), 3.675 (s, 3H), 3.45(s, 2H), 3.350 (t, J = 5.1 Hz, 2H), 2.784 (t, J = 5.3 Hz, 2H), 2.653 (t, J = 5.4 Hz, 2H) 2.581 (m, 2H), 1.851(t, J = 7.2 Hz, 2H). MS (m/z) 398 (MH^+). HRMS($C_{21}H_{24}N_2OBr$): 399.1070.

2.2.12. 2-[3-(5-Bromo-1H-indol-3-yl)-propyl]-5-methoxy-1,2,3,4- tetrahydro-isoquinoline (7)

^{1}H NMR (DMSO-d_6) δ 10.968 (s, NH), 7.663 (s, 1H), 7.293 (d, J = 2.1 Hz, 1H), 7.180 (m, 2H), 6.949 (d, J = 8.4 Hz, 1H), 6.669 (m, 2H), 3.677 (s, 3H), 3.443 (s, 2H), 3.152 (t, J = 5.3 Hz, 2H), 2.774 (t, J = 5.3 Hz, 2H), 2.703 (t, J = 5.4 Hz, 2H) 2.653 (m, 2H), 1.851 (t, J = 7.1 Hz, 2H). MS (m/z) 398 (MH^+), HRMS($C_{21}H_{24}N_2OBr$): 399.1072.

2.2.13. 2-[3-(5-Iodo-1H-indol-3-yl)-propyl]-6-methoxy-1,2,3,4-tetrahydro isoquinoline (8)

^{1}H NMR (DMSO-d_6) δ 10.969 (s, NH), 7.844 (s, 1H), 7.274 (m, 1H, J = 1.8 Hz), 7.195(m, 2H, J = 2.7 Hz), 6.931 (d, 3H, J = 3.0 Hz), 3.650 (s, 3H), 3.45(s, 2H), 3.350 (t, J = 5.1 Hz, 2H), 2.794 (t, J = 7.5 Hz, 2H), 2.643 (t, J = 6.6 Hz, 2H) 2.584 (m, J = 6.3 Hz, 2H), 1.831(t, J = 7.4 Hz, 2H). MS (m/z) 446 (MH^+), HRMS($C_{21}H_{24}N_2OI$): 447.0933.

2.2.14. 2-[3-(5-Iodo-1H-indol-3-yl)-propyl]-5-methoxy-1,2,3,4-tetrahydro-isoquinoline (9)

^{1}H NMR (DMSO-d_6) δ 10.945 (s, NH), 7.834 (s, 1H), 7.294 (m, 1H), 7.187 (m, 2H), 6.739 (d, J = 8.1 Hz, 3H), 3.736 (s, 3H), 3.481 (s, 2H), 3.314 (t, J = 1.8 Hz, 2H), 2.691 (t, J = 7.5 Hz, 4H), 2.481 (t, J = 6.5 Hz, 2H), 1.848 (t, J = 7.4 Hz, 2H). LCMS, m/z (relative intensity) MS (m/z) 446 (MH^+), HRMS($C_{21}H_{24}N_2OI$): 447.0928.

2.2.15. 6-Hydroxy-1,2,3,4-tetrahydroisoquinoline (10)

Compound **3a** (0.003 mol) was dissolved in hydrobromic acid (conc., 15 mL) and refluxed for 3 h. The reaction mixture was cooled and basified with sodium bicarbonate, extracted with ethyl acetate (2 × 50 mL), dried with $MgSO_4$, and evaporated. Crystallization from MeOH gave 0.045 g (10%) of **10**. ^{1}H NMR (DMSO-d_6) δ 6.812 (d, J = 7.5 Hz, 1H), 6.501 (d, J = 6.2 Hz,1H), 6.379 (d, J = 3.6 Hz, 1H), 3.729 (s, 2H), 2.881 (t, J = 1.4 Hz, 2H), 2.419 (t, J = 1.2 Hz, 2H). MS (m/z) 149 (MH^+).

2.2.16. 2-[3-(5-Fluoro-1H-indol-3-yl)-propyl]-6-hydroxy-1,2,3,4-tetrahydro-isoquinoline (11)

A mixture of 3-(3-bromo-propyl)-5-fluoro-1H-indole (0.0039 mol), 6-hydroxy-1,2,3,4-tetrahydro-isoquinoline (0.0039 mol), and triethylamine (0.8 mL, 0.0058 mol) in DMSO (10 mL) was stirred at 100 °C for 24 h. The reaction mixture was cooled and extracted with ethyl acetate (3 × 50 mL). The organic phase was washed with water (200 mL), dried with magnesium sulfate, and evaporated. Purification on silica gel (2% MeOH: 98% CH_2Cl_2) yielded 0.65 g (48%) of **11**. ^{1}H NMR (DMSO-d_6) δ 10.858 (s, NH), 9.089 (s, OH), 7.323 (m, J = 4.8 Hz, 3H), 6.907 (m, J = 8.1 Hz, 2H), 6.521 (m, J = 2.1 Hz, 2H), 3.160 (s, 2H), 2.711 (m, J = 4.5 Hz, 3H), 2.601 (t, J = 5.1 Hz, 2H), 2.486 (t, J = 1.5 Hz, 2H), 1.977 (t, J = 1.8 Hz, 3H). MS (m/z) 324 (MH^+).

2.3. Biological assay

2.3.1. Cell culture

HEK293 cells stably expressing human monoamine reuptake transporters were obtained from R. Blakely (hSERT-HEK293; Vanderbilt University, Nashville, USA), H. Boenisch (hNET-HEK293; University of Bonn, Germany), and A. Storch (hDAT-HEK293; Technical University Dresden, Germany). The transfected cells, maintained at 37 °C in a 5% CO_2 humidified atmosphere, were grown in complete medium (Dulbecco's modified Eagle's medium, 10% fetal calf serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin) supplemented with 250 μg/mL G418. The cells were grown to 95% confluency in 175 cm^2 cell culture flasks. Medium was aspirated, and cells were washed with phosphate-buffered saline and collected by scraping. The cells were sedimented by low-speed centrifugation at 800 rpm, resuspended in ice-cold buffer (50 mM Tris–HCl, pH 7.4) for cell disruption, and stored at −25 °C. The protein concentrations of the membrane suspensions were determined with the BCA assay.

2.3.2. Animals

Female SPRD rats (approximately 200 g., 10–12 weeks old; Medizinisch-Experimentelles Zentrum, University Leipzig) were anesthetized by carbon dioxide inhalation and killed by cervical dislocation. Brains were immediately excised and dissected on ice. The cortices were collected, homogenized in ice-cold 50 mM Tris–HCl (pH 7.4 at 4 °C) with a Potter Elvehjem homogenizer, and centrifuged (20.000g, 15 min, 4 °C). The membrane pellet was washed two times by rehomogenization and centrifugation as before. The final membrane pellet was dissolved with 50 mM Tris–HCl (pH 7.4 at 4 °C) at 1 g original wet weight/10 mL buffer, and stored in aliquots at −25 °C.

2.3.3. In vitro hSERT, hNET, hDAT, and rHT1A binding assays

For hSERT, hNET, and hDAT, the IC_{50} values of the test compounds were determined on preparations of HEK293 cells expressing the respective transporter protein and [^{3}H]paroxetine (SERT; PerkinElmer Life Sciences; A_S = 706 GBq/mmol), [^{3}H]citalopram (SERT; Amersham GE Healthcare; A_S = 3121 GBq/mmol), [^{3}H]nisoxetine (NET; Perkin-Elmer Life Sciences; A_S = 2960 GBq/mmol), and [^{3}H]WIN35,428 (DAT; PerkinElmer Life Sciences; A_S = 3145 GBq/mmol) as specific radioligands. The r5HT$_{1A}$ affinity was determined on rat cortical membrane homogenate and [^{3}H]-8-OH-DPAT (Amersham GE Healthcare; A_S = 8399 GBq/mmol) as specific radioligand.

For competitive binding experiments, membrane suspensions were thawed, diluted with the respective assay buffer (hSERT and hNET: 50 mM Tris–HCl, pH 7.4, 120 mM NaCl, and 5 mM KCl; hDAT: 50 mM Tris–HCl, pH 7.4, and 100 mM NaCl; r5HT$_{1A}$: 50 mM Tris–HCl, pH 7.4, 4 mM $CaCl_2$, 0.1% ascorbic acid) and homogenized by a 27-gauge needle. The particular membrane preparation (50–80 μg protein/assay tube) was incubated with the respective radioligand (∼working concentrations: 1 nM [^{3}H]paroxetine, 0.5 nM [^{3}H]citalopram, 0.5 nM [^{3}H]nisoxetine, 0.5 nM [^{3}H]WIN35,428, 0.3 nM [^{3}H]-8-OH-DPAT) and 6–12 concentrations of the test compounds in glass tubes. The test compounds (10 mM stock solutions in DMSO) were diluted with the respective assay buffer. Nonspecific binding was determined with 300 μM clomipramin (hSERT), 100 μM protriptyline (hNET), 10 μM GBR12909 (hDAT), or 10 μM serotonin hydrochloride (r5HT$_{1A}$). The tubes were gently mixed and incubated at 21 °C for 60 min (hSERT, hNET, and r5HT$_{1A}$), or on ice for 120 min (hDAT), rapidly filtered through Whatman GF/B glass-fibre filters, and washed four times with ice-cold 50 mM Tris–HCl, pH 7.4. Filter-bound radioactivity was determined by scintillation counting.

Saturation binding experiments of [^{3}H]paroxetine, [^{3}H]citalopram, [^{3}H]nisoxetine, and [^{3}H]WIN35,428 were performed by homologous competition according to the described protocols.

2.3.4. Data analysis

All test compounds were assayed in at least three independent experiments, each conducted in triplicate. The binding parameters IC_{50}, K_D, and B_{max} were estimated using iterative nonlinear curve fitting. The K_D of [^{3}H]-8-OH-DPAT was taken from literature (K_D = 3.1 nM on rat cortex; Newman-Tancredi et al., *Int. J. Neuropsychopharmacol. 8*, **2005**, 1–16). The Cheng–Prusoff equation was applied to calculate K_i values from estimated IC_{50} values.

2.4. Radiochemistry

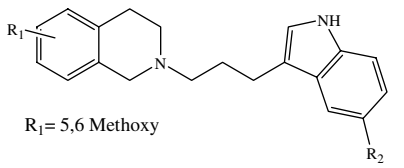
Carbon-11 MeI was prepared according to well-documented procedures. Briefly, [^{11}C]CO_2 (37 GBq, 1000 mCi) was trapped at −160 °C. The temperature of the cooling trap was increased to −50 °C, and the activity was transferred by a stream of argon (40 mL/min) to a reactor containing 300 μL of 0.25 N $LiAlH_4$ in THF at −50 °C. After 90 s, the solvent was removed under reduced pressure. In this manner, more than 80% of the activity was recovered. The reactor temperature was increased to 160 °C, HI was added, and [^{11}C]MeI was distilled (argon flow of 15 mL/min) through a NaOH column to a second reactor, containing the precursor, **11**, in 600 μL of dry DMF and 30 μL NaOH 5 N at −20 °C. After 1 min of distillation, an average of 550 ± 30 mCi (n = 4) was trapped in the second reactor. The reactor was sealed and heated to 80 °C for 5 min. The mixture was cooled to 40 °C, 600 μL CH_3CN/H_2O (1:1) was added, and the crude product (250 ± 20 mCi) was automatically injected to HPLC [Bischoff Nucleosil 100-7 C18 reverse phase preparative column (7 μm, 250× 16 mm, 55% ACN: 45% Acetate buffer, pH 3.8, flow rate of 10 ml/min]. The labeled compound was collected in a flask containing 85 mL of water. The solution was passed through a C-18 cartridge (Waters Sep-Pak Plus, pre-activated with 10 mL EtOH and 20 mL of water). The product was eluted with 0.75 mL of EtOH, followed by 4.25 mL of saline, and collected into the product vial after a total radiosynthesis time of 50 min, with total activity of 20 ± 6 mCi and radiochemical yield of 15 ± 3% decay corrected EOB (n = 4). Identification of the products and determination of chemical purity were obtained by reversed-phase HPLC C-18 analytical column in comparison with the standard and by co-injection.

3. Results and discussion

3.1. Chemistry

The chemical structure of indole derivatives and 5-HT serves as a template for the development of new chemical entities that could potentially target the 5-HT1A receptor and the SERT. In 2001, Meagher et al.[49] designed a new class of indole derivatives as a dual antidepressant drug targeting the SERT and the 5-HT1A receptor. The biological evaluation of these compounds indicated that some of these derivatives had high affinity and selectivity toward the SERT. Based on these results, we synthesized additional four new indole derivatives and evaluated all six compounds as potential selective SERT PET radiotracers. These compounds contain atoms such as fluorine, bromine, and iodine on the indole moiety to afford future labeling with PET isotopes (Fig. 1).

Scheme 1 shows the synthesis of the target molecules. The hydroxypropyl indole intermediates **1a–c** were obtained by reaction of 4*R* phenylhydrazine and dihydropyran in 43, 44, and 37% yields, respectively. The second step is bromination with carbon tetrabromide and triphenylphosphine to obtain **2a–c** in 80% yield.



R_1= 5,6 Methoxy

R_2= F, Br, I

4: F-6 Methoxy **5**: F-5 Methoxy
6: Br-6 Methoxy **7**: Br-5 Methoxy
8: I-6 Methoxy **9**: I- 5 Methoxy

Figure 1. Chemical structure of the SERT ligands used for PET radiotracer design.

The tetrahydroisoquinoline analogs, **3a–b**, were prepared by Pictet-Spengler cyclization of methoxy phenylethylamine with formaldehyde in acidic solution with a 10% yield. This procedure simplifies the multi-step synthesis via the chloroformate intermediate as described by Meagher et al.[49] In the case of 2-methoxy phenylethylamine, the cyclization with formaldehyde yielded only the 5-methoxy tetrahydroisoquinoline product, whereas, in the case of 3-methoxy phenylethylamine, cyclization yielded a mixture of 6- and 8-methoxy tetrahydroisoquinoline products. The 8-methoxy tetrahydroisoquinoline was obtained as the minor product, probably due to steric hindrance. A coupling reaction of methoxy-1,2,3,4-tetrahydroisoquinoline and 3-(3-bromo-propyl)-5-*R*-1H-indole yields the six final compounds **4–9**. The yield and selectivity of the final step are rather low (20%).

The synthesis of the precursor **11** for the radiolabeling is described in Scheme 2. The first step is demethylation with HBr conc. at 120 °C to obtain **10** in 10% yield, followed by coupling reaction in DMSO in the presence of triethylamine as base in 20% yield.

3.2. Affinity data

For [^{3}H]paroxetine, [^{3}H]citalopram, [^{3}H]nisoxetine, and [^{3}H]WIN35,428 the following K_D values were obtained on the human monoamine transporters hSERT, hNET, and hDAT by homologous competition experiments: 0.69 nM [^{3}H]paroxetine, 4.46 nM [^{3}H]citalopram, 6.77 nM [^{3}H]nisoxetine, and 24.1 [^{3}H]WIN35,428. The values correspond to previously published data on these monoamine transporters.[51,52]

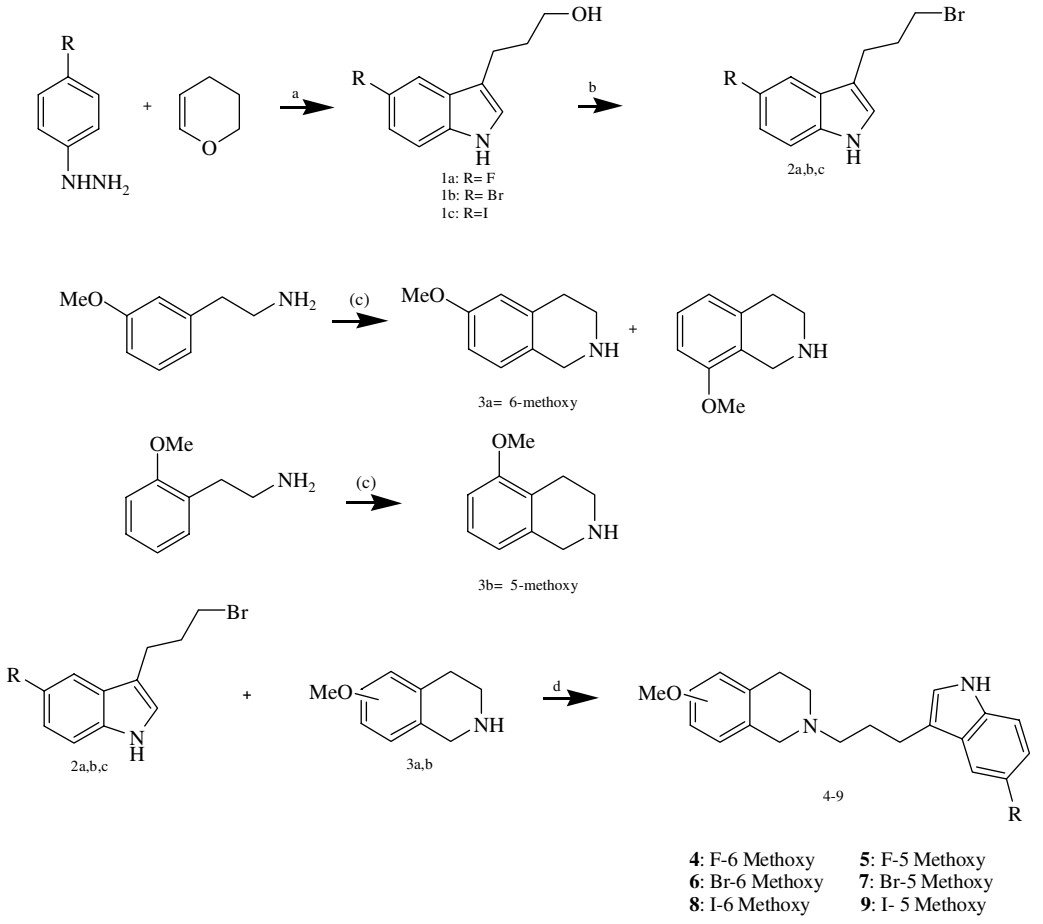
The specific K_i values of the two reference compounds **4, 5** and the four newly developed compounds **6, 7, 8, 9** (Fig. 1) are given in Table 1. While compounds **4, 5**, and **6** possess high affinity in the nanomolar range and 70- to 200-fold specificity for SERT over DAT and NET, compounds **7, 8**, and **9** are characterized by remarkably low SERT affinity and selectivity. Compounds **4** and **5** are particularly interesting in this series because they possess the highest SERT selectivity versus the $r5HT_{1A}$ receptor. Furthermore, in contrast to the diphenyl-thioether MADAM, compounds **4** and **5** discriminate significantly between the hSERT binding sites labeled with either [^{3}H]citalopram and [^{3}H]paroxetine, respectively, ($p < 0.05$, t test). This preliminary result may indicate the possibility to detect the integrity of SERT by investigating different parts of the protein conformation.

Due to its affinity and specificity profile, compound **4** was chosen as the most promising candidate for radiolabeling with the PET isotope carbon-11.

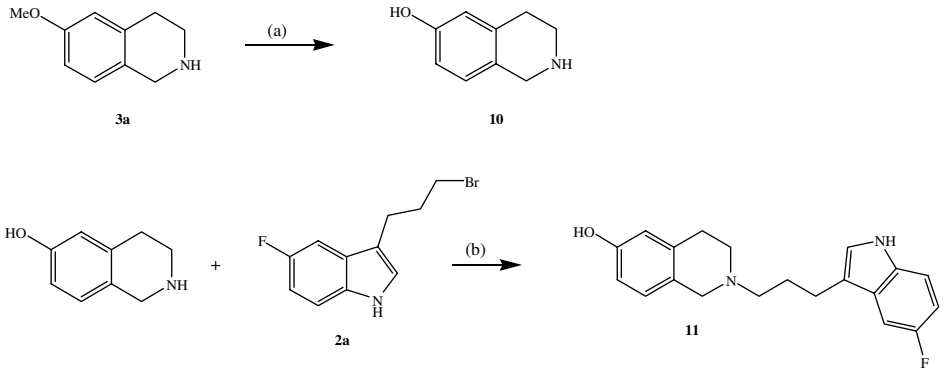
3.3. Radiochemistry

Based on the biological assay, compound **4** was chosen for labeling with C-11 on the hydroxy position at the isoquinoline ring.

The radiosynthesis is outlined in Scheme 3. The radiolabeling method was based on a C-11 methylation reaction on the phenolic



Scheme 1. Reagents and conditions: (a) H_2SO_4 (4%), 100 °C, 2 h; (b) CBr_4, PPh_3, CH_2Cl_2, 3 h; (c) HCHO (39%), 1 N HCl, rt, 24 h; (d) Et_3N, DMSO, 100 °C, 24 h.

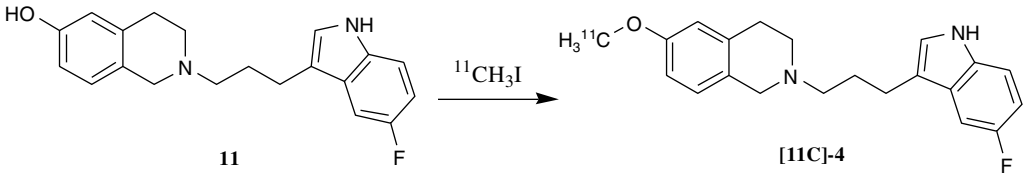


Scheme 2. Reagents and conditions: (a) HBr, 120 °C, 3 h; (b) Et_3N, DMSO, 100 °C, 24 h.

Table 1
Binding affinities of reference and test compounds at hSERT, $r5HT_{1A}$, hDAT, and hNET

Target/structure	hSERT; K_i (nM)		$r5HT_{1A}$; K_i (nM)	hNET; K_i (nM)	hDAT; K_i (nM)
Radioligand	[^{3}H]citalopram	[^{3}H]paroxetine	[^{3}H]-8-OH-DPAT	[^{3}H]nisoxetine	[^{3}H]WIN35,428
4	4.11 ± 1.34	25.3 ± 14.2	54.0 ± 5.40	1680 (1810; 1560)	2490 (2620; 2360)
6	11.4 ± 3.50	13.3 ± 7.22	50.0 ± 5.46	2770 ± 1940	1030 (1290; 769)
8	374 ± 226	1110 ± 617	685 ± 184	712 ± 524	3650 (1800; 5500)
5	6.46 ± 3.94	44.7 ± 6.19	164 ± 176	2880 (1990; 3770)	1485 (1700; 1270)
7	1910 ± 468	2980 ± 1320	1990 ± 157	6600 ± 2300	3490 ± 1820
9	213 ± 51.1	2660 ± 1480	1120 ± 99.9	1810 (2490; 1120)	1155 (1140; 1170)
Paroxetine	0.42 ± 0.13	0.38 ± 0.12	–	—	—
R/*S*-Citalopram	4.38 ± 1.17	32.8 ± 8.69	—	—	—
MADAM	0.73 ± 0.37	2.03 ± 1.08	—	—	—

IC_{50} values were obtained from triplicates in each experiment, and the calculated K_i values are given as single values ($n = 1$), means and single values ($n = 2$), or means ± SD ($n > 2$).



Scheme 3. Reagents and conditions: DMF, NaOH 5 N, 80 °C.

hydroxy group. C-11 methyliodide was produced using a commercial module (GE) and was prepared according to well documented procedures.[53] C-11 methyliodide was then reacted with 5 mg of precursor **11**. The optimal reaction temperature was found to be 80 °C. After 5 min reaction, HPLC solvent was added and the crude mixture was purified by a built-in HPLC to yield the final product [^{11}**C**]-**4**. The product was collected into a flask containing 85 ml of water in order to dilute the acetonitrile. Finally the product was eluted through C-18 cartridge with ethanol/saline solution. Total synthesis time was 50 min. HPLC analysis showed moderate radiochemical yield (15 ± 3% decay corrected EOB) and high chemical purity. The high specific activity (0.423 Ci/μmol) is an important factor for the development of PET biomarkers that target low capacity systems.

4. Conclusion

Six indolylpropylamine derivatives were synthesized and evaluated as SERT antagonists. The most promising compound, 2-[3-(5-fluoro-1H-indol-3-yl)-propyl]-6-methoxy-1,2,3,4-tetrahydroisoquinoline was identified and successfully labeled with C-11. This compound will be further evaluated in vivo using PET in animal models.

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