Synthesis, Sigma Receptor Binding Studies, and *In Vivo* Evaluation of Radioiodinated (*Z*)- and (*E*)-iodoallyl Analogs of SA4503

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Abstract

SA4503, a potent σ_1 receptor agonist, is under study for functional recovery after stroke, and has been tested for treatment of major depression. Recent behavioral studies indicate that SA4503 can also display antagonist properties, and attenuates psychostimulant-induced hyperactivity in animal models. Further, SA4503 labeled with carbon-11 (half-life 20.4 min), or analogs labeled with fluorine-18 (half-life 109.7 min), are useful for PET studies of the σ_1 receptor. Analogs labeled with iodine-123 (13.2 h half-life) would have potential as SPECT imaging agents, while analogs labeled with iodine-125 (60.1 d half-life) could be used routinely in laboratory studies. Toward these ends, we describe the synthesis and radiolabeling, as well as *in vitro* and *in vivo* binding studies, of two SA4503 analogs where the 4-methoxy group of the dimethoxyphenethyl moiety is replaced by either a (*Z*)- or (*E*)-iodoallyloxy substituent. The iodoallyl groups were introduced by base-promoted coupling of stannylated alkylating agents to 4-*O-des*-methyl-SA4503, followed by iododestannylation with retention of configuration. Both (*Z*)- and (*E*)-iodoallyl-SA4503 displayed moderately high affinities for σ_1 and σ_2 receptors *in vitro* (K_i values 11-18 nM). The corresponding radioiodinated ligands were prepared in good yields (57-58%), with high purities (>97%) and high specific activities (>2000 mCi/μmol). Both radioligands readily crossed the blood-brain-barrier of mice, although their log $D_{7.4}$ values of 3.6 were relatively high. Haloperidol pretreatment defined a modest degree of specific binding to σ_1 receptors, but only for the [^{125}I]-labeled (*E*)-isomer in mouse brain (28%) and liver (25%) at 60 min. Thus, these particular radioligands are not well suited to *in vivo* studies. More significantly, the work shows that σ receptors display substantial tolerance to bulky structural modifications of SA4503, a feature that might aid in the future development of possible therapeutics based on the SA4503 scaffold.

Keywords: Sigma receptor; SA4503; Structure activity relationship; Radioiodine; Lipophilicity

Abbreviations: SPECT: Single Photon Emission Computed Tomography; PET: Positron Emission Tomography; HPLC: High Performance Liquid Chromatography; t_R: Retention Time; HRMS-ESI: High Resolution Mass Spectrometry - Electrospray Ionization; NMR: Nuclear Magnetic Resonance; Tris-HCl: *Tris*(Hydroxymethyl) Aminomethane Hydrochloride; DMF: Dimethylformamide; %ID: Percent Injected Dose; SD: Standard Deviation; SEM: Standard Error of the Mean; SA4503: 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine; *o*-BON: 1-[2-(3,4-Dimethoxyphenyl)ethyl]-4-[3-(2-iodophenyl)propyl]piperazine; *m*-BON: 1-[2-(3,4-Dimethoxyphenyl) ethyl]-4-[3-(3-iodophenyl)propyl]piperazine

Introduction

SA4503 (Figure 1) has potent sigma$_1$ (σ_1) receptor agonist properties [1], and is under study in Phase II clinical trials for functional recovery after stroke [2]. SA4503 also has been tested for treatment of major depressive disorder (ClinicalTrials.gov Identifier: NCT00551109). Recent behavioral studies indicate that SA4503 can also display antagonist properties, and attenuates cocaine- and methamphetamine-induced hyperactivity in animal models [3,4]. Thus, SA4503 may be best described as a mixed agonist-antagonist. SA4503 has been labeled with carbon-11 (half-life 20.4 min), and used successfully for PET studies of the σ_1 receptor in human beings [5-7]. The fluoroethyl analog, FE-SA4503 (Figure 1), has been labeled with fluorine-18 (half-life 109.7 min) and also allows PET visualization of the σ_1 receptor in conscious monkey brain [8,9].

Our interest in the development of probes for σ receptors [10-14] has prompted us to investigate a pair of novel iodoallyl (IA) analogs of SA4503, (*Z*)- and (*E*)-IA-SA4503, that can readily incorporate radioiodine (Figure 1; 1, 2). [^{125}I]-Labeled receptor ligands (60.1 d half-life) have high specific activity (2175 mCi/μmol), are easy to detect without tissue processing, and can be used routinely for laboratory studies. Further, [^{123}I]-labeled ligands (13.2 h half-life) have the potential for use as SPECT imaging agents in nuclear medicine clinics, and offer some advantages over PET ligands labeled with short half-life radionuclides such as carbon-11 and fluorine-18. SA4503 analogs radioiodinated at the aromatic ring of the 3-phenylpropyl moiety, *o*- and *m*-BON (Figure 1), have been reported by others [15,16]. Here we describe the synthesis and radiolabeling, as well as *in vitro* and *in vivo* studies, of complementary SA4503 analogs where radioiodine is incorporated through (*Z*)- or (*E*)-iodoallyloxy substituents at the 4-position of the dimethoxyphenethyl moiety.
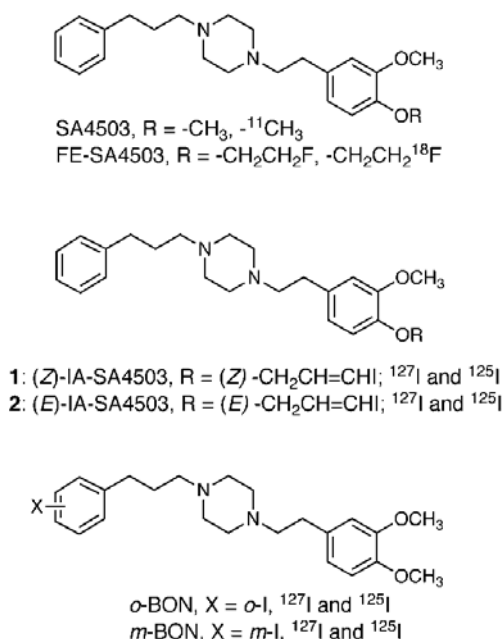
Materials and Methods

General information

Chemicals and solvents were reagent grade, and used as received from commercial sources. Literature procedures were used to

SA4503, R = -CH₃, -¹¹CH₃
FE-SA4503, R = -CH₂CH₂F, -CH₂CH₂¹⁸F

1: (*Z*)-IA-SA4503, R = (*Z*)-CH₂CH=CHI; ¹²⁷I and ¹²⁵I
2: (*E*)-IA-SA4503, R = (*E*)-CH₂CH=CHI; ¹²⁷I and ¹²⁵I

o-BON, X = o-I, ¹²⁷I and ¹²⁵I
m-BON, X = m-I, ¹²⁷I and ¹²⁵I

Figure 1: Structures of SA4503, FE-SA4503, novel (*Z*)- and (*E*)-iodoallyl analogs of SA4503, o-BON and m-BON.

prepare 4-*O*-*des*-methyl-SA4503 [8], as well as (*Z*)- and (*E*)-3-(tri-*n*-butylstannyl)prop-2-en-1-ol 4-methylbenzenesulfonate [17]. ¹H NMR spectra were obtained on Bruker ARX-250 (250 MHz) or DRX-300 (300 MHz) spectrometers. ¹³C NMR spectra were obtained on Bruker ARX-250 (62.5 MHz) or DRX-300 (75 MHz) spectrometers. Chemical shifts (¹H) are reported in ppm (δ) relative to internal tetramethylsilane in CDCl₃, or to the central peak of CDCl₃ (¹³C). HRMS-ESI analyses were obtained using a TSQ7000 mass spectrometer (Thermo Finnigan, San Jose, CA). Compounds 1 and 2 were converted to di-HCl salts for storage and ease of handling. Elemental analyses were determined by Atlantic Microlab, Inc., Norcross GA. Column Column chromatography was performed under N₂ pressure using 230-400 mesh Silicycle® UltraPure silica gel.

No-carrier-added [¹²⁵I] NaI, as well as [³H]DTG and [³H] (+)-pentazocine, were obtained from PerkinElmer, Inc. (Waltham, MA). A Brandel R48 manifold (Brandel Instruments, Gaithersburg, MD) was used for rapid filtrations of receptor binding assays. Tritium radioactivity was measured using a Wallac 1409 liquid scintillation counter and OptiPhase® HiSafe 2 cocktail at 44% efficiency. Radioiodine was measured during synthetic procedures with a dose calibrator (Capintec CRC-15W). Analytical radioiodine counting was performed with a Wallac (Turku, Finland) Wizard® 1480 automated gamma counter at 78% efficiency. A Waters (Milford, MA) C-18 Nova-Pak® radial compression module column (8×100 mm, 4 μm) was used for semi-preparative HPLC, and a Waters C-18 Nova-Pak® stainless steel column (3.9×150 mm, 4 μm) was used for analytical HPLC. Solid-phase extraction cartridges (Waters Sep-Pak® Light *t*-C-18) were activated prior to use by sequential elution with EtOH and H₂O.

Frozen Hartley guinea pig brains were obtained from Rockland Immunochemicals, Inc. (Gilbertsville, PA). Male CD1 mice were obtained from Charles River Laboratories International, Inc. (Wilmington, MA). All studies involving living animals were performed humanely in compliance with National Institutes of Health guidelines, and with prior approvals from the Institutional Animal Care and Use

Committees of the University of Missouri and the Harry S. Truman Memorial Veterans' Hospital.

Chemistry

(*Z*)-1-(3-methoxy-4-(3-(tri-*n*-butylstannyl)allyloxy)phenethyl)-4-(3-phenylpropyl)piperazine (4): NaH (6.3 g, 0.26 mol) was added to a stirred solution of 4-*O*-*des*-methyl-SA4503•2HCl (7.0 g, 0.016 mol) in 300 mL DMF under N₂ at room temperature. After 1 min, a solution of (*Z*)-3-(tri-*n*-butylstannyl)prop-2-en-1-ol 4-methylbenzenesulfonate (30.0 g, 0.06 mol) in 80 mL DMF was added. After 90 min, the reaction was quenched by slow addition of saturated NH₄Cl and extracted with CH₂Cl₂. The extracts were washed with saturated NaHCO₃ and concentrated under reduced pressure. Column chromatography (CHCl₃:MeOH, 20:1) gave 4 (7.69 g, 70%) as a yellow oil. ¹H NMR: (CDCl₃) 0.85-0.96 (m, 15H, *n*-Bu), 1.27-1.46 (m, 12H, *n*-Bu), 1.79-1.95 (m, 2H, CH₂), 2.56-2.63 (m, 16H, CH₂), 3.84 (s, 3H, CH₃), 4.49-4.51 (dd, 2H, CH₂, *J*=1.1, 5.9 Hz), 6.17-6.22 (dt, 1H, CH, *J*=1.1, 13.1 Hz), 6.68-6.82 (m, 4H, alkene CH, aromatic CH), 7.17-7.29 (m, 5H, aromatic CH).

(*Z*)-1-(4-(3-iodoallyloxy)-3-methoxyphenethyl)-4-(3-phenylpropyl)piperazine (1): A solution of 4 (2.0 g, 2.99 mmol) in 40 mL CH₂Cl₂ was added to I₂ (859 mg, 3.38 mmol) in 20 mL CH₂Cl₂. The reaction was stirred for 10 min at room temperature and quenched with an aqueous solution of Na₂S₂O₅. The organic layer was separated and the aqueous phase was extracted with CH₂Cl₂. The combined organic extracts were dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Dissolution in EtOH followed by precipitation with HCl gave 1 as the di-HCl salt (0.98 g, 55% yield). ¹H NMR: (CDCl₃) 1.81-1.90 (m, 2H, CH₂), 2.37-2.85 (m, 16H, CH₂), 3.87 (s, 3H, OCH₃), 4.66-4.68 (dd, 2H, CH₂, *J*=1.8, 5.2 Hz), 6.43-6.47 (dt, 1H, *J*=7.9, 1.8 Hz, CH), 6.59-6.66 (dt, 1H, *J*=7.9, 5.2 Hz, CH), 6.71-6.80 (m, 3H), 7.15-7.31 (m, 5H). ¹³C NMR: (CDCl₃) 28.56, 33.19, 33.68, 53.17, 55.83, 57.98, 60.55, 71.95, 83.09, 112.43, 113.66, 120.49, 125.69, 128.24, 128.32, 133.88, 137.60, 142.07, 145.76, 149.25; HRMS-ESI: *m/z* calcd, 520.1587; found 521.1482 [M + H]⁺; Anal. Calcd for the di-HCl salt (C₂₅H₃₅Cl₂IN₂O₂): C, 50.60; H, 5.95; N, 4.72. Found: C, 50.46; H, 5.92; N, 4.65.

(*E*)-1-(3-methoxy-4-(3-(tri-*n*-butylstannyl)allyloxy)phenethyl)-4-(3-phenylpropyl)piperazine (5): NaH (4.75 g, 198 mmol) was added to a stirred solution of 4-*O*-*des*-methyl-SA4503•2HCl (6.35 g, 12.4 mmol) in 300 mL DMF under N₂ at room temperature. After 1 min, a solution of (*E*)-3-(tri-*n*-butylstannyl)prop-2-en-1-ol 4-methylbenzenesulfonate (12.4 g, 24.7 mmol) in 72 mL DMF was added. After 90 min, the reaction was quenched by slow addition of saturated NH₄Cl and extracted with CH₂Cl₂. The extracts were washed with saturated NaHCO₃ and concentrated under reduced pressure. Column chromatography (CHCl₃:MeOH, 20:1) gave 5 (10.3 g, 80%) as a yellow oil. ¹H NMR: (CDCl₃) 0.86-0.93 (m, 15H, *n*-Bu), 1.26-1.51 (m, 12H, *n*-Bu), 1.79-1.90 (m, 2H, CH₂), 2.57-2.68 (m, 16H, CH₂), 3.87 (s, 3H, CH₃), 4.61-4.63 (dd, 2H, CH₂, *J*=1, 4.6 Hz), 5.95-6.45 (m, 2H, CH=CH), 6.71-6.81 (m, 3H, aromatic CH), 7.18-7.28 (m, 5H, aromatic CH). ¹³C NMR: (CDCl₃) 9.35, 13.60, 27.16, 29.01, 33.13, 33.63, 53.09, 55.78, 57.93, 60.57, 72.52, 112.18, 113.59, 120.33, 125.66, 128.28, 131.83, 132.96, 142.00, 143.04, 146.34, 149.11.

(*E*)-1-(4-(3-iodoallyloxy)-3-methoxyphenethyl)-4-(3-phenylpropyl)piperazine (2): To a solution of 5 (10.3 g, 15 mmol) in 200 mL CH₂Cl₂ was added I₂ (4.32 g, 17 mmol) in 100 mL CH₂Cl₂. The reaction was stirred for 10 min at room temperature and quenched with an aqueous solution of Na₂S₂O₅. The organic layer was separated and the aqueous phase was extracted with CH₂Cl₂. The combined organic

extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Dissolution in EtOH followed by precipitation with HCl gave 2 as the di-HCl salt (5.9 g, 56%). ^{1}H NMR: ($CDCl_3$) 1.78-1.88 (m, 2H, CH_2), 2.37-2.78 (m, 16H, CH_2), 3.85 (s, 3H,OCH_3), 4.45-4.47 (dd, 2H, CH_2, *J*=1.4, 5.5 Hz), 6.47-6.52 (dt, 1H, *J*=1.4, 14.6 Hz, CH), 6.69-6.80 (m, 4H), 7.15-7.30 (m, 5H). ^{13}C NMR: ($CDCl_3$) 28.53, 33.18, 33.64, 53.13, 55.80, 57.93, 60.48, 70.86, 79.86, 112.48, 113.99, 120.47, 125.68, 128.22, 128.30, 134.07, 140.85, 142.02, 145.65, 149.37; HRMS-ESI: *m/z* calcd, 520.1587; found 521.1278 [M + H]$^+$; Anal. Calcd for the di-HCl salt ($C_{25}H_{35}Cl_2IN_2O_2$): C, 50.60; H, 5.95; N, 4.72. Found: C, 50.50; H, 5.91; N, 4.70.

Radioiodination

[^{125}I]-1: Compound 4 (0.10 mg, 150 nmol) in MeOH (25 μL) was transferred to a glass vial sealed with a Teflon-faced septum. [^{125}I] NaI (15 μL, 1.55 mCi, 0.75 nmol), 75 μL MeOH containing 3% HOAc, and aqueous Chloramine-T (10 μL, 7.0 mM; 70 nmol) were added sequentially. After 1 min, the reaction was quenched by the addition of $Na_2S_2O_5$ (10 μl, 50 mM). Semi-preparative reverse-phase HPLC (280 nm UV detector; flow-through radioactivity detector) was run at a flow rate of 4 mL/min using the mobile phase MeOH (20%), CH_3CN (20%) and an aqueous solution (60%) of Et_3N (2.1% *v/v*) and HOAc (2.8% *v/v*). The *Z*-isomer [^{125}I]-1 (t_R=27.2 min) was obtained in a volume of 11 mL. Dilution with water (25 mL) followed by solid-phase extraction and elution of the cartridge with EtOH (1.0 mL), provided [^{125}I]-1 in 58% radiochemical yield. The radiochemical purity was 97%, and the specific activity was determined to be 2106 mCi/μmol (*vide infra*). Analytical HPLC, using the same detection methods and the same mobile phase at a flow rate of 2 mL/min, confirmed that [^{125}I]-1 co-eluted with non-radioactive 1 (t_R=21.2 min), and was resolved from *E*-isomer 2 (t_R=23.5 min) upon co-injection.

[^{125}I]-2: Similar treatment of 5 with [^{125}I] NaI and Chloramine-T gave [^{125}I]-2 (t_R=29.4 min) after semi-preparative reverse-phase HPLC under the conditions described above. Solid phase extraction provided a 57% radiochemical yield of material with high purity and specific activity (2097 mCi/ μmol). Analytical HPLC, as described above, showed that [^{125}I]-2 co-eluted with non-radioactive 2 (t_R=23.5 min), and was resolved from *Z*-isomer 1 (t_R=21.2 min) upon co-injection.

Specific activities, radioactivity per unit mass, were established for [^{125}I]-1 and [^{125}I]-2 by using HPLC to determine the mass associated with an aliquot of purified material having known radioactivity. To increase sensitivity, analytical HPLC conditions were chosen where isomers 1 and 2 were not fully resolved, but eluted rather quickly. Using the mobile phase MeOH (22.5%), CH_3CN (22.5%) and an aqueous solution (55%) of Et_3N (2.1% *v/v*) and HOAc (2.8% *v/v*) at 2 mL/min gave *Z*-isomer 1 with t_R=10.4 min and *E*-isomer 2 with t_R=11.4 min. Seven-point standard curves were generated for each isomer by relating HPLC peak heights for non-radioactive material over a 45-500 pmol mass range.

Lipophilicity determinations

Log $D_{7.4}$ measurements for [^{125}I]-1 and [^{125}I]-2 were determined by serial partitioning (n=6) of 4 μCi of each compound between equal volumes (3.5 mL) of *n*-octanol and Dulbecco's phosphate buffered saline (0.1 M, pH 7.4; DPBS) in 15 mL polypropylene centrifuge tubes as previously described [14]. Each phase was saturated with the other phase before use. Duplicate samples from each organic layer (100 μl) and each aqueous buffer layer (1.0 mL) were counted, and distribution coefficients at pH 7.4 (log $D_{7.4}$) were calculated by taking the log of [(organic counts×10)/buffer counts]. The algorithms for calculating ClogP for non-radioactive compounds [18] were developed by

BioByte Corp. (Claremont, CA), and were included as part of program ChemBioDraw Ultra, version 12.0 (CambridgeSoft Corporation, Cambridge, MA).

Receptor binding

Assays for σ receptor binding were performed in Tris-HCl buffers (50 mM) using 1.0 nM [^{3}H](+)-pentazocine (σ$_1$), 3.0 nM [^{3}H]ditolylguanidine ([^{3}H]DTG)/200 nM (+)-pentazocine (σ$_2$), and membranes from whole guinea pig brains as previously described [19]. Non-specific binding was defined by haloperidol (1.0 μM; σ$_1$) or by DTG (100 μM; σ$_2$). The IC$_{50}$ values were determined in four to six assays, each performed in duplicate, by non-linear regression of binding data using curve-fitting program Prism 5.0d (Graph-Pad Software, San Diego, CA). K_i values were calculated from IC$_{50}$ values using the Cheng-Prusoff relationship [20], and input K_d's of 2.3 nM for [^{3}H](+)-pentazocine and 23.9 nM for [^{3}H]DTG [19].

Biodistribution studies

Data for [^{125}I]-1 and [^{125}I]-2 were determined using non-fasted, adult male CD1 mice that had been housed on a 12 hr light-dark cycle in temperature and humidity controlled quarters. Each animal received radiotracer (2.5 – 3.0 μCi) in 0.9% saline (0.1 mL; 2% EtOH, *v/v*) by tail vein injection. Groups of mice (n=4, [^{125}I]-1; n=5, [^{125}I]-2) were euthanized by cervical dislocation at various intervals up to 2 hr, and bloods were obtained by cardiac puncture. Brain, heart, lung, liver, spleen and other organs were harvested and weighed. Tissue radioactivity was measured using an automated gamma counter, and %ID/g wet weight of tissue was calculated in relation to standard dilutions of the ID. Pharmacologic studies of [^{125}I]-1 and [^{125}I]-2 followed the same protocol, except haloperidol (2.5 μmol/kg; 0.1 mL) was given by tail vein 5 min prior to radioligand. Tissue radioactivity was determined for sets (n=5) of drug treated or saline control animals 60 min after radioligand administration. A two-tailed, unpaired *t*-test at the 95% confidence level (Prism 5.0d) was employed for statistical analysis.

Results and Discussion

Chemistry

The synthetic routes to 1 and 2, as well as to radioiodinated versions [^{125}I]-1 and [^{125}I]-2, are shown in Scheme 1. Alkylation of 4-*O*-*des*-methyl SA4503 (3) by (*Z*)- or (*E*)-3-(tri-*n*-butylstannyl)prop-2-en-1-ol 4-methylbenzenesulfonate was accomplished by treatment with NaH in DMF at room temperature for 90 min. Work-up and column chromatography provided precursors 4 and 5 in 70% and 80% yield, respectively. For the non-radioactive syntheses of 1 and 2, stereo specific iododestannylation was achieved using iodine in methylene
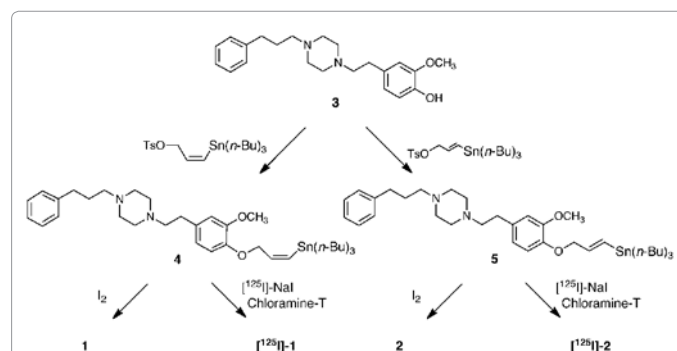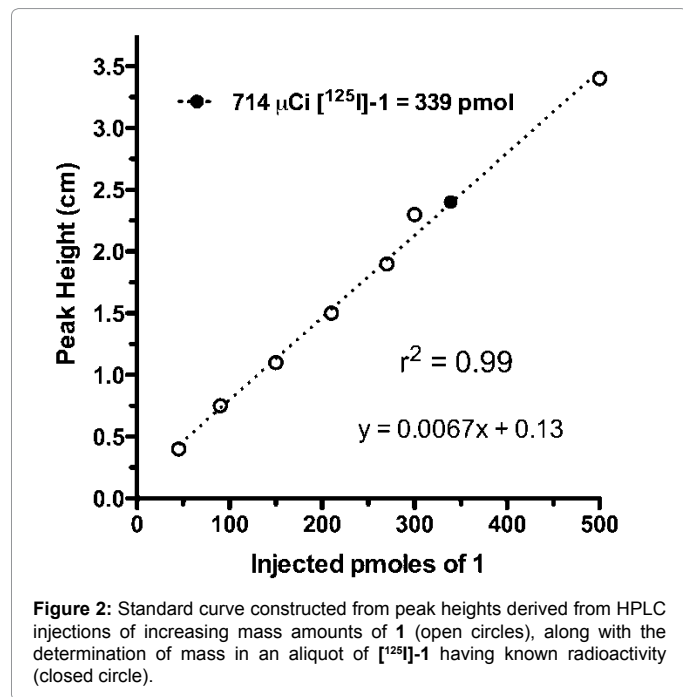

Scheme 1: Synthetic scheme for the preparation of **1** / [^{125}**I**]-**1** and **2** / [^{125}**I**]-**2**.

chloride at room temperature for 10 min. Column chromatography gave 1 and 2 in 55% and 56% isolated yield, respectively. These target compounds were analytically pure, and gave appropriate combustion analyses and spectroscopic data. The proton NMR data confirmed retention of configuration by the expected vicinal couplings for the ABX2 spin systems of (*Z*)-isomer 1 ($3J_{AB}$=7.9 Hz) and (*E*)-isomer 2 ($3J_{AB}$=14.6 Hz).
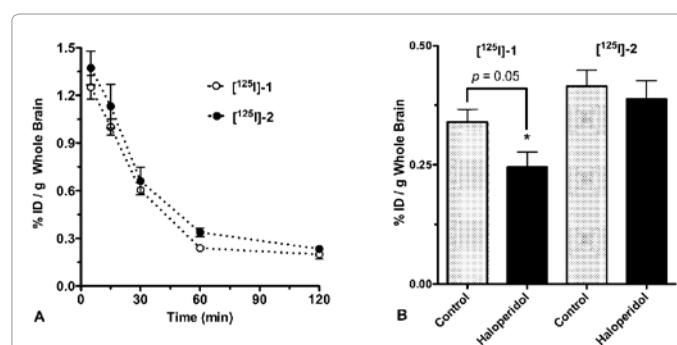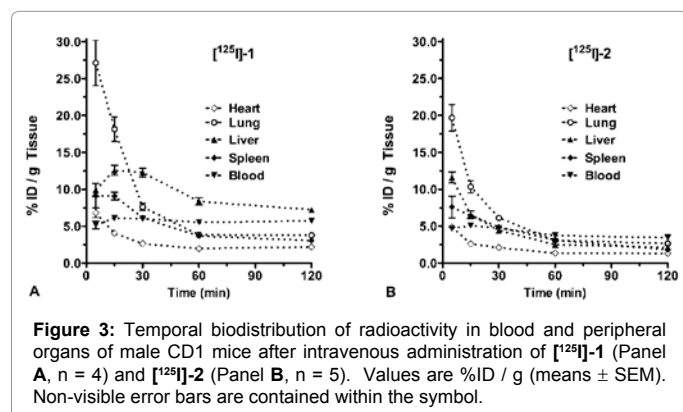
Radiochemistry

Radiolabeling was performed under mild conditions by

Figure 2: Standard curve constructed from peak heights derived from HPLC injections of increasing mass amounts of **1** (open circles), along with the determination of mass in an aliquot of **[125I]-1** having known radioactivity (closed circle).

Compound	aLog D$_{7.4}$	bClogP
[125I]-1, 1	3.60 ± 0.13	5.02
[125I]-2, 2	3.62 ± 0.14	5.02
o-BON	NR	4.29
m-BON	NR	4.59
SA4503	NR	3.47

aLog D$_{7.4}$ values (means ± SD, n = 6) were determined by partitioning between *n*-octanol and phosphate buffered saline (pH 7.4). bClogP values were calculated using a fragment-based approach by a subroutine of program ChemBioDraw Ultra. NR = not reported

Table 1: Lipophilicity measures for SA4503 and radioiodinated analogs of SA4503.

Figure 3: Temporal biodistribution of radioactivity in blood and peripheral organs of male CD1 mice after intravenous administration of **[125I]-1** (Panel **A**, n = 4) and **[125I]-2** (Panel **B**, n = 5). Values are %ID / g (means ± SEM). Non-visible error bars are contained within the symbol.

*Significantly reduced compared to saline-treated controls *t*-test).

Figure 4: (Panel **A**) Temporal biodistribution of radioactivity in whole CD1 mouse brain after intravenous administration of **[125I]-1** (n = 4) and **[125I]-2** (n = 5). Values are %ID / g (means ± SEM). Non-visible error bars are contained within the symbol. (Panel **B**) Effects of pretreatment with intravenous haloperidol (2.5 μmol / kg) on uptake of **[125I]-1** and **[125I]-2** at 60 min in whole CD1 mouse brain. Values are %ID / g (means ± SEM, n = 5).

electrophilic radioiododestannylation of 4 or 5 using no-carrier-added [125I]-NaI and Chloramine-T at room temperature in methanolic acetic acid for one min. Semi-preparative HPLC purification, followed by solid-phase extraction, gave good isolated radiochemical yields of [125I]-1 (58%) and [125I]-2 (57%). HPLC conditions were used where 1 and 2 were resolved, and retention of configuration upon radioiodination was confirmed. More stringent analytical HPLC studies showed that the purified [125I]-labeled isomers were resolved from each other ([125I]-1, t_R=21.2 min; [125I]-2, t_R=23.5 min), and co-eluted with their non-radioactive counterparts. Specific activities near the theoretical value for iodine-125 (2175 mCi/μmol) were established for **[125I]-1** (2106 mCi/μmol) and **[125I]-2** (2097 mCi/μmol) by determining the mass associated with an aliquot of purified material having known radioactivity. HPLC methods were used, where mass was related to peak height for 1 and 2 by linear standard curves ranging from 45 to 500 pmol to bracket the region of interest. Figure 2 shows the use of a standard curve to determine that an aliquot of 714 μCi of [125I]-1 contained 339 pmol 1 by HPLC, leading to the reported 2106 mCi/μmol specific activity.

Lipophilicity

Log D measurements were made for each radioiodinated compound by partitioning between *n*-octanol and pH 7.4 phosphate buffered saline (log D$_{7.4}$) using the shake-flask method. Values obtained (Table 1) were essentially identical for [125I]-1 (log D$_{7.4}$ 3.62 ± 0.14) and [125I]-2 (log D$_{7.4}$ 3.60 ± 0.13). Reversed-phase HPLC, however, could readily distinguish the two isomers through interactions with the stationary phase (*vide supra*). Computational methods using a fragment-based approach [18] were employed to calculate the lipophilicity (ClogP) of non-radioactive compounds (Table 1). Direct log D$_{7.4}$ values for [125I]-1 and [125I]-2 proved 1.42 log units lower than the ClogP values of 5.01. This difference can be attributed to the inability of the computer program to adjust for the protonation status of amines. Since the structures of piperazines SA4503, *o*-BON, *m*-BON, 1 and 2 are so similar (Figure 1), it is likely that extrapolated log D$_{7.4}$ values for the congeneric series would be near 2.87 for *o*-BON, 3.17 for *m*-BON, and 2.05 for SA4503. In our view, the lower hydrophobicity of *o*-BON compared to *m*-BON is an example of clustered lipophilic compensation, where the effect of the *o*-iodine substituent is moderated by its inclusion within the overall electronic cloud of the phenylpropyl moiety [21].

Compound	σ_1 Receptor Binding Parameters			σ_2 Receptor Binding Parameters		
	IC_{50} (nM)	K_i (nM)	Hill Slope	IC_{50} (nM)	K_i (nM)	Hill Slope
1, (*Z*)-IA-SA4503	15.45 ± 1.91	10.67 ± 1.32	1.01 ± 0.09	18.78 ± 1.24	16.71 ± 1.20	1.15 ± 0.04
2, (*E*)-IA-SA4503	24.48 ± 1.85	16.91 ± 1.28	1.06 ± 0.08	20.45 ± 2.23	18.20 ± 1.99	0.96 ± 0.01
[a]*o*-BON	6.4 ± 0.7	NR	NR	10.2 ± 1.9	NR	NR
[a]*m*-BON	8.9 ± 2.8	NR	NR	54.5 ± 6.6	NR	NR
[b]SA4503	6.67 ± 0.30	4.63 ± 0.21	1.23 ± 0.06	70.93 ± 4.86	63.09 ± 4.33	0.88 ± 0.05

Binding parameters for **1** and **2** are means ± SEM for four to six assays, each performed in duplicate. [a]Data from [15]. [b]Data from [19]. NR = not reported

Table 2: σ Receptor binding parameters for SA4503 and iodinated analogs of SA4503.

Sigma receptor binding

As shown in table 2, substitution of a (*Z*)- or (*E*)-iodoallyloxy substituent for the 4-methoxy group of SA4503 was well tolerated. Both stereoisomers displayed moderately high affinities for both σ_1 and σ_2 receptors in guinea pig brain membranes. The (*Z*)-isomer 1 (K_i=10.67 nM) showed 1.5-fold higher affinity for σ_1 sites than (*E*)-isomer 2 (K_i=16.91 nM), while the σ_2 receptor affinities were nearly equal for both isomers (17-18 nM). Introduction of the bulky iodoallyl group cut σ_1 affinities by 2.5- to 3.5-fold with respect to those previously reported for the parent SA4503 [19], while the σ_2 affinities were increased by about 3.5-fold. Thus, this particular structural modification yielded σ ligands 1 and 2 having high affinities, but low subtype selectivity. The affinities of *o*- and *m*-BON previously reported [15] were close to those of SA4503, and are about 2- to 4-fold higher for σ_1 sites than we find for (*Z*)- and (*E*)-IA-SA4503 (1, 2; Table 2).

Biodistribution studies

The biodistributions of [125I]-1 and [125I]-2 were examined in normal male CD1 mice from 5 to 120 min after tail vein injection. Temporal distributions of radioactivity for blood and major peripheral organs are shown in Figure 3. High lung uptake followed by rapid clearance, and high liver uptake followed by prolonged retention, were observed for [125I]-1 (Figure 3A). Lung clearance was fit by a one-phase exponential decay (r^2=0.91), with a half-life of 11.9 min (data not shown). Interestingly, [125I]-2 showed high lung uptake and rapid clearance (half-life 9.4 min), but relatively modest liver uptake and retention (Figure 3B). Other aspects of the peripheral biodistribution were unremarkable, and consistent with that which would be expected for lipophilic radioligands having no formal charge that clear through the hepatobiliary system.

A primary objective of this work was to determine the potential utility of [125I]-1 and [125I]-2 for *in vivo* studies of cerebral σ receptors. Figure 4A shows that initial radioligand uptakes in whole brain at 5 min were 1.37 ± 0.11 %ID/g for *Z*-isomer [125I]-1 and 1.25 ± 0.07 %ID/g for *E*-isomer [125I]-2. Brain radioactivity levels declined as single exponentials (r^2=0.88-95) over 120 min, with a similar half-life observed for [125I]-1 (19.8 min) and [125I]-2 (19.2 min). Thus, these radioligands readily cross the blood-brain-barrier. To determine if the radioactive signal could be associated with *in vivo* binding to σ receptors, we examined the effect of pretreatments with haloperidol (Figure 4B). Haloperidol is an antipsychotic that is often used to probe interactions of radioligands with σ and other receptors *in vivo* [22]. Haloperidol (2.5 μmol/kg) decreased the brain uptake of *E*-isomer [125I]-2 at 60 min by 28% (*p*=0.05), but had no effect on the brain uptake of *Z*-isomer [125I]-1. Thus, there appears to be some geometric discrimination between these two stereoisomers *in vivo*, and the data is consistent with a modest level of specific binding to the cerebral σ_1 receptor for [125I]-2.

In the periphery, haloperidol inhibited the uptake of [125I]-2 in liver by 25% (*p*=0.01) at 60 min, but not in other organs (data not shown). Haloperidol did not reduce the uptake of [125I]-1 in the periphery (data not shown). Liver is rich in σ receptors [23], but so are a number of other organs [14,16]. Taken with the brain data, failure to observe inhibition of [125I]-1 and [125I]-2 by haloperidol throughout the periphery indicates limited utility of these radioligands for *in vivo* studies of σ receptors.

The initial uptake of [125I]-1 and [125I]-2 at 5 min in whole mouse brain compares favorably to the results reported by Hirata and colleagues [16] for mouse brain uptake at 5 min for [125I]*o*-BON (1.86 ± 0.36 %ID/g) and [125I]*m*-BON (1.98 ± 0.13 %ID/g). However, haloperidol and other σ receptor ligands reduced brain uptake of [125I]*o*-BON and [125I]*m*-BON by approximately 70%. In addition, σ receptor ligands blocked the uptake of [125I]*o*-BON and [125I]*m*-BON in liver and several other peripheral organs [16]. These four radioiodinated ligands derived from the SA4503 scaffold have fairly similar σ_1 receptor affinities (Table 2), but *o*-BON/*m*-BON are 0.43-0.73 log units lower in lipophilicity than (*E*)-/(*Z*)-IA-SA4503 (Table 1). While log $D_{7.4}$ values up to about 4 are tolerated for passage across the blood-brain-barrier, higher lipophilicity usually makes it more difficult to detect the specific binding component of the total binding signal associated with a given receptor-binding radiotracer [24]. A number of other factors, such as degree of metabolic stability, also contribute to the differences observed *in vivo* between various radiotracers. The low level of specific binding observed *in vivo* for [125I]-1/[125I]-2 with respect to [125I]*o*-BON/[125I]*m*-BON seems due, at least in part, to a combination of higher lipophilicities with the lower σ_1 receptor affinities. In this regard, the present studies provided a springboard for the identification of [125I]-labeled *N*-(*E*-iodoallyl)-*N´*-(dimethoxyphenethyl)-piperazine, a metabolically stable radioligand with a log $D_{7.4}$ of 2.25, that exhibits suitable characteristics for binding to the σ_1 receptor *in vivo* [14].

Conclusions

(*Z*)- and (*E*)-IA-SA4503 were readily synthesized by coupling stannylated alkylating agents to 4-*O-des*-methyl-SA4503 followed by iododestannylation with retention of configuration. Substitution of (*Z*)- or (*E*)-iodoallyloxy substituents for the 4-methoxy group of SA4503 proved acceptable by the σ receptor pharmacophore. Both stereoisomers displayed moderately high affinities for σ_1 and σ_2 receptors *in vitro* (K_i values 11-18 nM). The corresponding radioiodinated ligands also could be prepared in good yields, with high purity and high specific activity. Log $D_{7.4}$ values for both radioligands were 3.6, which is higher than optimal for receptor-binding radiotracers for *in vivo* studies. Both radioligands readily cross the blood-brain-barrier of mice, but only the [125I]-labeled (*E*)-isomer showed a modest degree of specific binding to σ_1 receptors. The studies demonstrate that σ receptors have substantial tolerance toward bulky modifications of SA4503 at the 4-position of the dimethoxyphenethyl moiety. This structure activity relationship could aid in the future development of possible therapeutics based on the SA4503 framework.

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