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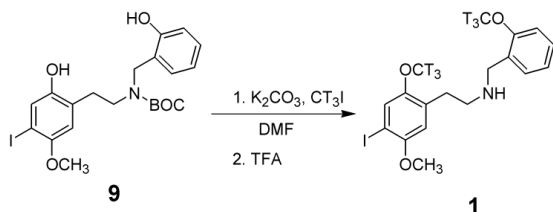
High Specific Activity Tritium-Labeled *N*-(2-methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine (INBMeO): A High Affinity 5-HT_{2A} Receptor-Selective Agonist Radioligand

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Abstract



The title compound (³H]INBMeO) was prepared by an *O,O*-dimethylation reaction of a *t*-BOC protected diphenolic precursor using no carrier added tritiated iodomethane in DMF with K₂CO₃. Removal of the *t*-BOC protecting group and purification by HPLC afforded an overall yield of 43%, with a radiochemical purity of 99% and specific activity of 164 Ci/mmol. The new radioligand was suitable for labeling human 5-HT_{2A} receptors in two heterologous cell lines and had about 20-fold higher affinity than [³H]ketanserin.

1. Introduction

The serotonin 2A (5-HT_{2A}) receptor is a member of the G protein-coupled receptor (GPCR) superfamily. Of the seven different subtypes of serotonin receptors, the 5-HT_{2A} receptor has been closely linked to complex behaviors. It is expressed at high density within the frontal cortex, but at lower levels in many other brain areas.¹⁻³ It has been implicated in working memory, cognitive processes, affective disorders such as schizophrenia, and mediates the primary effects of hallucinogenic drugs.⁴⁻⁷ In addition, many peripheral tissues also express the 5-HT_{2A} receptor. For example, 5-HT_{2A} receptors are involved in platelet function, and induce contraction within the vasculature.^{8,9} Thus, radiolabeled agonist and antagonist ligands are of high importance in the pharmacology community to measure density and changes in density of these receptors.

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Unfortunately, few ligands are highly specific for 5-HT_{2A} receptors, and those that are, such as M100907,¹⁰ are antagonists. It is desirable, therefore, to have available a radiolabeled agonist ligand. Furthermore, it is typical that agonist ligands label only a high-affinity subset of the total receptor population, so any useful radioligand should have high specific activity. One ligand that is widely used for this purpose is [¹²⁵I]-2,5-dimethoxy-4-iodoamphetamine ([¹²⁵I]DOI).^{11,12} Although this ligand does not discriminate between the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors,¹³ the high specific activity of ¹²⁵I makes it very useful for rapid assays of low density receptor populations. This radioligand is presently commercially available, but major drawbacks to the use of [¹²⁵I]DOI are its short half-life and high cost. In our own laboratory, for example, experiments have often been postponed until the need for results is sufficiently compelling, or until we have delayed a sufficient number of experiments to justify the cost of [¹²⁵I]DOI. Therefore, a high-specific activity radioligand with a longer half-life is very desirable. Recently, we reported on a series of high affinity *N*-benzyl phenethylamine ligands for the 5-HT_{2A} receptor.¹⁴ The most potent of the ones reported, *N*-(2-methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine (INBMeO, **1**), had exceptionally high affinity for the 5-HT_{2A} receptor, and was obviously suitable for the introduction of 3-9 tritium atoms by *O*-methylation. Based on preliminary work, we had concluded that six tritium atoms should provide sufficient radioactivity for a useful ligand. This report describes the synthesis and preliminary characterization of *N*-(2-[³H-OCH₃]-benzyl)-2-[³H-OCH₃]-5-methoxy-4-iodophenethylamine (**1**).

In order to prepare **1** with six tritium atoms it was planned to bis-methylate the diphenolic precursor **9** using carrier-free tritiated iodomethane. The overall synthesis of this precursor was carried out as shown in Scheme 1.

Preliminary studies of the methylation reaction, utilizing unlabeled iodomethane, demonstrated that treatment of the *N*-tert-BOC protected bisphenol **9** with sodium hydride in DMF, followed by the addition of a slight excess of iodomethane, afforded intermediate **10a** (Scheme 2). Partially methylated intermediates were likely by-products, so HPLC conditions were developed to distinguish between **1**, and **9-14**. Table 1 shows the retention parameters for the desired product **1**, the *N*-Boc protected bisphenol **9**, the *O,O*-dimethylated-*N*-Boc-protected intermediate **10**, the partially *O*-methylated *N*-Boc-protected potential byproducts **11** and **12**, and the deprotected analogs **13** and **14**.

Conditions for the deprotection of **10** and for isolation of pure INBMeO (**1**) were also developed. Thus, treatment of a mixture comprised of **10** and small amounts of **11** and **12**, with trifluoroacetic acid (TFA) for 15 min provided **1**, along with **13** and **14** (derived from **11** and **12**). Base treatment failed to remove the phenols **13** and **14**. Surprisingly, INBMeO was found to decompose in the presence of 1 N sodium hydroxide.

Unfortunately, when the *O*-methylation reaction conditions were applied on the scale that would be used in the radiosynthesis, only minute amounts of **10a**, **11a**, and **12a** were produced. Further experiments revealed that the starting material **9** and the monomethylated intermediate **11a** decomposed when a solution of **9-12a** in DMF was treated with sodium hydride for 15 h at room temperature. A similar experiment using potassium carbonate was more promising, showing the disappearance of intermediate **11a** only. Therefore, the methylation reaction using potassium carbonate to replace sodium hydride was examined. These conditions gave **10a**, accompanied by small amounts of **11a**, **12a**, and unreacted **9**. Deprotection gave INBMeO (**1b**) in 54% yield.

A similar reaction mixture was produced in the master radiosynthesis. Thus, treatment of a DMF solution of the bisphenol **9** with an 11-fold excess of iodomethane (100 mCi, specific activity 80 Ci/mmol) in the presence of potassium carbonate gave primarily **10b** (10.6 mCi),

accompanied by small amounts of **9**, **11b**, and **12b**. Because we had found that **10a** was more stable than **1a**, only a portion of **10b** (3.7 mCi) was deprotected. The product was purified by preparative HPLC to afford 1.8 mCi (49% yield) of [³H]INBMeO (**1b**) of nominal specific activity 160 Ci/mmol. A portion of **10b** was used to determine experimentally the specific activity, which was found to be 164 ± 1 Ci/mmol. Because high specific activity compounds are known to undergo radiolytically-promoted decomposition, the purity of [³H]INBMeO was monitored over a nine-month period. As shown in Figure 1, [³H]INBMeO is usable for a minimum of 60 days (~95% purity) and we have carried out successful experiments beyond 150 days (~90% purity).

We then used the new radioligand [³H]INBMeO **1b** to carry out receptor saturation isotherm experiments in two cells lines stably expressing human 5-HT_{2A} receptors. For a comparison, we used [³H]ketanserin (67 Ci/mmol, PerkinElmer). The results of that comparison are presented in Figure 2 and Table 2. The affinity of [³H]INBMeO was about 20-fold higher than that of ketanserin, and it labeled about 60% of the sites labeled by ketanserin. Use of [³H]INBMeO as the radioligand in competition binding assays with four ligands known to have high affinity for the 5-HT_{2A} receptor demonstrated that all of the ligands had comparable affinity, whether [³H]ketanserin or [³H]INBMeO was used (Table 3). Curiously, although INBMeO is an agonist, it labeled approximately 60% of the sites that were labeled by ketanserin.

4. Conclusions

We have prepared a new high specific activity tritiated ligand for the 5-HT_{2A/2C} receptors. It appears to have utility similar to ketanserin, but has about 20-fold higher affinity for 5-HT_{2A/2C} receptors. It may be an economical alternative to the use of [¹²⁵I]DOI as a radioligand for these receptors.

5. Experimental

5.1 Chemistry — General

The chemicals, reagents and solvents used in the synthesis were inspected and released for use based on visual inspection and conformance of the individual lot with the manufacturer's Certificate of Analysis. Nuclear magnetic resonance (NMR) spectra were determined on a Bruker Avance 300 MHz NMR spectrometer. HPLC analyses were performed on a dual pump system (Ranin HPXL solvent delivery system) equipped with a Rheodyne injector, a Varian ProStar 325 UV-Vis detector, β-RAM radio-detector, (IN/US and a Systems, Inc.) connected after the UV detector, controlled by Varian Star Workstation software. The columns and solvent systems are given below. The water used in HPLC solvent systems was obtained from a Millipore Milli-Q Plus Ultra-Pure Water System.

Radioactive samples were counted on Tri-Carb Liquid Scintillation Analyzer (Packard Bioscience Company, model: 2100TR) using IN-FLOW 3 as liquid scintillation counting cocktail (IN/US Systems, Inc.).

HPLC conditions for analysis: Phenomenex Synergy Fusion-RP column (3×150 mm, 4 μm), solvent A: [20 mM NaH₂PO₄-H₂O], solvent B: CH₃CN, gradient, 0-30 min, solvent B: 20-90%; 30-40 min, solvent B: 90%; flow rate: 0.6 ml/min, UV detector wavelength: 210 nm and 215 nm, β-RAM detector (for analyzing radio-chemical purity of samples).

HPLC conditions for purification of the final radioligand: Phenomenex Synergy Polar-RP column (3×150 mm, 4 μm), solvent A: 80% [20 mM NaH₂PO₄-H₂O]/20% EtOH, solvent B:

EtOH, gradient, 0-30 min, solvent B: 30-80%; 30-40 min, solvent B: 80%; flow rate: 0.4 mL/min, UV detector wavelength: 210 nm and 215 nm.

5.1.1 2-(2-Isopropoxy-5-methoxyphenyl)nitroethene 2—A solution of 2-isopropoxy-5-methoxybenzaldehyde^{15,16} (15.0 g, 72 mmol) and n-butylamine (6.84 g, 94 mmol) in 200 mL of toluene was heated at reflux for 45 min with H₂O removal using a Dean-Stark apparatus. The solvents were removed by rotary evaporation and the residue was dissolved in nitromethane (75 mL). This solution was stirred efficiently while glacial acetic acid (13.0 g, 216 mmol) was added dropwise. After stirring at room temperature for 12 h, the solvents were removed by rotary evaporation. The residue was dissolved in dichloromethane, washed with H₂O (2 × 100 mL), brine (100 mL), and dried over MgSO₄. After filtration to remove the drying agent, the filtrate was concentrated by rotary evaporation to afford a yellow solid that was recrystallized from methanol to provide 2-(2-isopropoxy-5-methoxyphenyl)-nitroethane (14.22 g, 83.3%) as bright yellow crystals, mp 65 °C. ¹H NMR (300 MHz, CDCl₃, δ ppm): 1.41 (s, 6H), 3.81 (s, 3H), 4.60 (m, 1H), 6.94 (m, 3H), 7.86 (d, *J* = 4.29 Hz, 1H), 8.14 (d, *J* = 4.29 Hz, 1H); CIMS *m/z* 238 (M+1), Anal. (C₁₂H₁₅NO₄) C, H, N.

5.1.2 2-(2-Isopropoxy-5-methoxyphenyl)ethylamine 3—To lithium aluminum hydride (9.07 g, 238 mmol) in a flame-dried 2 L reaction vessel, was added 600 mL of anhydrous tetrahydrofuran. A solution of 2-(2-isopropoxy-5-methoxyphenyl)-nitroethane (20.0 g, 84 mmol) in 150 mL of THF was then added dropwise over 45 min. The mixture was heated at 65 °C for 4 h, after which TLC analysis showed absence of starting material. After cooling to room temperature the reaction was quenched by the stepwise addition of 50% THF/H₂O (10 mL), 15% NaOH solution (10 mL), and H₂O (30 mL). The precipitated solids were removed by vacuum filtration and the filtrate was reduced under vacuum to afford a colorless oil. The oil was dissolved in Et₂O, washed with H₂O (2 × 100 mL), and then extracted into 1M HCl (2 × 200 mL). The acidic extract was washed with Et₂O (2 × 75 mL), and then made strongly basic with 5M NaOH. The basic solution was extracted with Et₂O (3 × 100 mL), the ether extract was washed with H₂O (2 × 75 mL), brine (75 mL), dried over MgSO₄, and filtered. The filtrate was concentrated by rotary evaporation and the residue was distilled at 97 °C and 0.17 torr, providing 13.32 g (75.5%) of the product as a clear oil. ¹H NMR (300 MHz, CDCl₃, δ ppm): 1.28 (s, 3H), 1.32 (s, 3H), 1.70 (bs, 2H), 2.73 (t, *J* = 6.67 Hz, 2H), 2.92 (t, *J* = 6.67 Hz, 2H), 3.75 (s, 3H), 4.42 (m, 1H), 6.74 (m, 3H); CIMS *m/z* 210 (M+1), Anal. (C₁₂H₁₉NO₂) C,H,N.

5.1.3 *N*-(2,2,2-Trifluoroacetyl)-2-(2-isopropoxy-5-methoxyphenyl)ethylamine 4—To a solution of the amine **3** (20.0 g, 96 mmol) in anhydrous THF (100 mL) and triethylamine (9.65 g, 96 mmol) in a dried 250 mL round bottomed flask was added ethyl trifluoroacetate (17.73 g, 125 mmol), and the solution was stirred efficiently at room temp overnight. TLC showed complete consumption of the starting material. The solvents were then removed by rotary evaporation and the residue was dissolved in 150 mL of CH₂Cl₂. The organic solution was washed with H₂O (2 × 100 mL), brine (100 mL), dried over MgSO₄, and filtered to remove the drying agent. The filtrate was concentrated by rotary evaporation to afford 23.42 g (80.3%) of amide **4** as a waxy white solid. This material could be recrystallized from methanol, distilled (155 °C at 0.2 torr), or, most advantageously, sublimed (30 °C at 0.2 torr), resulting in a crystalline product with a sharp melting point of 35 °C. ¹H NMR (300 MHz, CDCl₃, δ ppm): 1.32 (s, 3H), 1.34 (s, 3H), 2.87 (t, *J* = 6.15, 2H), 3.56 (q, *J* = 8.40, 2H), 3.71 (s, 3H), 4.46 (m, 1H), 6.74 (m, 2H), 6.87 (d, 1H), 7.32 (bs, 1H), CIMS *m/z* 306 (M+1), Anal. (C₁₄H₁₈F₃NO₃) C,H,N.

5.1.4 *N*-(2,2,2-Trifluoroacetyl)-2-(4-iodo-2-isopropoxy-5-methoxyphenyl)ethylamine 5—Acetic anhydride (25 mL) was added to a solution of trifluoroacetamide **4**

(5.0 g, 16.4 mmol) dissolved in glacial acetic acid (25 mL), and the reaction was cooled to 10 °C. A solution of iodine monochloride (2.92 g, 18 mmol) in acetic acid (5 mL) was added dropwise over 30 min to the vigorously stirred solution. The reaction was stirred for 6 h, and then poured onto ice. Excess saturated aqueous Na₂SO₃ was added to neutralize any free halogen. A white precipitate was collected from the aqueous mixture by vacuum filtration and washed on the filter with H₂O. The aqueous filtrate was extracted with CH₂Cl₂ (3 × 75 mL). The collected solid was dissolved in CH₂Cl₂ (125 mL), combined with the organic extracts, washed with H₂O (2 × 75 mL), brine (75 mL) and dried over MgSO₄. After filtration, the solvent was removed by rotary evaporation and the residue was recrystallized from methanol to provide the iodinated trifluoroacetamide **5** (5.59 g, 79.1%) as a white crystalline solid; mp 93 °C. ¹H NMR (300 MHz, CDCl₃, δ): 1.32 (s, 3H), 1.34 (s, 3H), 2.86 (t, *J* = 5.89 Hz, 2H), 3.56 (q, *J* = 9.06 Hz, 2H), 3.85 (s, 3H), 4.49(m, 1H), 6.60 (s, 1H), 7.06 (bs, 1H), 7.33 (s, 1H, *ArH*), CIMS *m/z* 432 (M+1), Anal. (C₁₄H₁₇F₃INO₃) C,H,N.

5.1.5 2-(4-Iodo-2-isopropoxy-5-methoxyphenyl)ethylamine 6—A solution of the trifluoroacetamide **5** (2.50 g, 58 mmol) in EtOH (50 mL) was cooled to 10 °C and aqueous 5M NaOH (10 mL) was added. After stirring overnight at RT H₂O (50 mL) was added and the solution was extracted with Et₂O (3 × 75 mL). The organic extracts were washed with H₂O (3 × 75 mL), brine (75 mL), dried with MgSO₄, and filtered. The filtrate was concentrated by rotary evaporation and the resulting golden oil (1.74 g, 89.3%) was used without further purification. An analytical sample was purified by radial TLC (Chromatotron, silica plate, CH₂Cl₂ as eluent, with nitrogen bubbled through conc NH₄OH as the atmosphere). ¹H NMR (300 MHz, CDCl₃, δ ppm): 1.29 (s, 3H), 1.31 (s, 3H), 1.65 (bs, 2H), 2.72 (t, *J* = 7.16 Hz, 1H), 2.92 (t, *J* = 7.16 Hz, 1H), 3.71 (q, *J* = 10.27 Hz, 2H), 3.82 (s, 3H), 4.42 (m, 1H), 6.65 (s, 1H), 7.25 (s, 1H), CIMS *m/z* 336(M+1), Anal. (C₁₂H₁₈INO₂) C,H,N.

5.1.6 N-(2-isopropoxybenzyl)-2-(4-iodo-2-isopropoxy-5-methoxyphenyl)ethylamine 7—To a solution of the crude amine **6** obtained above (0.99 g, 2.95 mmol) in toluene (30 mL) in a 100 mL round bottomed flask was added 0.629 g (3.83 mmol) of 2-isopropoxy-benzaldehyde,¹⁷ and the reaction was heated at reflux for one hour with H₂O removal using a Dean-Stark trap. When H₂O production ceased, the toluene was removed by rotary evaporation and the residue was dissolved in anhydrous EtOH (50 mL). Sodium borohydride (0.56 g, 15 mmol) was added portion-wise and the reaction was stirred at room temperature for 4 h. The excess NaBH₄ was decomposed by transferring the reaction to a 250 mL flask and adding methanol (50 mL). The solution was then heated at reflux until gas evolution ceased. The solvent was removed by rotary evaporation and the residue was dissolved in Et₂O (50 mL). The ether solution was extracted with 1M HCl (3 × 75 mL). The acidic aqueous extract was washed with Et₂O (3 × 25 mL), followed by basification with 2M NaOH. The liberated free base was extracted into Et₂O (3 × 75 mL), the organic layer was washed with H₂O (2 × 50 mL), brine (50 mL), dried over MgSO₄, and filtered. The filtrate was reduced to dryness by rotary evaporation and the resulting golden oil was purified using radial TLC (Chromatotron, 4 mm silica plate, CH₂Cl₂ as eluent, and nitrogen bubbled through conc. NH₄OH as the atmosphere), providing 0.86 g (60.1%) of the pure amine as a clear oil. ¹H NMR (300 MHz, CDCl₃, δ ppm): 1.24 (s, 3H), 1.25 (s, 3H), 1.26 (s, 3H), 1.27 (s, 3H), 1.80 (bs, 1H), 2.80 (m, 4H), 3.73 (s, 2H), 3.80 (s, 3H), 6.65 (s, 1H), 6.67-6.90 (m, 3H), 7.13-7.24 (m, 2H), HRMS (*ESI*): calculated for C₂₂H₃₀INO₃: 484.1349. Found: 484.1346

5.1.7 N-(2-hydroxybenzyl)-2-(4-iodo-2-hydroxy-5-methoxyphenyl)ethylamine 8—A solution of amine **7** obtained in the previous step (0.75 g, 1.55 mmol) in anhydrous CH₂Cl₂ (30 mL) in a 100 mL single neck round bottomed flask was cooled to -78 °C and a solution of 1M BCl₃ in CH₂Cl₂ (4.65 mL, 4.65 mmol) was added dropwise using a syringe. The reaction was allowed to warm to room temperature and was stirred overnight. The flask

was cooled in an ice bath and methanol (20 mL) was added dropwise. The solvent was then removed by rotary evaporation. Methanol (30 mL) was added to the residue, the solvent was removed by rotary evaporation, and the procedure was repeated using 25 mL of toluene. The resulting salt was basified with excess 1M NH₄OH, and the aqueous solution was extracted with CH₂Cl₂ (3 × 25 mL). The combined organic extract was washed with H₂O (25 mL), brine (25 mL), dried with MgSO₄, filtered, and the solvent was evaporated under vacuum. The residual free base was purified by radial TLC (Chromatotron, 4 mm silica plate, CH₂Cl₂ as eluent, with nitrogen bubbled through NH₄OH as the atmosphere) to provide 0.442 g (71.4%) of the *O,O*-dealkylated amine **8** as a golden oil. ¹H NMR (300 MHz, CDCl₃, δ ppm): 2.80 (t, *J* = 8.4 Hz, 2H), 2.95 (t, *J* = 8.4 Hz, 2H), 3.80 (s, 3H), 3.95 (s, 2H), 6.55 (s, 1H), 6.60–6.90 (m, 3H), 7.00–7.25 (m, 2H), HRMS (ESI): Calculated for C₁₆H₁₈INO₃: 400.0410. Found: 400.0412.

5.1.8 *N*-(*tert*-butoxycarbonyl)-*N*-(2-hydroxybenzyl)-2-(4-iodo-2-hydroxy-5-methoxyphenyl)ethylamine **9**—A solution of amine **8** prepared in the previous step (1.56 g, 3.59 mmol) in 10 mL of dry THF was cooled to 0 °C and a solution of di-*tert*-butyl dicarbonate (0.784 g, 3.59 mmol) in dry THF (2 mL) was added dropwise using a syringe. The reaction was allowed to warm to room temperature and was stirred for a total of 2 h. The THF was removed by rotary evaporation and the residue was dissolved in 20 mL CH₂Cl₂. This organic solution was washed with H₂O (2 × 25 mL), brine (25 mL), dried with MgSO₄, and filtered. The filtrate was concentrated by rotary evaporation, and the residual dark brown oil was purified using radial TLC (Chromatotron, 4 mm silica plate, CH₂Cl₂ as eluent, and nitrogen bubbled through NH₄OH as the atmosphere) to provide 0.984 g (54.9%) of the *t*-Boc protected amine **9** as a light golden oil. After drying under high vacuum the oil solidified to an easily handled glass. ¹H NMR (300 MHz, CDCl₃, δ ppm): 1.33 (s, 9H), 2.67 (t, *J* = 7.05 Hz, 2H), 3.38 (m, 2H), 3.70 (s, 3H), 4.25 (s, 2H), 4.45 (bs, 1H), 6.30 (s, 1H), 6.65–6.90 (m, 2H), 7.00–7.22 (m, 3H), 9.25 (bs, 1H), CIMS *m/z* 500 (M+1), Anal. C₂₁H₂₆INO₅ C,H,N.

5.1.9 *N*-(*tert*-butoxycarbonyl)-*N*-(2-methoxybenzyl)-2-(2,5-dimethoxy-4-iodophenyl)ethylamine **10a**—A mixture of the *t*-Boc-protected amine **9** (123 mg, 0.246 mmol) in anhyd DMF (20 mL), and NaH (60% in mineral oil, 275 mg, 6.89 mmol) was stirred under argon for 45 min. A solution of CH₃I (87 mg, 0.617 mmol) in toluene (4 mL) was added and the reaction was stirred overnight. TLC analysis showed total consumption of the starting material. The reaction was carefully quenched with H₂O and the solvent was removed by rotary evaporation under high vacuum. The resulting syrupy residue was dissolved in Et₂O (50 mL), washed with H₂O (2 × 20 mL), brine (20 mL), dried over MgSO₄, and filtered. The filtrate was concentrated by rotary evaporation and the residue was purified by radial TLC (Chromatotron, 2 mm silica plate, CH₂Cl₂ as eluent, with nitrogen bubbled through conc NH₄OH as the atmosphere) to provide 81 mg (63.0%) of chromatographically pure *O,O*-dimethylated *t*-Boc protected amine **10a** as a clear oil. ¹H NMR (300 MHz, CDCl₃, δ ppm): 1.33 (s, 9H), 2.66 (t, *J* = 6.93 Hz, 1H), 2.76 (t, *J* = 6.93 Hz, 1H), 3.25 (t, *J* = 6.93 Hz, 1H), 3.35 (t, *J* = 6.93 Hz, 1H), 3.65 (s, 3H), 3.72 (s, 3H), 3.74 (s, 3H), 4.28 (1, 1H), 4.40 (s, 1H), 6.44, 6.60 (2s, 1H), 6.77 (d, 1H), 6.83 (t, 3H), 7.00–7.20 (m, 3H). HRMS (ESI): Calculated for C₂₃H₃₀INO₅: 550.1066 (M + Na). Found: 550.1075.

5.1.10 *N*-(2-methoxybenzyl)-2-(2,5-dimethoxy-4-iodophenyl)ethylamine hydrochloride **1a**—The *t*-BOC protected amine **10a** from the previous step (50 mg, 94.8 μmol) was added to 2M HCl in methanol (3 mL), followed by stirring at room temperature for 12 h, a procedure that completely removed the *t*-BOC protecting group. The solvent was removed by rotary evaporation and the resulting white powder was recrystallized from acetonitrile to provide 43 mg (98%) of the desired deprotected amine (**1a**) hydrochloride; melting point 167 °C. ¹H NMR (300 MHz, CDCl₃, δ ppm): 3.11 (bs, 4H), 3.63 (s, 3H), 3.76

(s, 3H), 3.84 (s, 3H), 4.16 (t, $J = 4.90$ Hz, 2H), 6.79 (s, 1H), 6.84 (d, 1H), 6.95 (t, 1H), 7.28 (s, 1H), 7.33 (t, 1H), 7.39 (d, 1H), 9.45 (bs, 2H), CIMS m/z 428 (M+1).

5.2 Radiochemical Synthesis

5.2.1 N-(tert-butoxycarbonyl)-N-(2-[^3H -OCH $_3$]-benzyl)-2-(2-[^3H -OCH $_3$]-5-methoxy-4-iodophenyl)-ethylamine (10b**)**—A 0.2 mL aliquot (28.8 μg , 0.0577 μmol) was withdrawn from a solution of 3.5 mg (7.2 mmol) of *N*-(tert-butoxycarbonyl)-*N*-(2-hydroxybenzyl)-2-(4-iodo-2-hydroxy-5-methoxyphenyl)ethylamine (**9**) in anhyd DMF (25 mL). The aliquot was added to DMF (10 mL) containing potassium carbonate (30 mg, 0.22 mmol) in a pear-shaped flask and the solution was stirred under nitrogen for 30 min. The flask was then connected to a vacuum manifold and the mixture was de-gassed three times by freezing/evacuating/thawing. A sealed-tube containing [^3H]-methyl iodide (100 mCi, 80 Ci/mmol, 1.25 μmol , 11 eq., PerkinElmer Life Sciences) in toluene (2 mL) was connected to the vacuum manifold using a thermometer adapter that was connected to a double-end female adapter connected to an adapter containing P_2O_5 (429 mg, 3 mmol) and a cotton plug. The sealed-tube was evacuated for 5 min and the [^3H]methyl iodide/toluene solution was transferred to the reaction flask through the P_2O_5 layer over a 2 hour period. The reaction mixture was stirred at RT for an additional 5 min, cooled with liquid nitrogen, and then thawed. The stirring/freezing/thawing process was repeated three times. Finally, the reaction mixture was stirred at RT for 15 hours. The unreacted [^3H]methyl iodide was transferred back to the sealed-tube. H_2O (2 mL) was added to the reaction mixture followed by stirring at RT for 30 min. The mixture was transferred to a 15 mL centrifuge tube and extracted with Et_2O (5×2 mL). The combined ether extract was washed with H_2O (3×2 mL), dried over Na_2SO_4 , then filtered into a scintillation vial, providing crude intermediate (**10b**) (10.6 mCi, radiochemical purity: 77%).

5.2.2 Specific Activity Determination—A Beer's Law calibration curve for **10a** was generated by injection of aliquots (2, 3, 4, 5, 10, and 15 μL) of a standard solution (6.23×10^{-6} M) of **10a** in CH_3OH onto a Supelco Ascentis Express C18 HPLC column (2.1 \times 100 mm, 2.7 μm), eluting with a gradient of 55-90% B, over 20 min with solvent A: 0.05% NH_4OH - H_2O and solvent B: CH_3CN , flow rate: 0.4 mL/min, and monitoring at 200 nm. Plotting the amount (μmol , Y) versus the UV response (integrated area, X) gave a straight line ($Y = 2.0 \times 10^{-11} X$, $R^2 = 0.9984$). To determine the radiochemical purity of **10b** the percentage of radioactivity associated with **10b** (53.8%) was determined by integration of the counted (β -RAM) peaks and counting (scintillation counter) of the collected total effluent from a separate 8 μCi injection to confirm that no radioactivity remained on the column. To determine the specific activity, three injection of 8.0 μCi each (containing $0.538 \times 8 \mu\text{Ci} = 4.3 \mu\text{Ci}$) were made. The molar amounts of **10b**, determined from the calibration curve, were: 2.65×10^{-5} , 2.61×10^{-5} , and 2.62×10^{-5} μmol , giving values of 163, 165, and 164 Ci/mmol, respectively, for the specific activity of **10b**.

5.2.3 N-(2-[^3H -OCH $_3$]-benzyl)-2-(2-[^3H -OCH $_3$]-5-methoxy-4-iodophenyl)ethylamine ([^3H]INBMeO) (1b**)**—The crude intermediate **10b** (4.8 mCi) was stirred in 1:5 TFA: CH_2Cl_2 (2 mL) at RT for 1 hour. The solvent was evaporated under a stream of nitrogen and the crude produce (3.8 mCi) was purified by preparative HPLC (sample loaded in 0.5 mL 80% [20 mM NaH_2PO_4 - H_2O]/20% EtOH). The pure final product, [^3H]INBMeO (**1b**) (1.8 mCi, radiochemical purity: 99%, specific activity: 160 Ci/mmol) gave a single peak with R_t 18.2 min (UV detector), when coinjected with standard INBMeO **1a**.

5.3 Pharmacology

5.3.1 Materials—[^3H]ketanserin (67 Ci/mmol) was purchased from Perkin-Elmer (Boston, MA). Cinanserin HCl and ketanserin tartrate were purchased from Tocris Bioscience

(Ellisville, MO). DOI and LSD were synthesized in our own laboratory. Serotonin HCl and most other reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Cell culture reagents were purchased from Fisher Scientific (Pittsburgh, PA) or Invitrogen (Carlsbad, CA).

5.3.2 Cell Culture—A549 cells, stably expressing the human 5-HT_{2A} receptor and the ICAM-1 luciferase reporter gene (A20), were a generous gift from Dr. Ulrike Weyer-Czernilofsky of the Ernst Boehringer Institut, Bender and Co., Vienna, Austria.¹⁸ The creation of the HEK cells stably expressing the human 5-HT_{2A} receptor (Hh2A) has been previously reported.¹⁹ Both cell lines were grown in culture plates at 37 °C with 5% CO₂ in Dulbecco's modified Eagle's Medium (DMEM) containing 10% dialyzed fetal bovine serum (dFBS, Hyclone, Logan, UT), 2 mM L-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, and 300 µg/ml G-418 with 75 µg/ml hygromycin B (A20) or 20 µg/ml Zeocin (Hh2A).

5.3.3 Radioligand Assays—Methods for membrane preparation and radioligand assays were performed as previously described.²⁰ Briefly, cells were grown to confluency in 150 mm culture dishes before being lysed on ice with lysis buffer (1 mM HEPES, 2 mM EDTA, pH 7.4). After 10 min, the cells were scraped and centrifuged for 30 min at 30,000 × g at 4 °C. The supernatant was discarded and the pellets were resuspended in receptor binding buffer (RBB, 50 mM Tris, 4 mM MgCl₂, pH 7.4) by mechanical homogenization. This suspension was transferred to microcentrifuge tubes and centrifuged for 10 min at 13,000 × g and 4 °C. The supernatant was discarded and the pellets were frozen at -80 °C until use.

All radioligand binding experiments were performed in the presence of 1% bovine serum albumin in receptor binding buffer, and incubated for 60 minutes at 25 °C. For saturation binding experiments, 0.3-10.0 nM [³H]ketanserin and 0.03-4.0 nM [³H]INBMeO was used. Nonspecific binding was defined by 10 µM cinanserin in a total volume of 500 µL. Competition binding experiments were performed with 1.0 - 3.0 nM [³H]- ketanserin or 0.1 - 0.3 nM [³H] NBMeO in a total volume of 500 µL. Binding experiments were terminated by rapid filtration through FB glass fiber harvest plates with ice cold wash buffer (10mM TRIS, 0.9% NaCl) using a 96-well Packard FilterMate cell harvester. One well in each plate was spotted with 10 µL of radioligand stock solution to quantify total radioactivity, and the plates were dried overnight before 30 µL of Packard Microscint-O scintillation fluid was added to each well. Radioactivity per well was determined using a Packard TopCount scintillation counter. The saturation and competition binding curves were analyzed using GraphPad Prism (GraphPad Software, San Diego, CA).

5.3.4 Screening at Other Receptor Types—INBMeO was submitted to the NIMH-sponsored psychoactive drug screening program. The ligand had low affinity for most receptors, with the following reported K_i values (nM) for receptors where it had significant affinity: 5-HT_{2C} (2); 5HT₆ (73 ± 12); µ opiate (82 ± 14); H₁ (189 ± 35); 5-HT_{2B} (231 ± 73); kappa opiate (288 ± 50); all other K_i values were greater than 500 nM: 5-HT_{1A}, dopamine D₃, histamine H₂, 5-HT_{1D}, α_{1A} adrenergic, δ opiate, serotonin uptake transporter, 5-HT_{5A}, 5-HT_{1B}, dopamine D₂, 5-HT₇, dopamine D₁, 5-HT₃, 5-HT_{1E}, dopamine D₅, muscarinic M₁-M₅, histamine H₃, and the dopamine uptake transporter.

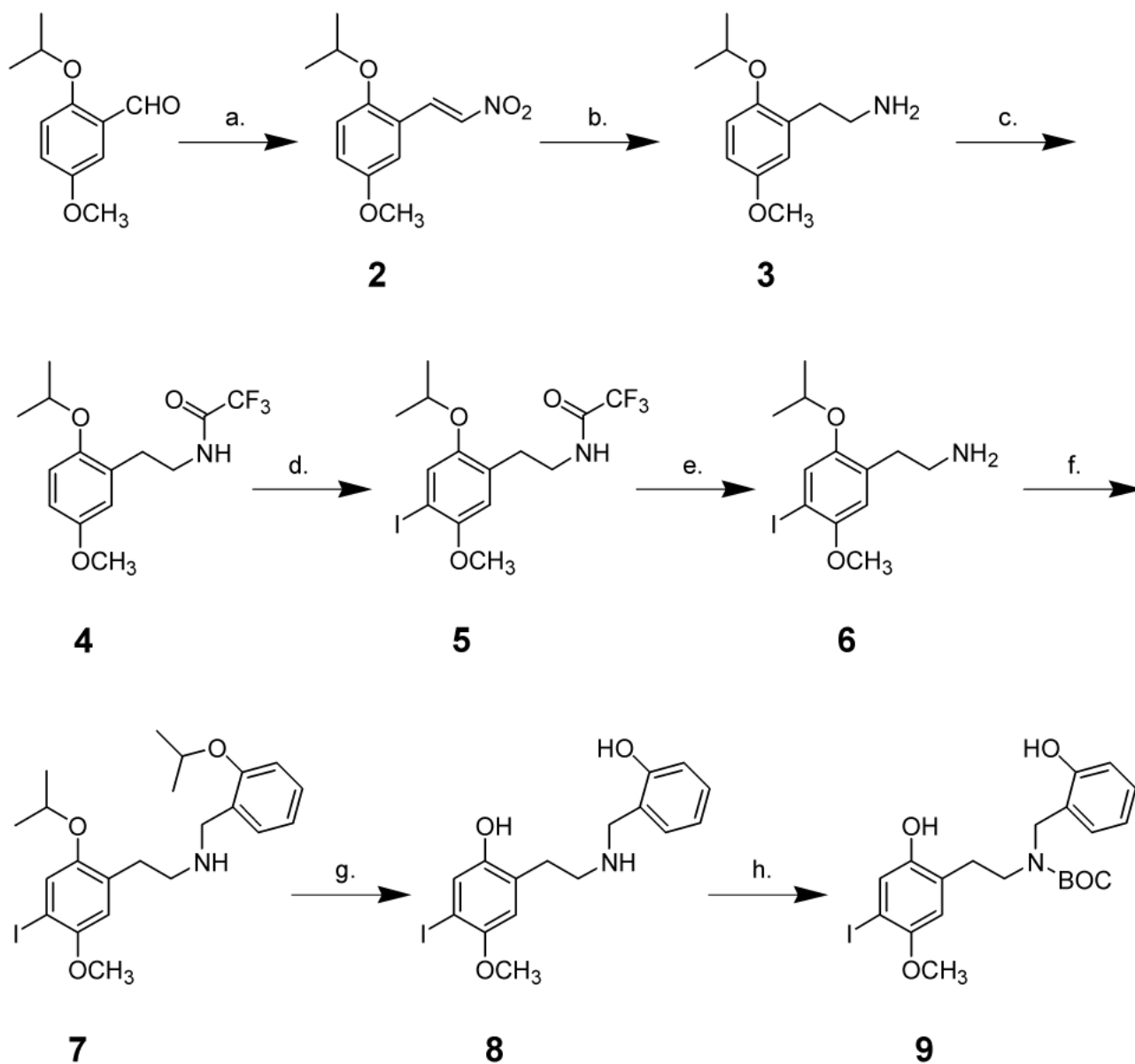
Acknowledgments

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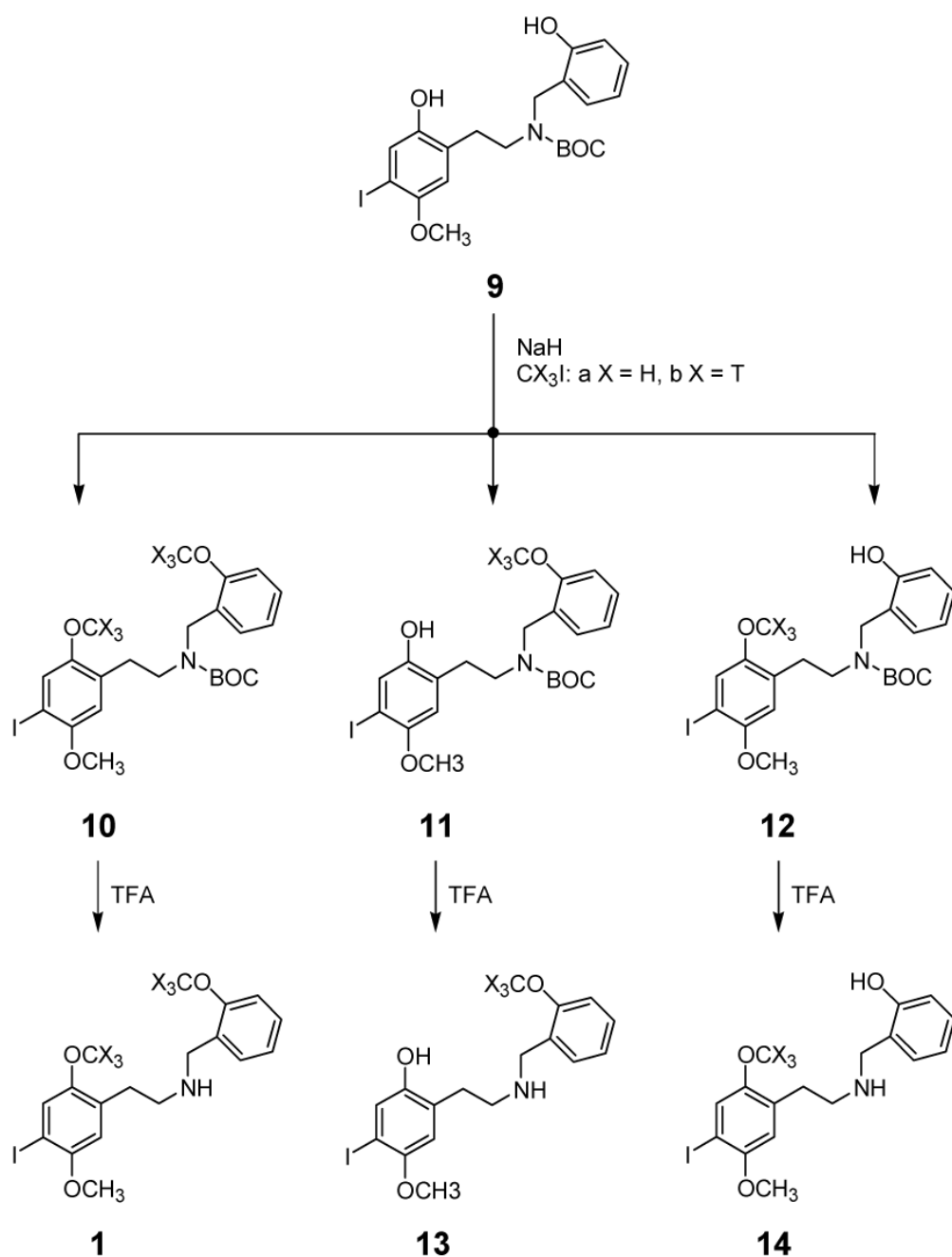
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**Scheme 1.**

Reagents and conditions: (a) EtNO₂, AcOH, reflux; (b) LiAlH₄; (c) CF₃COOEt, heat; (d) ICl, AcOH; (e) HCl; (f) *o*-isopropoxybenzaldehyde, NaCNBH₃; (g) BCl₃; (h) (*tert*-BOC)₂O.



Scheme 2.
Methylation and deprotection of precursor **9**, to produce **1**, as well as partially methylated byproducts **13** and **14**.

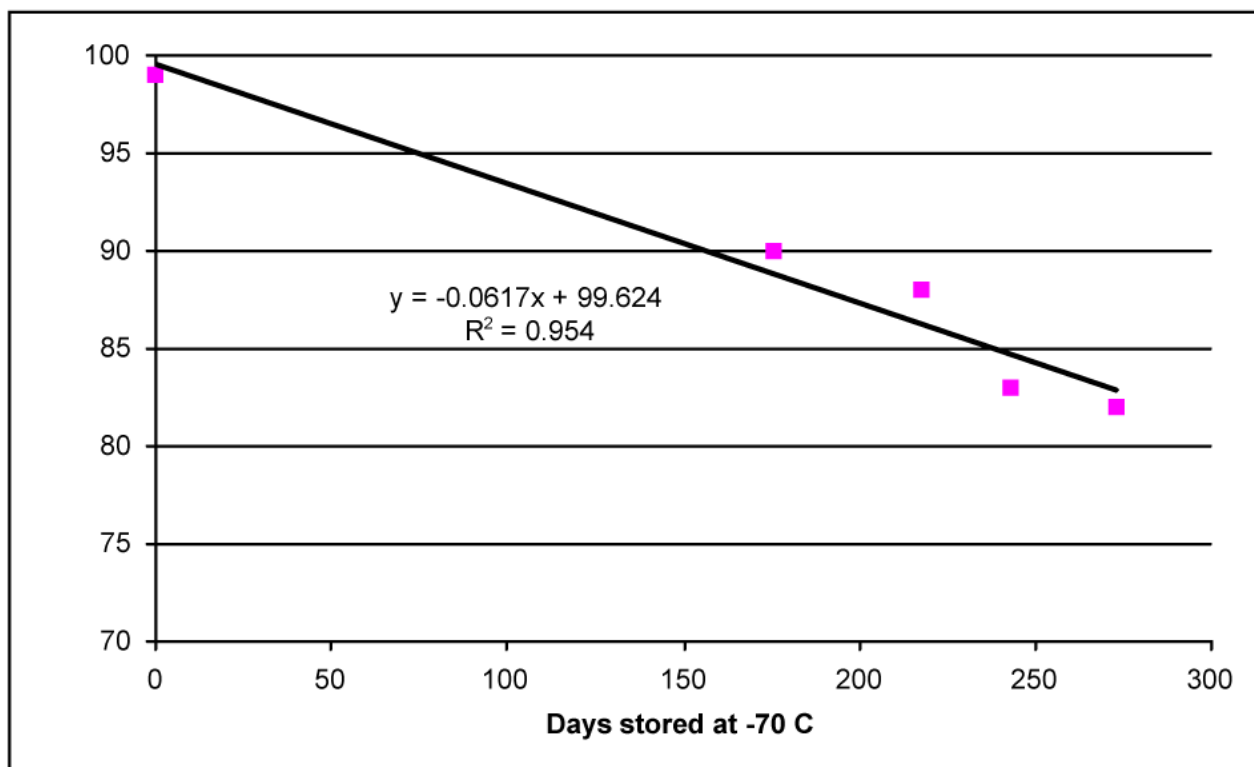


Figure 1.
Stability of [³H]INBMeO when stored at -70 °C in EtOH.

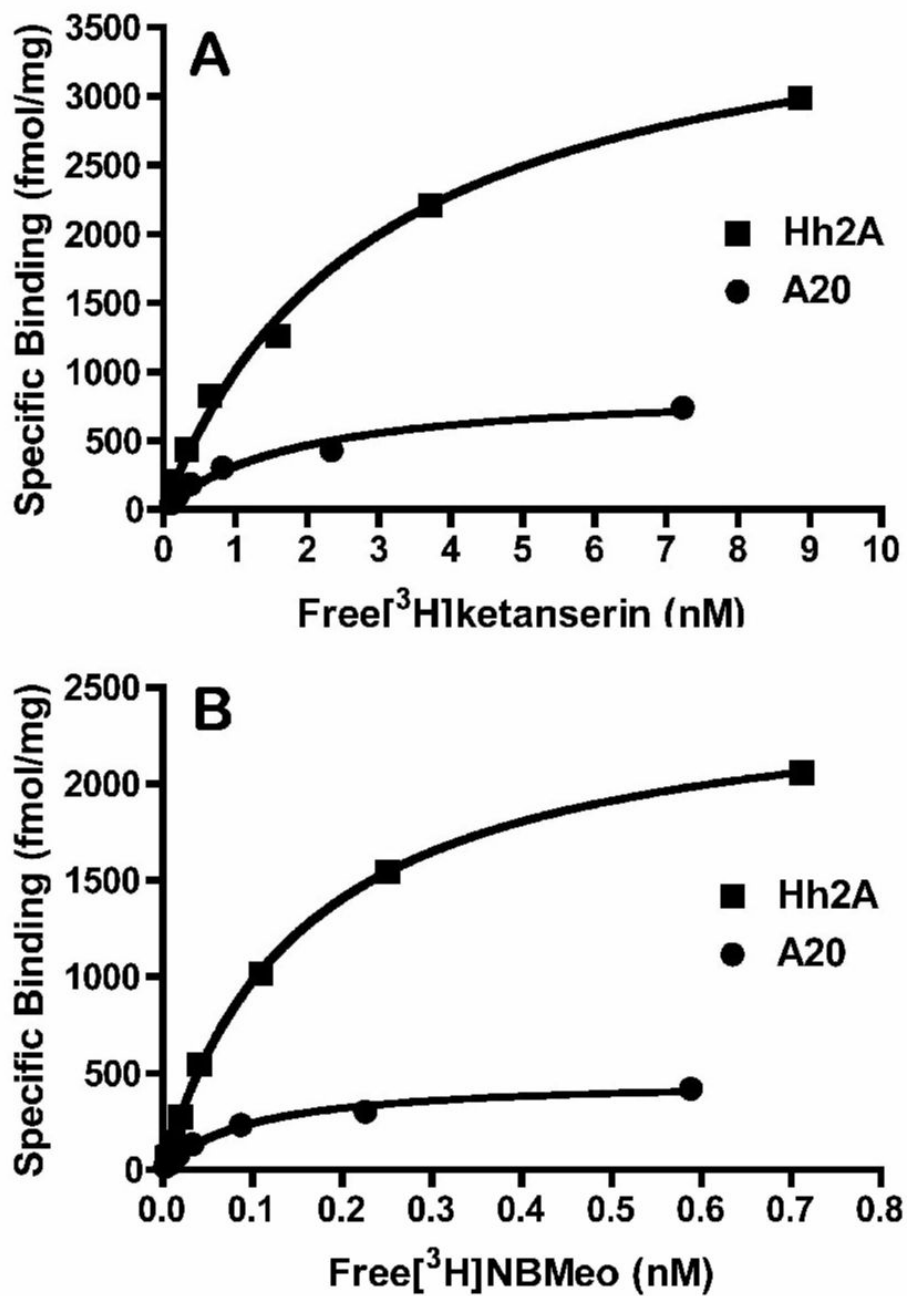


Figure 2. Representative Receptor Isotherm using **A.** [^3H]ketanserin and, **B.** [^3H]NBMeo to label 5-HT_{2A} receptors in Hh2A (HEK) and A20 (A549) cells.

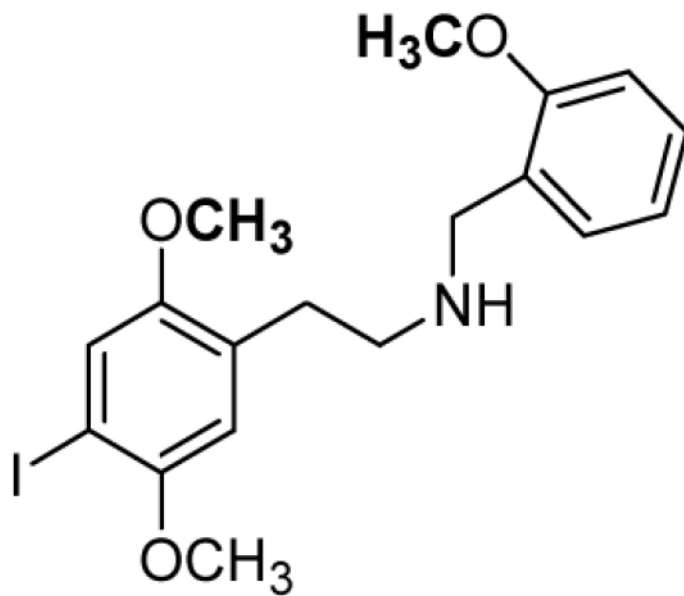
**1**

Table 1
HPLC Retention times for 1 and byproducts obtained in the radiochemical synthesis

Compound	9	10	11	12	13	14	INBMeO
Retention time (min)	26.3	31.7	27.7	27.7	14.7	15.5	17.5

Table 2A comparison of receptor saturation isotherm data for [³H]ketanserin and [³H]INBMeO

Cells	[³ H]ketanserin		[³ H]INBMeO	
	K _d (nM)	B _{max} (fmol/mg)	K _d (nM)	B _{max} (fmol/mg)
A20	2.2 ± 0.3	890 ± 160	0.097 ± 0.02	480 ± 70
Hh2A	2.7 ± 0.2	3260 ± 360	0.15 ± 0.01	2210 ± 100

(A20 cells n = 10, Hh2A cells n = 4)

Table 3

A comparison of competition binding for selected 5-HT_{2A} ligands using [³H]ketanserin and [³H]INBMeO. K_i values given are nM ± SEM.

Displacing Ligand ^a	A20 Cells		Hh2A cells	
	[³ H]ket ^b	[³ H]INB ^c	[³ H]ket ^b	[³ H]INB ^c
5-HT	120 ± 21	160 ± 17	130 ± 65	150 ± 20
Cinanserin	17 ± 2.8	24 ± 2.4	13 ± 5.1	22 ± 5.9
DOI	29 ± 5.2	28 ± 8	13 ± 2.0	14 ± 1.2
LSD	2.6 ± 0.9	4.0 ± 0.5	3.1 ± 0.7	3.5 ± 0.4

^a(n = 3)

^b[³H]ketanserin

^c[³H]INBMeO