Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **42**, 281-286 (1999)

Synthesis of 3β-(4-[^{125}I]Iodophenyl)tropane-2β-pyrrolidine Carboxamide ([^{125}I]RTI-229)

Desong Zhong,† Pravin Kotian,† Christopher D. Wyrick,† Herbert H. Seltzman,† John A. Kepler,† John W. Boja,§ Michael J. Kuhar,¶ and F. Ivy Carroll†,*

†Research Triangle Institute, Post Office Box 12194, Research Triangle Park, NC 27709

¶Yerkes Regional Primate Research Center, Emory University, 954 Gatewood NE, Atlanta, GA 30329

§Northeastern Ohio University College of Medicine, 4209 State Route 44, Rootstown, OH 44272

Summary

A WIN 35,065-2 analog, 3β-(4-iodophenyl)tropane-2β-pyrrolidine carboxamide (RTI-229), has been radiolabelled with iodine-125 by radioiododestannylation of the corresponding trimethyltin derivative using carrier-free sodium iodide-125 as the isotope source. Purification by reversed-phase HPLC gives [^{125}I]RTI-229 in good yield (89.4%) with high radiochemical purity (>99%) and high specific activity (2125 mCi/μmol, 78.6 GBq/μmol, based on the specific activity of the Na^{125}I used).

Key Words: Radioiodination, iodine-125 labelling, RTI-229, dopamine transporter, iodo-destannylation, chloramine-T

Introduction

Cocaine, a psychostimulant, has a variety of pharmacological effects on the central nervous system and the cardiovascular system (1, 2). Reports from several laboratories have shown that the psychostimulant properties of cocaine are related to its ability to inhibit dopamine (DA) reuptake by binding to a specific site on the dopamine transporter (2-6). Considerable effort has been directed to gaining information concerning the biochemical mechanism of action of cocaine. Part of this effort involved structure activity relationship (SAR) studies of the WIN 35,065-2 class of dopamine uptake inhibitors (7). These studies have been aided by the development and use of radiolabelled analogs. For example, 3β-(4-[^{123}I]iodophenyl)tropane-2β-carboxylic acid methylester ([^{123}I]RTI-55, also known as [^{123}I]-β-CIT), has been used as a novel ligand for labelling dopamine and serotonin reuptake sites in baboon in studies using Single Photon Emission Computed Tomography (SPECT)

* Author for correspondence

Received 5 July 1998
Revised 28 September 1998
Accepted 29 September 1998

imaging technology (8-10). The same technology has also been applied in other nonhuman primates (10, 11). [^{125}]RTI-55 shows high affinity for dopamine transporters in vitro and slightly less affinity for serotonin transporters (12, 13). 3β-(4-[^{123}I]Iodophenyl)tropane-2β-carboxylic acid isopropyl ester ([^{123}I] RTI-121, also known as [^{123}I] IPCIT) displays very good imaging properties in vivo in SPECT studies of the dopamine transporter where it is important to distinguish the dopamine transporter from the serotonin transporter (10, 14, 15). [^{125}I]RTI-121 has also been used in dopamine transporter studies in vitro, and the results indicate that [^{125}I]RTI-121 is a more selective ligand for the dopamine transporter than [^{125}I]RTI-55 (12, 16, 17).

In a continuation of our studies, we found that 3β-(4-iodophenyl)tropane-2β-pyrrolidine carboxamide (RTI-229) was a ligand that showed higher affinity for the dopamine transporter than RTI-55 or RTI-121 and was the most selective ligand reported that possessed an iodine substituent (Table 1) (13, 18). Even though studies have shown that the compound does not reach the brain after intravenous administration to rats (19), its high selectivity as indicated by the 2680 NE/DA ratio and 4670 5-HT/DA ratio suggested that [^{125}I]RTI-229 will be a highly useful ligand for in vitro studies of the dopamine transporter. In this paper, we report the radiolabelling of RTI-229 with iodine 125.

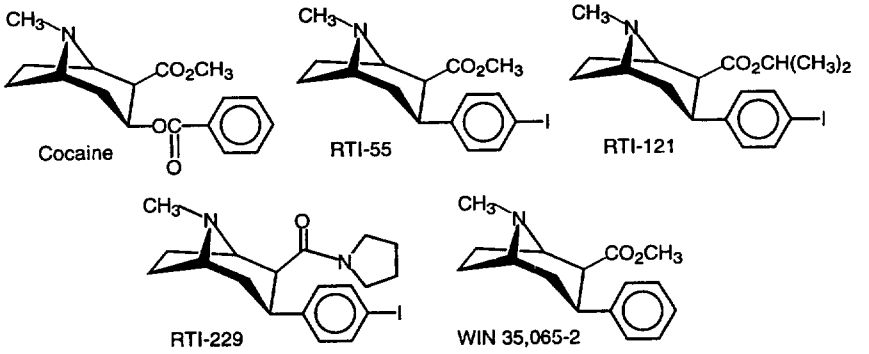

Figure 1. Cocaine, WIN 35,065-2, and Analogs

Table: Comparison of Transporter Binding Preparation of RTI-229 to Other Analogs

	IC_{50} (nM)				
Compd	DA [^{3}H]WIN 35,065-2	NE [^{3}H]Nisoxetine	5-HT [^{3}H]Paroxetine	NE/DA Ratio[a]	5-HT/DA Ratio[a]
RTI-55[b]	1.26 ± 0.04	36 ± 2.7	4.21 ± 0.34	29	3
RTI-121[b]	0.43 ± 0.05	285 ± 8	66.8 ± 6.5	662	155
RTI-229[c]	0.37 ± 0.04	991 ± 20.9	1728 ± 39	2680	4670

[a] Ratio of IC_{50} values; [b] IC_{50} values taken from ref. 18; [c] IC_{50} values taken from ref. 13.

Results and Discussion

The route used for the synthesis of [^{125}I]RTI-229 is shown in Figure 2. The reaction of 3β-(4-iodophenyl)tropane-2β-pyrrolidine carboxamide, RTI-229, **1**, with hexamethylditin in the presence of tetrakis(triphenylphosphine)palladium(0) followed by treatment with ammonium hydroxide, and recrystallization of the free base gave 3β-(4-trimethyltinphenyl)tropane-2β-pyrrolidine carboxamide (**2**). The structure of **2** was confirmed by ^{1}H NMR spectroscopy and MS analysis (M^+ 462). In addition, reaction of **2** with sodium iodide and chloramine-T regenerated **1**.

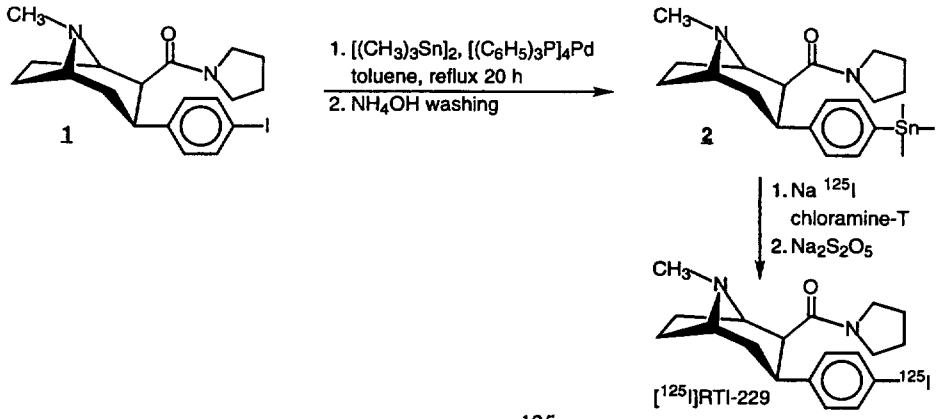

Figure 2. Synthesis of [^{125}I]RTI-229

Since the presence of residual **1** in the sample of **2** used for radioiodination will severely reduce the specific activity of the final radiolabelled product due to the large excess of **2** required, **2** was purified by HPLC to ensure the absence of **1** from the precursor. The contamination of RTI-229 in the precursor **2** was found to be under the detection limit of 0.01% (HPLC).

The radioiododestannylation of **2** was very rapid (1 min) at room temperature using chloramine-T as oxidant. The radiochemical yield was 89.4% with the only loss due to the handling of the radiolabelled product. Radiochemical purity was determined to be 99.8% by HPLC with βRAM detection and 99.2% by TLC radioscan with the t_R and R_f, respectively, being identical to an authentic sample of RTI-229.

Experimental

Materials and Methods

RTI-229 was synthesized as previously reported (13). Hexamethylditin, tetrakis(triphenylphosphine)palladium(0), and chloramine-T were purchased from Aldrich Chemical Company, sodium metabisulfite from Fisher Scientific Company, and carrier-free sodium iodide-125 from Amersham International. All the chemicals and solvents obtained from commercial sources were used without further purification. NMR spectra were obtained on a Bruker AM-250 spectrometer using

tetramethylsilane as an internal standard. Radiopreparative HPLC consisted of a Beckman HPLC pump, model 110A, Waters U6K universal injector. Analytical radio-HPLC and preparative HPLC for the precursor consisted of Rheodyne 7125 injector, Rainin HPXL pump, Rainin pressure module, Knauer variable wavelength monitor at 254 nm, and IN/US Systems βRAM flow-through radioactive monitor. Waters reversed-phase C18 Nova-Pak columns (RCM, 8 mm × 10 cm, 4 μm) were used for the purification of [^{125}I]RTI-229 and for the radiochemical purity analysis of the labelled product. The radiochemical purity of the product was also analyzed by a TLC radioscanner (Bioscan system 200 imaging scanner). Radio-TLC was carried out on a silica gel 60 F_{254} plate (EM Separations Technology). A γ-counter (Packard auto-gamma scintillation spectrometer 5135) was used to count the radioactivity of the labelled product. The specific activity of [^{125}I]RTI-229 was calculated from the specific activity of the Na^{125}I used in the synthesis.

3β-(4-Trimethyltinphenyl)tropane-2β-pyrrolidine carboxamide

Tetrakis(triphenylphosphine)palladium(0) (98.1 mg) was transferred to a round-bottomed flask (50-mL) containing **1** (1.018 g, 2.40 mmol) in a red-room under the protection of nitrogen gas. Toluene (18 mL) was added to the flask and followed by hexamethylditin (1.54 g, 4.69 mmol) using a syringe. The syringe was washed with toluene (2 mL), and the toluene solution was transferred to the flask. The mixture was stirred while heating at reflux under the protection of nitrogen gas for 20 h. The reaction mixture was then cooled and filtered through Celite. The reaction flask was washed with CH_2Cl_2 (10 mL), and the washings were filtered and combined with the filtrate. The solvents were removed using a rotary evaporator, and the product was dried under vacuum for 4 h. The crude product was crystallized twice with dichloromethane/hexane (1:1, 10 mL) to afford recrystallized 3β-(4-trimethyltinphenyl)tropane-2β-pyrrolidine carboxamide hydrogen iodide (0.673 g, 49% yield). ^{1}H NMR ($CDCl_3$) 0.02 (s, 9H), 1.05 (m, 1H), 1.45 (m, 1H), 1.65 (m, 2H), 1.9 (m, 1H), 2.15 (m, 2H), 2.4–2.9 (m, 4H), 3.0 (d, 3H), 3.15 (m, 1H), 3.3 (m, 1H), 3.45 (m, 3H), 4.05 (m, 1H), 4.55 (m, 1H), 7.3 (m, 2H), 7.4 (m, 2H), 11.95 (broad, 1H). The recrystallized salt (0.437 g, 0.74 mmol) was mixed with water (50 mL), basified to pH 9 with ammonium hydroxide, and extracted with CH_2Cl_2 (4 × 30 mL). The extract was washed with water (3 × 30 mL), dried over sodium sulphate, evaporated in vacuo, and dried under vacuum for 1 h, leaving 3β-(4-trimethyltinphenyl)tropane-2β-pyrrolidine carboxamide (0.310 g, 91% yield). The product was recrystallized three times with acetone-hexane (1:4), mp 176–177 °C. Mass spectrometry analysis: m/z (%) 462 (24) [M^+], 364 (32), 297 (3), 83 (100). ^{1}H NMR ($CDCl_3$) 0.2 (s, 9H), 1.52–1.90 (m, 7H), 1.94–2.17 (m, 2H), 2.21 (s, 3H), 2.82–2.98 (m, 3H), 3.11–3.40 (m, 6H), 7.21 (m, 2H), 7.41 (m, 2H). Crystallized 3β-(4-trimethyltinphenyl)tropane-2β-pyrrolidine carboxamide (1.2 mg) dissolved in methanol (100 μL) was injected onto a Waters reversed-phase C18 Nova-Pak column, RCM, 8 mm × 10 cm, 4 μm

(8NVC184μ), and eluted with 50% [CH_3OH-CH_3CN, 1:1], 50% [2% Et_3N-H_2O + HOAc to pH 7] at flow rate of 4 mL/min. The eluent was monitored with a UV detector at 254 nm. The eluent between t_R 16–27 min was collected. The solvent was evaporated, and the residue was dried under vacuum. The purified precursor was analyzed by HPLC under the conditions as those used in the preparative HPLC. The contamination of RTI-229 in the precursor was found to be under the detection limit of 0.01%, which was determined by integrating the peaks of standard samples under the same HPLC conditions.

3β-(4-[^{125}I]Iodophenyl)tropane-2β-pyrrolidine carboxamide ([^{125}I]RTI-229)

Sodium iodide-125 (2125 Ci/mmol, 15.52 mCi in NaOH solution, pH 8.5, 0.0073 μmol) was centrifuged for 1 min at 600 rpm, then transferred to a solution of 3β-(4-trimethyltinphenyl)tropane-2β-pyrrolidine carboxamide (0.54 mg, 1.2 μmol, purified by HPLC) in MeOH-HOAc (100 μL, 95/5 v/v) in a Reacti-vial (1 mL). The sodium iodide-125 vial was washed with the solution in the Reacti-vial (2 × 50 μL), and the washings were transferred back to the Reacti-vial. To this solution was added aqueous chloramine-T solution (80 μL, 10 mM, 0.8 μmol). The vial was capped, and the solution in the vial was stirred vigorously for 1 min with a vortex mixer. The reaction was then quenched by adding aqueous sodium metabisulfite ($Na_2S_2O_5$, 80 μL, 20 mM, 1.6 μmol) and stirred vigorously for 1 min with a vortex mixer. The entire reaction mixture was loaded onto a Waters reversed-phase C18 Nova-Pak column (RCM, 8 mm × 10 cm, 4 μm), and the Reacti-vial was washed with the HPLC eluent solvent (50 μL) and loaded onto the column. The column was eluted with 50% [CH_3OH-CH_3CN, 1:1], 50% [2% Et_3N-H_2O + HOAc to pH 7] at a flow rate of 2 mL/min. The eluent was collected as 2-mL fractions, and the eluent with the largest amount of radioactivity (fraction 6) was diluted to 25 mL with methanol as desired labelled product. HPLC analysis of 5 μL in the above eluent with a fresh column and a βRAM detector showed 99.8% purity (t_R 6.1 min, k' = 2.6). The radiochemical purity of the labelled product was also analyzed by TLC radioscan cospotted with unlabelled standard RTI-229 eluting with 30% [CH_3OH-CH_3CN, 1:1], 70% [1% Et_3N-H_2O + HOAc to pH 4] indicating a purity of 99.2% (R_f 0.38). The radioactivity of the purified labelled compound was counted by a γ-counter (Packard auto-gamma scintillation spectrometer 5135), which gave an overall radiochemical yield of 89.4%.

Acknowledgments

This work was supported by the National Institute on Drug Abuse, Grant DA05477.

References

1. Kuhar M.J. – Drug Abuse in the Decade of the Brain, Nahas, G.G. & Burks, T.F., eds., IOS Press, Amsterdam, 1997, 207
2. Ritz M.C., Lamb R.J., Goldberg S.R. and Kuhar M.J. – Science 237: 1219 (1987)

3. Wise R.A. and Bozarth M.A. – Psychol. Rev. 94: 469 (1987)
4. Koob G.F. and Bloom F.E. – Science 242: 715 (1988)
5. Bergman J., Madras B.K., Johnson S.E. and Spealman R.D. – J. Pharmacol. Exp. Ther. 251: 150 (1989)
6. Kuhar M.J., Ritz M.C. and Boja J.W. – Trends Neurosci. 14: 299 (1991)
7. Carroll F.I., Lewin A.H. and Kuhar M.J. – Neurotransmitter Transporters: Structure, Function, and Regulation, Reith, M.E.A., eds., Humana Press Inc., Totowa, NJ, 1997, 263
8. Carroll F.I., Rahman M.A., Abraham P., Parham K., Lewin A.H., Dannals R.F., Shaya E., Scheffel U., Wong D.F., Boja J.W. and Kuhar M.J. – Med. Chem. Res. 1: 289 (1991)
9. Carroll F.I., Scheffel U., Dannals R.F., Boja J.W. and Kuhar M.J. – Med. Res. Rev. 15: 419 (1995)
10. Kuhar M.J., Carroll F.I., Lewin A.H., Boja J.W., Scheffel U. and Wong D.F. – Neurotransmitter Transporters: Structure and Function, Reith, M.E.A., eds., Humana Press Inc., Totowa, NJ, 1997, 297
11. Baldwin R.M., Zea-Ponce Y., Zoghbi S.S., Laruelle M., Al-Tikriti M.S., Sybirska E.H., Malison R.T., Neumeger J.L., Milius R.A., Wang S., Stabin M., Smith E.O., Charney D.S., Hoffer P.B. and Innis R.B. – Nucl. Med. Biol. 20: 597 (1993)
12. Boja J.W., McNeill R.M., Lewin A.H., Abraham P., Carroll F.I. and Kuhar M.J. – NeuroReport 3: 984 (1992)
13. Carroll F.I., Kotian P., Dehghani A., Gray J.L., Kuzemko M.A., Parham K.A., Abraham P., Lewin A.H., Boja J.W. and Kuhar M.J. – J. Med. Chem. 38: 379 (1995)
14. Scanley B.E., Al-Tikriti M.S., Gandelman M.S., Laruelle M., Zea-Ponce Y., Baldwin R.W., Zoghbi S.S., Hoffer P.B., Charney D.S., Wang S., Neumeyer J.L. and Innis R.B. – Eur. J. Nucl. Med. 22: 4 (1995)
15. Boja J.W., Cadet J.L., Kopajtic T.A., Lever J., Seltzman H.H., Wyrick C.D., Lewin A.H., Abraham P. and Carroll F.I. – Mol. Pharmacol. 47: 779 (1995)
16. Lever J.R., Scheffel U., Stathis M., Seltzman H.H., Wyrick C.D., Abraham P., Parham K., Thomas B.F., Boja J.W., Kuhar M.J. and Carroll F.I. – Nucl. Med. Biol. 23: 277 (1996)
17. Staley J.K., Boja J.W., Carroll F.I., Seltzman H.H., Wyrick C.D., Lewin A.H., Abraham P. and Mash D.C. – Synapse 21: 364 (1995)
18. Boja J.W., Kuhar M.J., Kopajtic T., Yang E., Abraham P., Lewin A.H. and Carroll F.I. – J. Med. Chem. 37: 1220 (1994)
19. Kopajtic T.A., Boja J.W., Carroll F.I., Kotian P., Seltzman H.H., Wyrick C.B., Lewin A.H. and Kuhar M.J. – Soc. Neurosci. Abstr. 20: 992 (1994)

