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Radiosynthesis of (±)-1-(2-bromo-4,5-dimethoxybenzyl)-7-hydroxy-6-methoxy-2-[^{11}C]-methyl-1,2,3,4-tetrahydro-isoquinoline, [^{11}C]A-69024: A Non-benzazepine Antagonist for Studying Dopamine D1 Receptors *In Vivo* Using PET

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Summary

(±)-1-(2-bromo-4,5-dimethoxybenzyl)-7-hydroxy-6-methoxy-2-[^{11}C]-methyl-1,2,3,4-tetrahydroisoquinoline, [^{11}C]A-69024, a selective ligand for the D1 receptor was prepared by *N*-alkylation of (±)*N*-desmethyl A-69024 with [^{11}C]methyl iodide in DMF. The radiotracer was purified by semi-preparative reverse-phase HPLC. The average specific activity was 1950 mCi/μmol calculated at end-of-synthesis (EOS). The average time of synthesis including formulation was 20 minutes.

Key Words: A-69024, carbon-11, dopamine D1 receptor, PET, radiotracer.

Introduction

Dopamine receptors in the central nervous system are divided into two major subtypes, designated as D1 and D2. Activation of the D1 receptor subtype is linked biochemically to the cyclic adenosine monophosphate synthesis, whereas the D2 receptor subtype acts via inhibition of cAMP synthesis (1,2). It was the D2 receptor

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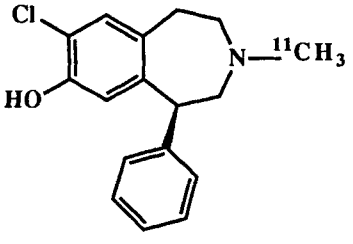
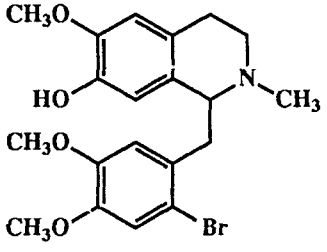
that was originally believed to be the site of antipsychotic drug action, thus early efforts focused on development of D2 radioligands. More recently, with the aid of selective D1 agonists and antagonists, the role of the D1 receptor has been documented, as well as the possibility that D1 and D2 receptors modulate each other (3-5). D1 receptors are attractive sites for the development of antipsychotic drugs and it has been suggested that, compared to classical neuroleptics, D1 antagonists might have advantages in the treatment of schizophrenia (6).

Introduction of non-invasive imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) has made possible the study of neuroreceptors in the living human brain. Such studies have proven useful in the localization and quantification of neuroreceptors and offer insight into the relationship of these receptors in normal and disease states (7-9). PET and SPECT studies of D1 receptors have been limited due to a lack of radioligands that display high affinity and selectivity for D1 receptors. The benzazepine SCH 23390 was the first reported antagonist with nanomolar affinity for the D1 receptor (10,11). This has resulted in the preparation of [^{11}C]SCH 23390 (Figure 1) (12-14) which has been used extensively for PET analysis of D1 receptor binding (12,15,16). However, *in vitro* binding experiments and functional studies revealed that SCH 23390 interacts with the 5-HT$_{2C}$ receptor (18); during the time course of a PET experiment [^{11}C]SCH 23390 was rapidly metabolized (19). Several radiohalogenated derivatives of SCH 23390 have also been reported (15-17). The structurally rigid benzazepine analogue, [^{11}C]SCH 39166 was prepared and demonstrated greater selectivity and nanomolar affinity for the D1 receptor (20). Thus, there is a continued need to develop more selective D1 ligands in order to aid the unambiguous determination of the physiological roles of the D1 receptor.



[^{11}C]SCH 23390



A-69024

Figure 1. Dopamine D1 Receptor Antagonists

More recently, selective non-benzazepine D1 ligands have been reported (21-24) including (±)-1-(2-bromo-4,5-dimethoxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline, A-69024 (Figure 1). *In vitro* binding experiments with A-69024 demonstrate that it is a selective D1 antagonist with nanomolar affinity for D1 receptors (K_i = 12.6 nM) labeled with [^{125}I]SCH 23982, and micromolar

affinity for D2 (K_i = 1.29 μM) and 5-HT_{2C} (K_i = 17.8 μM) receptors labeled with [^{3}H]spiroperidol and [^{125}I]lysergic acid diethylamide, respectively. In several *in vivo* assays A-69024 displayed characteristics indicative of neuroleptic activity (24). Based on these results and the need for a positron emitting non-benzazepine D1 ligand, we chose to radiolabel A-69024 with carbon-11 using [^{11}C]methyl iodide. In this paper we report the radiosynthesis, purification, and quality control of this D1 receptor ligand for PET.

Materials and Methods

N-desmethyl A-69024 was prepared by means of HBr acetic acid deprotection of the *O*-benzyl ether, the preparation of which has been described (25). Authentic A-69024 was obtained as a gift from Dr. Daniel J. Kerkman, Abbott Pharmaceuticals (Abbott Park, IL). [^{11}C]Carbon dioxide was produced by 16 MeV proton bombardment of a nitrogen gas target using a Scanditronix RNP-16 biomedical cyclotron. Conversion to [^{11}C]methyl iodide has been previously described (26). Radioactivity measurements were made using a Capintec CRC-12 dose calibrator.

Synthesis and purification of [^{11}C]A-69024.

The HBr salt of *N*-desmethyl A-69024 (3 mg, 6.1 μmol) was dissolved in H_2O (0.5 mL) to which was added K_2CO_3 (1 mg, 7.2 μmol). The mixture was extracted with diethyl ether (1 mL) and the solvent evaporated to give the free base of the *N*-desmethyl compound. This was taken up in dimethylformamide (200 μL) and transferred to a small septum sealed vessel. The vessel was cooled (-78 °C) and [^{11}C]methyl iodide was transferred into the reaction vessel by a stream of nitrogen carrier gas. When the radioactivity in the solution reached a plateau, the stream of nitrogen was stopped and the vessel submerged in a 80 °C water bath. After 5 minutes, 200 μL of HPLC solvent, consisting of 30:70 acetonitrile:water (0.1M ammonium formate), was added to the reaction solution. The mixture was injected onto a Alltech Econosil C18 10μ (250 mm x 10 mm) semi-preparative column and eluted at a rate of 10 mL/min. The effluent from the column was monitored with a UV detector (254 nm, Waters module 440) and an in-line radioactivity detector (Ortec 449 ratemeter, 575 amplifier, 550 single channel analyzer, with a NaI(Tl) crystal). The radioactive peak corresponding to [^{11}C]A-69024 (t_R = 8.2 minutes, k' = 5.8) was collected in a rotary evaporator and the solvent evaporated to dryness under reduced pressure. The residue was dissolved in sterile, normal saline (7 mL) and filtered through a sterile, 0.22 μm filter into a sterile, pyrogen-free evacuated vial. Sterile aqueous sodium bicarbonate (3 mL, 8.4%) was then added and the radioactivity was measured.

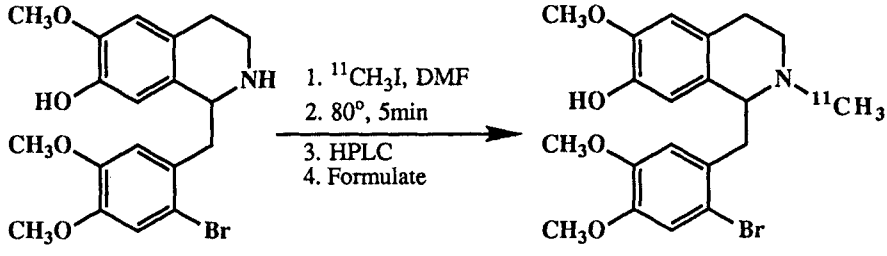
Determination of specific activity

An aliquot of the final solution of known volume and radioactivity was applied to an analytical reverse-phase HPLC column (Alltech Econosil C18 10μ 250 mm x

4.6 mm). A mobile phase of 40:60 acetonitrile:water (0.1 M ammonium formate) with a flow rate of 4 mL/min was used to elute the radioligand (t_R = 3.2 minutes, k' = 3.6). The area of the UV absorbance peak measured at 254 nm corresponding to carrier product was measured by an automated integrating recorder (Hewlett Packard 3390A) and compared to a standard curve relating mass to UV absorbance

Results and Discussion

The synthesis of [^{11}C]A-69024 involved the *N*-alkylation of *N*-desmethyl A-69024 with [^{11}C]methyl iodide (Scheme 1). A possible competing reaction is *O*-radioalkylation at the phenolic oxygen. To verify that this did not occur, an attempt was made to *O*-alkylate an authentic sample of A-69024 with [^{11}C]methyl iodide using the same reaction conditions as described for *N*-alkylation. No evidence for the formation of the radiolabeled *O*-alkylated product was found using analytical HPLC; the bulk of radioactivity corresponded to volatile material.



Scheme 1.

Reverse-phase semi-preparative HPLC was used to purify [^{11}C]A-69024 (Figure 2). *N*-desmethyl A-69024 eluted at 5.4 minutes (k' = 3.2) while [^{11}C]A-69024 eluted at 8.2 minutes (k' = 5.3). The radioactive peak corresponding to the product was collected remotely and the HPLC solvent removed via rotary evaporation under high vacuum. [^{11}C]A-69024 was formulated in sterile saline, microfiltered into a sterile, evacuated dose-vial, and diluted with sterile sodium bicarbonate. Using this procedure, the formulated radiotracer proved to be sterile and pyrogen-free.

The average time of synthesis of [^{11}C]A-69024 from end of bombardment was 20 minutes with a non-decay corrected radiochemical yield of 20%. To determine specific activity and final radiochemical purity of the radioligand, a known aliquot of radioactivity was injected onto an analytical reverse-phase HPLC column. Comparison of the carrier peak associated with the radioactivity to that of a standard sample of A-69024 enabled calculation of the specific activity. The resulting chromatogram showed [^{11}C]A-69024 to be of high radiochemical while its chemical purity determined at 254 nm was greater than 99%. The radioactive product co-eluted with an authentic sample of A-69024 confirming its identity, while the specific

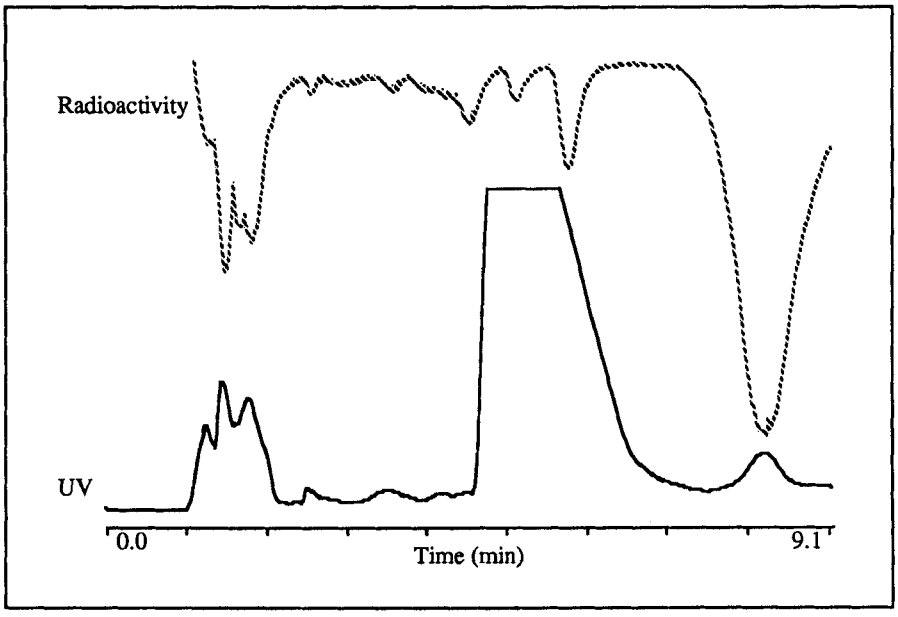


Figure 2. Preparative chromatogram of [^{11}C]A-69024

activity calculated at the end of synthesis was 1950 mCi/μmol.

Future efforts will include radiolabeling of the resolved enantiomers of A-69024. This should improve affinity and selectivity for D1 receptors as its believed that the D1 activity is associated with the (S) - enantiomer as described for other tetrahydroisoquinolines (21).

Conclusions

[^{11}C]A-69024 was prepared in high specific activity by *N*-alkylation of *N*-desmethyl A-69024 with [^{11}C]methyl iodide. The synthesis produced radiochemically pure product in reasonable quantities. A sufficient amount of the radioligand can be prepared to allow *in vivo* studies of D1 receptors in the brain with PET.

Acknowledgments

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