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Research Article

Synthesis of *N*-(piperidin-1-yl)-5-(4-methoxyphenyl)-1-(2-chlorophenyl)-4-[^{18}F]fluoro-1*H*-pyrazole-3-carboxamide by nucleophilic [^{18}F] fluorination: a PET radiotracer for studying CB_1 cannabinoid receptors

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Summary

The feasibility of nucleophilic displacement of bromide in the 4-bromopyrazole ring with [^{18}F]fluoride has been demonstrated by the synthesis of two radiolabeled compounds: *N*-(piperidin-1-yl)-5-(4-methoxyphenyl)-1-(2-chlorophenyl)-4-[^{18}F]fluoro-1*H*-pyrazole-3-carboxamide, ([^{18}F] NIDA-42033) **1b** and 1-(2-chlorophenyl)-4-[^{18}F]fluoro-5-(4-methoxyphenyl)-1*H*-pyrazole-3-carboxylic acid, ethyl ester **4**. The radiochemical yields were in the range of 1–6%. [^{18}F]NIDA-42033, a potential radiotracer for the study of CB_1 cannabinoid receptors in the animal brain by positron emission tomography, has been synthesized in sufficient quantities with specific radioactivity greater than 2500 mCi/μmol and radiochemical purity > 95%. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: positron emission tomography; cannabinoid receptors; radiosynthesis; ^{18}F; nucleophilic radiofluorination

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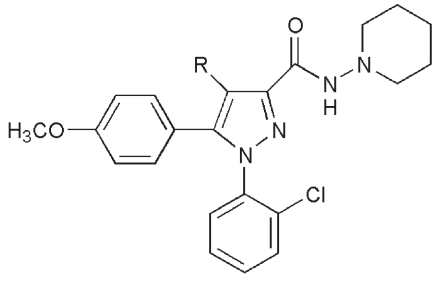


1a R = F (NIDA-42033)

1b R = ^{18}F ([^{18}F] NIDA-42033)

2 R = Br (NIDA-42055)

Figure 1.

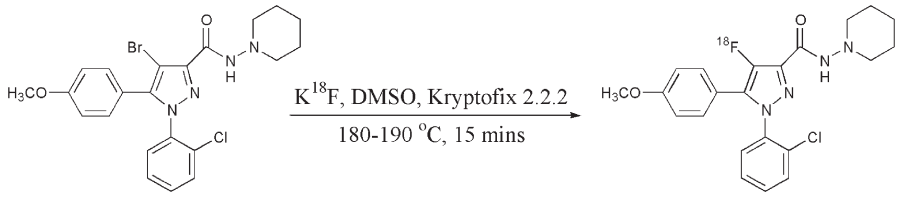
Introduction

Non-invasive *in vivo* imaging of the central CB_1 receptors by positron emission tomography (PET) would enable the investigation of the pharmacological and physiological role played by the cannabinoids in normal and disease states. Recently, several PET and SPECT radiotracers based on the selective and potent CB_1 antagonist SR141716 have been reported.[1–4] Unfortunately, these radiotracers display very high non-specific binding due to their high lipophilicity.

We have recently synthesized NIDA-42033, *N*-(piperidin-1-yl)-5-(4-methoxyphenyl)-1-(2-chlorophenyl)-4-fluoro-1*H*-pyrazole-3-carboxamide, an SR141716[5] analogue with lower lipophilicity (clog $D = 3.43$) and high affinity at CB_1 receptors ($K_i = 18$ nM) in *in vitro* studies (Figure 1).[6] Herein, we report the radiosynthesis of ^{18}F labeled NIDA-42033 by no-carrier-added nucleophilic [^{18}F]fluorination of NIDA-42055, *N*-(piperidin-1-yl)-5-(4-methoxyphenyl)-1-(2-chlorophenyl)-4-bromo-1*H*-pyrazole-3-carboxamide. The presented radiosynthesis utilizes a novel nucleophilic radiofluorination reaction with 4-bromopyrazole precursors[7] yielding 4-[^{18}F]fluoropyrazole derivatives.

Results and discussion

In the past, synthesis of non-labeled fluorinated pyrazoles has been performed mainly via three routes: (i) condensation of fluorinated diketo substrates with substituted hydrazines,[8] (ii) direct ring



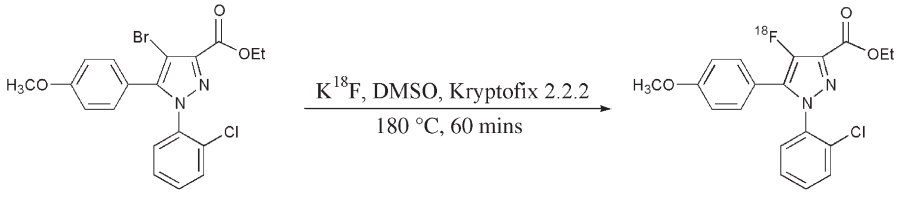
2
NIDA-42055

1b
[18F] NIDA-42033

Scheme 1.

fluorination using gaseous fluorine,[9] and (iii) diazotization and photochemical irradiation of the diazonium salts in tetrafluoroboric acid.[10] Any examples of nucleophilic fluorination of 4-substituted pyrazole derivatives were not found even after an exhaustive literature search. Because the pyrazole moiety can be found in many CNS drugs, a practical method of nucleophilic radiofluorination of pyrazole ring in position C-4 can be potentially beneficial for the development of novel PET radiotracers including novel cannabinoid ligands. As a result, we proposed to synthesize a 4-[18F]fluoropyrazole derivative by nucleophilic radiofluorination of the corresponding 4-bromopyrazole. The previously synthesized cannabinoid ligands NIDA-42055 and NIDA-42033 by our group[6] appeared to be interesting objects for such a study.

No-carrier-added radiofluorination of NIDA-42055 in DMSO solution in the presence of Kryptofix® 222 at 180–190°C for 15 min gave the corresponding [18F]NIDA-42033 (Scheme 1). The average radiochemical yield (not decay corrected) was 1% based on five syntheses and the average specific radioactivity was greater than 2500 mCi/μmol calculated at the end of the synthesis. There were two radioactive hydrophilic by-products in the reaction mixture as determined by the preparative HPLC. The first peak was [18F] fluoride and the second peak was unknown. The purification of the product was performed with reverse phase semi-preparative HPLC. The total time of the synthesis including purification was 45–60 min. After the evaporation of HPLC solvent, the final product was formulated as a saline solution (5 ml) with the addition of 10 μl Tween 80 (Aldrich). Tween 80 was added to improve the solubility of the radiotracer in saline. Microwave heating at 250°C for 2 min gave a similar radiochemical yield. The final product was shown to be radiochemically pure (>95% by radioHPLC) and coeluted with the standard.[6, 7] The relatively low yield of [18F]NIDA-42033 may be due to the instability of the hydrazide



3 **4**

Scheme 2.

moiety under the drastic radiofluorination conditions. Under similar conditions, 1-(2-chlorophenyl)-4-[^{18}F]fluoro-5-(4-methoxyphenyl)-1*H*-pyrazole-3-carboxylic acid ethyl ester, **4** was obtained by radiofluorination of the corresponding bromo analogue **3** with a radiochemical yield of 6% (Scheme 2) (3 runs, experiment not presented). The higher yield in case of **4** as opposed to **1b** suggests that the nucleophilic radiofluorination reaction may prove more or less efficient based on the stability of other substituents attached to the pyrazole backbone and/or activation of 4-position for the nucleophilic substitution.

Conclusion

In summary, a simple one-step radiosynthesis of a high specific activity radioligand for studying the cerebral cannabinoid receptors by PET has been developed. The synthesis yields sufficient quantities of the radioligand for future studies of CB_1 receptors by PET. A novel nucleophilic radiofluorination at C-4 position of the pyrazole ring has been reported. This nucleophilic radiofluorination procedure may prove useful for the preparation of various 4-[^{18}F]fluoropyrazole based radiotracers. Potentially, the yield of 4-[^{18}F]fluoropyrazole derivatives may be improved by employing precursors with a better leaving group, such as, 4-trimethylammoniumpyrazole derivatives.

Experimental

Materials and Methods

All reagents were ACS or HPLC grade and were purchased from Aldrich. HPLC analyses and purification were performed with two

Waters 600/610 HPLC pumps, an in-line UV-detector (Waters, 254 nm), and a flow-count radioactivity detector (Bioscan 3200). HPLC chromatograms were recorded on a Rainin Dynamax dual channel control/ interface module connected to a Macintosh computer with Dynamax v.1.4.2 software. A dose calibrator (Capintec CRC-35R) was used for all radioactivity measurements. [^{18}F]Fluoride was prepared using an RDS111 cyclotron (CTI, Knoxville, TN). The radiofluorination was performed either with an automated radiochemistry module (CPCU, CTI, Knoxville, TN) or, manually, with a microwave oven (CEM, Matthews, NC). All precursors and standards were prepared in our lab.[6, 7]

Radiochemistry

N-(piperidin-1-yl)-5-(4-methoxyphenyl)-1-(2-chlorophenyl)-4-[^{18}F] *fluoro-*1*H-pyrazole-3-carboxamide* ***(1b)***. *N*-(piperidin-1-yl)-5-(4-methoxyphenyl)-1-(2-chlorophenyl)-4-bromo-1*H*-pyrazole-3-carboxamide (NIDA-42055) (3 mg, 6 μmol) was dissolved in anhydrous DMSO (0.5 ml) and transferred to a reaction vessel containing K[^{18}F]F/Kryptofix®222/K_2CO_3 complex prepared by the Hamacher method[11] with 24 mg Kryptofix®222 and 4.5 mg K_2CO_3. The reaction mixture was heated at 182°C for 15 min, cooled down, diluted with 0.5 ml water and injected onto the Waters PrepPak NOVA C18 cartridge (25 × 100 mm^2). The reaction mixture was eluted with mixture of acetonitrile : 0.1 M ammonium formate buffer (53:47) at a flow rate of 8 ml/min. The radioactive peak with a retention time of 14.2 min ($k' = 3.05$) corresponding to the NIDA-42033 standard was collected. The retention time and the capacity factor of the precursor, NIDA-42055, under the separation conditions were 17.25 min and 3.93, respectively. There were three more substantial unidentified non-radioactive peaks in the reaction mixture with retention times of 4.7, 5.0 and 8.8 min.

An aliquot of the final solution of known volume and radioactivity was applied to an analytical HPLC column (Waters Symmetry C18 column, 4.6 × 250 mm). A mobile phase of acetonitrile: 0.1 M ammonium formate buffer (50:50) at a flow rate of 3 ml/min was used to elute the radioligand, which had a retention time of 4.8 min. The area of the UV absorbance peak measured at 254 nm corresponding to carrier product was measured and compared to the cold standard for specific radioactivity calculation. The radiochemical product coeluted with a sample of authentic NIDA-42033.

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