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Synthesis and in vitro autoradiographic evaluation of a novel high-affinity radioiodinated ligand for imaging brain cannabinoid subtype-1 receptors

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ABSTRACT

There is strong interest to study the involvement of brain cannabinoid subtype-1 (CB_1) receptors in neuropsychiatric disorders with single photon emission computed tomography (SPECT) and a suitable radioligand. Here we report the synthesis of a novel high-affinity radioiodinated CB_1 receptor ligand ([^{125}I]**8**, [^{125}I]1-(2-iodophenyl)-4-cyano-5-(4-methoxyphenyl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxylate, [^{125}I]SD7015). By autoradiography in vitro, [^{125}I]**8** showed selective binding to CB_1 receptors on human brain postmortem cryosections and now merits labeling with iodine-123 for further evaluation as a SPECT radioligand in non-human primate.

Cannabis sativa (marijuana) is one of the oldest known plant derived-therapeutics, owing mainly to its anti-nociceptive properties.[1] Other beneficial effects of cannabis intake may include anti-emesis and appetite stimulation. Conversely, the accompanying psychological ‘high’ and memory impairment have limited its therapeutic value. The most abundant and psychoactive cannabinoid, Δ^9-tetrahydrocannabinol (Δ^9-THC, **1**), binds with high affinity to two known body receptor systems. These receptors have been named cannabinoid subtype-1 (CB_1) and cannabinoid subtype-2 (CB_2).[2–4] CB_1 receptors have high-densities in brain and are implicated in a number of neuropsychiatric disorders[5] such as, schizophrenia[6,7] and depression.[8,9] CB_2 receptors are located mainly in the periphery and are of less interest to neuropsychiatric research.[10,11]

Ability to image and measure brain CB_1 receptors non-invasively with radiation computed tomography would assist neuropsychiatric research and drug development. The development of radioligands suitable for imaging brain CB_1 receptors is therefore of strong importance. Recently, there has been a great deal of progress in the development of useable radioligands for positron emission tomography (PET). Some of the more notable PET radioligands (Fig. 1) are [^{11}C]MePPEP ([^{11}C]**2**),[12,13] [^{18}F]PipISB ([^{18}F]**3**),[14,15] [^{18}F]MK-9470 ([^{18}F]**4**),[16,17] (−)-[^{11}C]SD5014 ((−)-[^{11}C]**5**),[18] [^{11}C]-OMAR ([^{11}C]**6**),[19,20] and [^{11}C]JHU75575 ([^{11}C]**7**).[20,21] In comparison, the development of radioligands for more widespread single-photon computed tomography (SPECT) is much less advanced. Due to the longer half-life of radioiodine for SPECT imaging (e.g., ^{123}I, $t_{1/2}$ = 13.2 h), an on-site cyclotron is not required for radiolabeling, as with PET, which allows for more widespread use in pharmaceutical, academic, and hospital settings. Here we report the synthesis, receptor screening, radioiodination, and in vitro autoradiographic evaluation of a novel radioiodinated CB_1 receptor radioligand ([^{125}I]4-cyano-1-(2-iodophenyl)-5-(4-methoxyphenyl) -*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide, [^{125}I]**8**, [^{125}I]SD 7015). Our results strongly suggest that [^{123}I]**8** would merit evaluation as a SPECT radioligand in vivo.

Compound **8** was synthesized according to the procedure depicted in Scheme 1. Conversion of 2-iodoaniline into a diazonium salt, followed by treatment with ethyl 2-chloroacetoacetate in ethanol–water solution under basic conditions gave chloro[(2-iodophenyl)hydrazono]ethyl acetate (**9**). Heating of a solution of **9**, 4-methoxybenzoylacetonitrile and *N*,*N*-diisopropylethylamine in *tert*-butanol to reflux gave **10** in low but adequate yield. Hydrolysis of **10** with LiOH in aq-tetrahydrofuran gave the corresponding carboxylic acid, which was then converted into the acyl chloride with oxalyl chloride and a catalytic amount of *N*,*N*-dimethylformamide. The synthesis of **8** was completed by coupling the acyl chloride with 1-aminopiperidine under basic conditions.

The trimethylstannylated precursor, **11**, required for radiolabeling, was synthesized by refluxing a solution of the known bromo compound **7**[20] and hexamethylditin in the presence of palladium catalyst (Scheme 2).

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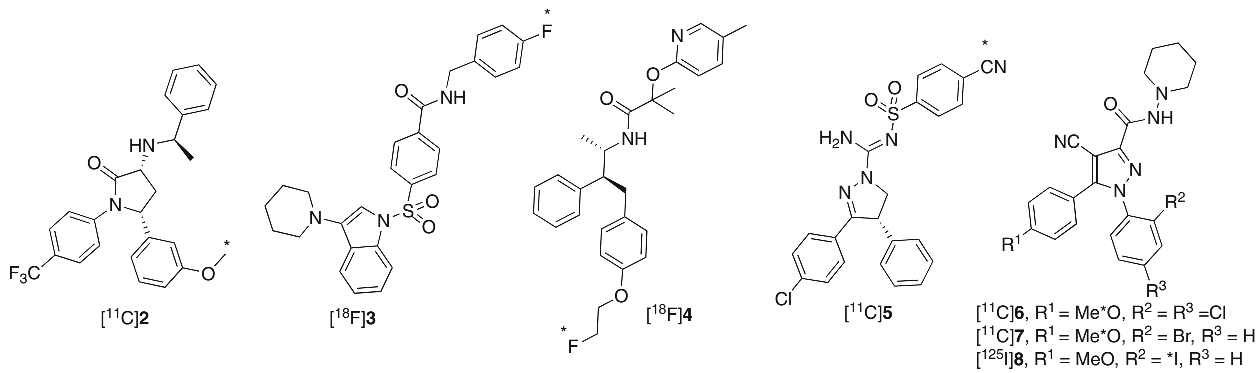
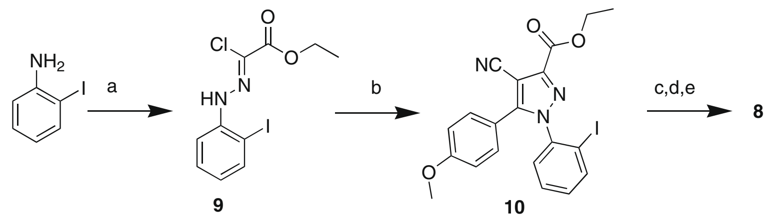


Figure 1. Structures of [^{11}C]**2**, [^{18}F]**3**, [^{18}F]**4**, [^{11}C]**5–7**, and [^{125}I]**8**. Asterisks denote positions of radiolabels.

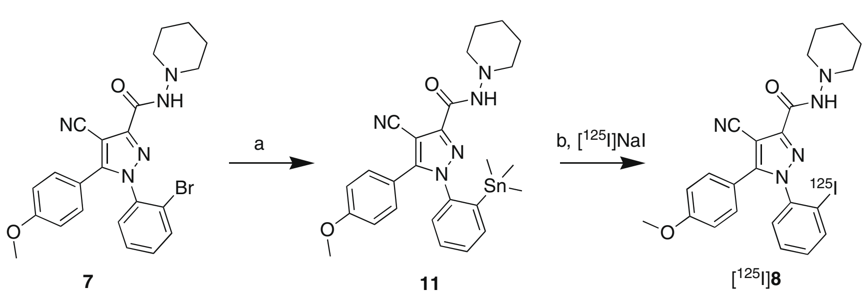


Scheme 1. Synthesis of **8**. Reagent, conditions and yields: (a) concentrated HCl, $NaNO_2$, ethyl 2-chloroacetoacetate, NaOAc, EtOH–H_2O, 16 h, 72%; (b) 4-methoxybenzoylacetonitrile, DIPEA, *tert*-BuOH, reflux, 16 h, 6%; (c) aq-LiOH, THF, 65 °C, 4 h; (d) $DMF_{(cat)}$, $(COCl)_2$, DCM; (e) 1-aminopiperidine, DIPEA, DCM, 2 h, 73%.

A radioligand for imaging brain CB_1 receptors would ideally possess adequately high affinity and moderate lipophilicity to promote rapid development of a high ratio of receptor-specific binding to non-specific binding in brain in vivo, that might then allow accurate computation of output measures, such as binding potential.[22,23] CB_1 receptors are one of the most abundant G protein-coupled receptors in brain, reaching a concentration (B_{max}) of 1752 fmol/mg protein (175 nM) in rat cerebellum.[24] Generally, a SPECT radioligand should show a substantial B_{max}/K_d value (>5) to be successful. Hence, an effective CB_1 receptor radioligand might require only a moderately high affinity (K_i or K_d <35 nM). Ligand **8** was found to have about 10-fold higher affinity (3.4 nM; Table 1) than perhaps needed. Calculated ligand lipophilicity ($cLog\,D_{7.4}$) can be an important predictor of blood–brain barrier penetration and brain non-specific binding. Moderate lipophilicity ($cLog\,D_{7.4}$ in the range 2.0–3.5) is usually preferred for adequate brain entry without incurring excessive non-specific binding in brain.[23] However, when target binding sites exist in high concentration, higher lipophilicity may be tolerated. For example, [^{11}C]MePPEP is a successful PET radioligand for brain CB_1 receptors despite having a high $cLog\,D_{7.4}$ value of 5.42.[12,13] The $cLog\,D_{7.4}$ value of **8** is 4.14 and appears more favorable for a radioligand than that of [^{11}C]MePPEP. Furthermore, the physiochemical and pharmacological properties of **8** compare well with other successful PET radioligands.[20,21] Therefore, ligand **8** presents acceptable CB_1 receptor affinity and lipophilicity for development as a SPECT radioligand (Table 1).

In addition to acceptable binding affinity and lipophilicity a candidate SPECT radioligand should be selective for binding to the target protein. Ligand **8**, at 10 μM concentration, showed <50% inhibition ($n = 4$) of radioligand binding to the sites: 5-$HT_{1B–E}$, 5-$HT_{2A–C}$, 5-HT_3, 5-HT_{5A}, 5-HT_6, 5-HT_7, $\alpha_{1A,B}$, $\alpha_{2A,B}$, $\beta_{1–3}$, $D_{1–4}$, DOR, $H_{1–4}$, $M_{1–5}$, NET, SERT, $\sigma_{1,2}$, $V_{1A,1B,2}$. K_i values ($n = 3$) of 93.5 ± 20.4 nM (5-HT_{1A}), >10,000 nM (α_{2C}), 1477 ± 148 nM (KOR), 1496 ± 216 nM (MOR), and 3166 ± 586 nM (TSPO) were found. Details of the employed binding assays may be found at the NIMH PDSP web site: http://pdsp.med.unc.edu. Hence, **8** was found to have excellent CB_1 receptor selectivity for development as a SPECT radioligand.

[^{125}I]**8** was prepared by [^{125}I]iodo-destannylation of the corresponding trimethylstannyl precursor (**11**) with [^{125}I]NaI, aq-HCl and chloramine-T (oxidizer) in methanol (Scheme 2). The crude product was purified with high-performance liquid chromatography (HPLC) as described in Supplementary data. The decay-corrected radiochemical yield of [^{125}I]**8** ranged from 48% to 59%. The specific radioactivity of [^{125}I]**8** was 81.4 GBq/μmol and the radio-



Scheme 2. Radiosynthesis of [^{125}I]**8**. Reagents, conditions and yields: (a) hexamethylditin, $Pd(PPh_3)_4$, toluene, 20 h, 32%; (b), chloramine-T, aq-HCl, 5 min, 48–59%.