Development and Evaluation of Novel PET Tracers for Imaging Cannabinoid Receptor Type 2 in Brain

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Abstract: The cannabinoid receptor type 2 (CB2) has a very low expression level in brain tissue under basal conditions, but it is up-regulated in diverse pathological conditions. Two promising lead structures from the literature, *N*-((3S,5S,7S)-adamantan-1-yl)-8-methoxy-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide and 8-butoxy-*N*-(2-fluoro-2-phenylethyl)-7-methoxy-2-oxo-1,2-dihydroquinoline-3-carboxamide – designated KD2 and KP23, respectively – were evaluated as potential PET ligands for imaging CB2. Both KD2 and KP23 were synthesized and labeled with carbon-11. *In vitro* autoradiographic studies on rodent spleen tissues showed that [^{11}C]KD2 exhibits superior properties. A pilot study using [^{11}C]KD2 on human post mortem ALS spinal cord slices indicated high CB2 expression level and specific binding, a very exciting finding if considering the future diagnostic application of CB2 ligands and their utility in therapy monitoring. *In vivo* blocking studies in rats with [^{11}C]KD2 showed also high specific uptake in spleen tissue. Although the protein-bound fraction is relatively high, KD2 or KD2 derivatives could be very useful tools for the non-invasive investigation of CB2 levels under various neuroinflammatory conditions.

Keywords: Autoradiography · Cannabinoid receptor type 2 ligand · Neuroinflammation · Positron emission tomography · Radiolabeling

1. Introduction

Cannabinoid receptor types 1 (CB1) and 2 (CB2) were discovered about 30 years ago as G protein-coupled receptors containing seven transmembrane spanning domains. CB1 is expressed throughout the body, but mainly in brain regions (cerebellum, basal ganglia, hippocampus, and neocortex). CB2 is predominantly expressed on cells related to the immune system (spleen, lymphocytes) and also on keratinocytes. The presence of CB2 in the central nervous system has been controversially discussed in literature since 1996.[1] Today CB2 is generally accepted to be present in brain tissues, although in very low concentrations.

However, under neuroinflammatory conditions, a CB2 level increase is observed. For instance in the brain of Alzheimer patients where activated microglia cluster at β-amyloid plaques.[2–5] CB2 increase is also reported for the rat chronic lesion model of Huntington's disease, especially in the striatum.[6] Furthermore, both hypoxia-ischemia and middle cerebral artery occlusion induced an up-regulation in the expression of CB2 in proliferating microglia in rat brain.[7] Increased CB2 levels were also reported in post mortem spinal cord of human patients of Amyotrophic Lateral Sclerosis (ALS) and corresponding mouse models and Multiple Sclerosis.[4,8]

A large number of structurally diverse CB2 ligands have been synthesized and characterized in the past decade.[9] Among all these classes of CB2 ligands, 2-oxoquinoline and 4-quinolone-3-carboxamide derivatives appear to be the most efficient inverse agonists, with high binding affinity and selectivity towards CB2. Currently, there are no PET (positron emission tomography) ligands for imaging CB2 expression in humans. A suitable PET radioligand for imaging CB2 would be an invaluable research tool to explore the role of CB2 receptor expression in peripheral and/or central inflammatory disorders.

Herein, we report the *in vitro/in vivo* evaluation of two oxo-quinoline derivatives, designated [^{11}C]KD2 and [^{11}C]KP23, as potential radiotracers for imaging CB2 receptor by PET.

2. Synthesis and Radiolabeling

2.1 *Synthesis of KD2*

The synthesis of the reference compound, KD2, followed synthetic pathways already described.[10] In brief, the quinoline structure **2** was prepared *via* the Gould–Jacobs reaction as outlined in Scheme 1 by reacting anisidine with diethyl 2-(ethoxymethylene)malonate and subsequent benzannulation at high temperature. N-alkylation with 1-bromopentane followed by hydrolysis of the ethyl ester gave the free quinoline acid **4**, which was coupled to aminoadamantane using HBTU as the coupling reagent to yield KD2. Phenolic precursor compound **5** for radiolabeling was synthesized from the reference compound by demethylation using boron tribromide.

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2.2 *Synthesis of KP23*

The synthesis of the non-radioactive reference compound, KP23, was accomplished based on the modified procedure described by Turkman *et al.*[11] as outlined in Scheme 2. Briefly, compound **7** was obtained by aromatic nitration of 3-hydroxy-4-methoxybenzaldehyde (**6**) with nitric acid. O-alkylation with 1-bromobutane afforded intermediate **8**. Reduction using iron powder followed by reaction *in situ* with dimethyl malonate yielded compound **9**. Hydrolysis under basic conditions to the free acid **10** and amide bond formation using 2-fluoro-2-phenylethanamine free base led to the final reference compound KP23. The phenolic precursor **11** for radiolabeling was directly synthesized from KP23 by demethylation using lithium chloride.

2.3 *Radiolabeling Procedure*

The phenolic precursor **5** or **11**, was reacted with [¹¹C]-methyl iodide to afford [¹¹C]KD2 or [¹¹C]KP23, respectively (Scheme 3). Both tracers were obtained in 99% radiochemical purity after semi-HPLC purification in yields of 2–5 GBq. The total radiolabeling time was around 40 min after [¹¹C]CO_2 delivery from the cyclotron. Specific activity was high and ranged from 80 to 350 GBq/µmol at the end of synthesis.

3. *in vitro/in vivo* Evaluation

3.1 *Autoradiography Experiments*

To confirm specificity of binding of the radiotracers, rat and mouse spleens known to have a high physiological expression of CB2 were investigated in autoradiography experiments (Fig. 1). Specific binding of [¹¹C]KD2 and [¹¹C]KP23 was determined in blocking experiments (B1, B2) using the CB2 specific partial agonist GW405833 in 5000-fold excess.

Although high *in vitro* specific binding was observed for both radiotracers, [¹¹C]KD2 showed superior properties and has greater potential to become a successful PET tracer. Consequently, we selected [¹¹C]KD2 for further evaluation *in vitro* and *in vivo*.

In a preliminary autoradiographic study, we investigated whether [¹¹C]KD2 binds to human post mortem ALS spinal cord slices, as literature reports suggest CB2 overexpression in the spinal cord of ALS patients.[12–14] Fig. 2 confirms not only the presence of CB2 but also specific binding of [¹¹C]KD2. These are encouraging results towards non-invasive imaging of CB2 in ALS patients.

To exclude KD2 as a substrate for efflux transporter P-gp within the blood brain barrier, the permeation of [¹¹C]KD2 across
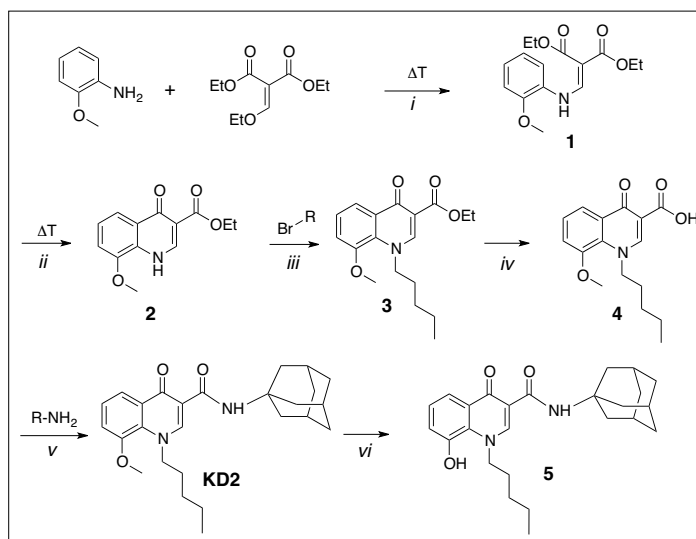
Scheme 1. Synthesis of KD2: (i) 110 °C for 1 h, 88%, (ii) diphenylether, 250 °C for 1 h, 70%, (iii) DMF, K_2CO_3, 90 °C for 4 h, 95%, (iv) NaOH 10%, reflux for 3.5h, 94%, (v) DIPEA, HBTU in DMF, RT for 4 h, quant., (vi) BBr_3 in DCM, RT for 18 h, 87%.
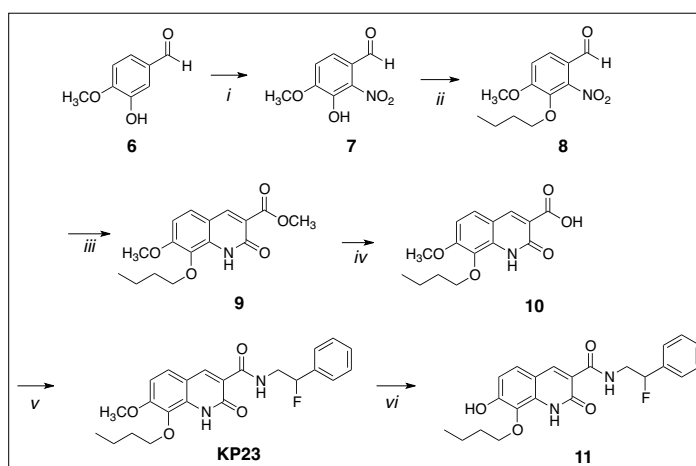
Scheme 2. Synthesis of KP23: (i) HNO_3 in EtOAc, 2 °C for 2 h, 30%, (ii) K_2CO_3 in DMF, 80° for 16 h, 89%, (iii) (a) piperidine, AcOH in toluene, reflux for 4 h, (b) AcOH, Fe, 100 °C for 2 h, 34%, (iv) 2M KOH in EtOH, RT for 6 h, 96%, (v) DIPEA, HBTU in DMF, RT for 8 h, 46%, (vi) LiCl in DMF, reflux for 4 h, 72%.
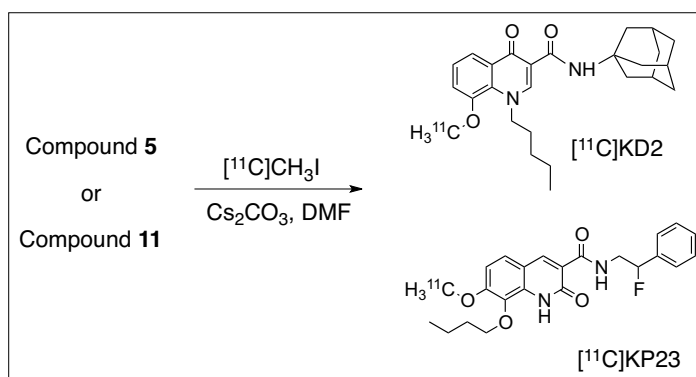
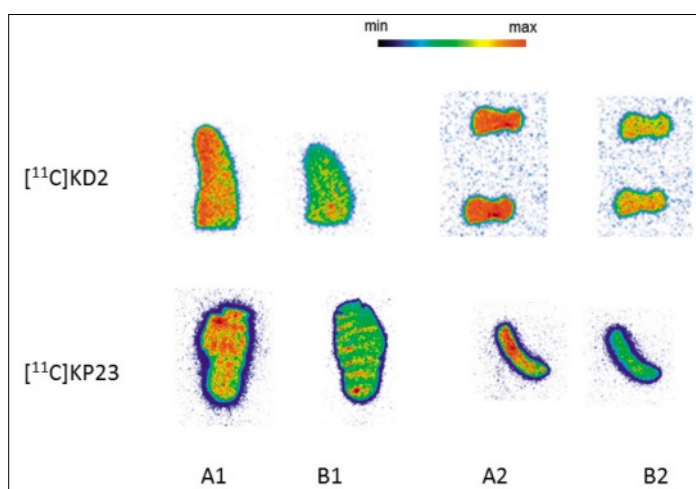Scheme 3. Radiosynthetic scheme towards [¹¹C]KD2 and [¹¹C]KP23.

Fig. 1. A) 0.6 nM [¹¹C]KD2 or [¹¹C]KP23 on rat (A1) and mouse (A2) spleen. B) Blocking with 5 µM GW405833 on rat (B1) and mouse (B2) spleen.
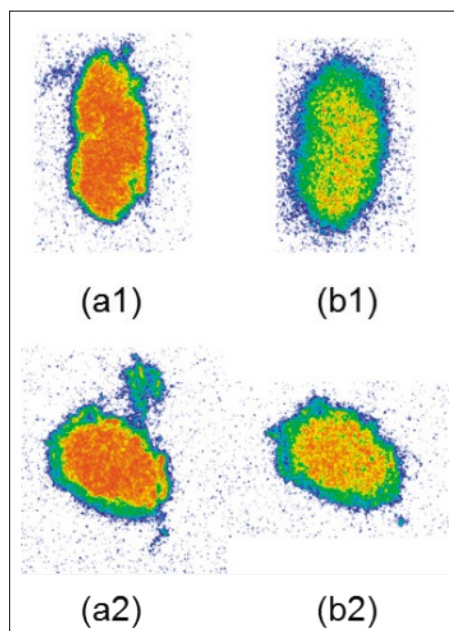
Fig. 2. *In vitro* autoradiography with slices from cervical spinal cord from two ALS patients. Incubation with 0.2 nM [¹¹C]KD2 in the absence (a) and presence (b) of 1 µM GW405833.
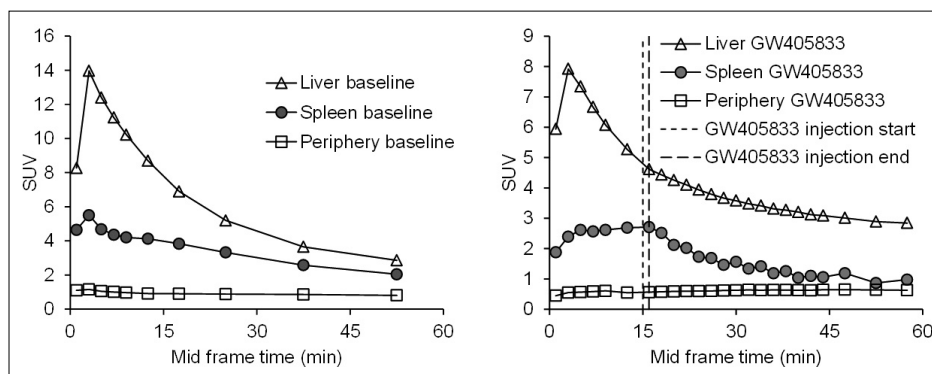

Fig. 3. Time activity curves of [¹¹C]KD2 from an *in vivo* displacement PET study. CB2 selective GW4058233 was injected *i.v.* from 15 to 16 min (dashed lines) after injection of 20 MBq [¹¹C]KD2.

MDCK cells transfected with human P-gp was investigated. The respective ratio for [¹¹C]KD2 of the transport basal to apical and apical to basal, respectively, was 0.5, indicating no net efflux transport by P-gp.

Lipophilicity (logD) as an important physicochemical attribute for a CNS penetrating compound was determined by the shake-flask method in octanol/buffer at pH 7.4.[15] The logD value of [¹¹C]KD2 was found to be 3.29 ± 0.04 ($n = 6$), and suggested that [¹¹C]KD2 would be a good blood-brain barrier penetrant.

The free fraction of [¹¹C]KD2 in human plasma was determined in a protein binding assay displaying a free fraction f_u of 0.001 and in 4% BSA 0.017. These findings were confirmed in an equilibrium dialysis experiment. The low free fraction is probably a consequence of the relatively high lipophilicity of [¹¹C]KD2. Increase of free fraction could be subject of future optimization.

3.2 *in vivo PET studies*

PET scans with Wistar rats showed high accumulation of [¹¹C]KD2 in liver, spleen and intestine. The accumulation in spleen was displaced by injection of the CB2 specific partial agonist GW405833 (1.5 mg/kg), indicating *in vivo* specific binding (Fig. 3). As expected, radioactivity in the brain was significantly lower than in the periphery (data not shown). Uptake in pons and cerebellum was higher than in hippocampus and caudate/putamen, in agreement with the expression pattern of CB2 in the rat brain.[16–18]

We are currently investigating the utility of [¹¹C]KD2 in mouse models of neuroinflammation where CB2 receptors are upregulated, *e.g.* the lipopolysaccharide LPS mouse model as described by Qin *et al*.[19,20]

4. Conclusion

In the process of developing a suitable CNS radiotracer for imaging CB2 receptors we evaluated the potential of two lead compounds KD2 and KP23. We have established the syntheses of non-radioactive reference compounds using modified synthetic routes based on literature procedures. A robust and reliable radiosynthetic procedure for both novel CB2 radiotracers, [¹¹C]KD2 and [¹¹C]KP23, was established and afforded products with high specific activity, high radiochemical yields and excellent radiochemical purity of ≥99%. *In vitro* autoradiography of both radiotracers on rodent spleen tissues and post mortem ALS spinal cord slices indicated specific binding to CB2. The results from the pilot study using human post mortem ALS spinal cord tissue sections showing high CB2 expression level are very exciting if one considers the future diagnostic application of CB2 ligands and their utility in therapy monitoring. Specific uptake into spleen tissue was also observed in dynamic PET studies using Wistar rats. [¹¹C]KD2 is a very promising radiotracer for CB2, but with good potential for improvement towards reduced protein binding. The syntheses of KD2 derivatives with improved physicochemical properties are currently ongoing in our laboratory.

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