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Original article

Synthesis, radiolabeling and evaluation of novel 4-oxo-quinoline derivatives as PET tracers for imaging cannabinoid type 2 receptor

Roger Slavik [a], Adrienne Müller Herde [a], Daniel Bieri [a], Markus Weber [b], Roger Schibli [a], Stefanie D. Krämer [a], Simon M. Ametamey [a, **], Linjing Mu [c, *]

[a] Institute of Pharmaceutical Sciences, ETH Zürich, 8093, Zürich, Switzerland
[b] Neuromuscular Diseases Unit/ALS Clinic, Kantonsspital St. Gallen, 9007, St. Gallen, Switzerland
[c] Department of Nuclear Medicine, University Hospital Zürich, 8091, Zürich, Switzerland

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ABSTRACT

Our goal is to develop a highly specific and selective PET brain tracer for imaging CB2 expression in patients with neuroinflammatory diseases. Based on our previous findings on a carbon-11 labeled 4-oxo-quinoline structure, designated KD2, further structural optimizations were performed, which led to the discovery of *N*-(1-adamantyl)-1-(2-ethoxyethyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxamide (**RS-016**). Compared to KD2, **RS-016** exhibits a higher binding affinity towards CB2 (K_i = 0.7 nM) with a selectivity over CB1 of >10,000 and lower lipophilicity ($logD_{7.4}$ = 2.78). **[^{11}C]RS-016** was obtained in ≥99% radiochemical purity and up to 850 GBq/μmol specific radioactivity at the end of synthesis. *In vitro* autoradiography on rodent spleen tissue showed high specific binding to CB2. **[^{11}C]RS-016** was stable *in vitro* in rodent and human plasma over 40 min, whereas 47% intact compound was found *in vivo* in rat blood plasma 20 min post injection (p.i.). High specific binding to CB2 was observed in murine spleen tissues and postmortem ALS patient spinal cord tissues *in vitro* autoradiography, *ex vivo* biodistribution data confirmed the high and specific uptake of **[^{11}C]RS-016** in spleen region in rats. *In vivo* specificity of **[^{11}C]RS-016** could also be shown in brain by PET imaging using a murine neuroinflammation model, which has higher CB2 receptor expression level in the brain induced by lipopolysaccharide (LPS) application.

1. Introduction

The endocannabinoid system is activated on demand to preserve cell homeostasis in both periphery and brain. Its receptors, cannabinoid receptor 1 and 2 (CB1 and CB2), were first identified in 1990 [1] and 1993 [2], respectively. Both are $G_{i/o}$-protein coupled receptors with seven transmembrane helices. CB1 receptors are mainly expressed on neurons in cortex, hippocampus, amygdale, basal ganglia and cerebellum, but to a less extend in peripheral tissues [3,4]. CB2 receptors are abundant on immune cells such as B-lymphocytes, natural killer cells or monocytes [2] and have very low concentration in brain tissue under basal conditions [5–7]; however, it is up-regulated in cerebellum, cortex and brainstem in pathological conditions such as in multiple sclerosis, traumatic brain injury, HIV-induced encephalitis, Alzheimer's disease, Parkinson's disease and Huntington's disease [8,9]. Upregulation of CB2 in spinal cord was detected in a mouse model of Amyotrophic lateral sclerosis (ALS) as well as in human spinal cord tissues from ALS patients [10,11]. ALS is a progressive neurodegenerative disorder affecting both upper and lower motor neurons in spinal cord, brain stem and motor cortex. Of all ALS cases, 90–95% occur in a sporadic form, whereas 5–10% are inheritable familial cases, partly due to mutations in superoxide dismutase 1 gene (SOD1) [12]. Patients suffer from high symptom burdens including pain, fatigue, dyspnea, and sialorrhea and the average life span after diagnosis is about three years. There is no effective treatment available today, although a lot of progress has been made in this direction over the past few years (for review article see Ref. [13]).

Non-invasive imaging of CB2 upregulation *via* Positron emission

Abbreviations: ALS, Amyotrophic lateral sclerosis; HBTU, O-(Benzotriazol-1-yl)-N,N,N′,N'-tetramethyluronium hexafluorophosphate; hCB2, human cannabinoid receptor type 2; PET, Positron emission tomography.

* Corresponding author. NUK D2, 8091, Zürich, Switzerland.
** Corresponding author. HCI H427, 8093, Zürich, Switzerland.
E-mail addresses: simon.ametamey@pharma.ethz.ch (S.M. Ametamey), linjing.mu@usz.ch (L. Mu).

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tomography (PET) could help to understand pathology that involves CB2 and explore the role and importance of CB2 in (neuro) inflammation and to evaluate the therapeutic value of new CB2-related drugs [14–16].

Several CB2 PET radioligands have been reported by different groups (Fig. 1). Among these, [^{11}C]NE40 is the only CB2 radioligand which has been evaluated in healthy volunteers [17]. Expected uptake in lymphoid tissue and appropriate brain kinetics were observed. Recently, we evaluated [^{11}C]KD2 as an imaging agent for CB2 receptor [18], and it is not optimal yet probably due to its high lipophilicity. This prompted us to search for suitable candidate compounds that retain the high affinity and selectivity profile of KD2 for CB2 but show reduced lipophilicity. We report herein the design and synthesis of a series of novel 4-oxo-quinoline derivatives based on the lead structure of KD2. Structure–activity relationship (SAR) studies revealed compound **RS-016** (Scheme 1) as a very promising ligand, therefore, **RS-016** was selected for radiolabeling with carbon-11 and its utility as an imaging agent was examined *in vitro*/*in vivo* studies.

2. Results

2.1. Chemistry

Novel derivatives based on the structure of KD2 were synthesized starting from commercially available anisidine and diethyl 2-(ethoxymethylene)malonate as depicted in Scheme 1. Compound **2** was prepared *via* the Gould–Jacobs reaction and subsequent benzannulation at high temperatures as previously described [18,25]. *N*-alkylation of quinolone ester **2** afforded compounds **3a**–**c** under basic conditions in 83–96% yields. Free quinoline acids **4a**–**c** were obtained by saponification of their corresponding ethyl esters with 10% sodium hydroxide. Reacting of **4a**–**c** with different amines using HBTU as the coupling reagent afforded to the eight final compounds **RS-005**, **RS-006**, **RS-007**, **RS-008**, **RS-011**, **RS-016**, **RS-022** and **RS-028** in 69–92% yield after the purification by flash column chromatography.

3-Hydroxy-1-aminoadamantane (**5**), which was used for the synthesis of compound **RS-028**, was obtained by nitration of 1-aminoadamantane using 65% nitric acid in conc. sulfuric acid followed by hydrolysis using KOH (Scheme 2) [26].

2.2. Structure–activity relationship (SAR) study

Eight novel compounds were submitted to the SAR studies for their binding affinities towards CB2 and selectivities over CB1. *In vitro* competitive binding assays were performed with human CB1 and CB2 membranes using [^{3}H]-CP-55940, a non-specific CB agonist, as the radioligand. All compounds exhibited excellent selectivity over CB1 and K_i values towards the CB2 receptor ranged from 0.7 to 750 nM (Fig. 2, Table 1). Pasquini et al. [25] reported that a class of 4-quinolone-3-carboxamides with pentyl group in the R_1 position (Fig. 2) showed high binding affinities towards CB2, we found that replacing pentyl to butyl group in the R_1 position as shown in **RS-005** had no significant impact on its binding affinity towards CB2, and with a positive influence on its lipophilicity. A K_i value of 3.3 nM was obtained for the butyl derivative **RS-005** whereas for the pentyl derivative KD2 a value of 1.7 nM was obtained. Keeping the shorter butyl side chain and introducing less bulky groups such as *tert*-butyl, cyclopentyl, methylcyclopropyl in the R_2 position gave rise to three novel compounds **RS-006**, **RS-007** and **RS-008**, respectively. Replacement of the adamantyl moiety with a smaller but still bulky *tert*-butyl moiety resulted in an approximately 26 times less lipophilic compound **RS-006** based on the calculated clogP values of 2.87 compared to **RS-005** (clogP 4.29), while its K_i value was still at the level of single digit nanomolar range. In general, variation of the substituents in the R_2 position could influence both the lipophilicity and their binding affinities. As introduction of a fluorine atom is of high interest for imaging agent, therefore, **RS-011** was synthesized with a fluoropropyl side chain in the R_1 position and keeping the *tert*-butyl group in R_2 position, however, it showed the weakest binding affinity among all the tested compounds. The reason might be due to the relative short alkyl chain length in the R_1 position of **RS-011**

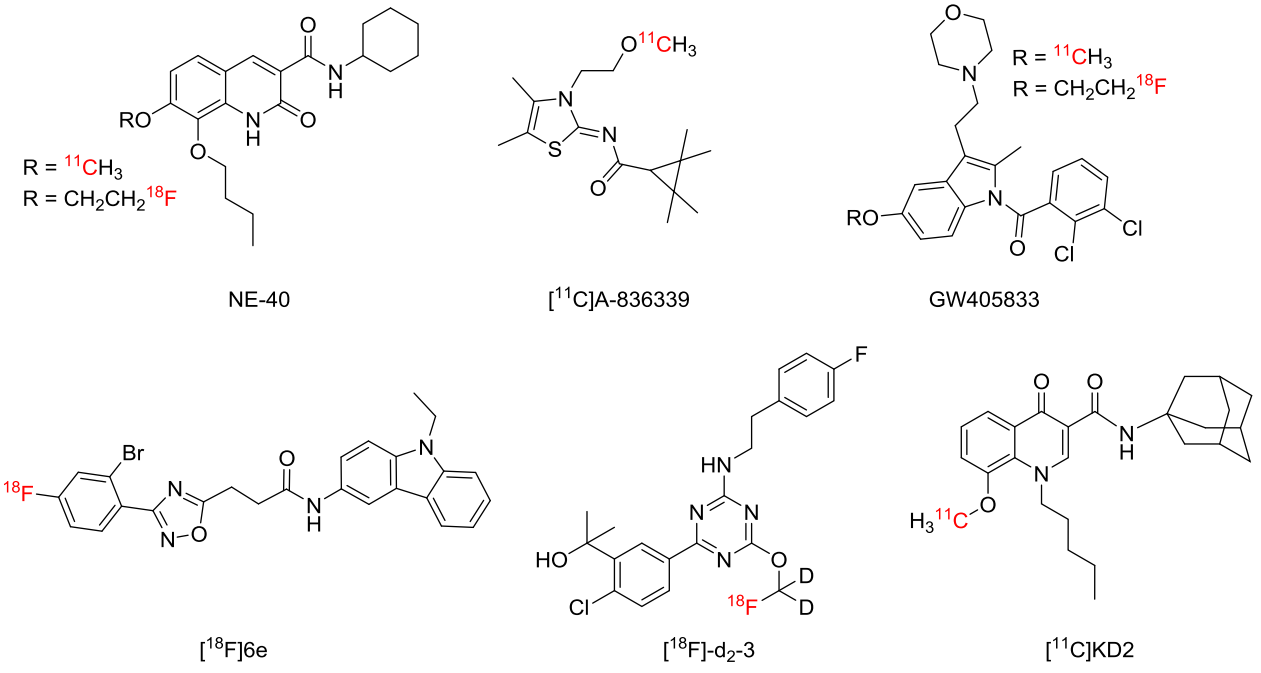

Fig. 1. Representative CB2 PET ligands NE-40 [19], [^{11}C]A-836339 [20], GW405833 [21,22], [^{18}F]6e [23], [^{18}F]d_2-3 [24] and [^{11}C]KD2 [18].