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Design, synthesis and preliminary evaluation of ^{18}F-labelled 1,8-naphthyridin- and quinolin-2-one-3-carboxamide derivatives for PET imaging of CB2 cannabinoid receptor

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ABSTRACT

In the present work, we report the synthesis of new aryliodonium salts used as precursors of single-stage nucleophilic ^{18}F radiofluorination. The corresponding unlabelled fluorinated derivatives showed to be CB2 cannabinoid receptor specific ligands, with K_i values in the low nanomolar range and high CB2/CB1 selectivity. The radiolabelled compound [^{18}F]**CB91**, was successfully formulated for in vivo administration, and its preliminary biodistribution was assessed with microPET/CT. This tracer presented a reasonable in vivo stability and a preferential extraction in the tissues that constitutionally express CB2 cannabinoid receptor. The results obtained indicate [^{18}F]**CB91** as a possible candidate marker of CB2 cannabinoid receptor distribution. This study would open the way to further validation of this tracer for assessing pathologies for which the expression of this receptor is modified.

CB2 cannabinoid receptor (CB2R) belongs to the rhodopsin-like family class A of G-protein-coupled receptors (GPCRs) and constitutes, with CB1 cannabinoid receptor (CB1R), the restricted family of cannabinoid receptors (CBRs).[1] Even if CB1R is expressed throughout the body, it is found in higher concentrations in the brain and its activation is mainly associated with psychotropic and behavioural actions of cannabinoid drugs.[2] The CB2R is expressed in peripheral cells and tissues derived from the immune system[1] even if some recent studies showed that CB2R has a limited central nervous system distribution. In pathological conditions, the CB2R can be up-regulated and recent studies have highlighted that neuroinflammation, for example, related to neurodegenerative (e.g. Alzheimer's) or autoimmune disorders (multiple sclerosis), stroke, trauma or brain tumors can lead to an over-expression of CB2R.[3] Therefore, interest in developing Positron Emission Tomography (PET) radioligands for non-invasive imaging of the CB2R in neurological diseases and cancer, and in monitoring the therapeutic efficacy of new anti-inflammatory drugs is growing. A number of radioligands with affinity for the human CB2R for in vitro use are available. Non-selective cannabinoid radioligands such as [^{3}H]CP55,940 or [^{3}H]WIN55,212 are extensively used in binding analyses of the CB2 receptor.[4] Even if ^{18}F-labelled tracers are preferred for PET imaging due to the longer radionuclide half-life (109 min), the majority of papers dealing with CB2R utilizes ^{11}C-radioligands. Carbon-11 is a very short-lived positron emitting radionuclide (20 min half-life), and was used in the preparation of the first CB2-selective ligand, the dimethoxytriaryl bis-sulfone [^{11}C]methoxy-Sch225336.[5] Subsequently, other [^{11}C]-labelled compounds have been synthesized and tested in animals, such as [^{11}C]A-836339[3] and [^{11}C]KD2,[6] and also in healthy volunteers, such as [^{11}C]NE40.[7] Most of these radioligands were obtained by using ^{11}C-methylation reactions, usually on demethylated substrates. A similar alkylation-based approach has been extended to fluorine-18 in the synthesis of a dideuterofluoromethyl derivative of a new CB2R scaffold based on a triazine moiety.[8] The use of deuterium was conceived to achieve improved stability towards in vivo defluorination. Indeed, this fact may represent a problem with ^{18}F-radiolabelled tracers based on alkylfluorides, as the bone seeking fluoride anion may be removed in vivo from the scaffold and jeopardize tracer exploitability in terms of image reading and subject exposure to unnecessary radiations.

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Extensive research has been dedicated to a novel class of 2-oxoquinoline derivatives,[4,9–12] which have been radiolabelled with either carbon-11 or fluorine-18. In the quest for an optimal ^{18}F-labelled CB2R tracer, other scaffolds, with different structures, have been synthesized and evaluated for the in vivo visualization of CB2R, such as the *N*-dichlorobenzoyl fluoroethoxyindole [^{18}F]FE-GW405833.[13] To circumvent the in vivo defluorination of scaffolds being labelled via the ^{18}F-fluoroethylation reaction, the route to the synthesis of a more stable aromatic fluoride was attempted by using an aromatic nucleophilic substitution on a *N*-aryl-oxadiazolyl-proprionamide,[14] designed to bear a deactivated aromatic ring and a trimethylammonium leaving group. Although in vitro biological data of these radioligands were promising, unsatisfactory results were obtained in vivo because of metabolic instability,[4,13] poor solubility[12] and limited potential usefulness from biodistribution studies.[5]

In a research program aimed at obtaining CB2R selective ligands, we described the synthesis and pharmacological characterization of several fluorinated derivatives possessing the 1,8-naphthyridin-, and quinolin-2-one- central scaffold.[15,16] Among these, **CB91** (*cis* or *trans N*-4-methyl cyclohexyl isomers) and **VL22** (Fig. 1) demonstrated to be CB2R specific ligands with K_i values in the low nanomolar range and interesting CB2R/CB1R selectivity, while bearing the fluorine atom on the aromatic ring, which in principle should ensure high in vivo stability of the label.

As a general rule, the most convenient way of ^{18}F-labelling is to introduce the radionuclide in the final step of the synthetic process by using a nucleophilic substitution reaction.[17] In our case, we could not exploit the effect of electron-withdrawing groups. Therefore, an alternative route was identified in the use of aryliodonium salt precursors,[18] which represent a useful way to introduce a fluorine atom even into an electron-rich aromatic ring. Hypervalent iodine bonded to two aromatics rings leads to the formation of a fluoroaryl and an iodoaryl derivative when reacted with fluoride in nucleophilic conditions; the former (that represent the product of interest) is usually generated on the less electron-rich ring.

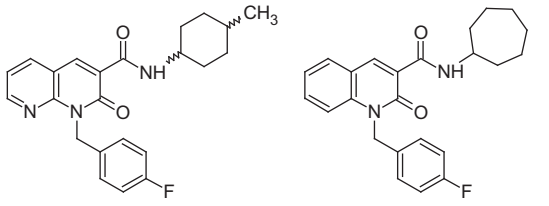


Figure 1. Structures of fluorinated 1,8-naphthyridin-(**CB91**) and quinolin-2-one (**VL22**) derivatives.

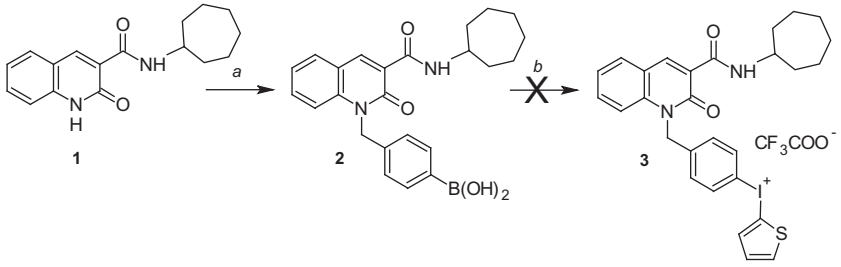
In this work we report the synthesis of the aryliodonium salts related to **CB91** and **VL22** and their radiofluorination in microfluidic conditions, to obtain the corresponding ^{18}F-labelled compounds. The CB2R affinities of *cis*- and *trans*-**CB91** were both extremely high and not very different from each other (*trans*/*cis* 1:13),[15] therefore we decided to synthesize the aryliodonium salt precursor and [^{18}F]**CB91** as a mixture of *cis*- and *trans*-isomers. [^{18}F]**CB91**, whose corresponding unlabelled derivative shows promising *c*log*P* value and good Papp value for intestinal and blood-brain barrier (BBB) permeability, was also tested in microPET to assess its in vivo biodistribution in rodents.

Incorporation of ^{18}F into aryliodonium salts occurs preferentially on the less electron-rich ring. Therefore, aryliodonium salts bearing a thienyl sacrificial portion were developed in order to direct the fluorination towards the relatively deactivated benzylic group. Precursors were synthesised by reaction of a boronic acid and a diacetoxyiodo derivative in the presence of a suitable organic acid.

In a first attempt, the boronic derivative **2** was prepared by reaction of carboxamide **1**[16] with 4-(bromomethyl)phenyl boronic acid (Scheme 1). Then, the boronic acid **2** was reacted with 2-(diacetoxyiodo)thiophene, synthesized according to a published method.[19] Unfortunately this approach was unsuccessful in obtaining the desired derivative **3**. This phenomenon could be related to the electron rich character of thiophene: an high level of impurities and side-products might originate from internal redox processes and other side-reactions of the highly reactive 2-(diacetoxyiodo)thiophene.[20]

The synthetic approach was then modified creating the diacyloxy function on the benzylic fragment, which was reacted with the commercially available thienyl boronic acid (Scheme 2). As reported in Scheme 2, the N1-alkylation of carboxamides **1** and **4**[15,16] was performed in anhydrous THF/DMF with *p*-iodobenzylbromide in presence of NaH at room temperature and afforded the desired iodobenzyl derivatives **5** and **6**. Oxidation of the iodine atom with $NaBO_3{\cdot}4H_2O$ in glacial acetic acid led to the corresponding diacetoxyiodo derivatives. These compounds are characterized by high reactivity/instability; therefore, they were not isolated and rapidly reacted with the 2-thienylboronic acid in presence of a Lewis acid (trifluoroacetic acid) to obtain the desired iodonium salts **3** and **7**.[21]

The aryliodonium salts **3** and **7**, were radiolabelled, following classical nucleophilic fluorination conditions, by using potassium ^{18}F-fluoride/kryptofix (K_{222}) complex in DMSO (Scheme 3). The radiofluorination was optimized employing an Advion Nanotek microfluidic system; this apparatus allows delivering discrete amounts of both aryliodonium DMSO solution and the radiofluorination complex, prepared using the traditional azeotropic distillation,[21,22] into a tubular flow reactor of 15.6 μL internal volume.



Scheme 1. Reagents and conditions: (a) (1) NaH, anhydrous THF, rt, 1 h; (2) anhydrous DMF, 4-(bromomethyl)phenylboronic acid, anhydrous THF, rt, 24 h; (b) (1) 2-(diacetoxyiodo)thiophene, CH_2Cl_2, −30 °C; (2) TFAA, rt, 1 h; (3) −30 °C, addition of **2** → rt, 16 h.