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N-(4-[^{18}F]-fluoropyridin-2-yl)-*N*-{2-[4-(2-methoxyphenyl) piperazin-1-yl]ethyl}carboxamides as analogs of WAY100635. New PET tracers of serotonin 5-HT$_{1A}$ receptors

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ABSTRACT

N-(4-[^{18}F]-Fluoropyridin-2-yl)-*N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-carboxamides were prepared by labeling their 4-nitropyridin-2-yl precursors through nitro substitution by the ^{18}F anion. *In vitro* and *in vivo* tests showed that the cyclohexanecarboxamide derivative is a reversible, selective and high affinity 5-HT$_{1A}$ receptor antagonist ($IC_{50} = 0.29$ nM, $k_i = 0.18$ nM) with high brain uptake, slow brain clearance and stability to defluorination when compared with conventional standards. This PET radioligand is a promising candidate for an improved *in vivo* quantification of 5-HT$_{1A}$ receptors in neuropsychiatric disorders.

1. Introduction

Molecular imaging techniques such as PET (Positron Emission Tomography) are useful tools for translational neuroscience from animal models to man. Observations carried out by this technique have suggested that there is a relationship between the serotonin 5-HT$_{1A}$ receptor and human cognition by the determination of alterations in receptor binding in patients through the imaging of receptor densities [1]. The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) and its receptors have been shown to be involved in the pathophysiology of psychiatric diseases such as schizophrenia [2], depression [3], mood disorders [4–7], and neurodegenerative processes such as Alzheimer's and Parkinson's diseases [8,9]. The 5-HT$_{1A}$ receptor has therefore been proposed as a target for the development of cognitive enhancers in the treatment of numerous cognitive dysfunction-related disorders.

An array of PET tracers has been developed for the imaging of 5-HT$_{1A}$ receptors (Fig. 1) [1,10–12]. Two antagonists have been principally studied in human diseases, namely [*carbonyl*-^{11}C]

Abbreviations: PET, positron emission tomography; 5-HT$_{1A}$, receptor 1A of 5-hydroxytryptamine; 5-HT, 5-hydroxytryptamine; WAY100635, *N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)cyclohexanecarboxamide; MPPF, 4-fluoro-*N*-{2-[1-(2-methoxyphenyl)-piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl) benzamide; k_D, apparent equilibrium dissociation constant; WAY100634, *N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}pyridin-2-amine; 5-HT$_{2B}$, receptor 2BA of 5-hydroxytryptamine; D$_{4,4}$, receptor 4,4 of dopamine; SPECT, single-photon emission computed tomography; EtOH, ethyl alcohol; MW, microwaves; dba, tris(dibenzylideneacetone)dipalladium(0); BINAP, 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl; IPrHCl, 1,3-bis(2,4,6-trimethylphenyl)imidazolium chloride; *t*-BuONa, sodium butoxide; CsOAc, cesium acetate; DMSO, dimethyl sulfoxide; Chx, cyclohexyl; 1-Ad, adamant-1-yl; 2-py, pyridin-2-yl; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; HPLC, high-performance liquid chromatography; Ph, phenyl; IC_{50}, concentration producing 50% inhibition; k_i, inhibition constant; EOB, end-of-bombardment; 8-OH-DPAT, 2-(dipropylamino)-8-hydroxytetraline; BBB, blood–brain barrier; BP, binding potential; ID, injected dose.

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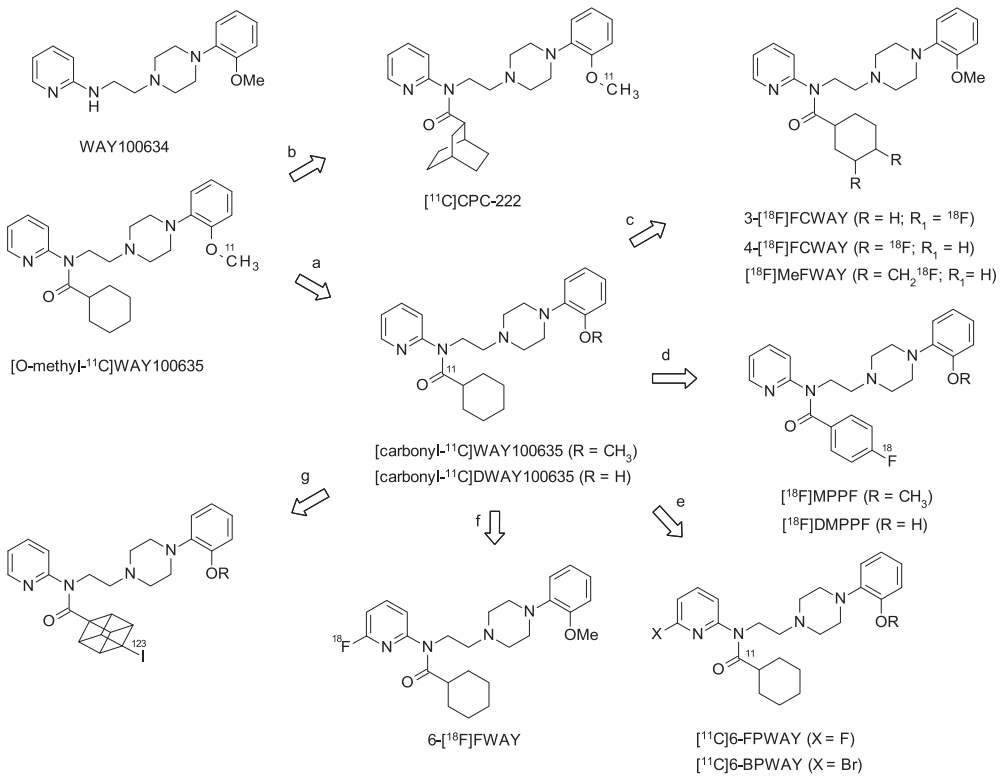

Fig. 1. Evolution of structures of PET radiotracers of 5-HT_{1A} receptors, key metabolite WAY100634 and radiolabeling methods: (a) carbonyl labeling; (b) bulky acyl group; (c) fluorine labeling on the cyclohexane ring; (d) fluorine labeling on the aromatic acyl group; (e) halogen on the pyridine C6 atom; (f) fluorine labeling on the pyridine C6 atom; (g) iodine labeling on the bulky acyl group.

WAY100635 and [^{18}F]MPPF [13,14]. WAY100635 is potent, selective and shows high affinity (k_D = 0.2 nM) [15]. Recent *in vitro* studies showed that WAY100635 and its major metabolite WAY100634 also have affinity for other receptors such as 5-HT_{2B} and $D_{4,4}$ [16], although this finding has not subsequently been confirmed [17]. In addition, radiolabeling with carbon-11, which has a very short half life, is a challenge to radiochemical yields, purities and specific activities [18]. [^{18}F]MPPF is a selective antagonist (k_D = 0.34 nM) with a longer half-life, but it shows low brain uptake compared to [*carbonyl*-^{11}C]WAY100635 (0.05% and 0.45% ID/g at 30 min in rats, respectively) and rapid metabolism *in vivo* [14,19]. In spite of its drawbacks, [*carbonyl*-^{11}C]WAY100635 remains the 'gold standard' for PET imaging of the 5-HT_{1A} receptor [20,21]. However, [^{18}F]MPPF has the advantage of being simpler to synthesize and the longer half-life of ^{18}F allows for distribution to remote facilities that do not have an in-house cyclotron. The synthesis of this compound is based on the displacement of a nitro group on a benzene ring by a weak nucleophile such as the ^{18}F anion, a process that suffers from low efficiency. However, the efficiency can be substantially improved by changing the position of the leaving group – the nitro group in this case – on the molecule.

Numerous modifications to the structures of WAY100635 and MPPF have been reported as part of the development of new PET and SPECT (Single-Photon Emission Computed Tomography) radiolabeled ligands for 5-HT_{1A} with improved specificity [22], *in vivo* kinetics [22–26] and metabolic stability [27–33] (Fig. 1). In spite of the improved parameters, the aforementioned tracers also suffer from poor radiosynthetic yields [22,23,34], low brain uptake [33], extensive *in vivo* defluorination [32,35] and hydrolysis of the amide bond [36,37]. There is therefore a need for new 5-HT_{1A} radiotracers that combine an optimal radiosynthesis process with improved pharmacological profiles and imaging properties.

We report here a series of novel analogs of WAY100634 and MPPF labeled with fluorine-18 for the quantification of 5-HT_{1A} receptors by PET imaging. These tracers all bear a [^{18}F]fluorine label on the pyridine C4 atom, which *a priori* would allow an improvement in radiolabeling efficiency, and they possess different aliphatic (cyclohexyl, adamant-1-yl), aromatic (phenyl) and heteroaromatic (pyridine-2-yl) radicals at the acyl group. The chemical synthesis of the precursors is described and we report the first radiosynthesis of these derivatives. The results of *in vitro* and *in vivo* assays of binding specificity and kinetics are also reported. One analog, the 4-[^{18}F]fluoropyridine derivative of WAY100635, was found to be a potent 5-HT antagonist and it showed good selectivity for 5-HT_{1A} receptors, higher brain uptake and slower washout than [^{18}F]MPPF, a commonly used fluorine-18 PET tracer for 5-HT_{1A}.

2. Chemistry

The synthetic route for the precursors was designed in order to build molecules with a 4-nitropyridin-2-yl moiety on which the fluorination step would subsequently be carried out (Scheme 1). Commercially available piperazine **1** was transformed into **2** and **3** in 98% yield using modifications of previously reported methods [38]. 2-Chloro-4-nitropyridine-1-oxide (**4**) was used to link amine **3** by nucleophilic substitution to give pyridine *N*-oxide **5**. Compound **5** was the only reaction product but it was obtained in low yield (31%) in spite of the harsh conditions applied (1.5 equiv of **4**, EtOH, 100 °C, 1 h, microwaves). The yield was not improved by carrying out the reaction in the presence of a stoichiometric amount of tertiary amine