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^{11}C-labelled PIB analogues as potential tracer agents for in vivo imaging of amyloid β in Alzheimer's disease

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ABSTRACT

Pittsburgh Compound-B (PIB) is currently being evaluated clinically for in vivo visualization of amyloid plaques in patients with Alzheimer's disease (AD). We have synthesized three structural isomers of 6-hydroxy-2-(4′-aminophenyl)-1,3-benzothiazole, performed radiolabelling with carbon-11 and investigated their in vivo and in vitro properties. Specific binding to amyloid plaques was demonstrated in vitro using post-mortem brain homogenates of AD patients, transgenic AD mice brain sections and post-mortem human AD brain sections. In normal mice, initial brain uptake (at 2 min p.i.) was high and was followed by a fast wash-out. The three structural analogues have a high potential as tracer agents for in vivo visualization of amyloid plaques in AD patients.

1. Introduction

The increasing mean life span of the global population emphasizes the need for accurate diagnostic tools and therapies for age-related diseases such as Alzheimer's disease (AD) which has an exponentially increasing incidence with age [1]. Alzheimer's disease is the most frequent form of dementia and is characterized by a progressive neurodegeneration. The hallmarks of AD are an extracellular deposition of amyloid β (Aβ) in the form of neuritic plaques, an intraneuronal cytoplasmatic deposition of neurofibrillary tangles (NFTs), activated microglia and reactive astrocytes [2]. Although scientists are uncovering more and more of the pathogenesis of AD, it is still not entirely clear what the exact correlation is between the neuritic plaques and the NFTs [3–5]. Nevertheless, the amyloid cascade hypothesis presented by Hardy and Higgins in 1991 [6] is still up-to-date and highlights the importance of Aβ deposits and NFTs as potential targets for therapies against AD and as diagnostic tracer agents.

Until now, post-mortem histological staining of amyloid plaques with specific dyes (silver staining, Congo red, thioflavin-S, thioflavin-T (ThT),…) or using amyloid β antibodies is the only way to obtain a definitive diagnosis of AD. To allow non-invasive in vivo diagnosis of AD, the search for a radiolabelled derivative of one of these dyes has become subject of worldwide research. There are, however, a number of challenges to reach this goal. First of all, the tracer agent has to be able to cross the blood-brain barrier (BBB). This requires a neutral molecule with a molecular mass not exceeding 600 [7]. Furthermore, the log octanol-buffer partition coefficient (log *P*), which is a measure of the lipophilicity of the compound, should be preferably between 1 and 2.5 [8]. The ability to pass the BBB has to be combined with a high affinity for amyloid β plaques and the compound must be radiolabelled with a suitable radionuclide to allow detection of the radiation emitted by the

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β; APP, amyloid precursor protein; BBB, blood-brain barrier; ESI, electrospray ionization; FCS, fetal calf serum; ID, injected dose; IMPY, 6-iodo-2-(4′-dimethylamino)phenyl-imidazo[1,2]pyridine; LC–MS, liquid chromatography hyphenated to mass spectrometry; Mp, melting point; MPLC, medium pressure liquid chromatography; NFT, neurofibrillary tangle; NMP, *N*-methyl-2-pyrrolidone; NMR, nuclear magnetic resonance; *P*, partition coefficient; PBS, phosphate buffered saline; PBST, PBS containing 0.05% Tween® 20; PET, positron emission tomography; PIB, Pittsburgh Compound-B, 6-hydroxy-2-(4′-*N*-[^{11}C]methylaminophenyl)-1,3-benzothiazole; PPA, polyphosphoric acid; RP-HPLC, reversed phase high performance liquid chromatography; RT, room temperature; ThT, thioflavin-T; TLC, thin layer chromatography.

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tracer agent outside the patient's body using a gamma or positron emission tomography (PET) camera ultimately providing quantitative in vivo images of amyloid β plaque deposition in the brain.

In the past years, radiolabelled derivatives of several of the higher mentioned dyes have been tested and reported [9–18]. Up to now, by far the most promising and clinically useful results have been obtained with [11C]PIB (6-hydroxy-2-(4′-N-[11C]methylaminophenyl)-1,3-benzothiazole, Fig. 1), but early clinical evaluation of fluorine-18 labelled derivatives is also ongoing [12,19]. In a search for derivatives with improved characteristics we synthesized and labelled in the present study three structure analogues of this compound, namely the 4-hydroxy, 5-hydroxy and 7-hydroxy structural isomers (Fig. 1). We compared their in vivo and in vitro biological characteristics with those of the parent compound [11C]PIB to obtain structure activity information related to the position of the OH-group which would also be relevant for the development of new 18F-labelled PIB derivatives.

2. Results and discussion

2.1. Synthesis

2.1.1. Synthesis of 4-hydroxy-, 5-hydroxy- and 7-hydroxy-2-(4′-aminophenyl)-1,3-benzothiazole (5a–c)

The three compounds were synthesized using a similar pathway (Scheme 1) [20]. For the synthesis of 4-hydroxy-2-(4′-aminophenyl)-1,3-benzothiazole (**5a**), ring closure of the benzothiazole does not yield two isomers and further separation was not required. Briefly, *o*-anisidine was first reacted with *p*-nitrobenzoyl chloride to form *N*-2′-methoxyphenyl-4-nitrobenzamide (**1a**). The amide was then converted to the thiobenzamide (**2a**) using Lawesson's reagent (2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulphide) which is a useful thiation reagent to replace the carbonyl oxygen atoms of ketones, amides and esters by sulphur. In the presence of potassium ferricyanide, **2a** was cyclized to the 2-(4′-nitrophenyl)-benzothiazole **3a**. After reduction of the nitro to an amine group using $SnCl_2$, the methyl ether was demethylated using boron tribromide (BBr_3) in dichloromethane at 70 °C, yielding **5a**. For the synthesis of 5-hydroxy-2-(4′-aminophenyl)-1,3-benzothiazole (**5b**) and 7-hydroxy-2-(4′-aminophenyl)-1,3-benzothiazole (**5c**), ring closure can lead to the formation of two isomers and therefore the procedure described by Hutchinson [21] was used. When a halogen atom (typically chlorine or bromine) is present in the position where the ring closure should occur and sodium hydride (NaH) or sodium methoxide (NaOMe) is used in combination with *N*-methyl-2-pyrrolidone (NMP) as a solvent, the ring closure is specific for the intended position. Using this procedure, we were able to obtain **5b** and **5c** in sufficient yield. For the synthesis of 7-hydroxy-2-(4′-aminophenyl)-1,3-benzothiazole (**5c**) the starting compound 2-bromo-3-aminoanisole was obtained following a reported procedure [22]. However, for the synthesis of **5b**, the commercially available hydrochloride salt of 6-chloro-*m*-anisidine was used as starting product resulting in a much higher overall yield.

2.1.2. Synthesis of 4-hydroxy-2-(4′-methylaminophenyl)-1,3-benzothiazole (6a) and 7-hydroxy-2-(4′-methylaminophenyl)-1,3-benzothiazole (6c)

A small amount of **6a** and **6c** was synthesized by methylation of **5a** and **5c**, respectively, using iodomethane (Scheme 2). Mass spectrometric analysis of the reaction mixture showed the presence of both the *N*-dimethylated and *N*-monomethylated **6**. Reversed phase high performance liquid chromatography (RP-HPLC) analysis further showed that the obtained monomethylated compound had a longer retention time than the methoxy-isomer (**4**), indicating that methylation had occurred at the amino group. *O*-methylation normally requires deprotonation of the phenol and thus requires more alkaline reaction conditions. Purification was done using medium pressure liquid chromatography (MPLC).

2.1.3. Synthesis of 5-hydroxy-2-(4′-methylaminophenyl)-1,3-benzothiazole (6b)

To avoid a difficult MPLC separation of the mono- and dimethylamino derivative resulting from the methylation reaction, **6b** was prepared using the method described by Lin (Scheme 3) [23]. The benzothiazole ring of commercially available 5-methoxy-2-methyl-benzothiazole was opened using 10 M sodium hydroxide in order to obtain 2-amino-4-methoxy-thiophenol (**7**), which then was reacted with 4-(methylamino)benzoic acid in polyphosphoric acid (PPA) to obtain **8** after further purification using column chromatography. The methoxy group was then converted to a hydroxy group using BBr_3 to obtain **6b** in a 69% yield.

2.2. Radiolabelling

2.2.1. Synthesis of 4-hydroxy-2-(4′-[11C]methylaminophenyl)-1,3-benzothiazole ([11C]6a), 5-hydroxy-2-(4′-[11C]methylaminophenyl)-1,3-benzothiazole ([11C]6b) and 7-hydroxy-2-(4′-[11C]methylaminophenyl)-1,3-benzothiazole ([11C]6c)

11C-methylation of precursor **5a** was performed with a 25% yield by bubbling [11C]$CH_3OSO_2CF_3$ (methyltriflate) with a stream of helium through a solution of the precursor (Scheme 4). Pre-purification of the reaction mixture was done with the aid of an activated C-18 Sep-Pak® cartridge, which retained [11C]**6a** whereas more hydrophilic compounds (such as [11C]methyltriflate) were eluted from the cartridge. After rinsing the cartridge with water, the labelled compound [11C]**6a** was eluted with methanol. The eluate containing [11C]**6a** was further purified by RP-HPLC on a semi-preparative C18 column. The use of a semi-preparative column was necessary to allow injection of the entire prepurified reaction mixture. During isolation of the desired 11C-labelled benzothiazole care was taken to start the collection of the peak only after the UV-signal had returned to baseline, in order to prevent contamination of the carbon-11 labelled benzothiazole with the non-radioactive precursor. Identity confirmation was done using radio-HPLC combined with mass spectrometry (radio-LC–MS) (experimental mass: 257 Da, theoretical mass: 257 Da in ES^+, data not shown) and by comparison of the retention times of the authentic analogue **6a** and the 11C-labelled [11C]**6a** on RP-HPLC. Starting from **5b** and **5c**,

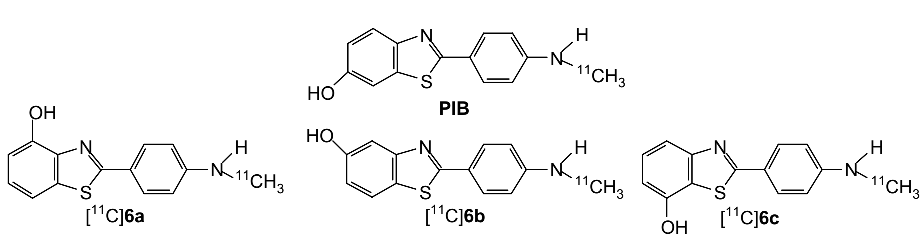

Fig. 1. Structure of PIB, 4-hydroxy-2-(4′-[11C]methylaminophenyl)-1,3-benzothiazole [11C]**6a**, 5-hydroxy-2-(4′-[11C]methylaminophenyl)-1,3-benzothiazole [11C]**6b** and 7-hydroxy-2-(4′-[11C]methylaminophenyl)-1,3-benzothiazole [11C]**6c**.