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Aminothienopyridazines as imaging probes of tau pathology: a patent evaluation of WO2013090497

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Abstract

Small-molecule ligands, amenable to positron emission tomography (PET) imaging of different types of neurodegenerative disease-associated amyloid deposits in the CNS of living patients, hold considerable promise for diagnostic purposes, as well as for monitoring disease progression and the effectiveness of treatments. The patent entitled ‘Heterocyclic Compounds as Imaging Probes of Tau Pathology’ (WO2013090497) discloses the identification of a novel class of tau imaging agents, the aminothienopyridazines. Selected compounds from this class are described that can stain selectively tau pathology in brain tissue sections. Moreover, examples of this class of compounds exhibit absorption, distribution, metabolism, excretion and pharmacokinetics (ADME-PK) properties that are appropriate for CNS PET ligands.

Keywords

Alzheimer’s disease; aminothienopyridazine; positron emission tomography; tau

1. Introduction

Insoluble proteinaceous aggregates known as neurofibrillary tangles (NFTs), neuropil threads and dystrophic neurites, comprising the microtubule-associated protein tau, constitute pathological hallmarks of several neurodegenerative diseases, collectively termed *tauopathies*. These include Alzheimer’s disease (AD), Pick’s disease, progressive supranuclear palsy and other forms of frontotemporal lobar degeneration^[1]. In the case of AD, the most common cause of dementia that primarily affects the elderly, senile plaques (SPs) made of A β peptides, are also abundant in brain regions affected by tau pathology. Unambiguous diagnosis of AD calls for the postmortem detection of both plaques and

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Declaration of interest

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tangles in the brain. However, the deposition of tau and A β in tangles and plaques, respectively, is believed to take place at different time points or stages of AD as well during normal aging. Although circumscribed medial temporal limbic tau deposits are thought to occur very early, the most dramatic presymptomatic AD pathology appears to be an accumulation of A β aggregates. The development of increased tau aggregate burden is believed to occur after substantial A β deposition has occurred and may be more closely associated with neurodegeneration and cognitive decline^[2]. This is consistent with the observation that tau burden in the AD brain correlates much better with cognitive decline than does SP pathology^[2–4], and implies that noninvasive detection and quantification of tau aggregates could be useful to enable i) accurate diagnosis of AD during life; ii) staging of AD, especially early in the disease course; and iii) monitoring the effectiveness of therapies or target engagement of a potential therapy targeting tau pathology. Thus far, the most promising techniques for noninvasive brain imaging have been positron emission tomography (PET) and single-photon emission computed tomography (SPECT). As a result, considerable effort has been devoted in identifying PET/SPECT ligands that could be utilized to visualize pathology in the brains of living patients^[5]. Among such ligands, the first notable examples were 2-(1-{6-[(2-[fluorine-18] fluoroethyl)(methyl)amino]-2-naphthyl}-ethylidene)malono nitrile (FDDNP) (Figure 1)^[6], which detects both SP and NFTs, as well as the thioflavin-T analog known as Pittsburgh Compound-B (or PIB, Figure 1)^[7–9], which exhibits preferential binding to A β plaques relative to tau aggregates. Since the discovery of these two imaging agents, other research groups have focused on the development of second-generation PET ligands that could be used to visualize selectively specific types of disease-associated protein aggregates. In the field of tau-selective imaging agents, a number of promising compounds have been reported, including arylquinoline derivatives such as THK-5105^[10], pyridindole derivatives such as T-807^[11] and several phenyl- or pyridinyl-butadienyl-benzothiazoles such as PBB-3 (Figure 1)^[12].

The patent entitled ‘Heterocyclic compounds as imaging probes of tau pathology’ (WO2013090497)^[13] now discloses the identification of a novel class of tau imaging agents, the aminothienopyridazines (ATPZs, Figure 2).

2. Chemistry, biology and action

Central to the discovery of the ATPZs as potential tau imaging agents, as disclosed in this patent^[13], is the observation that ATPZ **15** (Table 1), a compound possessing intrinsic fluorescence that was originally identified as a brain-penetrant tau aggregation inhibitor^[14], could preferentially stain NFTs in comparison to plaques in human AD brain tissue sections. As shown in Table 1, tissue-binding studies reveal that compound **15** produced pronounced staining of NFTs at 100 μ M, while staining of SP appeared to be comparatively weak at the same compound concentration. This differential ATPZ staining of tau NFTs relative to A β plaques became even more pronounced at lower compound concentration (i.e., 10 μ M), where no A β staining was observed (Table 1). In contrast, neither FDDNP nor PIB exhibited tau-selective staining, as expected from prior reports.

The discovery that **15** could preferentially bind to tau aggregates led to the synthesis and evaluation of several related ATPZs, in particular, analogs carrying a fluorine atom that

could be conveniently replaced with the ^{18}F isotope. Compound synthesis was conducted for the most part by adaptation of a previously reported synthetic strategy^[15] for the construction of the ATPZ scaffold, as highlighted in Scheme 1. Thus, starting from the appropriate aniline, diazonium coupling reaction was used to generate hydrazones that were then converted to corresponding pyridazines via a Knoevenagel-type condensation with ethyl cyanoacetate. Finally, Gewald aminothiophene synthesis^[16] was employed to complete the ATPZ scaffold. Further manipulations of the compounds so obtained permitted the construction of a series of compounds possessing substitutions around the ATPZ heterocycle (see selected examples in Table 1).

Interestingly, evaluation of these compounds in tissue-binding experiments reveals a highly discontinuous SAR, as exemplified by the dramatic changes in the intensity of staining observed for closely related congeners (cf. Table 1, **38** with **61**, and **14** with **15**). This is in sharp contrast with the SAR that was established previously for the ATPZ in the tau fibrillization assays, where the ATPZ heterocycle was found to be both necessary and sufficient for effective inhibition of tau fibrillization^[17].

Among all analogs tested, the most promising derivative was compound **38**, a close derivative of **15**, in which the para-chloro substituent is replaced with the 2-fluoroethanol fragment. Furthermore, not only was **38** capable of discriminating NFTs from SP, but this fluorinated derivative of **15** was also found to exhibit significantly improved *in vitro* ADME-PK properties relative to **15**, including a noticeable reduction in nonspecific protein binding in rat brain homogenates (Table 2). These promising results thus led to the synthesis and preliminary evaluation of the corresponding [^{18}F]-labeled derivative, [^{18}F]-**38**, prepared by Kryptofix-mediated direct nucleophilic fluorination of tosylated precursor **39** (Scheme 2).

Evaluation of the biodistribution of [^{18}F]-**38** in normal mice (Table 3) revealed relatively rapid brain uptake of the compound (i.e., > 1% ID/g at 2 min post-injection), as well as a rapid clearance as indicated by the clearance ratio > 2, both of which are important prerequisites for effective CNS PET imaging agents^[18].

3. Expert opinion

That the ATPZs could be tau-interacting molecules was suggested initially by the discovery that compounds of this class could disrupt heparin-induced tau aggregation *in vitro*^[19]. Furthermore, it was found that these ATPZ inhibitors were essentially devoid of activity against the *in vitro* fibrillization of other peptides, including A β , thereby suggesting that this class of compounds may be potentially selective for tau^[19]. Meanwhile, several prototypic ATPZ derivatives, including compound **15**, were identified with ADME-PK properties that are potentially favorable for CNS indications^[14,17]. In addition, one of these ATPZs was found to be an effective inhibitor of tau aggregation in a tau transgenic *Caenorhabditis elegans* model that expresses the aggregation-prone κ 280 tau mutant^[20]. Subsequent investigations into the mode of action of this class of compounds in the tau fibrillization assay, however, revealed that active ATPZ inhibitors act by promoting the spontaneous oxidation of cysteine residues in tau monomers, which inhibits the fibrillization of 4R tau isoforms but promotes the formation of 3R tau fibrils^[21]. Moreover, the ability of the

ATPZs to promote cysteine oxidation was not exclusive to tau, as it was also observed with an unrelated peptide and the reducing agent dithiothreitol^[21]. Thus, while the activity of ATPZs in the tau fibrillization assay, coupled with the favorable ADMEPK properties, initially indicated that this class of compounds might be a promising starting point for the development of tau-directed therapeutics and/or diagnostic agents, the above-mentioned studies of the mode of action of this class of heterocycles indicate that they may act on tau via a relatively nonspecific oxidative mechanism and, as a result, may not be selective for tau. In this context, the disclosure in this patent that selected ATPZs can bind preferentially to tau aggregates is of interest. Additional studies are clearly needed to confirm and extend these findings, as well as to compare the ATPZs with previously disclosed tau ligands, some of which are now being tested in human subjects^[22]. For example, it would be of interest to determine the binding affinity of ATPZ ligands for NFTs, and establish whether these ATPZs share the same binding site with any of the existing classes of tau imaging agents. Furthermore, as there is growing evidence suggesting that different tau conformers may be responsible for the pathogenesis of distinct sporadic tauopathies, the potential of these new tau ligands to bind preferentially to particular strains of tau aggregates should be explored. Finally, most important will be the evaluation of ¹⁸F-**38**, or other related congeners, in PET imaging of tau pathology in tauopathy and control patients. Collectively, these studies will be necessary to compare the ATPZs with the state of the art in tau PET imaging and determine the potential of these compounds as candidates for PET imaging of tau pathology.

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Figure 1.
Representative structures of PET ligands used to visualize protein aggregates.

Aminothienopyridazines

Figure 2.
Structure of theaminothienopyridazine heterocycle.

Scheme 1.

General synthetic scheme for the synthesis of the aminothienopyridazines.

Scheme 2.
Synthesis of ^{18}F -derivative of ATPZ **38**.

Table 1

Tissue-binding data for representative compounds.

Compound	Structure	Concentration	NFTs	Plaques
PIB		100	+++	+++
		10	+	++
		1	–	++
FDDNP		100	+++	+++
		10	++	++
		1	+	+
ATPZ 15		100	+++	+
		10	++	–
		1	+	–
ATPZ 38		100	+++	++
		10	++	+
		1	+	–
ATPZ 14		100	+	+
		10	–	–
		1	–	–
ATPZ 61		100	–	–

+++ : Intense staining;

++ : Moderate staining;

+ : Weak staining;

– : No staining.

Table 2

In vitro ADME-PK properties of selected compounds.

Compound	PAMPA log Pe	Protein binding		Liver stability		
		Human plasma	Rat brain	Rat (% PCP)		Human (% PCP)
Desirable values	> -6	< 99	< 99	> 50% 5 min	> 50% 30 min	> 50% 5 min > 50% 30 min
PIB	-5.41	99.82	98.26	57.18	5.01	66.99 14.54
ATPZ 15	-5.24	99.02	90.94	97.06	70.19	98.9 68.29
ATPZ 38	-5.02	93.58	57.2	81.7	56.4	87.2 74.4

Table 3

Results of biodistribution of radiolabeled ATPZ [¹⁸F]-38 in mouse.

Compound	Brain uptake %ID/g p.i (min)			Clearance ratio 2.30 min
	2	10	30	60
[¹⁸ F]-38	6.50	3.26	2.83	2.35
				2.3