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(71) Applicant: UCB BIOPHARMA SPRL [BE/BE]; 60, Allée de la Recherche, 1070 Brussels (BE).

(72) Inventors: MERCIER, Joël; c/o IP Department, 60, Allée de la Recherche, 1070 Brussels (BE). VERMEIREN, Celine; c/o IP Department, 60, Allée de la Recherche, 1070 Brussels (BE).

(74) Agent: UCB INTELLECTUAL PROPERTY; c/o UCB Biopharma SPRL, 60, Allée de la Recherche, 1070 Brussels (BE).

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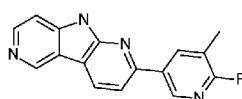
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(54) Title: 9H-PYRROLO-DIPYRIDINE DERIVATIVES



(I)

(57) Abstract: The invention relates to 9H-pyrrolo-dipyridine derivatives of formula (I), processes for preparing them, pharmaceutical compositions containing them and their use as radiopharmaceuticals in particular as imaging agents for the detection of Tau aggregates.



9H-PYRROLO-DIPYRIDINE DERIVATIVES

FIELD OF THE INVENTION

The invention relates to 9H-pyrrolo-dipyridine derivatives, processes for preparing them,
5 pharmaceutical compositions containing them and their use as radiopharmaceuticals in particular
as imaging agents for the detection of Tau aggregates.

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) and progressive supranuclear palsy (PSP) are neurodegenerative
10 diseases with high medical unmet needs that cause substantial morbidity and mortality, high
healthcare costs, and high burden for the families and caregivers of the affected individuals. AD
initially causes impaired cognition, especially memory, but eventually AD leads to impairments in
multiple domains and the need for patients to live in a nursing home. Ultimately, AD causes death.
PSP initially causes symptoms that are often misdiagnosed for Parkinson's disease, affecting
15 balance, gait and eye movement. The disease progresses rapidly, with patients falling, being
wheelchair bound and requiring nursing home care. Ultimately, PSP causes death.

Symptomatic treatments for AD and PSP provide limited benefit and there are currently no
disease-modifying treatments available.

The brain pathology observed in AD includes amyloid plaques and neurofibrillary tangles.
20 Neurofibrillary tangles are also observed in PSP. The main protein component of neurofibrillary
tangles is hyperphosphorylated, aggregated microtubule-associated protein tau (Tau) forming
paired helical filaments (PHF).

Tau is a neuronal protein that is unfolded under physiological conditions, associated with
microtubules, and which may play a role with their assembly and stabilization (Clavaguera et al.
25 Brain Pathol. 2013 2013 23(3):342-9). Six isoforms were described, three containing three
microtubule binding regions (MTBR), three containing four MTBR; the longest form comprises
441 amino acids.

In pathological conditions, Tau undergoes post-translational modifications (hyper-
phosphorylation, acetylation, nitrosylation, glycosylation, etc) and self-aggregates on its MTBR.
30 This aggregated post-translationally modified protein is the major component of paired helical
filament (PHF) which is the building block of neurofibrillary tangles observed in a range of
tauopathy diseases.

The following tauopathies have been described to contain Tau inclusions (Clavaguera et al. Brain
Pathol. 2013 2013 23(3):342-9) and may be caused by Tau accumulation:

35 Alzheimer's disease; Amyotrophic lateral sclerosis/parkinsonism-dementia complex; Argyrophilic
grain disease; Chronic traumatic encephalopathy; Corticobasal degeneration; Diffuse
neurofibrillary tangles with calcification; Down syndrome; Familial British dementia; Familial
Danish dementia; Frontotemporal dementia and parkinsonism linked to chromosome 17 caused

by MAPT mutations; Frontotemporal lobar degeneration (caused by C9ORF72 mutations); Gerstmann–Sträussler–Scheinker disease; Guadeloupean parkinsonism; Myotonic dystrophy; Neurodegeneration with brain iron accumulation; Niemann–Pick disease, type C; Non-Guamanian motor neuron disease with neurofibrillary tangles; Pick disease; Post-encephalitic parkinsonism; Prion protein cerebral amyloid angiopathy; Progressive subcortical gliosis; Progressive supranuclear palsy; SLC9A6-related mental retardation; Subacute sclerosing panencephalitis; Tangle-only dementia; White matter tauopathy with globular glial inclusions.

Direct correlation was shown between tau aggregates in cortical areas and severity of dementia (Braak et al. Acta Neuropathol. 1991 82(4):239-59) suggesting that Tau aggregation might be a potential marker of neurodegenerative disease progression.

An imaging agent that is selective for Tau aggregates compared to other aggregated pathological proteins (beta-amyloid, α -synuclein, TDP-43, ...) would allow *in-vivo* visualization of Tau aggregates in patients therefore allowing a more accurate diagnosis and monitoring of treatment effects. Additionally it would better define the time course of the disease in each individual patient, and assess the efficacy of disease-modifying, tau-targeted treatments.

SUMMARY OF THE INVENTION

The present invention relates to 9H-pyrrolo-dipyridine derivatives, compositions, methods and use as imaging agents for the *in vivo* detection of Tau aggregates in the brain.

A further aspect of the present invention consists of novel agents that demonstrate high binding to Tau aggregates and have low non-specific binding and high selectivity compared to other unrelated proteins.

Further aspects of the invention will become apparent from the detailed specification.

DETAILED DESCRIPTION OF THE INVENTION

DESCRIPTION

In vivo imaging of Tau pathology would provide novel insights into the time course of deposition of Tau aggregates in the human brain, associations between Tau load and symptoms and between changes in Tau load and symptoms over time, and changes in Tau load when testing novel tau-targeting disease-modifying treatments.

Potential ligands for detecting Tau aggregates in the living brain must be brain penetrant and possess high affinity for Tau aggregates and specificity, especially compared to other aggregated proteins (beta-amyloid, α -synuclein, TDP-43, ...) and compared to other unrelated proteins. To achieve this objective, it is known that successful neuroimaging radiotracers must have appropriate lipophilicity (logD 1-3), low non-specific brain tissue binding ($F_u \geq 5\%$), low molecular weight (< 450) and show rapid clearance from blood. (Zhang et al J Med Chem. 2013 56(11):4568-4579).

Potential Tau PET ligands have been described for example in Chien et al. J Alzheimers Dis. 2013;34(2):457-68 and Maruyama et al. Neuron. 2013 79(6):1094-108. However, it is reported that they may have insufficient sensitivity and specificity to detect changes in Tau load (Villemagne et al. Lancet Neurol. 2015 (1):114-124). The object of the present application is to
5 identify a Tau PET ligand that will improve the identification of potential patients with excess of Tau aggregates in the brain.

The present invention describes compounds that may be used for binding and imaging Tau aggregates, especially for diagnostic and monitoring imaging of Tau aggregates in neurodegenerative diseases such as Progressive supranuclear palsy, Alzheimer's patients,
10 Pick's disease, chronic traumatic encephalopathy, corticobasal degeneration, Frontotemporal dementia and parkinsonism linked to chromosome 17 caused by MAPT mutations, Frontotemporal lobar degeneration, Amyotrophic lateral sclerosis/parkinsonism-dementia complex, Down syndrome and related tauopathies as listed in the background section.

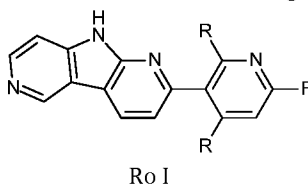
Tricyclic carboline and carbazole compounds are described for example in US-6,177,440 as
15 inhibitors of the human non-pancreatic secretory phospholipase A2 (sPLA₂) for the treatment of septic shock and in WO 2013/176698 and US-8,491,869 as senile plaques and neurofibrillary tangles binders for the imaging of β -Amyloid deposits and Tau aggregates.

WO 2009/102498 describes compounds and methods of diagnosing Alzheimer's Disease or a predisposition thereto in a mammal, the method comprising administering to the mammal a
20 diagnostically effective amount of a radiolabeled compound, wherein the compound is selected from the group consisting of radiolabeled flavones, coumarins, carbazoles, quinolinones, chromenones, imidazoles and triazoles derivatives, allowing the compound to distribute into the brain tissue, and imaging the brain tissue, wherein an increase in binding of the compound to the brain tissue compared to a normal control level of binding indicates that the mammal is suffering
25 from or is at risk of developing Alzheimer's Disease.

It has now surprisingly been found that certain tricyclic analogs described hereafter have high affinity to Tau aggregates and are markedly more specific than the compounds described in WO2013/176698, WO 2009/102498 and US-8,491,869. The invention provides imaging agents having a higher selectivity over unrelated targets compared to WO2013/176698, WO
30 2009/102498 and US-8,491,869. The compounds of the present invention display significantly less non-specific binding to brain tissue proteins as demonstrated by the significantly higher rat brain free fractions (Fu). Importantly, the compounds of the present invention are more specific when tested on large variety of unrelated targets comprising proteins highly expressed in the brain. Specifically, they are characterized by a 10-100 fold lower affinity for the monoamine
35 oxidase-A enzyme (MAO-A) and therefore produce significantly less background signal due to MAO-A binding. Because MAO-A is found at higher levels in regions where Tau accumulates initially in PSP (Saura et al. J Neurosci. 1992 (5):1977-1999, Williams et al. Brain. 2007 130(Pt

6):1566-1576), the use of PET tracers for Tau with affinity for MAO-A may not provide useable information about Tau load in PSP. Whether affinity for MAO-A is a potential problem for Tau imaging in AD is not known.

- 5 WO 2015/052105 describes diazacarbazole derivatives of general formula Ro I. as follows



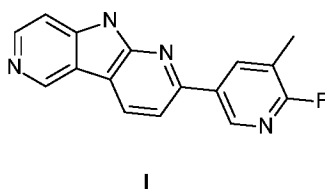
wherein R is hydrogen or tritium; and F is fluoro or ^{18}F fluoro or to a pharmaceutically acceptable acid addition salt.

WO 2015/052105 describes specifically 2-(6-fluoro-pyridin-3-yl)-9H-dipyrido[2,3-b;3',4'-d]pyrrole (IUPAC name : 2-(6-fluoropyridin-3-yl)-9H-pyrrolo[2,3-b:4,5-c']dipyridine) ;

- 10 ^3H -2-(6-fluoro-pyridin-3-yl)-9H-dipyrido[2,3-b;3',4'-d]pyrrole (IUPAC names: 2-[6-fluoro(2,4- $^3\text{H}_2$)pyridin-3-yl]-9H-pyrrolo[2,3-b:4,5-c']dipyridine ; 2-[6-fluoro(2- ^3H)pyridin-3-yl]-9H-pyrrolo[2,3-b:4,5-c']dipyridine ; 2-[6-fluoro(4- ^3H)pyridin-3-yl]-9H-pyrrolo[2,3-b:4,5-c']dipyridine) and [^{18}F]-2-(6-fluoro-pyridin-3-yl)-9H-dipyrido[2,3-b;3',4'-d]pyrrole 2-(6-fluoropyridin-3-yl)-9H-pyrrolo[2,3-b:4,5-c']dipyridine (IUPAC name : 2-[6-(^{18}F)fluoropyridin-3-yl]-9H-pyrrolo[2,3-b:4,5-c']dipyridine). These compounds may be used for binding and imaging tau aggregates and related beta-sheet aggregates including besides others beta-amyloid aggregates or alpha-synuclein aggregates.
- 15

In one aspect, the present invention relates to compounds of formula I, or a pharmaceutically acceptable acid addition salt,

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wherein any H of the formula is H or its ^2H or ^3H isotope ; any C of the general formula is C or its radioactive isotope ^{14}C , or ^{11}C ; any F of the formula is F or its radioactive isotope ^{18}F

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Usually any H of the general formula I is H or is its ^2H or ^3H isotope.

Usually C of the general formula on a benzylic methyl is C or is its radioactive isotope ^{14}C or ^{11}C .

Usually any F of the general formula is F or is its radioactive isotope ^{18}F .

30

Best results have been obtained with the compound 2-(6-fluoro-5-methylpyridin-3-yl)-9H-pyrrolo[2,3-b:4,5-c']dipyridine.

The term "pharmaceutically acceptable salt" or "pharmaceutically acceptable acid addition salt" according to the invention embraces therapeutically active, non-toxic acid or base salt forms which the compounds of formula I are able to form.

The acid addition salt form of a compound of formula I that occurs in its free form as a base can be obtained by treating the free base with an appropriate acid such as an inorganic acid, for example, a hydrohalic such as hydrochloric or hydrobromic, sulfuric, nitric, phosphoric and the like; or an organic acid, such as, for example, acetic, trifluoroacetic, oxalic, hydroxyacetic, propanoic, lactic, pyruvic, malonic, succinic, maleic, fumaric, malic, tartaric, citric, methanesulfonic, ethanesulfonic, benzenesulfonic, p-toluenesulfonic, cyclamic, salicylic, p-aminosalicylic, pamoic and the like.

With respect to the present invention reference to a compound or compounds is intended to encompass that compound in each of its possible isomeric forms and mixtures thereof, unless the particular isomeric form is referred to specifically.

For imaging studies, compounds of formula I or their pharmaceutically acceptable salts may be administered in the form of a pharmaceutical composition.

Therefore, another embodiment of the present invention concerns a pharmaceutical composition comprising a detectable amount of a compound of formula I or a pharmaceutically acceptable salt thereof in combination with a pharmaceutically acceptable diluent or carrier.

The compounds of formula I may be used for diagnostic imaging of Tau-aggregate deposits in the brain of a mammal.

Therefore, another embodiment of the present invention is a method of imaging Tau aggregates, including introducing into a mammal a detectable quantity of a pharmaceutical composition of a compound of formula I ; allowing sufficient time for the compound of formula I to be associated with Tau aggregates in the mammal brain ; and detecting the compound of formula I associated with Tau aggregates.

Preferably, the compounds of formula I can be used for diagnostic and monitoring imaging of Tau aggregates in the brain of human patients suffering from a tauopathy as listed above.

In another embodiment, the present invention concerns a compound as listed above for use as diagnostic and monitoring imaging tool of Tau aggregates in the brain.

In another embodiment, the present invention concerns a radiolabeled compound containing an isotope compound of formula I for use as diagnostic and monitoring imaging tool of tau aggregates in the brain.

In another embodiment, the present invention concerns a compound as listed above for use as a medicament.

In a specific embodiment, the present invention concerns a compound as listed above for use as a medicament in the treatment of neurodegenerative diseases.

In another embodiment, the present invention concerns a pharmaceutical composition containing a compound as listed above as well as pharmaceutically acceptable excipients.

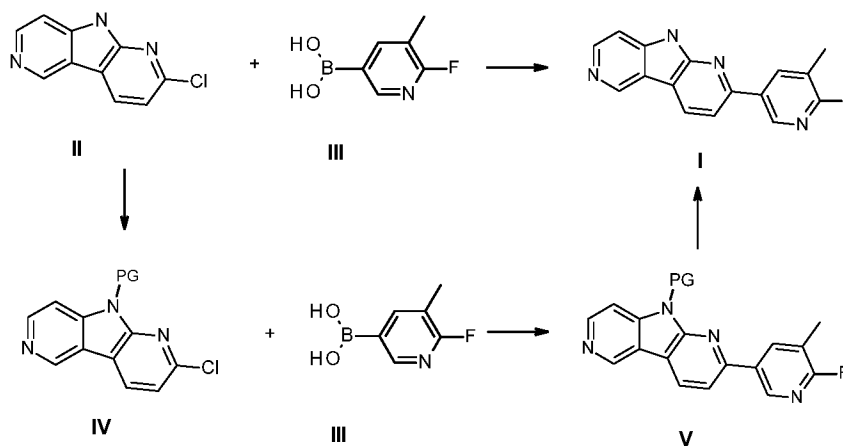
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SYNTHETIC METHODS

The compounds of formula I according to the invention can be prepared analogously to conventional methods as understood by the person skilled in the art of synthetic organic chemistry.

According to one embodiment, compounds of general formula I may be prepared by a Suzuki coupling reaction of a chloropyridine intermediate II and a boronic acid (or its corresponding boronic ester or trifluoroborate salt) III:

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This reaction may be performed in the presence of classical palladium catalytic systems such as [1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) or $\text{Pd}_2(\text{dba}_3)_2/\text{Xantphos}$ or other catalytic system known by the person skilled in the art, in the presence of a base such as Na_2CO_3 or K_3PO_4 in a solvent such as dioxane or n-butanol at a temperature ranging from 80 to 120°C. Alternatively, compounds of general formula I may be prepared by a Suzuki coupling reaction of a chloropyridine intermediate IV protected by a suitable group (PG) known from the person skilled in the art and a boronic acid (or its corresponding boronic ester or trifluoroborate salt) III, followed by protecting group removal.

Protection of intermediates II may for example be performed in the presence of SEM-Cl with a suitable base such as NaH in a solvent such as DMF at a temperature ranging from 0°C to 25°C. The Suzuki reaction may then be performed as described above while the SEM protecting group

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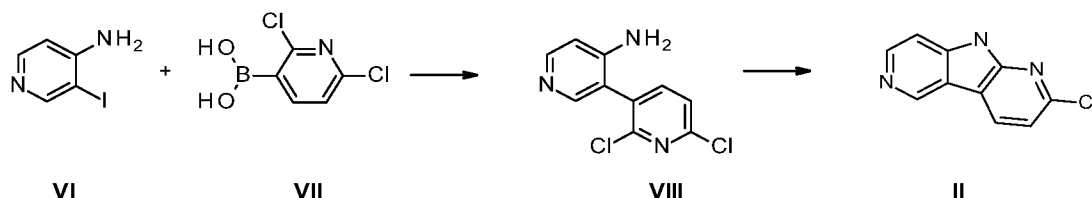
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may typically be removed in a 1 to 1 TFA/DCM mixture at room temperature or in any other conditions known by the person skilled in the art.

Compounds of formula **III** are commercially available or may be prepared according to any procedure known to the person skilled in the art.

5

Tricyclic chloro-intermediates of formula **II** may be prepared by Suzuki coupling of a suitable amino-iodo-pyridine **VI** with the boronic acid **VII**, followed by intramolecular cyclization of intermediate **VIII** according to the equation:



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This Suzuki coupling reaction may be performed in the presence of classical palladium catalytic systems such as Bis(triphenylphosphine)palladium(II) dichloride or other catalytic system known by the person skilled in the art, in the presence of a base such as Na_2CO_3 or K_3PO_4 in a solvent

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such as dioxane or n-butanol at a temperature ranging from 80 to 120°C. Iodopyridine of formula **VI** is commercially available or may be prepared according to any procedure known to the person skilled in the art.

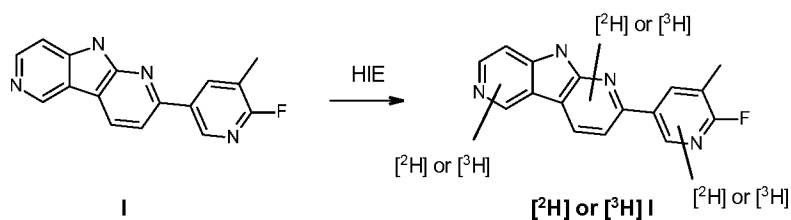
Boronic acid of formula **VII** is commercially available.

Intermediates of formula **VIII** may then be cyclized into compounds of formula **II** in the presence of a base such as LiHMDS or any similar base known from the person skilled in the art, in a solvent such as THF at a temperature of 90°C.

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The deuterated or tritiated compounds of formula **I** may be prepared by direct Hydrogen isotopic Exchange (HIE) using methods known from the people skilled in the art:

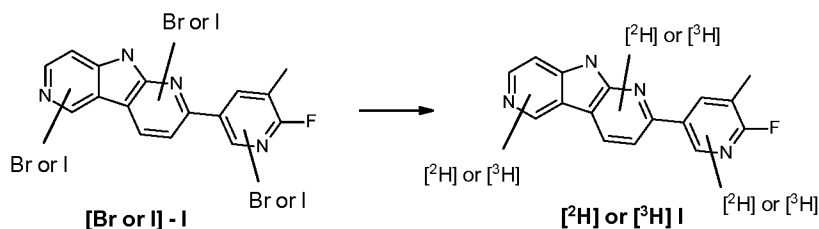
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This HIE reaction may be performed in the presence of the well known Crabtree's iridium catalyst, $[(\text{COD})\text{Ir}(\text{py})\text{PCy}_3]\text{PF}_6$, Kerr's iridium-carbene catalysts or any similar catalyst known

from the person skilled in the art, in a solvent such as THF or DMF in the presence of deuterium or tritium gas.

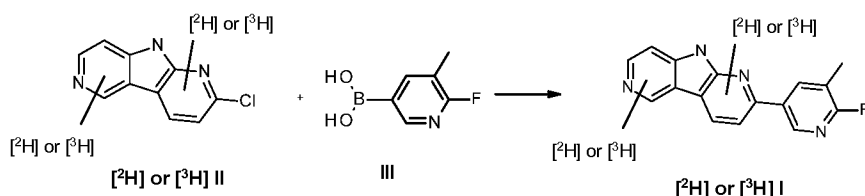
Alternatively, the deuterated or tritiated compounds of formula I may be prepared by reduction of the corresponding mono-, di- or tri-iodide or bromide using methods known from the people skilled in the art:



This reduction reaction may be performed in the presence of various palladium catalysts or any similar catalyst known from the person skilled in the art, in a solvent such as THF or DMF in the presence of deuterium or tritium gas.

The iodo- or bromo-substituted I may be prepared by any of the aforementioned method starting from the corresponding iodo- or bromo-substituted reagents or intermediates or by bromination/iodination using brominating agents, such as N-bromosuccinimide, known to the person skilled in the art.

Alternatively, the deuterated or tritiated compounds of formula I may be prepared by a Suzuki coupling reaction of a deuterated or tritiated chloropyridine intermediate II and a boronic acid (or its corresponding boronic ester or trifluoroborate salt) III:



The deuterated or tritiated chloropyridine intermediates II may be prepared by any of the aforementioned methods.

PREPARATION OF COMPOUNDS
MATERIALS AND METHODS

Starting materials are either commercially available or can be prepared by methods analogous to the methods given below or by methods known in the art.

Compounds were named with the aid of ACD/Name Batch (Network) ver. 11.01

All reactions involving air-or moisture-sensitive reagents were performed under a nitrogen or argon atmosphere using dried solvents and glassware. Commercial solvents and reagents were generally used without further purification, including anhydrous solvents when appropriate (usually Sure-Seal™ products from Aldrich Chemical Company or AcroSeal™ from ACROS Organics). In general reactions were followed by thin layer chromatography, HPLC or mass spectrometry analyses.

Crude materials could be purified by normal phase chromatography, (acidic or basic) reverse phase chromatography, chiral separation or recrystallization.

Products were generally dried under vacuum before final analyses and submission to biological testing.

Tritium labeling of the compounds has been performed by Asclepia MedChem Solutions through direct hydrogen-tritium exchange according to the general method described hereafter.

HPLC analysis:

HPLC chromatograms are recorded as follows,

Method A: Acidic

HPLC analysis is performed with Shimadzu HPLC system equipped with LC-2010 CHT module, SPD-M20A photodiode array detector (210-400 nm), by using column YMC Triart C-18 (150 X 4.6)mm 3μ. Gradient elution is done with 5 mM ammonium formate in water +0.1% formic acid (Phase A), and Acetonitrile+5%solvent A+0.1% formic acid (Phase B), with gradient 5-95% B in 8.0 min hold till 13.0 min, 5%B at 15.0 min hold till 18.0 min. HPLC flow rate: 1.0 ml/min, injection volume: 10 μL.

Method B: Basic

HPLC analysis is performed with Shimadzu HPLC system equipped with LC-2010 CHT module, SPD-M20A photodiode array detector (210-400 nm), by using column YMC Triart C-18 (150 X 4.6)mm 3μ. Gradient elution is done with 5 mM ammonium formate in water +0.1% Ammonia (Phase A), and Acetonitrile+5%solvent A+0.1% Ammonia (Phase B), with gradient 5-95% in 8.0 min hold till 13.0 min, 5%B at 15.0 min hold till 18.0 min. HPLC flow rate.

LCMS Analysis:

LCMS analyses are performed as follows,

Method A: Acidic

Shimadzu 2010EV single quadrupole mass spectrometer is used for LC-MS analysis. This spectrometer is equipped with an ESI source and LC-20AD binary gradient pump, SPD-M20A photodiode array detector (210-400 nm). Data is acquired in a full MS scan from m/z 70 to 1200 in positive and negative mode. The reverse phase analysis is carried out by using Waters XBridge C 18 (30 X 2.1)mm 2.5 μ column. Gradient elution is done with 5 mM ammonium formate in water +0.1% formic acid (Phase A) and Acetonitrile +5% solvent A +0.1% formic acid (Phase B), with gradient 5-95%B in 4.0 min hold till 5.0 min, 5% B at 5.1 min hold till 6.5 min. HPLC flow rate: 1.0 ml/min, injection volume: 5 μ L.

MS parameters: Detector voltage 1.5 kV. Source block temperature 200°C. Desolvation temperature 240°C. nebulizing gas flow 1.2 L/min (Nitrogen). Data is acquired in a full MS scan from m/z 70 to 1200 in positive and negative mode.

15 Method B : Basic

Shimadzu 2010EV single quadrupole mass spectrometer is used for LC-MS analysis. This spectrometer is equipped with an ESI source and LC-20AD binary gradient pump, SPD-M20A photodiode array detector (210-400 nm). Data is acquired in a full MS scan from m/z 70 to 1200 in positive and negative mode. The reverse phase analysis is carried out by using Waters XBridge C 18 (30 X 2.1)mm 2.5 μ column Gradient elution is done with 5 mM ammonium formate in water +0.1% Ammonia (solvent A),or Acetonitrile +5% solvent A+0.1% Ammonia (solvent B), with gradient 5-95% B in 4.0 min hold till 5.0 min, 5%B at 5.1 min hold till 6.5 min. HPLC flow rate: 1.0 ml/min, injection volume: 5 μ L.

MS parameters: Detector voltage 1.5 kV. Source block temperature 200°C. Desolvation temperature 240°C. Nebulising gas flow 1.2 L/min (Nitrogen). Data is acquired in a full MS scan from m/z 70 to 1200 in positive and negative mode.

NMR :

NMR spectra are recorded on a Varian MR 400 MHz NMR Spectrometer fitted with a Linux 3.2 software with operating system Redhat enterprise Linux 5.1. and 5 mm inverse $^1\text{H}/^{13}\text{C}$ probe head, or Varian VNMR 400 MHz NMR fitted with Linux 3.2 software with operating system Redhat enterprise Linux 6.3 and 5 mm inverse $^1\text{H}/^{13}\text{C}/^{19}\text{F}$ triple probe head. The compounds are studied in deuterated solvents such as DMSO- d_6 , CDCl_3 , MeOD or D_2O at a probe temperature of 300 K and at a concentration around 4-5 mg/mL. The instrument is locked on the deuterium signal of the deuterated solvent used. Chemical shifts are given in ppm downfield from TMS (tetramethylsilane) taken as internal standard.

ABBREVIATIONS

- ACN: Acetonitrile
Brine: Saturated aqueous sodium chloride solution
- 5 DCM: Dichloromethane
DMF: *N,N*-Dimethylformamide
DMSO: Dimethylsulfoxide
ES⁺: Electrospray Positive Ionisation
EtOH : Ethanol
- 10 Et₂O: Diethyl ether
EtOAc: Ethyl acetate
ESI : Electrospray Ionization
h: Hour
HCl: Hydrochloric acid
- 15 HIE: Hydrogen Isotope Exchange
K₂CO₃: Potassium carbonate
LC: Liquid Chromatography
LCMS: Liquid Chromatography Mass Spectrometry
MeOH: Methanol
- 20 MgSO₄: Magnesium sulfate
min.: minutes
Na₂CO₃: Sodium carbonate
NaOH: Sodium hydroxide
Na₂SO₄: Sodium sulfate
- 25 NMR: Nuclear magnetic resonance
PdCl₂(dppf): [1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II)
Pd₂(dba)₃: Tris(dibenzylideneacetone)dipalladium(0)
PG: protecting group
iPrOH: isopropanol
- 30 PTSA: *p*toluenesulfonic acid
py: pyridine
RCP: radiochemical Purity
RT: room temperature
SA: specific activity
- 35 SEM : [2-(Trimethylsilyl)ethoxy]methyl
SEM-Cl : [2-(Trimethylsilyl)ethoxy]methyl Chloride
TEA: Triethyl amine

TFA: Trifluoroacetic acid

THF: Tetrahydrofuran

TLC: Thin Layer Chromatography

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EXAMPLES

The following examples illustrate how the compounds covered by formula I may be synthesized. They are provided for illustrative purposes only and are not intended, nor should they be construed, as limiting the invention in any manner. Those skilled in the art will appreciate that routine variations and modifications of the following examples can be made without exceeding the spirit or scope of the invention.

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Tritium labeling procedure

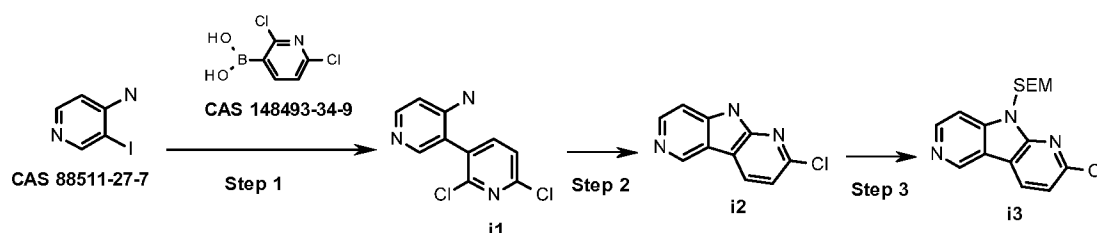
This procedure exemplarily describes the preparation of [³H]-labeled compounds by direct Hydrogen Isotope Exchange.

5 mg of the unlabeled compound and 6 mg of rhodium black were suspended in 0.9 ml of a mixture of THF and DMF (1/8). The suspension was degassed three times at the high vacuum manifold and stirred under an atmosphere of tritium gas (9 Ci) for 3 h at room temperature. The solvent was removed under vacuum, and labile tritium was exchanged by adding 1 ml of methanol, stirring the solution, and removing the solvent again under vacuum. This process was repeated three times. Finally, the well-dried solid was extracted with 5 ml of ethanol containing 0.1% of trifluoroacetic acid. The suspension was filtered through a 0.2 μm nylon membrane, obtaining a clear solution.

Purification of 100 mCi (3.70 GBq) of the crude compound was performed on a Macherey + Nagel Nucleodur Gravity C18, 5 μm, 8 x 150 mm; solvents A: 10 mM NH₄OAc; B: acetonitrile; 35% B; 254 nm and 220 nm; 3.1 ml/min; 20°C to afford the radiolabeled product with a radiochemical purity >98%. The specific activity was determined for each synthesis.

30

Compounds of Formula I



2',6'-dichloro-[3,3'-bipyridin]-4-amine (i1):

To a solution of 3-iodopyridin-4-amine (6 g, 27.2 mmol) in dioxane (135 mL), (2,6-dichloropyridin-3-yl)boronic acid (7.29 g, 38.1 mmol), and 1M Na₂CO₃ aqueous solution (3 eq) were added and the reaction mixture was degassed with argon for 20 min. Then Bis(triphenylphosphine)palladium(II) dichloride (3.79 g, 5.4 mmol) was added and the reaction mixture was heated at 100°C for 16h. After completion of reaction, the reaction mixture was filtered through a celite pad and the filtrate was concentrated under reduced pressure to afford a residue that was dissolved in water and extracted with ethyl acetate. The organic layer was separated, dried over sodium sulphate and concentrated under reduced pressure to afford the crude product, which was further purified by silica gel (100:200 mesh) column chromatography to afford 2',6'-dichloro-[3,3'-bipyridin]-4-amine (**i1**) (2.9 g, Yield 44%).

¹H NMR (400 MHz, DMSO-d₆) δ 6.04 (s, 2H), 6.62 (d, *J* = 5.8 Hz, 1H), 7.71 – 7.55 (m, 1H), 7.94 – 7.75 (m, 2H), 8.03 (d, *J* = 5.7 Hz, 1H).

MS (ESI) *m/e* (M+1)⁺: 240.05

2-chloro-9*H*-pyrrolo[2,3-*b*:4,5-*c'*]dipyridine (i2):

To a solution of 2',6'-dichloro-[3,3'-bipyridin]-4-amine (**i1**) (1 g, 8.0 mmol) in THF (100 mL), LiHMDS (12 eq) was added drop wise at 0°C and the reaction mixture was heated at 90°C for 2h in a sealed tube. The reaction mixture was quenched with water and extracted with ethyl acetate. The organic layer was separated dried over sodium sulphate and concentrated under reduced pressure, which was purified by repeated washing with pentane to afford 2-chloro-9*H*-pyrrolo[2,3-*b*:4,5-*c'*]dipyridine (**i2**) (1.5 g, Yield 89%).

¹H NMR (400 MHz, DMSO-d₆) δ 7.37 (d, *J* = 8.1 Hz, 1H), 7.50 (d, *J* = 5.6, 1.1 Hz, 1H), 8.49 (d, *J* = 5.6 Hz, 1H), 8.66 (d, *J* = 8.1 Hz, 1H), 9.37 (s, 1H), 12.45 (s, 1H).

MS (ESI) *m/e* (M+1)⁺: 204.05

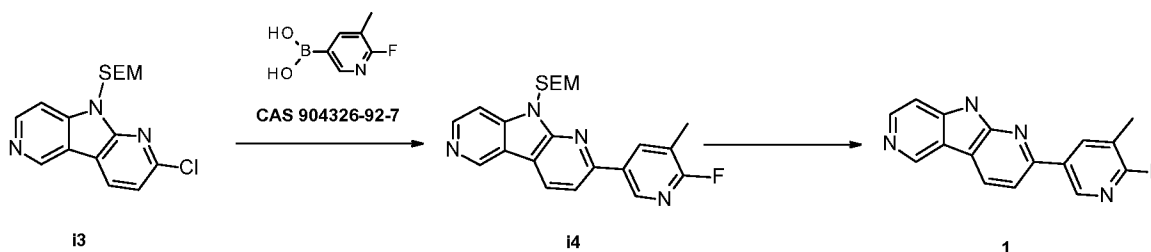
2-chloro-9-((2-(trimethylsilyl)ethoxy)methyl)-9*H*-pyrrolo[2,3-*b*:4,5-*c'*]dipyridine (i3):

To a solution of 2-chloro-9*H*-pyrrolo[2,3-*b*:4,5-*c'*]dipyridine (**i2**) (0.5 g, 2.46 mmol) in DMF (10 mL), NaH (60%, 118 mg, 2.95 mmol) was added at 0°C and the reaction mixture was stirred at the same temperature for 30 min. SEM-Cl (0.49 g, 2.95 mmol) was then added dropwise at 0°C. The reaction was stirred at room temperature for 3h. The progress of the reaction was monitored by TLC. After completion, the mixture was quenched with water and extracted with ethyl acetate. The organic layer was separated, dried over sodium sulphate and concentrated under reduced pressure. The crude product was purified by silica gel (100-200 mesh) column chromatography

using 2% methanol in dichloromethane as eluent to afford 2-chloro-9-((2-(trimethylsilyl)ethoxy)methyl)-9H-pyrrolo[2,3-*b*:4,5-*c'*]dipyridine (**i3**) (0.26 g, Yield 31%).

¹H NMR (400 MHz, DMSO-*d*₆) δ -0.15 (s, 9H), 0.89 – 0.79 (m, 2H), 3.60 – 3.49 (m, 2H), 5.85 (s, 2H), 7.50 (d, *J* = 8.1 Hz, 1H), 7.80 (d, *J* = 5.7 Hz, 1H), 8.62 (d, *J* = 5.7 Hz, 1H), 8.74 (d, *J* = 8.1 Hz, 1H), 9.45 (s, 1H).

MS (ESI) *m/e* (*M*+1)⁺: 334.35



2-(6-fluoro-5-methylpyridin-3-yl)-9-((2-(trimethylsilyl)ethoxy)methyl)-9H-pyrrolo[2,3-*b*:4,5-*c'*]dipyridine (**i4**):

To a solution of 2-chloro-9-((2-(trimethylsilyl)ethoxy)methyl)-9H-pyrrolo[2,3-*b*:4,5-*c'*]dipyridine (**i3**) and 2-Fluoro-3-Methylpyridine-5-Boronic Acid (0.13 g, 0.84 mmol) in dioxane (4 mL) and H₂O (1 mL) was added Na₂CO₃ (0.19 g, 1.80 mmol) and reaction was purged with argon for 30 min. PdCl₂(dppf).DCM (0.05 g, 0.06 mmol) was added, purged with argon for 5 min. The reaction mixture was heated at 100°C for 2h. Progress of the reaction was monitored by TLC and LCMS. After completion, the reaction mixture was concentrated in vacuo. The crude obtained was purified by column chromatography (silica, 230-400 mesh, 0 to 1% MeOH in DCM) to afford 2-(6-fluoro-5-methylpyridin-3-yl)-9-((2-(trimethylsilyl)ethoxy)methyl)-9H-pyrrolo[2,3-*b*:4,5-*c'*]dipyridine (**i4**; 0.24 g, 94%) as a brown solid.

MS (ESI) *m/e* (*M*+1)⁺: 409.00

¹H NMR (400 MHz, DMSO-*d*₆) δ -0.21 (s, 9H) 0.83 – 0.92 (m, 2H) 2.36 (s, 3H) 3.55 – 3.64 (m, 2H) 5.98 (s, 2H) 7.75 (d, *J* = 5.6 Hz, 1H) 8.08 (d, *J* = 8.1 Hz, 1H) 8.59 (d, *J* = 5.6 Hz, 1H) 8.67 (d, *J* = 9.5 Hz, 1H) 8.78 (d, *J* = 8.1 Hz, 1H) 8.92 (s, 1H) 9.43 (s, 1H).

2-(6-fluoro-5-methylpyridin-3-yl)-9H-pyrrolo[2,3-*b*:4,5-*c'*]dipyridine (**1**):

To a solution of 2-(6-fluoro-5-methylpyridin-3-yl)-9-((2-(trimethylsilyl)ethoxy)methyl)-9H-pyrrolo[2,3-*b*:4,5-*c'*]dipyridine (**i4**) (0.23 g, 0.61 mmol) in DCM (2.5 mL) was added TFA (2.5 mL)

at 0°C and reaction mixture was stirred at room temperature for 16h. Progress of the reaction was monitored by TLC and LCMS. After completion, the reaction mixture was concentrated in vacuo. The residue was dissolved in CH₃CN (2 mL) and aq NH₄OH (3 mL) and stirred at room temperature for 2h. The reaction mixture was filtered, washed with H₂O (20 mL) and dried in vacuo. The crude obtained was purified by triturating with CH₃CN (20 mL), pentane (20 mL) and dried in vacuo to afford 2-(6-fluoro-5-methylpyridin-3-yl)-9H-pyrrolo[2,3-b:4,5-c']dipyridine **1** (0.14 g, 89%) as off-white solid.

HPLC Purity: 96.5%

MS (ESI) m/e (M+1)⁺: 279.00

¹H NMR (400 MHz, DMSO-d₆) δ 2.37 (s, 3H) 7.49 (d, J = 5.6 Hz, 1H) 7.99 (d, J = 8.1 Hz, 1H) 8.50 (d, J = 5.6 Hz, 1H) 8.59 (d, J = 8.6 Hz, 1H) 8.74 (d, J = 8.1 Hz, 1H) 8.83 (s, 1H) 9.39 (s, 1H) 12.38 (s, 1H).

BIOLOGICAL RESULTS OF COMPOUNDS MATERIALS AND METHODS

Biological samples

Human K18/K19 recombinant Tau fibrils were prepared as follow described. His-tagged K18 and K19 Tau containing respectively four and three repeats microtubule binding domain were cloned into a pET expression vector. Expression vectors were transformed into E.coli strain BL21 (DE3), cultured and expression induced with isopropyl β-D-thiogalactoside. Harvested E. Coli were lysed mechanically before purification of His-tagged proteins by fast protein liquid chromatography (nickel charged resin Ni-NTA superflow kit from Qiagen, Venlo, Netherlands). His-Tag was removed from K18 by cleavage with Tobacco Etch Virus protease. K18 and K19 His-tagged Tau were suspended into phosphate buffered saline solution (PBS, pH 7.4), flash frozen and stored at -80°C. Tau K18 and Tau K19-His were thawed and mixed (both at ~300 μM). Mixture was filtered through a 0.22 μm membrane. Sample was shaken in thermomixer (Eppendorf, Rotselaar, Belgium) at 750 rpm, 37°C for 96 hours. Fibril mixture was recovered, aliquotted and stored at -80°C until use.

Human recombinant monoamine oxidase (MAO) A and B proteins was expressed in baculovirus infected BTI insect cells and obtained from Sigma-Aldrich (Diegem, Belgium).

OFA Sprague-Dawley rats (200 to 300 g) were purchased from Charles River (Saint-Germain-Nuelles, France). Animals were sacrificed by decapitation and brain was quickly removed and dissected. The reported experiments were carried out in accordance with the UCB ethical

committee for animal experimentation. Crude cerebral cortex membranes were prepared as described in Gillard et al. (Eur J Pharmacol. 2003 Sep 30;478(1):1-9).

Radioligand *in vitro* binding assays

- 5 Biological samples as described above (0.2 - 50 µg proteins per assays) were incubated for 60 min at 25°C with tritiated appropriate ligand in 0.2 ml of PBS containing 0.1% bovine serum albumin. At the end of the incubation period, the protein-bound radioligand was recovered by reduced pressure filtration through glass fiber filters pre-soaked in 0.1% polyethyleneimine. Filters were washed with at least 4 times the assay volume of ice-cold phosphate buffered saline
10 solution (pH 7.4). The entire filtration step did not exceed 10 sec. The filters were dried and the radioactivity determined by liquid scintillation. Compound affinity was measured using radioligand saturation or competitive binding assays. Saturation binding assays were carried out using increasing concentrations of tritiated compound (0.5 to 100 nM). Competitive binding experiments were carried out at constant radioligand concentration and increasing concentrations
15 of test compound and reference ligand (10 concentrations data point from 10 µM to 0.1 nM). When appropriate, pIC₅₀ were corrected to pK_i according to Cheng and Prusoff (Biochem Pharmacol. 1973 Dec 1;22(23):3099-108). In all experiments, the non specific binding was defined as the residual binding of radioligand observed in the presence of 10 µM reference ligand. Binding data analysis was performed by computerized curve fitting (Graphpad Prism® software,
20 version 4.0, San Diego, CA) according to equations describing specific saturation binding in one or two sites, and competitive binding model.

Selectivity profile

- 25 Compound selectivity for Tau protein was assessed at CEREP (Celle-l'Evescault, France) as compared to a broad panel of receptors, enzymes and ion channels. Compound was tested at 10 µM in radioligand competitive binding assays for most of the targets except for MAO-A, MAO-B, COMT, COX1 and 2, AChE, GABA transaminase, LCK, cNOS, PDE3A, 4D2 and 7A and PLA2 in which case functional enzyme assays were performed.
30 Compound affinity was measured using radioligand *in vitro* binding assays as described above.

Rat brain free fraction (F_u) measurement

The Brain free fraction was carried out in duplicate at a single concentration of 1 µM after 4h of equilibrium dialysis.

- 35 Male Sprague-Dawley Rat (Harlan, Bresso, Italy) brain homogenate were prepared in PBS, pH 7.4 at 25% w/vy using a Precellys 24-dual tissue homogenizer (BERTIN technologies, Montigny-le Bretonneux, France).

200 µL brain homogenate was incubated with 1 µM test compound or reference compound (propranolol, Sigma, St Louis, United states) (1% DMSO final) for at least 30 min at 37°C under agitation before loading in a retentate chamber of a RED Device insert (8K MWCO, Thermo Scientific™ Pierce™ RED Device, Waltham, United states). 350 µL PBS pH 7.4 was loaded in the other chamber of the insert. The Red device reusable base counting insert containing insert with both samples and buffer was sealed and incubated during 4h at 37°C, 300 rpm, on an orbital shaker.

At the end of the incubation, all brain samples were diluted 1:1 with PBS and PBS samples were diluted 1:1 with control brain homogenate. All samples were then diluted 1:3 with an internal standard (dextromethorphan 10 ng/mL in acetonitrile, Sigma, St Louis, United states), mixed and centrifuged 5 min at 3000 rpm at 4°C. Supernatant was diluted 1:2 with 0.1% formic acid in water (Biosolve, Dieuze, France) before analysis by LC/MS/MS. The LC system used was an Agilent 1290 (Agilent, Santa Clara, United states) coupled with a API5000 mass spectrometer (ABSciex, Framingham, United states) . The software was analyst 1.5.2. (Agilent, Santa Clara, United states), the analytical column was an Aquity UPLC HSS T3 (30x2.1mm, 1.8µm, Waters, Saint-Quentin , France) operated at 40°C. Analysis were performed in the gradient described below. Gradient used for LC MS/MS :

<i>Total Time (min)</i>	<i>Flow Rate (µl/min)</i>	<i>B (%)</i>
0.00	1000	5
0.50	1000	5
1.96	1000	70
1.97	1000	95
2.50	1000	95
2.51	1000	5
3.00	1000	5

Where eluent A was 0.1% formic acid in H₂O (Biosolve, Dieuze, France), eluent B was 0.1% formic acid in acetonitrile (Biosolve, Dieuze, France).

The flow was directly injected into the electrospray source.

The Fu brain (%) was calculated using the following equation :

$$\text{Fu brain (\%)} = (1 / (1 + ((1 / ((\text{fu homogenate}) - 1) \times D))) \times 100$$

Where Fu homogenate = peak area ratio buffer/ peak area ratio brain and D = dilution factor of the homogenate.

Table 1

BINDING RESULTS

Human recombinant Tau fibrils affinity and rat brain free fraction

EXAMPLE #	IUPAC NAME	pIC ₅₀ K18/K19 Tau fibrils (-log M)	Fu Rat Brain (%)
1	2-(6-fluoro-5-methylpyridin-3-yl)- 9H-pyrrolo[2,3-b:4,5-c']dipyridine	7.6	1.7

5

Table 2

10

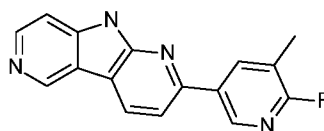
Human MAO-A binding Results

Example #	IUPAC_NAME	Human MAO-A Binding at 100nM (% inhibition)	Human MAO-A Binding at 1 µM (% inhibition)
1	2-(6-fluoro-5-methylpyridin-3-yl)- 9H-pyrrolo[2,3-b:4,5-c']dipyridine	4.0	24.0

15

CLAIMS

1 – A compound of general formula I, or a pharmaceutically acceptable acid addition salt,



I

5 wherein any H of the formula is H or its ^2H or ^3H isotope ; any C of the general formula is C or its radioactive isotope ^{14}C , or ^{11}C ; any F of the formula is F or its radioactive isotope ^{18}F .

2 – A compound according to claim 1 wherein any H of the general formula is H or its ^2H or ^3H isotope .

10

3 – A compound according to claim 1 or 2 wherein C of the general formula on a benzylic methyl is C or is its radioactive isotope ^{14}C or ^{11}C .

15

4 - A compound according to claim 1, 2 or 3 wherein any F of the general formula is F or is its radioactive isotope ^{18}F .

5 - A compound according to claim 1 which is 2-(6-fluoro-5-methylpyridin-3-yl)-9H-pyrrolo[2,3-b:4,5-c']dipyridine.

20

6 - A radiolabeled compound containing an isotope according to any of claims 1 to 5 for use as diagnostic and monitoring imaging tool of tau aggregates in the brain.

7 - A compound according to any of claims 1 to 5 for use as a medicament.

25

8 - A compound according to any of claims 1 to 5 for use as a medicament in the treatment of neurodegenerative diseases.

9 - Pharmaceutical composition containing a compound according to any of claims 1 to 5 as well as pharmaceutically acceptable excipients.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/069234

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D471/14 A61K31/4375 A61P25/28 A61K51/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/052105 A1 (HOFFMANN LA ROCHE [CH]; HOFFMANN LA ROCHE [US]) 16 April 2015 (2015-04-16) cited in the application claims 1, 5, 7; examples -----	1-9
A	WO 2009/102498 A1 (SIEMENS MEDICAL SOLUTIONS [US]; KOLB HARTMUTH C [US]; WALSH JOSEPH C [US]) 20 August 2009 (2009-08-20) cited in the application claims 1, 33; examples -----	1-9



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

10 August 2017

Date of mailing of the international search report

22/08/2017

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Miniejew, Catherine

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/069234

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