

DIHYDROPYRROLO[3,4C]-PYRAZOLE DERIVATIVES AND THEIR USE IN DIAGNOSIS

5

FIELD OF THE INVENTION

The present invention relates to novel compounds of formula (I), or a detectably labelled compound, stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, that can be employed in the imaging of alpha-synuclein aggregates and determining an amount thereof. Furthermore, the compounds can be used for diagnosing a disease, disorder or abnormality associated with alpha-synuclein (α -synuclein, A-synuclein, aSynuclein, A-syn, α -syn, aSyn, a-syn) aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites (such as Parkinson's disease), determining a predisposition to such a disease, disorder or abnormality, prognosing such a disease, disorder or abnormality, monitoring the evolution of the disease in a patient suffering from such a disease, disorder or abnormality, monitoring the progression of such a disease, disorder or abnormality, and predicting responsiveness of a patient suffering from such a disease, disorder or abnormality to a treatment thereof. The present invention also relates to processes for the preparation of the compounds and their precursors, diagnostic compositions comprising the compounds, methods of using the compounds, kits comprising the compounds and their uses thereof.

BACKGROUND OF THE INVENTION

Many diseases of aging are based on or associated with extracellular or intracellular deposits of amyloid or amyloid-like proteins that contribute to the pathogenesis as well as to the progression of the disease. The best characterized amyloid protein that forms extracellular aggregates is amyloid beta (A β or Abeta).

Amyloid-like proteins that form mainly intracellular aggregates include, but are not limited to, Tau, alpha-synuclein, and huntingtin (HTT). Diseases involving alpha-synuclein aggregates are generally listed as synucleinopathies (or alpha-synucleinopathies) and these include, but are not limited to, Parkinson's disease (PD). Synucleinopathies with primarily neuronal aggregates include, but are not limited to, Parkinson's disease (sporadic, familial with SNCA (the gene encoding for the alpha-synuclein protein) mutations or SNCA gene duplication or triplication, familial with mutations in other genes than SNCA, pure autonomic failure and Lewy body dysphagia), SNCA duplication carrier, Lewy Body dementia (LBD), dementia with Lewy bodies (DLB) ("pure" Lewy body dementia), Parkinson's disease dementia (PDD), diffuse Lewy body disease (DLBD), Alzheimer's disease,

sporadic Alzheimer's disease, familial Alzheimer's disease with APP mutations, familial Alzheimer's disease with PS-1, PS-2 or other mutations, familial British dementia, Lewy body variant of Alzheimer's disease and normal aging in Down syndrome. Synucleinopathies with neuronal and glial aggregates of alpha synuclein include, but are not limited to, multiple system atrophy (MSA) (Shy-
 5 Drager syndrome, striatonigral degeneration and olivopontocerebellar atrophy). Other diseases that may have alpha-synuclein-immunoreactive lesions are, but are not limited to, traumatic brain injury, chronic traumatic encephalopathy, dementia puglistica, tauopathies (Pick's disease, frontotemporal dementia, progressive supranuclear palsy, corticobasal degeneration and Niemann-Pick type C1 disease, frontotemporal dementia with Parkinsonism linked to chromosome 17), motor neuron
 10 disease, Huntington's disease, amyotrophic lateral sclerosis (sporadic, familial and ALS-dementia complex of Guam), neuroaxonal dystrophy, neurodegeneration with brain iron accumulation type 1 (Hallervorden-Spatz syndrome), prion diseases, Creutzfeldt-Jakob disease, ataxia telangiectatica, Meige's syndrome, subacute sclerosing panencephalitis, Gerstmann-Straussler-Scheinker disease, inclusion-body myositis, Gaucher disease, Krabbe disease as well as other lysosomal storage
 15 disorders (including Kufor-Rakeb syndrome and Sanfilippo syndrome) and rapid eye movement (REM) sleep behavior disorder (Jellinger, *Mov. Disord.* 2003, 18 Suppl. 6, S2-12; Galvin et al. *JAMA Neurology* 2001, 58 (2), 186-190; Kovari et al., *Acta Neuropathol.* 2007, 114(3), 295-8; Saito et al., *J. Neuropathol. Exp. Neurol.* 2004, 63(4), 323-328; McKee et al., *Brain*, 2013, 136(Pt 1), 43-64; Puschmann et al., *Parkinsonism Relat. Disord.* 2012, 18S1, S24-S27; Usenovic et al., *J. Neurosci.*
 20 2012, 32(12), 4240-4246; Winder-Rhodes et al., *Mov. Disord.* 2012, 27(2), 312-315; Ferman et al., *J. Int. Neuropsychol. Soc.* 2002, 8(7), 907-914; Smith et al., *J. Pathol.* 2014; 232:509-521, Lippa et al., *Ann Neurol.* 1999 Mar; 45(3):353-7; Schmitz et al., *Mol. Neurobiol.* 2018 Aug 22; Charles et al., *Neurosci. Lett.* 2000 Jul 28; 289(1):29-32; Wilhelmsen et al., *Arch Neurol.* 2004 Mar; 61(3):398-406; Yamaguchi et al., *J. Neuropathol. Exp. Neurol.* 2004, 80th annual meeting, vol.63; Askanas et al., *J.*
 25 *Neuropathol. Exp. Neurol.* 2000 Jul; 59(7):592-8).

Alpha-synuclein is a 140 amino acid natively unfolded protein (Iwai et al., *Biochemistry* 1995, 34(32), 10139-10145). The sequence of alpha-synuclein can be divided into three main domains: 1) the N-terminal region comprising of residues 1-60, which contains the 11-mer amphipathic imperfect repeat
 30 residues with highly conserved hexamer (KTKEGV). This region has been implicated in regulating alpha-synuclein binding to membranes and its internalization; 2) the hydrophobic Non Amyloid beta Component (NAC) domain spanning residues 61-95; which is essential for alpha-synuclein fibrillization; and 3) the C-terminal region spanning residues 96-140 which is highly acidic and proline-rich and has no distinct structural propensity. Alpha-synuclein has been shown to undergo several
 35 post translational modifications, including truncations, phosphorylation, ubiquitination, oxidation and/or transglutaminase covalent cross linking (Fujiwara et al., *Nat. Cell. Biol.* 2002, 4(2); 160-164;

Hasegawa et al., J. Biol. Chem. 2002, 277(50), 49071-49076; Li et al., Proc. Natl. Acad. Sci. U S A 2005, 102(6), 2162-2167; Oueslati et al, Prog. Brain Res. 2010, 183, 115-145; Schmid et al., J. Biol. Chem. 2009, 284(19), 13128-13142). Interestingly, the majority of these modifications involve residues within the C-terminal region.

5

Several phosphorylation sites have been detected in the carboxyl-terminal region on Tyr-125, -133, and -136, and on Ser-129 (Negro et al., FASEB J. 2002, 16(2), 210-212). Tyr-125 residues can be phosphorylated by two Src family protein tyrosine kinases, c-Src and Fyn (Ellis et al., J. Biol. Chem. 2001, 276(6), 3879-3884; Nakamura et al., Biochem. Biophys. Res. Commun. 2001, 280(4), 1085-1092). Phosphorylation by Src family kinases does not suppress or enhance the tendency of alpha-synuclein to polymerize. Alpha-synuclein has proved to be an outstanding substrate for protein tyrosine kinase p72^{syk} (Syk) *in vitro*; once it is extensively Tyr-phosphorylated by Syk or tyrosine kinases with similar specificity, it loses the ability to form oligomers, suggesting a putative anti-neurodegenerative role for these tyrosine kinases (Negro et al., FASEB J. 2002, 16(2), 210-212). Alpha-synuclein can be Ser-phosphorylated by protein kinases CKI and CKII (Okochi et al., J. Biol. Chem. 2000, 275(1), 390-397). The residue Ser-129 is also phosphorylated by G-protein-coupled receptor protein kinases (Pronin et al., J. Biol. Chem. 2000, 275(34), 26515-26522). Extensive and selective phosphorylation of alpha-synuclein at Ser-129 is evident in synucleinopathy lesions, including Lewy bodies (Fujiwara et al., Nat. Cell. Biol. 2002, 4(2), 160-164). Other post-translational modifications in the carboxyl-terminal, including glycosylation on Ser-129 (McLean et al., Neurosci. Lett. 2002, 323(3), 219-223) and nitration on Tyr-125, -133, and -136 (Takahashi et al., Brain Res. 2002, 938(1-2), 73-80), may affect aggregation of alpha-synuclein. Truncation of the carboxyl-terminal region by proteolysis has been reported to play a role in alpha-synuclein fibrillogenesis in various neurodegenerative diseases (Rochet et al., Biochemistry 2000, 39(35), 10619-10626). Full-length as well as partially truncated and insoluble aggregates of alpha-synuclein have been detected in highly purified Lewy bodies (Crowther et al., FEBS Lett. 1998, 436(3), 309-312).

Abnormal protein aggregation appears to be a common feature in aging brain and in several neurodegenerative diseases (Trojanowski et al., 1998, Cell Death Differ. 1998, 5(10), 832-837, Koo et al., Proc. Natl. Acad. Sci. 1999, 96(18), 9989-9990, Hu et al., Chin. Sci. Bull. 2001, 46, 1-3); although a clear role in the disease process remains to be defined. In *in vitro* models, alpha-synuclein (or some of its truncated forms) readily assembles into filaments resembling those isolated from the brain of patients with Lewy Body (LB) dementia and familial PD (Crowther et al., FEBS Lett. 1998, 436(3), 309-312). Alpha-synuclein and its mutated forms (A53T and A30P) have a random coil conformation and do not form significant secondary structures in aqueous solution at low concentrations; however, at higher concentrations they are prone to self-aggregate, producing

amyloid fibrils (Wood et al., J. Biol. Chem. 1999, 274(28), 19509-19512). Several differences in the aggregation behavior of the PD-linked mutants and the wild-type protein have been documented. Monomeric alpha-synuclein aggregates *in vitro* form stable fibrils via a metastable oligomeric (i.e., protofibril) state (Volles et al., Biochemistry 2002, 41(14), 4595-4602).

5

Parkinson's disease (PD) is the most common neurodegenerative motor disorder. PD is mainly an idiopathic disease, although in at least 5% of the PD patients the pathology is linked to mutations in one or several specific genes. Several point mutations have been described in the alpha-synuclein gene (A30P, E46K, H50Q, G51D, A53T) which cause familial PD with autosomal dominant inheritance. Furthermore, duplications and triplications of the alpha-synuclein gene have been described in patients that developed PD, underlining the role of alpha-synuclein in PD pathogenesis (Lesage et al., Hum. Mol. Genet., 2009, 18, R48-59). The pathogenesis of PD remains elusive. However, growing evidence suggests a role for the pathogenic folding of the alpha-synuclein protein that leads to the formation of amyloid-like fibrils. Indeed, the hallmarks of PD are the presence of intracellular alpha-synuclein aggregate structures called Lewy Bodies and neurites mainly in the nigral neurons, as well as the death of dopaminergic neurons in the substantia nigra and elsewhere. Alpha-synuclein is a natively unfolded presynaptic protein that can misfold and aggregate into larger oligomeric and fibrillar forms which are linked to the pathogenesis of PD. Recent studies have implicated small soluble oligomeric and protofibrillar forms of alpha-synuclein as the most neurotoxic species (Lashuel et al., J. Mol. Biol., 2002, 322, 1089-102). However, the precise role of alpha-synuclein in the neuronal cell toxicity remains to be clarified (review: Cookson, Annu. Rev. Biochem., 2005, 74, 29-52).

Besides Parkinson's disease, the accumulation of aggregated alpha-synuclein into Lewy bodies is a characteristic of all Lewy body diseases, including Parkinson's disease with dementia (PDD), and dementia with Lewy bodies (DLB) (Capouch et al., Neurol. Ther. 2018, 7, 249-263). In DLB, Lewy Bodies are diffusely distributed throughout the cortices of the brain and in addition to Lewy Bodies and neurites, more threads and dot-like structures (Lewy dots) were found to be immunopositive for alpha-synuclein phosphorylated at Ser-129 (Outeiro et al., Mol. Neurodegener. 2019, 14, 5). Alpha-synuclein aggregates are also found in multiple system atrophy (MSA). MSA is a rare and sporadic neurodegenerative disorder that manifests with rapidly progressive autonomic and motor dysfunction, as well as variable cognitive decline. Such disorders include Shy-Drager syndrome, striatonigral degeneration and olivopontocerebellar atrophy. The disease can be clinically subclassified in parkinsonian (MSA-P) or cerebellar (MSA-C) variant, depending on the predominant motor phenotype (Fanciulli et al., N. Engl. J. Med. 2015; 372, 249-63). It is characterized by the aggregation of alpha-synuclein in the cytoplasm of oligodendrocytes, forming glial cytoplasmic

inclusions (GCIs). GCIs, consisting primarily of fibrillary forms of alpha-synuclein, are the neuropathological hallmark of MSA and are found throughout the neocortex, hippocampus, brainstem, spinal cord and dorsal root ganglia (Galvin et al., Arch Neurol. 2001, 58,186-90). GCIs are considered a central player in the pathogenesis of MSA. A correlation between the GCI load and the degree of neuronal loss has been reported in both the striatonigral and the olivopontocerebellar regions (Stefanova et al., Neuropathol. Appl. Neurobiol. 2016, 42, 20-32). Furthermore, a causative link between GCIs and the induction of neuronal loss has been shown in transgenic mice overexpressing human alpha-synuclein in oligodendrocytes under various oligodendroglia-specific promoters. A key event in the pathophysiological cascade is considered to be the permissive templating ('prion-like' propagation) of misfolded alpha-synuclein.

The diagnosis of Parkinson's disease is largely clinical and depends on the presence of a specific set of symptoms and signs (the initial core feature being bradykinesia, rigidity, rest tremor and postural instability), the absence of atypical features, a slowly progressive course, and the response to a symptomatic drug therapy, mainly limited to a dopamine replacement therapy. The accurate diagnosis requires sophisticated clinical skills and is open to a degree of subjectivity and error, as several other degenerative and non-degenerative diseases can mimic PD symptoms (multiple system atrophy (MSA), progressive supranuclear palsy (PSP), Alzheimer's disease (AD), essential tremor, dystonic tremor), (Guideline No. 113: Diagnosis and pharmacological management of Parkinson's disease, January 2010. SIGN). The final confirmation of the pathology can only be made by post-mortem neuropathological analysis.

Computed tomography (CT) and conventional magnetic resonance imaging (MRI) brain scans of people with Parkinson's disease (PD) usually appear normal. These techniques are nevertheless useful to rule out other diseases that can be secondary causes of parkinsonism, such as basal ganglia tumors, vascular pathology and hydrocephalus. A specific technique of MRI, diffusion MRI, has been reported to be useful at discriminating between typical and atypical parkinsonism, although its exact diagnostic value is still under investigation. Dopaminergic function in the basal ganglia can be measured with different PET and SPECT radiotracers. Examples are ioflupane (^{123}I) (trade name DaTSCAN) and iometopane (Dopascan) for SPECT or fluorodeoxyglucose (^{18}F) (^{18}F -FDG) and dihydrotetrabenazine (^{11}C) (^{11}C -DTBZ) for PET. A pattern of reduced dopaminergic activity in the basal ganglia can aid in diagnosing PD, particularly in the symptomatic stage (Brooks, J. Nucl. Med., 2010, 51, 596–609; Redmond, Neuroscientist, 2002, 8, 457–88; Wood, Nat. Rev. Neurol., 2014, 10, 305).

Strategies are being developed to apply recent advances in understanding the potential causes of Parkinson's disease to the development of biochemical biomarkers (Schapira Curr. Opin. Neurol. 2013; 26(4):395-400). Such biomarkers that have been investigated in different body fluids (cerebrospinal fluid (CSF), plasma, saliva) include alpha-synuclein levels but also DJ-1, Tau and Abeta, as well as neurofilaments proteins, interleukins, osteopontin and hypocrontin (Schapira Curr. Opin. Neurol. 2013; 26(4):395-400), but so far none of these biomarkers alone or in combination can be used as a determinant diagnostic test. To our knowledge, no approved alpha-synuclein diagnostic agent is currently on the market or available for clinical trials despite a crucial need for Parkinson's disease research and drug development (Eberling et al., J Parkinsons Dis. 2013; 3(4):565-7).

The ability to image alpha-synuclein deposition in the brain would be a huge achievement for alpha-synucleopathies research, including Parkinson's disease research, diagnosis, and drug development. The accumulation of aggregated alpha-synuclein in the brain is considered a key pathological hallmark of Parkinson's disease (PD) and can start many years before the appearance of the symptoms. Therefore, alpha-synuclein is a priority target for drug development given not only its likely contribution to neurodegeneration but also because it can offer the possibility to treat the disease while still in the asymptomatic or prodromal stages. *In vivo* imaging of alpha-synuclein pathology could be useful as a biomarker to (i) detect the presence of the disease potentially in early stages, (ii) to evaluate disease progression and (iii) to be used as a pharmacodynamics tool for drug development. The development of an alpha-synuclein PET imaging agent is considered nowadays key for an accurate diagnosis of synucleinopathies as well as to support the clinical development of therapeutics targeting alpha-synuclein, starting from the optimal selection of the trial population (Eberling, Dave and Frasier, J. Parkinson's Disease, 3, 565-567 (2013)). Despite a huge effort to identify an alpha-synuclein PET ligand, so far only compounds that bind with reasonably high affinity to artificial alpha-synuclein fibrils were identified but none of them were confirmed in human clinical trials. They are not optimal for a number of reasons: low affinity or no binding was observed on pathological aggregates of alpha-synuclein present in the diseased brains, low or no selectivity for alpha-synuclein over other aggregated proteins was reported and inappropriate physicochemical properties for their use as brain-penetrant PET agents (Eberling et al., J Parkinsons Dis. 2013; 3(4):565-7; Neal et al., Mol. Imaging Biol. 2013, 15:585-595; Bagchi et al., PLoS One 2013, 8(2):e55031; Yu et al., Bioorganic and Medicinal chemistry 2012, 20:4625-4634; Zhang et al., Appl Sci (Basel) 2014, 4(1):66-78; Chu et al., J. Med. Chem., 2015, 58 (15):6002-17).

WO 2011/128455 refers to specific compounds which are suitable for treating disorders associated with amyloid proteins or amyloid-like proteins. US 2012/0302755 relates to certain imaging agents

for detecting neurological dysfunction. Further compounds for the diagnosis of neurodegenerative disorders on the olfactory epithelium are discussed in WO 2012/037928.

5 WO 2010/063701 refers to a certain *in vivo* imaging agent for use in a method to determine the presence of, or susceptibility to, Parkinson's disease, wherein the *in vivo* imaging agent comprises an alpha-synuclein binder labelled with an *in vivo* imaging moiety, and wherein the *in vivo* imaging agent binds to alpha-synuclein with a binding affinity.

10 US 2014/0142089 relates to a method for preventing or treating a degenerative brain disease, the method comprising administering to a subject in need thereof an effective amount of a pharmaceutical composition comprising a specific compound, a pharmaceutically acceptable salt, an isomer, a solvate, a hydrate, and a combination thereof.

15 WO 2009/155017 describes aryl or heteroaryl substituted azabenzoxazole derivatives, which are stated to be useful as tracers in positron emission tomography (PET) imaging to study amyloid deposits in the brain *in vivo* to allow diagnosis of Alzheimer's disease.

WO 2016/033445 refers to a specific compound for imaging huntingtin protein.

20 WO 2017/153601, WO 2019/234243 and WO2021/224489 refer to bicyclic compounds for diagnosing a-synuclein aggregates.

Therefore, there is a need for a new class of imaging compounds that bind with reasonably high affinity to alpha-synuclein.

25

SUMMARY OF THE INVENTION

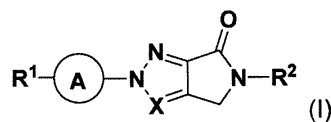
The present invention provides compounds that can be employed in diagnosing a disease, disorder or abnormality associated with alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites (such as Parkinson's disease), prognosing such a disease, disorder or abnormality, and monitoring the progression of such a disease, disorder or abnormality. In particular, the compounds should be suitable for determining a predisposition to such a disease, disorder or abnormality, monitoring the progression of the disease, disorder or abnormality, or predicting the responsiveness of a patient who is suffering from such a disease, disorder or abnormality to the treatment with a certain medicament. Furthermore, the compounds should be suitable for diagnosing a disease, disorder or abnormality associated with alpha-synuclein aggregates and / or detecting and optionally quantifying alpha-synuclein aggregates.

30

35

Various embodiments of the invention are described herein.

Within a certain aspect, provided herein is a compound of formula (I):



- 5 or a detectably labelled compound, stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

(A) is a 6-membered heteroaryl;

X is CH or N;

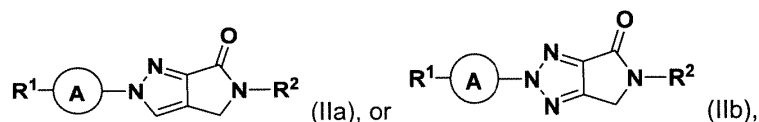
R¹ is a 4- to 7-membered heterocyclyl which is optionally substituted with at least one halo; or

- 10 R¹ is -NH₂; -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl); wherein the C₁-C₄alkyl is optionally substituted with at least one halo or wherein at least one H which is attached to N in -NH₂ or -NH(C₁-C₄alkyl) is replaced by halo; or

R¹ is haloC₁-C₄alkoxy; and

- 15 R² is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl.

In another aspect the invention is also directed to a compound having the following subformulae



- 20 or a detectably labelled compound, stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof.

- In one aspect, the present invention provides a diagnostic composition comprising a compound of formula (I), and optionally at least one pharmaceutically acceptable excipient, carrier, diluent and/or adjuvant.
- 25

- In one aspect, the present invention provides a compound of formula (I), or a diagnostic composition as defined herein, which can be use in the imaging of alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites. In another aspect the compound of formula (I), or the diagnostic composition can be for use in positron emission tomography imaging of alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites. In another aspect, the
- 30

compound of formula (I) or the diagnostic composition, as defined herein, can be for use for in vitro imaging, ex vivo imaging, or in vivo imaging, preferably the use is for in vivo imaging, more preferably the use is for brain imaging. In yet another aspect, the compound of formula (I) or the diagnostic composition, as defined herein, can be use in diagnostics.

5

In a further aspect, the present invention refers to a method of diagnosing a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites, in a subject, the method comprising the steps:

- 10 (a) Administering a compound of the formula (I), or a diagnostic composition which comprises a compound of formula (I), as defined herein, to the subject;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites; and
- (c) Detecting the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites.

15

In another aspect, the present invention refers to a method of positron emission tomography (PET) imaging of alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites, in a tissue of a subject, the method comprising the steps:

- 20 (a) Administering a compound of the formula (I), or a diagnostic composition which comprises a compound of formula (I), as defined herein to the subject;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites; and
- (c) Detecting the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites by collecting a positron emission tomography (PET) image of the tissue of the subject.

25

In a further aspect, the present invention is directed to a method for the detection and optionally quantification of alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites, in a tissue of a subject, the method comprising the steps:

- 30 (a) Bringing a sample or a specific body part or body area suspected to contain alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites, into contact with a compound of formula (I), or a diagnostic composition which comprises a compound of formula (I), as defined herein;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites;

35

- (c) Detecting the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites; and
- (d) Optionally quantifying the amount of the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites.

5

The present invention is also directed to a method of collecting data for the diagnosis of a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites, wherein the method comprises the steps:

- (a) Bringing a sample or a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with a compound of the formula (I), or a diagnostic composition which comprises a compound of formula (I), as defined herein;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites; and
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area.

The present invention also refers to a method of collecting data for determining a predisposition to a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites, the method comprising the steps:

- (a) Bringing a sample or a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with a compound of the formula (I), or a diagnostic composition which comprises a compound of formula (I), as defined herein;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites; and
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area.

In a further aspect the present invention also relates to a method of collecting data for prognosing a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites, wherein the method comprises the steps:

- 5 (a) Bringing a sample, a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with a compound of the formula (I), or a diagnostic composition which comprises a compound of formula (I), as defined herein;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to,
10 Lewy bodies and/or Lewy neurites;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence
15 or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area; and
- (e) Optionally repeating steps (a) to (c) and, if present, optional step (d) at least one time.

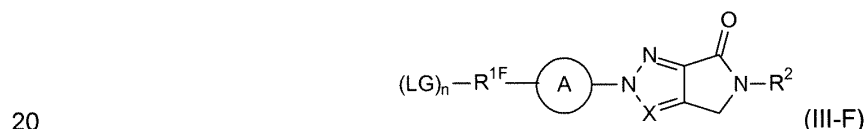
In another aspect the present invention is directed to a method of collecting data for monitoring the
20 progression of a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in a patient, the method comprising the steps:

- (a) Bringing a sample, a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with the
25 compound of the formula (I), or a diagnostic composition which comprises a compound of formula (I), as defined herein;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to,
30 Lewy bodies and/or Lewy neurites;
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area; and
- 35 (e) Optionally repeating steps (a) to (c) and, if present, optional step (d) at least one time.

In a further aspect, the present invention relates to a method of collecting data for predicting responsiveness of a patient suffering from a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites to a medicament, the method comprising the steps:

- 5 (a) Bringing a sample, a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with a compound of formula (I), or a diagnostic composition which comprises a compound of formula (I), as defined herein;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to,
- 10 Lewy bodies and/or Lewy neurites;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence
- 15 or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area; and
- (e) Optionally repeating steps (a) to (c) and, if present, optional step (d) at least one time.

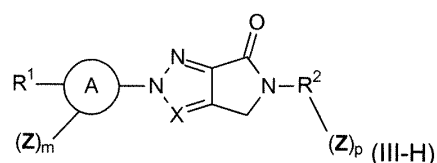
In another aspect the invention is further directed to a compound of formula (III-F):



or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

- (A) is a 6-membered heteroaryl;
- X is CH or N;
- 25 R^{1F} is a 4- to 7-membered heterocyclyl; or
- R^{1F} is $-N(C_1-C_4alkyl)_2$, $-NH(C_1-C_4alkyl)$; or
- R^{1F} is $C_1-C_4alkoxy$;
- R^2 is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from $-halo$, $-OH$, $-CN$, $-N(C_1-C_4alkyl)_2$, $-NH(C_1-C_4alkyl)$, $-N(haloC_1-$
- 30 $C_4alkyl)_2$, $-NH(haloC_1-C_4alkyl)$, $haloC_1-C_4alkyl$, $haloC_1-C_4alkoxy$, $-C_1-C_4alkoxy$, and $-C_1-C_4alkyl$;
- LG is a leaving group; and
- n is at least 1, preferably 1.

In another aspect the invention is further directed to compound of formula (III-H)



or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

- 5 **(A)** is a 6-membered heteroaryl;
X is CH or N;
R¹ is a 4- to 7-membered heterocyclyl which is optionally substituted with at least one halo; or
R¹ is -NH₂; -N(C₁-C₄alkyl)₂; -NH(C₁-C₄alkyl); or
R¹ is fluoroC₁-C₄alkoxy; and
 - 10 **R²** is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂; -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;
m is 0, 1, or 2;
p is 0, 1, or 2; and
 - 15 **Z** is bromo, chloro or iodo;
 wherein -N(C₁-C₄alkyl)₂; -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, or -C₁-C₄alkyl optionally comprise at least one **Z**,
 with the proviso that the compound of formula (III-H) comprises at least one **Z**.
 - 20 In another aspect, the invention is further directed to a method of preparing a compound of formula (I-F), by reacting a compound of formula (III-F) with a ¹⁸F-fluorinating agent.
- In another aspect, the invention is further directed to a method of preparing a compound of formula (I-H), by reacting the compound of formula (III-H) with a ³H radiolabeling agent.
- 25 In another aspect, the invention is further directed to the use of the compound of formula (I) as an *in vitro* analytical reference or an *in vitro* screening tool.
 - 30 In another aspect, the invention is further directed to a test kit for detection and/or diagnosis of a disease, disorder or abnormality associated with alpha-synuclein aggregates, wherein the test kit comprises at least one compound of formula (I) as defined herein.

The invention is further directed to a kit for preparing a radiopharmaceutical preparation, wherein the kit comprises a sealed vial containing at least one compound of formula (III-F) or (III-H).

DEFINITIONS

5 For the purpose of interpreting this specification, the following definitions will apply unless specified otherwise, and when appropriate, terms used in the singular will also include the plural and vice versa. It must also be noted that as used herein and in the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "the compound" includes reference to one or more compounds; and so forth.

10

The term "C₁-C₄alkyl" refers to a saturated straight or branched hydrocarbon chain consisting solely of carbon and hydrogen atoms, containing no unsaturation, having from one to four carbon atoms, and which is attached to the rest of the molecule by a single bond. Examples of suitable alkyl groups having 1 to 4 carbon atoms include, but are not limited to, methyl, ethyl, propyl, isopropyl, 15 1-methylethyl, n-butyl, t-butyl and isobutyl.

The term "C₁-C₄alkoxy" refers to a radical of the formula -OR_a where R_a is a C₁-C₄alkyl radical as generally defined above. Examples of C₁-C₄alkoxy include, but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, butoxy, and isobutoxy.

20

The term "halogenC₁-C₄alkyl" or "haloC₁-C₄alkyl" refers to a C₁-C₄alkyl radical, as defined above, substituted with one or more halo radicals, as defined below. Examples of "haloC₁-C₄alkyl" include, but are not limited to, trifluoromethyl, difluoromethyl, fluoromethyl, trichloromethyl, 2,2,2-trifluoroethyl, 1,3-dibromopropan-2-yl, 3-bromo-2-fluoropropyl and 1,4,4-trifluorobutan-2-yl.

25

The term "halogenC₁-C₄alkoxy" refers to a C₁-C₄alkoxy radical as defined above, substituted with one or more halo radicals as defined below. Examples of "haloC₁-C₄alkoxy" include, but are not limited to, trifluoromethoxy, difluoromethoxy, fluoromethoxy, 2,2,2-trifluoroethoxy, 3,3,3-trifluoropropoxy, 4,4,4-trifluorobutoxy, 2,2-difluorobutoxy, and 4-bromobutoxy.

30

The term "heterocyclyl" refers to a stable 4- to 6-membered non-aromatic monocyclic ring radical which comprises 1 or 2 heteroatoms which are, e.g., selected from N, O or S. The heterocyclyl group can be unsaturated or saturated. The heterocyclyl radical may be bonded via a carbon atom or a heteroatom. Examples include, but are not limited to, azetidiny, oxetanyl, pyrrolidiny, pyrrolidyl, 35 tetrahydrofuryl, tetrahydrothienyl, piperidyl, piperaziny, tetrahydropyranyl, or morpholinyl, preferably azetidiny, pyrrolidiny, or piperidyl.

The term "heteroaryl" refers to a 6-membered aromatic monocyclic ring, which comprises 1, 2, or 3 heteroatoms independently selected from N, O and S. The heteroaryl radical may be bonded via a carbon atom or heteroatom selected from N, O and S. Examples of heteroaryl include, but are not limited to, thiopyranyl, dioxanyl, pyranyl, pyrazinyl, pyridazinyl, pyrimidyl or pyridyl.

The term "Hal" or "halogen" or "Halo" refers to F, Cl, Br, and I. With respect to diagnostic and pharmaceutical applications, F (e.g., ^{19}F and ^{18}F) is particularly preferred.

10 The term "leaving group" (LG) as employed herein is any leaving group and means an atom or group of atoms that can be replaced by another atom or group of atoms. Examples are given e.g. in Synthesis (1982), p. 85-125, table 2, Carey and Sundberg, Organische Synthese, (1995), page 279-281, table 5.8; or Netscher, Recent Res. Dev. Org. Chem., 2003, 7, 71-83, schemes 1, 2, 10 and 15 and others). (Coenen, Fluorine-18 Labeling Methods: Features and Possibilities of Basic
15 Reactions, (2006), in: Schubiger P.A., Friebe M., Lehmann L., (eds), PET-Chemistry - The Driving Force in Molecular Imaging. Springer, Berlin Heidelberg, pp.15-50, explicitly: scheme 4 pp. 25, scheme 5 pp 28, table 4 pp 30, Figure 7 pp 33). Preferably, the "leaving group" (LG) is selected from halogen, C₁-C₄alkylsulfonate and C₆-C₁₀arylsulfonate, wherein C₆-C₁₀arylsulfonate can be optionally substituted with -CH₃ or -NO₂.

20 Unless specified otherwise, the term "compound of the invention" refers to a compound of formula (I), or of subformulae thereof (e.g. (IIa), (IIb), (IIa'), (IIb'), (IIa''), (IIb''), (I-F), (I-H)), or a detectably labelled compound, stereoisomer (including diastereomeric mixture and individual diastereomer, enantiomeric mixture and single enantiomer, mixtures of conformers and single conformer), racemic
25 mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof. It is understood that every reference to a compound of formula (I), as defined herein, also covers the subformulae thereof (e.g. (IIa), (IIb), (IIa'), (IIb'), (IIa''), (IIb''), (I-F), (I-H)). The compounds of the formulae (III-F) and (III-H) will be referred to as the precursors of the compounds of the present invention.

30 Compounds of the present invention and their precursors having one or more optically active carbons can exist as racemates and racemic mixtures, stereoisomers (including diastereomeric mixtures and individual diastereomers, enantiomeric mixtures and single enantiomers, mixtures of conformers and single conformers), tautomers, atropoisomers, and rotamers. All isomeric forms are included in the present invention.

35

"Pharmaceutically acceptable salts" are defined as derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as, but not limited to, hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as, but not limited to, acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pantoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like. The pharmaceutically acceptable salts of the compounds of the present invention and their precursors can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two. Organic solvents include, but are not limited to, nonaqueous media like ethers, ethyl acetate, ethanol, isopropanol, or acetonitrile. Lists of suitable salts can be found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Company, Easton, PA, 1990, p. 1445, the disclosure of which is hereby incorporated by reference.

"Pharmaceutically acceptable" is defined as those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication commensurate with a reasonable benefit/risk ratio.

"Solvates" can be formed from the compound of the present invention and any suitable pharmaceutically acceptable solvent. Examples include C1–4 alcohols (such as methanol or ethanol).

The patients or subjects in the present invention are typically animals, particularly mammals, more particularly humans.

Alpha-synuclein aggregates are multimeric beta-sheet rich assemblies of alpha-synuclein monomers that can form either soluble oligomers or soluble/insoluble protofibrils or mature fibrils which coalesce into intracellular deposits detected as a range of Lewy pathologies in Parkinson's disease and other

synucleinopathies. Alpha-synuclein aggregates that are composing Lewy pathologies can be detected as having the following morphologies: Lewy bodies, Lewy neurites, premature Lewy bodies or pale bodies, perikaryal deposits with diffuse, granular, punctate or pleomorphic patterns. Moreover, alpha-synuclein aggregates are the major component of intracellular fibrillary inclusions
5 detected in oligodendrocytes (also referred to as glial cytoplasmic inclusions) and in neuronal somata, axons and nuclei (referred to as neuronal cytoplasmic inclusions) that are the histological hallmarks of multiple system atrophy. Alpha-synuclein aggregates in Lewy pathologies often display substantial increase in post-translational modifications such as phosphorylation, ubiquitination, nitration, and truncation.

10 Lewy bodies are abnormal aggregates of protein that develop inside nerve cells in Parkinson's disease (PD), Lewy body dementia and other synucleinopathies. Lewy bodies appear as spherical masses that displace other cell components. Morphologically, Lewy bodies can be classified as being brainstem or cortical type. Classic brainstem Lewy bodies are eosinophilic cytoplasmic inclusions
15 consisting of a dense core surrounded by a halo of 5-10-nm-wide radiating fibrils, the primary structural component of which is alpha-synuclein; cortical Lewy bodies differ by lacking a halo. The presence of Lewy bodies is a hallmark of Parkinson's disease.

Lewy neurites are abnormal neuronal processes in diseased neurons, containing granular material,
20 abnormal alpha-synuclein (a-syn) filaments similar to those found in Lewy bodies, dot-like, varicose structures and axonal spheroids. Like Lewy bodies, Lewy neurites are a feature of α -synucleinopathies such as dementia with Lewy bodies, Parkinson's disease, and multiple system atrophy.

25 The terms "disease", "disorder" or "abnormality" are used interchangeably herein.

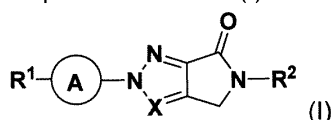
The compounds of formula (I) can bind to alpha-synuclein aggregates. The type of bonding with the compounds of formula (I) has not been elucidated and any type of bonding is covered by the present invention. The wording "compound bound to the alpha-synuclein aggregates" and the like are used
30 interchangeably herein and are not considered to be limited to any specific type of bonding.

The preferred definitions given in the "Definition"-section apply to all of the embodiments described below unless stated otherwise. Various embodiments of the invention are described herein, it will be recognized that features specified in each embodiment may be combined with other specified
35 features to provide further embodiments of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

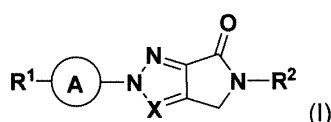
The compounds of the present invention and their precursors are described in the following. It is to be understood that all possible combinations of the following definitions are also envisaged.

- 5 The present invention relates to a compound of formula (I):



or a detectably labelled compound, stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

- (A) is a 6-membered heteroaryl;
- 10 X is CH or N;
- R¹ is a 4- to 7-membered heterocyclyl which is optionally substituted with at least one halo; or R¹ is -NH₂; -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl); wherein the C₁-C₄alkyl is optionally substituted with at least one halo or wherein at least one H which is attached to N in -NH₂ or -NH(C₁-C₄alkyl) is replaced by halo; or
- 15 R¹ is haloC₁-C₄alkoxy; and
- R² is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl.
- 20 In an embodiment, the present invention relates to a compound of formula (I):

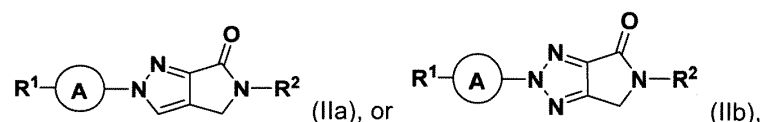


or a detectably labelled compound, stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

- (A) is a 6-membered heteroaryl;
- 25 X is CH or N;
- R¹ is a 4- to 6-membered heterocyclyl which is optionally substituted with at least one halo; or R¹ is -NH₂; -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl); wherein the C₁-C₄alkyl is optionally substituted with at least one halo, and

R² is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl.

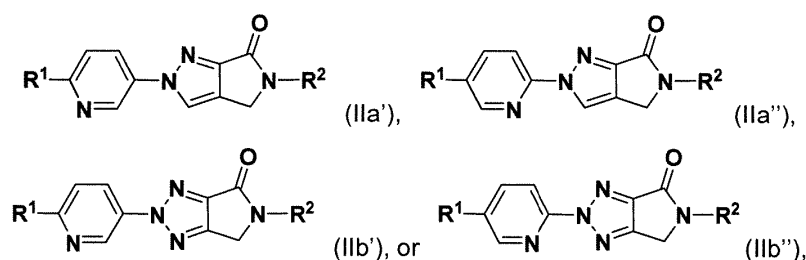
- 5 In another embodiment, the invention provides a compound of formula (I), having a formula (IIa) or (IIb):



or a detectably labelled compound, stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof.

10

Preferably, the invention provides a compound of formula (I), having a formula (IIa'), (IIa''), (IIb'), or (IIb''):



- 15 or a detectably labelled compound, stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof.

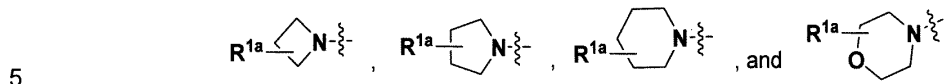
In one embodiment, **R**¹ is -NH₂; -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl); wherein the C₁-C₄alkyl is optionally substituted with at least one halo or wherein at least one H which is attached to N in -NH₂ or -NH(C₁-C₄alkyl) is replaced by halo. In a further embodiment, **R**¹ is -NH₂; -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl), wherein the C₁-C₄alkyl is optionally substituted with at least one halo (e.g., 1, 2 or 3 halo, preferably 1 or 2 halo). In one embodiment, **R**¹ is -NH₂; -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl). In a preferred embodiment, **R**¹ is -N(C₁-C₄alkyl)₂ or -NH(C₁-C₄alkyl).

- 25 In another preferred embodiment, **R**¹ is haloC₁-C₄alkoxy.

In another embodiment, **R**¹ is 4- to 7-membered heterocyclyl which is optionally substituted with at least one halo. In another embodiment, **R**¹ is 4- to 6-membered heterocyclyl which is optionally substituted with at least one halo. Preferably, the heterocyclyl is substituted with at least one halo,

more preferably with one or two halo, even more preferably with one halo. In one embodiment halo is F, and more preferably F is ^{19}F or ^{18}F , even more preferably ^{18}F .

In another embodiment, R^1 is a 4- to 6-membered heterocyclyl selected from the following:



wherein R^{1a} is H or halo, preferably halo.

In a preferred embodiment, R^1 is a 4- or 5-membered heterocyclyl selected from the following:



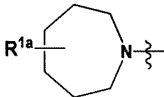
wherein R^{1a} is H or halo, preferably halo.

In a preferred embodiment, halo in R^1 and R^{1a} are F. Preferably, F is ^{19}F or ^{18}F , more preferably ^{18}F .

In yet another embodiment R^1 is a 4- or 5-membered heterocyclyl selected from the following:



—preferably, F is ^{19}F or ^{18}F , more preferably ^{18}F .

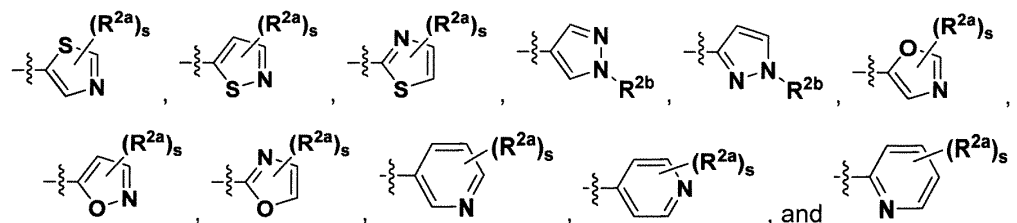
In yet another embodiment, R^1 is a 7-membered heterocyclyl selected from , wherein R^{1a} is H or halo, preferably halo. In a preferred embodiment, halo in R^{1a} is F. Preferably, F is ^{19}F or ^{18}F , more preferably ^{18}F .



In yet another embodiment R^1 is $-\text{O}-(\text{CH}_2)_m-\text{halo}$, wherein m is an integer from 1 to 4, preferably 1 or 2, more preferably 2.

25 In one embodiment, R^2 is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, $-\text{N}(\text{C}_1\text{-C}_4\text{alkyl})_2$, $-\text{NH}(\text{C}_1\text{-C}_4\text{alkyl})$, $-\text{N}(\text{haloC}_1\text{-C}_4\text{alkyl})_2$, $-\text{NH}(\text{haloC}_1\text{-C}_4\text{alkyl})$, $\text{haloC}_1\text{-C}_4\text{alkyl}$, $\text{haloC}_1\text{-C}_4\text{alkoxy}$, $-\text{C}_1\text{-C}_4\text{alkoxy}$, and $-\text{C}_1\text{-C}_4\text{alkyl}$.

In a preferred embodiment, R^2 is a 5-membered or 6-membered heteroaryl selected from the following:



5 wherein

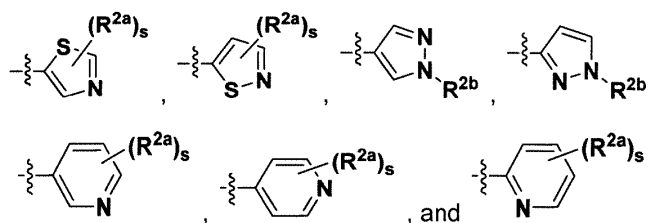
R^{2a} is independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;

R^{2b} is selected from -H, haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl; and

s is 0, 1 or 2, preferably 0 or 1.

10

Preferably, R^2 is a 5-membered or 6-membered heteroaryl selected from the following:



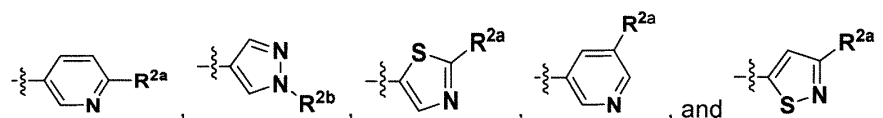
wherein

15 R^{2a} is independently selected from haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;

R^{2b} is selected from -H, and C₁-C₄alkyl; and

s is 0, 1 or 2 (preferably 0 or 1).

More preferably, R^2 is selected from the following:



20

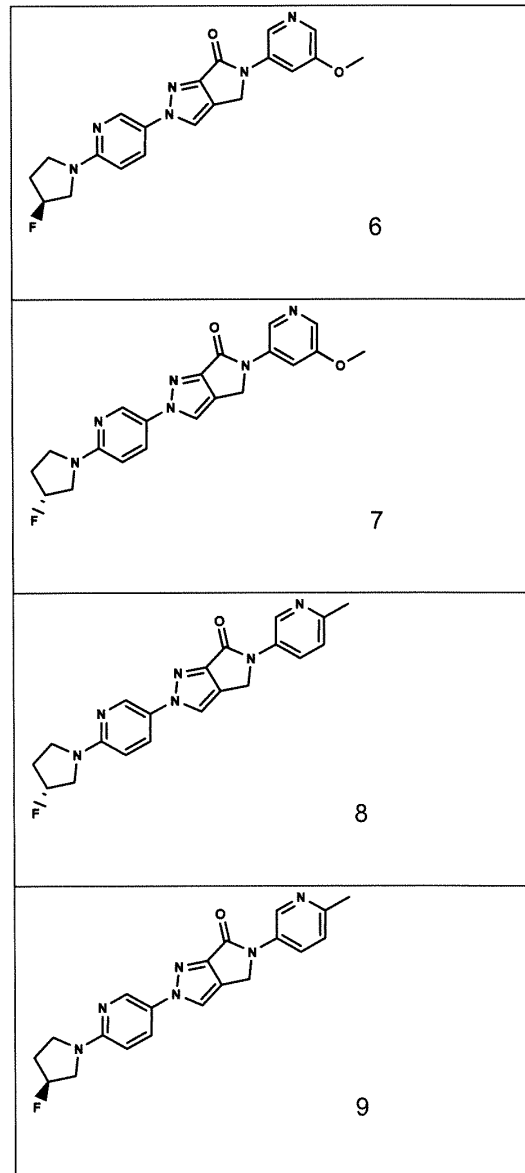
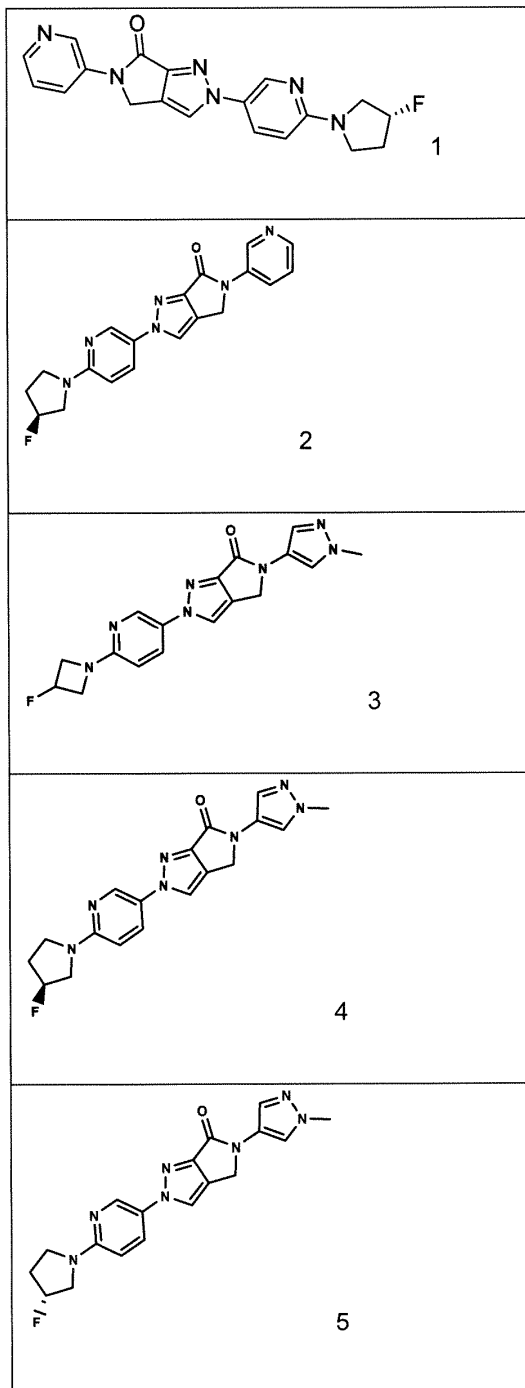
wherein

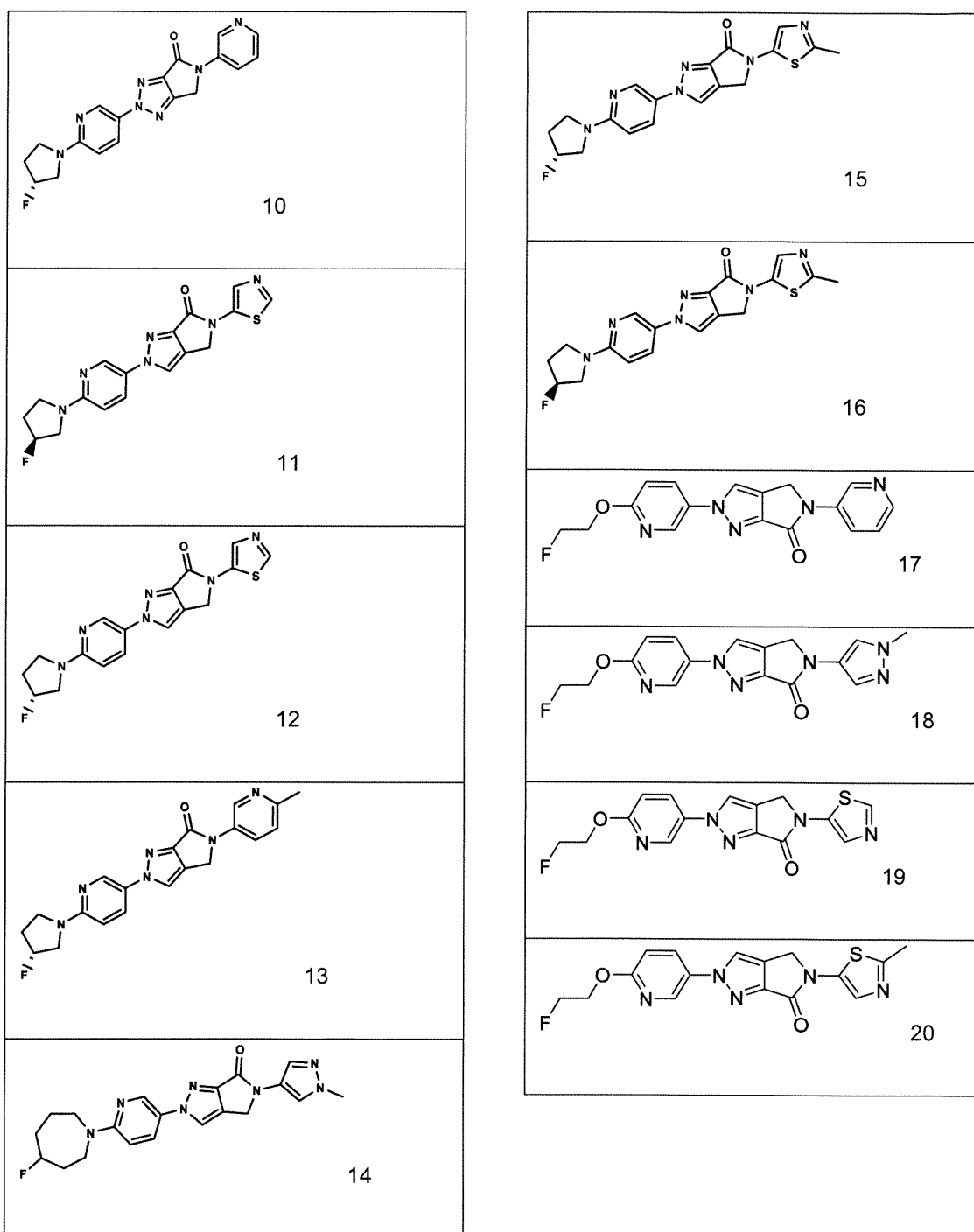
R^{2a} is selected -H, haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl; and

R^{2b} is selected from -H, and -C₁-C₄alkyl.

25 Preferably, R^{2a} is independently -OCH₃, -CH₃, or -H; and preferably, R^{2b} is -H or -CH₃.

In one embodiment, the present invention provides a compound of formula (I), as defined herein above, wherein the compound is selected from:

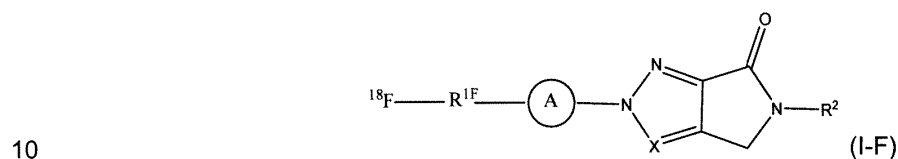




or a detectably labelled compound, stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof.

In one embodiment the present invention provides a compound of formula (I), wherein the compound of formula (I) is a detectably labelled compound. The detectable label can be a radioisotope. In one embodiment, the compound of formula (I) comprises at least one radioisotope. Preferably, the detectable label is a radioisotope selected from ^{18}F , ^2H and ^3H . Most preferably, the radioisotope is selected from ^{18}F and ^3H .

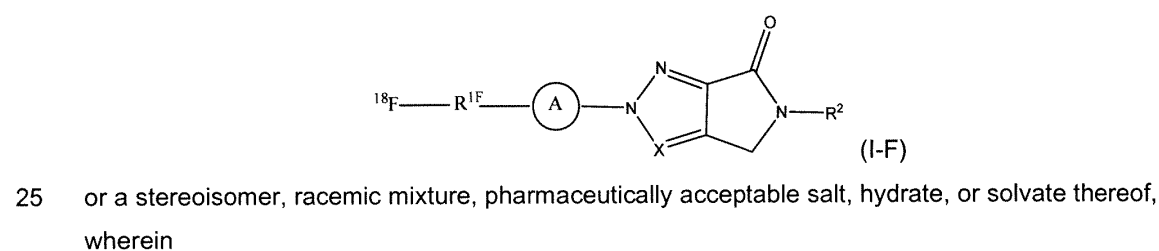
In one embodiment the present invention provides a compound of formula (I), wherein the compound is a detectably labelled compound of formula (I-F):



or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

- (A) is a 6-membered heteroaryl;
 X is CH or N;
 15 $\text{R}^{1\text{F}}$ is a 4- to 6-membered heterocyclyl or a 7-membered heterocyclyl; or
 $\text{R}^{1\text{F}}$ is $-\text{N}(\text{C}_1\text{-C}_4\text{alkyl})_2$; or $-\text{NH}(\text{C}_1\text{-C}_4\text{alkyl})$; or
 $\text{R}^{1\text{F}}$ is $\text{C}_1\text{-C}_4\text{alkoxy}$ and
 R^2 is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, $-\text{N}(\text{C}_1\text{-C}_4\text{alkyl})_2$, $-\text{NH}(\text{C}_1\text{-C}_4\text{alkyl})$, $-\text{N}(\text{haloC}_1\text{-C}_4\text{alkyl})_2$, $-\text{NH}(\text{haloC}_1\text{-C}_4\text{alkyl})$, $\text{haloC}_1\text{-C}_4\text{alkyl}$, $\text{haloC}_1\text{-C}_4\text{alkoxy}$, $-\text{C}_1\text{-C}_4\text{alkoxy}$, and $-\text{C}_1\text{-C}_4\text{alkyl}$.
- 20

In one embodiment the compound of formula (I) is a detectably labelled compound of formula (I-F):



- (A) is a 6-membered heteroaryl;
 X is CH or N;
 $\text{R}^{1\text{F}}$ is a 4- to 6-membered heterocyclyl; or

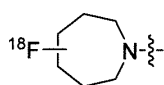
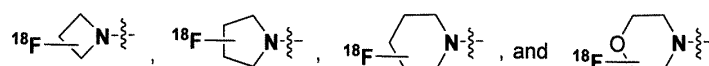
R^{1F} is $-N(C_1-C_4alkyl)_2$; or $-NH(C_1-C_4alkyl)$; or R^{1F} is $C_1-C_4alkoxy$; and

R^2 is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from $-halo$, $-OH$, $-CN$, $-N(C_1-C_4alkyl)_2$, $-NH(C_1-C_4alkyl)$, $-N(haloC_1-C_4alkyl)_2$, $-NH(haloC_1-C_4alkyl)$, $haloC_1-C_4alkyl$, $haloC_1-C_4alkoxy$, $-C_1-C_4alkoxy$, and $-C_1-C_4alkyl$.

5

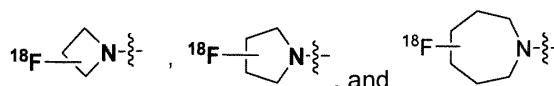
In a preferred embodiment, R^{1F} is a 4- to 6-membered heterocyclyl or a 7-membered heterocyclyl.

Preferably, $-R^{1F-18F}$ is selected from the following:



10 $-R^{1F-18F}$ can also be selected from

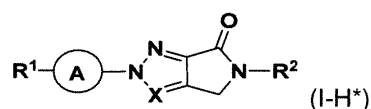
More preferably, R^{1F} is 4- or 5-membered or 7-membered heterocyclyl. For example, $-R^{1F-18F}$ is selected from the following:



In another preferred embodiment, $-R^{1F-18F}$ is $-O-(CH_2)_m-^{18F}$

15 The detectably labelled compound of formula (I-F) comprises at least one 18F . Preferably, the detectably labelled compound of formula (I-F) comprises one 18F .

In one embodiment the present invention provides a compound of formula (I), wherein the compound is a detectably labelled compound of formula (I-H*)



20

or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof; wherein

(A) is a 6-membered heteroaryl;

X is CH or N;

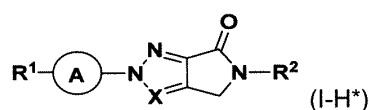
25 R^1 is a 4- to 7-membered heterocyclyl which is optionally substituted with at least one halo; or

R^1 is $-NH_2$; $-N(C_1-C_4alkyl)_2$; $-NH(C_1-C_4alkyl)$; or

R^1 is $haloC_1-C_4alkoxy$ and

- R²** is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl; and with the proviso that the compound of formula (I-H*) comprises at least one ²H (deuterium "D") or ³H (Tritium "T"), preferably **T** (preferably 1, 2, or 3 **D** or **T**).

In one embodiment the present invention provides a compound of formula (I), wherein the compound is a detectably labelled compound of formula (I-H*)



- or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof; wherein

(A) is a 6-membered heteroaryl;

X is CH or N;

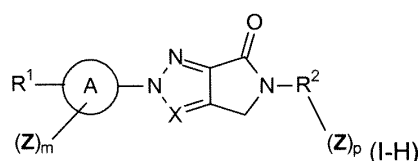
R¹ is a 4- to 6-membered heterocyclyl which is optionally substituted with at least one halo; or

- R¹** is -NH₂; -N(C₁-C₄alkyl)₂; -NH(C₁-C₄alkyl); and

R² is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl; and with the proviso that the compound of formula (I-H*) comprises at least one ²H (deuterium "D") or ³H

- (Tritium "T"), preferably **T** (preferably 1, 2, or 3 **D** or **T**).

In a preferred embodiment, the compound is a detectably labelled compound of formula (I-H)



- or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof; wherein

(A) is a 6-membered heteroaryl;

X is CH or N;

R¹ is a 4- to 6-membered or a 7-membered heterocyclyl which is optionally substituted with at least one halo; or

- R¹** is -NH₂; -N(C₁-C₄alkyl)₂; -NH(C₁-C₄alkyl); or

R¹ is haloC₁-C₄alkoxy and

R² is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;

5 **Z** is **T** or CT₃;

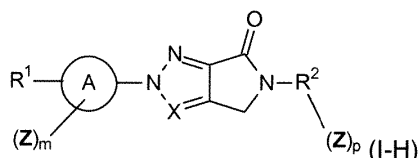
m is 0, 1, 2 or 3;

p is 0, 1, 2 or 3;

with the proviso that the compound of formula (I-H) comprises at least one **T** or CT₃, wherein **T** is ³H (Tritium).

10

In a preferred embodiment, the compound is a detectably labelled compound of formula (I-H)



or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof; wherein

15 **(A)** is a 6-membered heteroaryl;

X is CH or N;

R¹ is a 4- to 6-membered heterocyclyl which is optionally substituted with at least one halo; or

R¹ is -NH₂; -N(C₁-C₄alkyl)₂; -NH(C₁-C₄alkyl); and

R² is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents

20 independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;

Z is **T** or CT₃;

m is 0, 1, 2 or 3;

p is 0, 1, 2 or 3;

25 with the proviso that the compound of formula (I-H) comprises at least one **T** or CT₃, wherein **T** is ³H (Tritium).

It is understood that the tritium can present at any available position at which a hydrogen is present.

For instance, in the group **R²** tritium can be present either directly bound to the 5-membered or

30 6-membered heteroaryl (such as in the form of **T**) or can be present in the -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy,

and -C₁-C₄alkyl (such as in the form of CT₃). In the 4- to 6-membered heterocyclyl of R¹ tritium can be, e.g., directly bound to the 4- to 6-membered heterocyclyl.

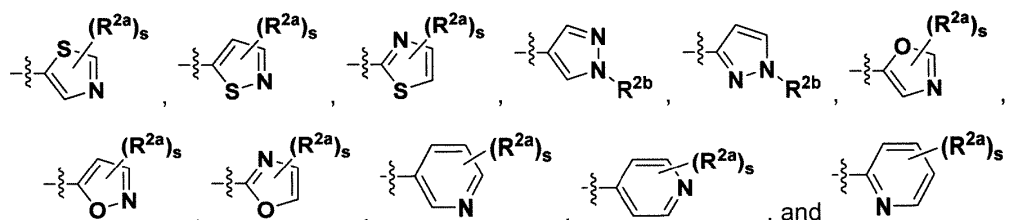
In one embodiment, $\textcircled{A}$ is a 6-membered heteroaryl and m is 1, 2 or 3, e.g., 1.

5

In one embodiment, R² is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl; and , and p is 1, 2 or 3, e.g., 1.

10

In a preferred embodiment, R² is a 5-membered or 6-membered heteroaryl selected from the following:



15

wherein R^{2a} is independently selected from -H, -T, -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;

R^{2b} is selected from -H, -T, haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl (e.g., CT₃);

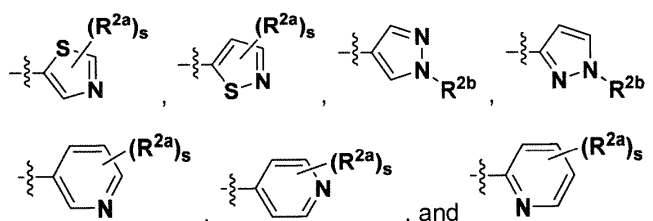
20

s is 0, 1 or 2 (preferably 0 or 1); and

wherein -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkyl, or -C₁-C₄alkoxy optionally comprise one or more T.

25

Preferably, R² is a 5-membered or 6-membered heteroaryl selected from the following:



wherein

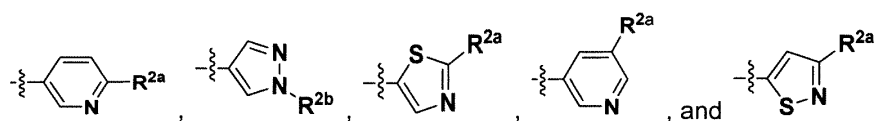
R^{2a} is independently selected from -H, -T, haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;

R^{2b} is selected from -H, -T, -C₁-C₄alkyl (e.g., CT₃) (preferably R^{2b} is selected from T or CT₃);

s is 0, 1 or 2 (preferably 1); and

- 5 wherein haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkyl, or -C₁-C₄alkoxy optionally comprise one or more T.

More preferably, R^2 is selected from the following:



- 10 wherein

R^{2a} is selected -T, -H, haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl (e.g., CT₃); and

R^{2b} is selected from -H, -T, and -C₁-C₄alkyl (e.g., CT₃) (preferably R^{2b} is selected from T or CT₃), wherein haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkyl, or -C₁-C₄alkoxy optionally comprise one or more T.

- 15

Preferably, R^{2a} is -T, -OCH₃, -CH₃, or -H; and R^{2b} is selected from -H or -CT₃.

- 20 In a preferred embodiment, the detectably labelled compound of formula (I-H*) or (I-H) comprises one, two or three T. Preferably, the detectably labelled compound of formula (I-H*) or (I-H) comprises one T. More preferably, the detectably labelled compound of formula (I-H*) or (I-H) comprises two T. Even more preferably, the detectably labelled compound of formula (I-H*) or (I-H) comprises three T such as -CT₃.

- 25 In another embodiment, the invention provides a detectably labelled compound of formula (I-H*) or (I-H), wherein ³H Tritium ("T") can be replaced by ²H Deuterium ("D"). The deuterated compound can be prepared by reacting a compound of formula (III-H) with a ²H radiolabelling agent.

- 30 The compounds of the present invention and their precursors can be detectably labelled. The type of the label is not specifically limited and will depend on the detection method chosen. Examples of possible labels include isotopes such as radionuclides, positron emitters, and gamma emitters, preferably the detectable label is a radioisotope. With respect to the detectably labelled compounds of the present invention and their precursors which include a radioisotope, a positron emitter, or a gamma emitter, it is to be understood that the radioisotope, positron emitter, or gamma emitter is to

be present in an amount which is not identical to the natural amount of the respective radioisotope, positron emitter, or gamma emitter. Furthermore, the employed amount should allow detection thereof by the chosen detection method. Examples of suitable isotopes such as radionuclides, positron emitters and gamma emitters include ^2H , ^3H , ^{18}F , ^{11}C , ^{13}N , and ^{15}O , preferably ^2H , ^3H , ^{11}C ,
5 ^{13}N , ^{15}O , and ^{18}F , more preferably ^2H , ^3H and ^{18}F , even more preferably ^3H and ^{18}F .

^{18}F -labelled compounds are particularly suitable for imaging applications such as PET. The corresponding compounds which include fluorine having a natural ^{19}F isotope are also of particular interest as they can be used as analytical standards and references during manufacturing, quality
10 control, release, and clinical use of their ^{18}F -analogs.

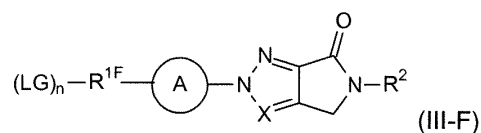
Further, substitution with isotopes such as deuterium, i.e., ^2H , may afford certain diagnostic and therapeutic advantages resulting from greater metabolic stability by reducing for example defluorination, increased *in vivo* half-life or reduced dosage requirements, while keeping or improving
15 the original compound efficacy.

Isotopic variations of the compounds of the invention and their precursors can generally be prepared by conventional procedures such as by the illustrative methods or by the preparations described in the Examples and Preparative Examples hereafter using appropriate isotopic variations of suitable
20 reagents, which are commercially available or prepared by known synthetic techniques.

Radionuclides, positron emitters and gamma emitters can be included into the compounds of the present invention and their precursors by methods which are usual in the field of organic synthesis. Typically, they will be introduced by using a correspondingly labelled starting material when the
25 desired compound of the present invention and its precursor is prepared. Illustrative methods of introducing detectable labels are described, for instance, in US 2012/0302755.

The position at which the detectable label is to be attached to the compounds of the present invention and their precursors is not particularly limited. The radionuclides, positron emitters and gamma
30 emitters, for example, can be attached at any position where the corresponding non-emitting atom can also be attached. For instance, ^{18}F can be attached at any position which is suitable for attaching F. The same applies to the other radionuclides, positron emitters and gamma emitters. Due to the ease of synthesis, preferably R^1 is substituted with ^{18}F . ^3H can be attached at any available position at which H is present. If ^2H is employed as a detectable label it can be attached at any available
35 position at which H is present.

In another embodiment, the present invention relates further to a compound of formula (III-F) that is a precursor of the compound of formula (I-F)



or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

(A) is a 6-membered heteroaryl;

X is CH or N;

R¹ᶠ is a 4- to 6-membered or a 7-membered heterocyclyl; or

R¹ᶠ is -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl); or

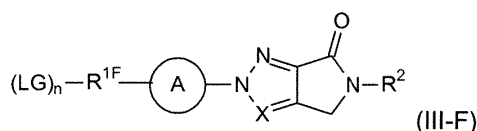
10 **R¹ᶠ** is C₁-C₄alkoxy and

R² is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;

LG is a leaving group; and

15 **N** is at least 1.

In another embodiment, the present invention relates further to a compound of formula (III-F) that is a precursor of the compound of formula (I-F)



20 or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

(A) is a 6-membered heteroaryl;

X is CH or N;

R¹ᶠ is a 4- to 6-membered heterocyclyl; or

25 **R¹ᶠ** is -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl); and

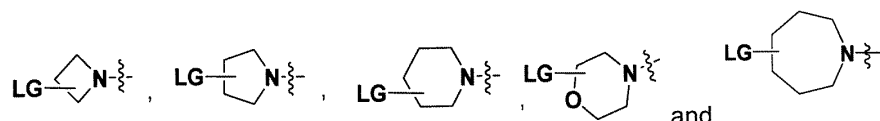
R² is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;

LG is a leaving group; and

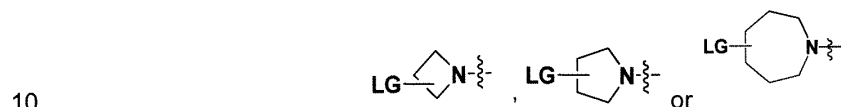
30 **n** is at least 1.

Preferably, R^{1F} is $-N(C_1-C_4\text{alkyl})_2$, or $-NH(C_1-C_4\text{alkyl})$. More preferably, R^{1F} is $-N(C_1-C_4\text{alkyl})_2$, or $-NH(C_1-C_4\text{alkyl})$ or $C_1-C_4\text{alkoxy}$.

- 5 In another preferred embodiment, R^{1F} is a 4- to 6-membered or a 7-membered heterocyclyl. Preferably, $(LG)_n-R^{1F}$ is selected from the following:



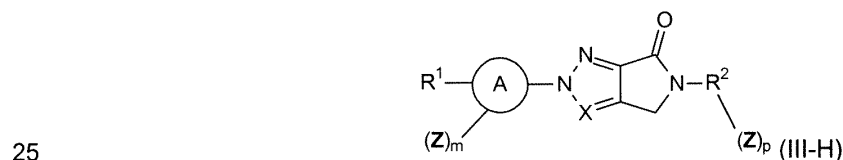
More preferably, $(LG)_n-R^{1F}$ is selected from the following:



In yet another embodiment R^1 is $-O-(CH_2)_m-LG$, wherein m is an integer from 1 to 4, preferably 1 or 2, more preferably 2.

- 15 Preferably, the Leaving Group (LG) is halogen, C_1-C_4 alkylsulfonate, C_1-C_4 alkyl ammonium, or C_6-C_{10} arylsulfonate, wherein the C_6-C_{10} arylsulfonate can be optionally substituted with $-CH_3$ or $-NO_2$. More preferably, the Leaving Group (LG) is bromo, chloro, iodo, C_6-C_4 alkylsulfonate, or C_6-C_{10} arylsulfonate, wherein the C_6-C_{10} arylsulfonate can be optionally substituted with $-CH_3$ or $-NO_2$. Even more preferably, the Leaving Group (LG) is mesylate, tosylate or nosylate. Even more preferably, the Leaving Group (LG) is mesylate, or nosylate. More preferably the Leaving Group (LG) is mesylate.
- 20

In another embodiment, the present invention relates further to a compound of formula (III-H), a precursor of the compound of formula (I-H):



or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

(A) is a 6-membered heteroaryl;

X is CH or N;

R¹ is a 4- to 6-membered or a 7-membered heterocyclyl which is optionally substituted with at least one halo; or

R¹ is -NH₂; -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl); or

5 **R¹** is haloC₁-C₄alkoxy;

R² is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂; -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;

m is 0, 1, or 2;

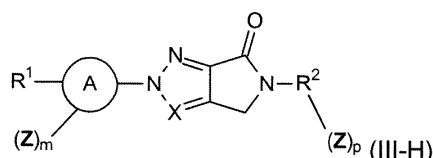
10 **p** is 0, 1, or 2; and

Z is bromo, chloro or iodo;

with the proviso that the compound of formula (III-H) comprises at least one **Z** (e.g., 1, 2 or 3 **Z**, preferably 1 or 2 **Z**).

In another embodiment, the present invention relates further to a compound of formula (III-H), a

15 precursor of the compound of formula (I-H):



or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

A is a 6-membered heteroaryl;

20 **X** is CH or N;

R¹ is a 4- to 6-membered heterocyclyl which is optionally substituted with at least one halo; or

R¹ is -NH₂; -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl) ;

R² is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂; -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;

25 **m** is 0, 1, or 2;

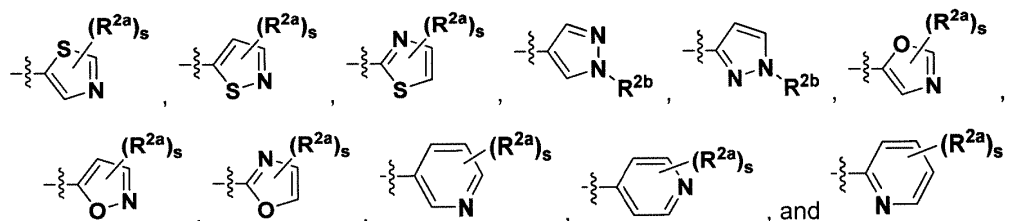
p is 0, 1, or 2; and

Z is bromo, chloro or iodo;

with the proviso that the compound of formula (III-H) comprises at least one **Z** (e.g., 1, 2 or 3 **Z**,

30 preferably 1 or 2 **Z**).

In a preferred embodiment, (Z)_p-**R²** is selected from the following:



wherein

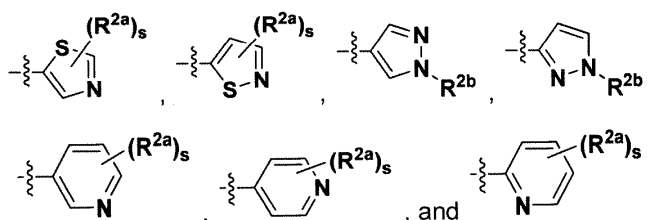
R^{2a} is independently selected from -H, -Z, -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;

R^{2b} is selected from -H, -Z, haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;

s is 0, 1 or 2 (preferably 0 or 1); and

wherein -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkyl, or -C₁-C₄alkoxy optionally comprise one or more Z.

Preferably, (Z)_p-R² is selected from the following:



wherein

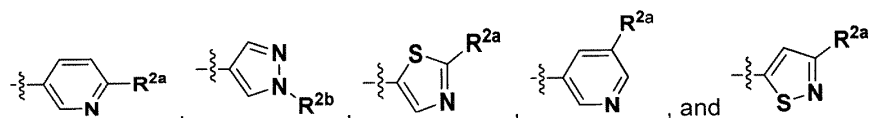
R^{2a} is independently selected from -H, -Z, haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkyl, and -C₁-C₄alkoxy;

R^{2b} is selected from -H, -Z, and -C₁-C₄alkyl, preferably -Z;

s is 0, 1 or 2 (preferably 0 or 1); and

wherein haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkyl, or -C₁-C₄alkoxy optionally comprise one or more Z.

More preferably, (Z)_p-R² is selected from the following:



wherein

R^{2a} is selected from -H, -Z, haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkyl, and -C₁-C₄alkoxy,

R^{2b} is selected from -H, -Z, and -C₁-C₄alkyl;

s is 0, 1 or 2 (preferably 0 or 1); and

wherein haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkyl, or -C₁-C₄alkoxy optionally comprise one or more Z.

5

In a preferred embodiment, the detectably labelled compound of formula (III-H) comprises one, two or three Z. In a preferred embodiment, the detectably labelled compound of formula (III-H) comprises one Z. In another preferred embodiment, the detectably labelled compound of formula (III-H) comprises two Z. In one embodiment, Z is selected from bromo, chloro and iodo. In a preferred

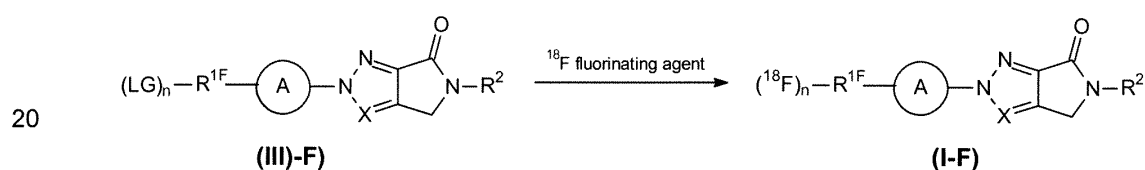
10

METHODS OF SYNTHESIS OF DETECTABLY LABELLED COMPOUNDS

The present invention relates further to a method for preparing a compound of formula (I), or of subformulae thereof (e.g. (IIa), (IIb), (IIa'), (IIb'), (IIa''), (IIb''), (I-F), (I-H*), (I-H)), and in particular a

15

In one embodiment, the present invention relates to a method for preparing a compound of formula (I-F), by reacting a compound of formula (III-F) with a ¹⁸F-fluorinating agent.

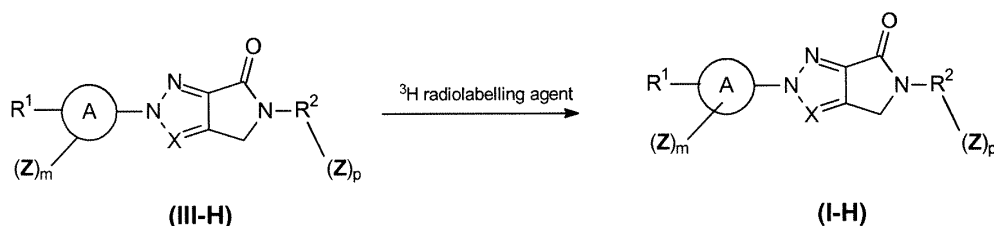


wherein $\textcircled{A}$, R^{1F} , R^2 , X, n and LG are as defined herein above.

Suitable solvents for the ¹⁸F-fluorination comprise DMF, DMSO, acetonitrile, DMA, or mixtures thereof, preferably acetonitrile or DMSO. Suitable agents for the ¹⁸F-fluorination are selected from K¹⁸F, Rb¹⁸F, Cs¹⁸F, Na¹⁸F, tetra(C₁₋₆alkyl)ammonium salt of ¹⁸F, Kryptofix[222]¹⁸F and tetrabutylammonium [¹⁸F]fluoride.

In one embodiment, the present invention relates to a method of preparing a compound of formula (I-H), by reacting a compound of formula (III-H) with a ³H radiolabeling agent.

30



wherein $\textcircled{\text{A}}$, R^1 , R^2 , X , Z , p , and m are as defined herein above.

- 5 The ^3H radiolabeling agent can be tritium gas. The method can be conducted in the presence of a catalyst such as palladium on carbon (Pd/C), a solvent such as dimethylformamide (DMF) and a base such as N,N-diisopropylethylamine (DIEA).

- Alternatively, in another embodiment, the present invention relates to a method for preparing a compound of formula (I-H), by radiolabeling a compound of formula (III-H) with a CT_3 radiolabeling agent, wherein T is ^3H . The CT_3 radiolabeling agent can be ICT_3 (derivative of iodomethane with ^3H). The method can be conducted in the presence of a solvent such as dimethylformamide (DMF) and a base such as cesium carbonate or sodium hydride.

15 RADIOPHARMACEUTICAL PREPARATIONS

- The compounds of the present invention can also be employed in kits for the preparation of radiopharmaceutical preparations. Due to the radioactive decay, the radiopharmaceuticals are usually prepared immediately before use. The kit typically comprises a precursor of the compound of the present invention, and an agent which reacts with the precursor to introduce a radioactive label
- 20 into the compound of the present invention. The precursor of the compound of the present invention, can, for example, be a compound having the formula (III-F), or (III-H). The agent can be an agent which introduces a radioactive label such as ^{18}F , or ^3H .

- In one embodiment, the kit of part is a test kit for the detection and/or diagnosis of a disease, disorder or abnormality associated with alpha-synuclein aggregates, wherein the test kit comprises at least one precursor of the compound of the present invention (e.g. a compound having the formula (III-F), or (III-H)).

- In another embodiment, the kit of part is a kit for preparing a radiopharmaceutical preparation, wherein the kit comprises a sealed vial containing at least one precursor of the compound of the present invention (e.g. a compound having the formula (III-F), or (III-H)).

DIAGNOSTIC COMPOSITIONS

The compounds of the present invention are particularly suitable for imaging of alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites. With respect to alpha-synuclein protein, the compounds are particularly suitable for binding to various types of alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites. The imaging can be conducted in mammals, preferably in humans. The imaging is preferably *in vitro* imaging, *ex vivo* imaging, or *in vivo* imaging. More preferably the imaging is *in vivo* imaging: Even more preferably, the imaging is preferably brain imaging. The imaging can also be eye/retinal imaging. The compounds of the present invention are particularly suitable for use in diagnostics.

The diagnostics can be conducted for mammals, preferably for humans. The tissue of interest on which the diagnostics is conducted can be brain, tissue of the central nervous system (CNS), tissue of the eye (such as retinal tissue), tissue of peripheral organs such as the gut or other tissues, or body fluids such as cerebrospinal fluid (CSF) or blood. The tissue is preferably brain tissue.

In one embodiment, the present invention provides a diagnostic composition comprising a compound of the invention, and optionally at least one pharmaceutically acceptable excipient, carrier, diluent and/or adjuvant.

Due to their design and to the binding characteristics, the compounds of the present invention are suitable for use in the diagnosis of diseases, disorders and abnormalities associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites. In another embodiment, the diagnostic composition which comprises a compound of the present invention is also suitable for use in the diagnosis of diseases, disorders and abnormalities associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites.

In yet another embodiment, the compound of the present invention, or the diagnostic composition comprising a compound of the invention, is suitable for use in imaging, such as *in vitro* imaging, *ex vivo* imaging, or *in vivo* imaging, preferably the use is for *in vivo* imaging, more preferably the use is for brain imaging. In particular, the use is in humans.

In another embodiment, the compounds of the present invention, or the diagnostic composition are particularly suitable for use in positron emission tomography imaging of alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites.

Diseases involving alpha-synuclein aggregates are generally listed as synucleinopathies (or α -synucleinopathies). The compounds of the present invention are suitable for use in the diagnosis of diseases, disorders or abnormalities including, but not limited to, Parkinson's disease (sporadic, familial with alpha-synuclein mutations, familial with mutations other than alpha-synuclein, pure autonomic failure and Lewy body dysphagia), SNCA duplication carrier, dementia with Lewy bodies ("pure" Lewy body dementia), Alzheimer's disease, sporadic Alzheimer's disease, familial Alzheimer's disease with APP mutations, familial Alzheimer's disease with PS-1, PS-2 or other mutations, familial British dementia, Lewy body variant of Alzheimer's disease and normal aging in Down syndrome). Synucleinopathies with neuronal and glial aggregates of alpha synuclein include multiple system atrophy (MSA) (Shy-Drager syndrome, striatonigral degeneration and olivopontocerebellar atrophy). Other diseases that may have alpha-synuclein-immunoreactive lesions include traumatic brain injury, chronic traumatic encephalopathy, tauopathies (Pick's disease, frontotemporal dementia, progressive supranuclear palsy, corticobasal degeneration and Niemann-Pick type C1 disease), motor neuron disease, amyotrophic lateral sclerosis (sporadic, familial and ALS-dementia complex of Guam), neuroaxonal dystrophy, neurodegeneration with brain iron accumulation type 1 (Hallervorden-Spatz syndrome), prion diseases, ataxia telangiectatica, Meige's syndrome, subacute sclerosing panencephalitis, Gaucher disease as well as other lysosomal storage disorders (including Kufor-Rakeb syndrome and Sanfilippo syndrome) and rapid eye movement (REM) sleep behavior disorder (Jellinger, *Mov Disord* 2003, 18 Suppl. 6, S2-12; Galvin et al. *JAMA Neurology* 2001, 58 (2), 186-190; Kovari et al., *Acta Neuropathol.* 2007, 114(3), 295-8; Saito et al., *J Neuropathol Exp Neurol.* 2004, 63(4), 323-328; McKee et al., *Brain*, 2013, 136(Pt 1), 43-64; Puschmann et al., *Parkinsonism Relat Disord* 2012, 18S1, S24-S27; Usenovic et al., *J Neurosci.* 2012, 32(12), 4240-4246; Winder-Rhodes et al., *Mov Disord.* 2012, 27(2), 312-315; Ferman et al., *J Int Neuropsychol Soc.* 2002, 8(7), 907-914). Preferably, the compounds of the present invention are suitable for use in the diagnosis of Parkinson's disease, multiple system atrophy, dementia with Lewy bodies, Parkinson's disease dementia, SNCA duplication carrier, or Alzheimer's disease, more preferably Parkinson's disease (PD).

In the methods of diagnosing a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites (e.g. Parkinson's disease), or a predisposition therefor in a subject, the method comprises the steps of:

- (a) administering to the subject a diagnostically effective amount of a compound of the present invention, or a diagnostic composition which comprises a compound of the present invention;
- (b) allowing the compound of the present invention to distribute into the tissue of interest (such as brain tissue, tissue of the central nervous system (CNS), tissue of the eye, tissue of peripheral organs or other tissues), or body fluid (such as cerebrospinal fluid (CSF) or blood); and

(c) imaging the tissue of interest or body fluid.

If the amount of the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites is increased compared to a normal control level the subject is suffering from or is at risk of developing a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites.

The compounds of the present invention can be used for imaging of alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in any sample or a specific body part or body area of a patient which is suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites. The compounds are able to pass the blood-brain barrier. Consequently, they are particularly suitable for imaging of alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the brain, tissue of the central nervous system (CNS), tissue of the eye (such as retinal tissue), tissue of peripheral organs such as the gut or other tissues, or body fluids such as cerebrospinal fluid (CSF) or blood.

In diagnostic applications, the compounds of the present invention are preferably administered in the form of a diagnostic composition comprising the compound of the invention. A "diagnostic composition" is defined in the present invention as a composition comprising one or more compounds of the present invention in a form suitable for administration to a patient, e.g., a mammal such as a human, and which is suitable for use in the diagnosis of the specific disease, disorder or abnormality at issue. Preferably a diagnostic composition further comprises a pharmaceutically acceptable excipient, carrier, diluent or adjuvant. Administration is preferably carried out as defined below. More preferably by injection of the composition as an aqueous solution. Such a composition may optionally contain further ingredients such as buffers; pharmaceutically acceptable solubilizers (e.g., cyclodextrins or surfactants such as Pluronic, Tween or phospholipids); and pharmaceutically acceptable stabilisers or antioxidants (such as ascorbic acid, gentisic acid or para-aminobenzoic acid). The dose of the compound of the present invention will vary depending on the exact compound to be administered, the weight of the patient, and other variables as would be apparent to a physician skilled in the art.

While it is possible for the compounds of the present invention to be administered alone, it is preferable to formulate them into a diagnostic composition in accordance with standard pharmaceutical practice. Thus, the invention also provides a diagnostic composition which comprises a diagnostically effective amount of a compound of the present invention in admixture with, optionally, at least one pharmaceutically acceptable excipient, carrier, diluent or adjuvant.

Pharmaceutically acceptable excipients are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, 15th Ed., Mack Publishing Co., New Jersey (1975). The pharmaceutical excipient can be selected with regard to the intended route of administration and standard pharmaceutical practice. The excipient must be acceptable in the sense of being not deleterious to the recipient thereof.

Pharmaceutically useful excipients, carriers, adjuvants and diluents that may be used in the formulation of the diagnostic composition of the present invention may comprise, for example, solvents such as monohydric alcohols such as ethanol, isopropanol and polyhydric alcohols such as glycols and edible oils such as soybean oil, coconut oil, olive oil, safflower oil cottonseed oil, oily esters such as ethyl oleate, isopropyl myristate, binders, adjuvants, solubilizers, thickening agents, stabilizers, disintegrants, glidants, lubricating agents, buffering agents, emulsifiers, wetting agents, suspending agents, sweetening agents, colorants, flavors, coating agents, preservatives, antioxidants, processing agents, drug delivery modifiers and enhancers such as calcium phosphate, magnesium stearate, talc, monosaccharides, disaccharides, starch, gelatin, cellulose, methylcellulose, sodium carboxymethyl cellulose, dextrose, hydroxypropyl- β -cyclodextrin, polyvinylpyrrolidone, low melting waxes, and ion exchange resins.

The routes for administration (delivery) of the compounds of the invention include, but are not limited to, one or more of: intravenous, gastrointestinal, intraspinal, intraperitoneal, intramuscular, oral (e. g. as a tablet, capsule, or as an ingestible solution), topical, mucosal (e. g. as a nasal spray or aerosol for inhalation), nasal, parenteral (e. g. by an injectable form), intrauterine, intraocular, intradermal, intracranial, intratracheal, intravaginal, intracerebroventricular, intracerebral, subcutaneous, ophthalmic (including intravitreal or intracameral), transdermal, rectal, buccal, epidural and sublingual. Preferably, the route of administration (delivery) of the compounds of the invention is intravenous.

For example, the compounds can be administered orally in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavoring or coloring agents, for immediate-, delayed-, modified-, sustained-, pulsed- or controlled-release applications.

The tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycolate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose

(HPMC), hydroxypropylcellulose (HPC), sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included. Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include starch, a cellulose, milk sugar (lactose) or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the agent may be combined with various sweetening or flavoring agents, coloring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

Preferably, in diagnostic applications, the compounds of the present invention are administered parenterally. If the compounds of the present invention are administered parenterally, then examples of such administration include one or more of: intravenously, intraarterially, intraperitoneally, intrathecally, intraventricularly, intraurethrally, intrasternally, intracranially, intramuscularly or subcutaneously administering the compounds; and/or by using infusion techniques. For parenteral administration, the compounds are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

As indicated, the compounds of the present invention can be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurized container, pump, spray or nebulizer with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA134AT) or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA), carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container, pump, spray or nebulizer may contain a solution or suspension of the active compound, e. g. using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, e. g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of the compound and a suitable powder base such as lactose or starch.

Alternatively, the compounds of the present invention can be administered in the form of a suppository or pessary, or it may be applied topically in the form of a gel, hydrogel, lotion, solution,

cream, ointment or dusting powder. The compounds of the present invention may also be dermally or transdermally administered, for example, by the use of a skin patch.

They may also be administered by the pulmonary or rectal routes. They may also be administered
5 by the ocular route. For ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH was adjusted, sterile saline, or, preferably, as solutions in isotonic, pH was adjusted, sterile saline, optionally in combination with a preservative such as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

10 For application topically to the skin, the compounds of the present invention can be formulated as a suitable ointment containing the active compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream,
15 suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Typically, a physician will determine the actual dosage which will be most suitable for an individual subject. The specific dose level and frequency of dosage for any particular individual may be varied
20 and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the individual undergoing diagnosis.

25 The diagnostic compositions of the invention can be produced in a manner known per se to the skilled person as described, for example, in Remington's Pharmaceutical Sciences, 15th Ed., Mack Publishing Co., New Jersey (1975).

The compounds of the present invention are useful as an in vitro analytical reference or an in vitro
30 screening tool. They are also useful in in vivo diagnostic methods.

The compounds according to the present invention can also be provided in the form of a mixture, a pharmaceutical composition, or a combination, comprising a compound according to the present invention and at least one compound selected from an imaging agent different from the compound
35 according to the invention, a pharmaceutically acceptable excipient, carrier, diluent or adjuvant. The imaging agent different from the compound according to the invention is preferably present in a

diagnostically effective amount. More preferably the imaging agent different from the compound according to the invention is an Abeta or Tau imaging agent.

METHODS

- 5 In one embodiment, the invention provides a method of diagnosing a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites, in a subject, the method comprising the steps:
- (a) Administering a compound of the invention, or a diagnostic composition which comprises a compound of the invention to the subject;
 - 10 (b) Allowing said compound to bind to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites; and
 - (c) Detecting the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites.
- 15 Optionally, said method may further comprise the step of:
- (d) Generating an image representative of the location and/or amount of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites.

- In another embodiment, the invention provides a method of positron emission tomography (PET) imaging of alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites, in a tissue of a subject, the method comprising the steps:
- 20 (a) Administering a compound of the invention, or a diagnostic composition which comprises a compound of the invention to the subject;
 - (b) Allowing the compound to bind to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites; and
 - 25 (c) Detecting the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites by collecting a positron emission tomography (PET) image of the tissue of the subject;
- 30 In another embodiment, the invention relates to a method for the detection and optionally quantification (e.g., an in vivo or in vitro method) of alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites, in a tissue of a subject, the method comprising the steps:
- 35 (a) Bringing a sample or a specific body part or body area suspected to contain alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites, into contact with a

compound of the invention, or a diagnostic composition which comprises a compound of the invention;

- (b) Allowing the compound to bind to the alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites;
- 5 (c) Detecting the compound bound to the alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites; and
- (d) Optionally quantifying the amount of the compound bound to the alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites.

10

In an embodiment, the present invention refers to a method of collecting data for the diagnosis of a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites, the method comprising the steps:

- (a) Bringing a sample or a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with a compound according to the present invention, or a diagnostic composition which comprises a compound according to the present invention;
- 15 (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- 20 (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites; and
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or
- 25 Lewy neurites in the sample or specific body part or body area.

If the amount of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites is higher than a normal control value it can be assumed that the patient is suffering from a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites.

Yet another embodiment of the present invention refers to a method of collecting data for determining a predisposition to a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites, the method comprising the steps:

- 35 (a) Bringing a sample or a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with a

compound according to the present invention, or a diagnostic composition which comprises a compound according to the present invention;

- (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- 5 (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites; and
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or
- 10 Lewy neurites in the sample or specific body part or body area.

If the amount of the compound bound to the alpha-synuclein aggregates is higher than a normal control value of a healthy/reference subject this indicates that the patient is suffering from or is at risk of developing a disease, disorder or abnormality associated with alpha-synuclein aggregates. In particular, if the amount of the compound bound to the alpha-synuclein aggregates is higher than what expected in a person showing no clinical evidence of a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites, it can be assumed that the patient has a disposition to a disease, disorder or abnormality associated with alpha-synuclein aggregates.

20

In a further aspect, the present invention relates to a method of collecting data for prognosing a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites, wherein the method comprises the steps:

- (a) Bringing a sample, a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with a compound according to the present invention, or a diagnostic composition which comprises a compound according to the present invention;
- 25 (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- 30 (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or
- 35 Lewy neurites in the sample or specific body part or body area; and
- (e) Optionally repeating steps (a) to (c) and, if present, optional step (d) at least one time.

The progression of a disease, disorder or abnormality and/or the prospect (e.g., the probability, duration, and/or extent) of recovery can be estimated by a medical practitioner based on the presence or absence of the compound bound to the alpha-synuclein aggregates, the amount of the compound
5 bound to the alpha-synuclein aggregates or the like. If desired, steps (a) to (c) and, if present, optional step (d) can be repeated over time to monitor the progression of the disease, disorder or abnormality and to thus allow a more reliable estimate.

A further aspect is directed to a method of collecting data for monitoring the progression (or evolution)
10 of a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in a patient, the method comprising the steps:

- (a) Bringing a sample, a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with the compound according to the present invention, or a diagnostic composition which comprises a
15 compound according to the present invention;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- 20 (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area; and
- (e) Optionally repeating steps (a) to (c) and, if present, optional step (d) at least one time.

25 In the method for monitoring the progression the amount of the compound bound to the alpha-synuclein aggregates can be optionally compared at various points of time during the treatment, for instance, before and after onset of the treatment or at various points of time after the onset of the treatment.

30 Typically, the patient is or has been undergoing treatment of the disease, disorder or abnormality associated with alpha-synuclein aggregates or is/has been undergoing treatment of the synucleinopathy. In particular, the treatment can involve administration of a medicament which is suitable for treating the disease, disorder or abnormality associated with alpha-synuclein aggregates.

35

In another embodiment, the invention relates to a method of collecting data for predicting responsiveness of a patient suffering from a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites to a treatment with a medicament, the method comprising the steps of

- 5 (a) Bringing a sample, a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with a compound of the invention, or a diagnostic composition which comprises a compound of the invention;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to,
10 Lewy bodies and/or Lewy neurites;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence
15 or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area; and
- (e) Optionally repeating steps (a) to (c) and, if present, optional step (d) at least one time.

In the method for predicting the responsiveness, the method can further comprises steps (i) to (vi) before step (a):

- 20 (i) bringing a sample or specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with the compound of the present invention, which compound specifically binds to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- 25 (ii) allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (iii) detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (iv) correlating the presence or absence of the compound bound to the alpha-synuclein aggregates
30 including, but not limited to, Lewy bodies and/or Lewy neurites with the presence or absence of alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area;
- (v) optionally comparing the amount of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites to a normal control value; and
- 35 (vi) treating the patient with the medicament.

Optionally the method can further comprise step (A) after step (d) or step (e):

- (A) comparing the amount of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites determined in step (iv) to the amount of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy
5 bodies and/or Lewy neurites determined in step (d).

In the method for predicting responsiveness the amount of the compound/protein aggregate complex can be optionally compared at various points of time during the treatment, for instance, before and after onset of the treatment or at various points of time after the onset of the treatment. A change,
10 especially a decrease, in the amount of the compound/protein aggregate complex may indicate that the patient has a high potential of being responsive to the respective treatment.

If the amount of the compound bound to the alpha-synuclein aggregates decreases over time, it can be assumed that the patient is responsive to the treatment. If the amount of the compound bound to
15 the alpha-synuclein aggregates is essentially constant or increases over time, it can be assumed that the patient is non-responsive to the treatment.

Alternatively, the responsiveness can be estimated by determining the amount of the compound bound to the alpha-synuclein aggregates. The amount of the compound bound to the alpha-synuclein
20 aggregates can be compared to a control value such as a normal control value, a preclinical control value or a clinical control value. Alternatively, the control value may refer to the control value of subjects known to be responsive to a certain therapy, or the control value may refer to the control value of subjects known to be non-responsive to a certain therapy. The outcome with respect to responsiveness can either be "responsive" to a certain therapy, "non-responsive" to a certain therapy
25 or "response undetermined" to a certain therapy. Response to the therapy may be different for the respective patients.

Optionally, the diagnostic composition can be used before, during and after, surgical procedures (e.g. deep brain stimulation (DBS)) and non-invasive brain stimulation (such as repetitive transcranial
30 magnetic stimulation (rTMS)), for visualizing alpha-synuclein aggregates before, during and after such procedures. Surgical techniques, including DBS, improve advanced symptoms of PD on top of the best currently used medical therapy. During the past 2 decades, rTMS has been closely examined as a possible treatment for PD (Ying-hui Chou et al. JAMA Neurol. 2015 April 1; 72(4): 432–440).

35 In any of the above methods, the step of optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or

Lewy neurites with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area; comprises

- 5 – determining the amount of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy;
- correlating the amount of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the amount of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area; and
- 10 – optionally comparing the amount of the compound bound with the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area to a normal control value in a healthy control subject.

15 The control value can be, e.g., a normal control value, a preclinical control value and/or a clinical control value.

20 A “healthy control subject” or “healthy volunteer (HV) subject” is a person showing no clinical evidence of a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites.

 If in any of the above summarized methods the amount of the compound bound with the alpha-synuclein aggregates is higher than the normal control value, then it can be expected that the patient is suffering from or is likely to from a disease, disorder or abnormality associated with alpha-synuclein aggregates or from a synucleinopathy.

25 A sample or a specific body part or body area suspected to contain an alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites is brought into contact with a compound of the present invention.

30 Any of the compounds of the present invention can be used in the above summarized methods. Preferably detectably labelled compounds of the present invention are employed in the above summarized methods.

35 The specific body part or body area is preferably of a mammal, more preferably of a human, including the full body or partial body area or body part of the patient suspected to contain alpha-synuclein

aggregates. The specific body part or body area can be brain, the central nervous system, eye or a peripheral organ such as the gut, preferably brain.

5 The tissue can be brain tissue, tissue of the central nervous system (CNS), tissue of the eye (such as retinal tissue), tissue of peripheral organs such as the gut or other tissues, or body fluids such as cerebrospinal fluid (CSF) or blood. The tissue is preferably brain tissue. Preferably, the sample is an in vitro sample from a patient.

10 In the above methods, the compound of the present invention can be brought into contact with the sample or the specific body part or body area suspected to contain the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites by any suitable method.

In *in vitro* methods the compound of the present invention and a liquid sample can be simply mixed.

15 In an *in vivo* method, the specific body part or body area can be brought into contact with a compound of the invention by administering an effective amount of a compound of the invention to the patient.

20 The effective amount of a compound of the invention is an amount which is suitable for allowing the presence or absence of alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample, specific body part or body area to be determined using the chosen analytical technique. The amount is not particularly limited and will depend on the compound of the formula (I), the type of detectable label, the sensitivity of the respective analytical method and the respective device. The amount can be chosen appropriately by a skilled person.

25 The compound is then allowed to bind to the alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites. The step of allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites includes allowing sufficient time for the compound of the invention to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites. The amount of time required for
30 binding will depend on the type of test (e.g., *in vitro* or *in vivo*) and can be determined by a person skilled in the field by routine experiments. In an *in vivo* method, the amount of time will depend on the time which is required for the compound to reach the specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites. The amount of time should not be too extended to avoid washout and/or metabolism of the compound
35 of the invention.

The compound which has bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites, can be subsequently detected by any appropriate method. The method of detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites is not particularly limited and depends, among others, on the detectable label, the type of sample, specific body part or body area and whether the method is an *in vitro* or *in vivo* method. Examples of possible methods include, but are not limited to, a fluorescence imaging technique or a nuclear imaging technique such as positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), and contrast-enhanced magnetic resonance imaging (MRI). These have been described and enable visualization of alpha-synuclein biomarkers. The fluorescence imaging technique and/or nuclear imaging technique can be employed for monitoring and/or visualizing the distribution of the detectably labelled compound within the sample or a specific body part or body area. The imaging system provides an image of bound detectable label such as radioisotopes, in particular positron emitters or gamma emitters, as present in the tested sample, the tested specific body part or the tested body area. Preferably, the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites is detected by an imaging apparatus such as PET or SPECT scanner, more preferably PET.

The amount of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites can be determined by visual or quantitative analysis, for example, using PET scan images.

A compound according to the present invention or its precursor can also be incorporated into a test kit for detecting alpha-synuclein protein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites. The test kit typically comprises a container holding one or more compounds according to the present invention or its precursor(s) and instructions for using the compound for the purpose of binding to alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites and detecting the formation of the compound bound to the alpha-synuclein aggregates such that presence or absence of the compound bound to the alpha-synuclein aggregates correlates with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites.

The term "test kit" refers in general to any diagnostic kit known in the art. More specifically, the latter term refers to a diagnostic kit as described in Zrein et al., Clin. Diagn. Lab. Immunol., 1998, 5, 45-49.

The dose of the detectably labelled compounds of the present invention, preferably compounds of formula (I-F) labelled with ^{18}F or compounds of formula (I-H*) or (I-H) labelled with ^3H , will vary depending on the exact compound to be administered, the weight of the patient, size and type of the sample, and other variables as would be apparent to a physician skilled in the art. Generally, the dose could preferably lie in the range 0.001 $\mu\text{g/kg}$ to 10 $\mu\text{g/kg}$, preferably 0.01 $\mu\text{g/kg}$ to 1.0 $\mu\text{g/kg}$. The radioactive dose can be, e.g., 100 to 600 MBq, more preferably 150 to 450 MBq.

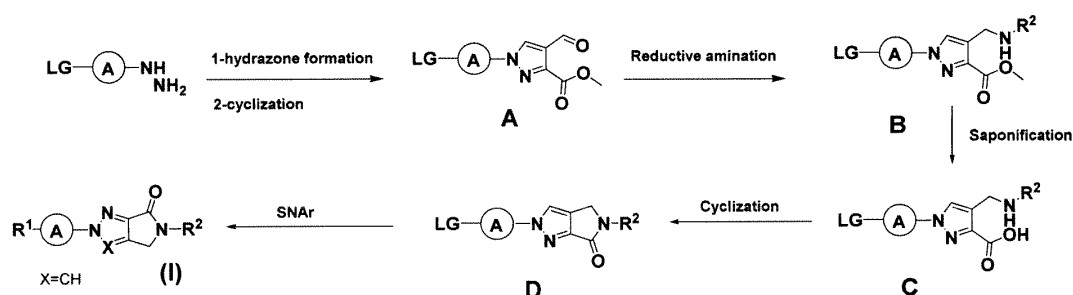
METHODS OF SYNTHESIZING THE COMPOUNDS OF THE INVENTION

The compounds of the present invention may be prepared in accordance with the definition of compound of formula (I), by the routes described in the following Schemes or the Examples. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g. "such as") provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. In the following general methods,

R^1 , R^2 , A , X , LG , and n are as previously defined in the above embodiments or limited to designations in the Schemes. Unless otherwise stated, starting materials are either commercially available or are prepared by known methods.

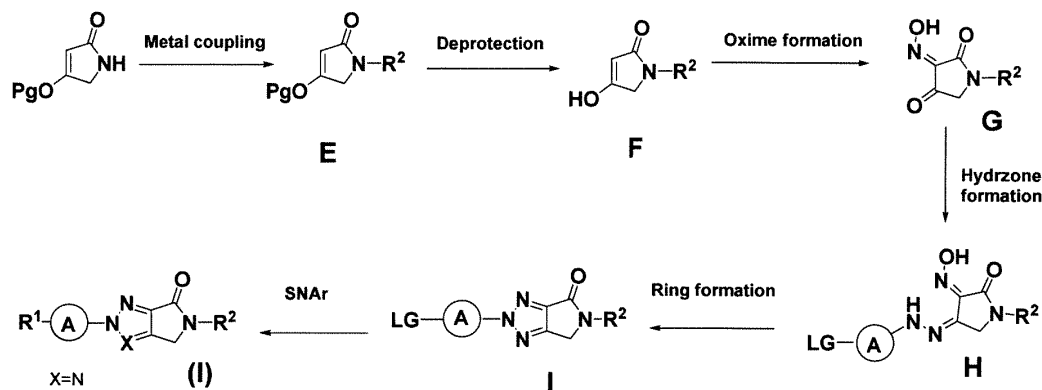
General synthetic scheme for the preparation of compounds and precursors of this invention:

Scheme 1



Commercially available hydrazine can be condensed with the appropriate ketone to afford the corresponding hydrazone. The crude hydrazone can be subjected to ring cyclization using POCl_3/DMF to give intermediate A. Reductive amination with amine in presence of reductive reagent can afford intermediate B. A saponification reaction can be conducted in either basic or acidic conditions to give intermediate C. Ring closure can be then performed using standard conditions such as HATU or POCl_3 or NMI/TCFH in a suitable solvent. Finally, intermediate D can be further functionalized using a SNAr reaction to give compounds of formula (I).

Scheme 2



The R^2 group can be introduced into protected pyrrolone using metal coupling such as an Ullmann reaction. Intermediate E can be deprotected using acidic conditions. Then, oxime formation using for example a nitrite source under acidic conditions can deliver the intermediate G. Hydrazone formation can be conducted using intermediate G and commercially available hydrazine, followed by ring cyclized by activating the hydroxyl of the oxime with for example acetic anhydride to deliver intermediate I. Finally, intermediate I can be further functionalized using a SNAr reaction to give compounds of formula (I).

General synthesis of ^{18}F -labelled compounds of the present invention

Compounds having the formula (I) which are labelled by ^{18}F can be prepared by reacting a precursor compound, as described below, with an ^{18}F -fluorinating agent, so that the LG comprised in the precursor compound is replaced by ^{18}F .

The reagents, solvents and conditions which can be used for the ^{18}F -fluorination are well-known to a skilled person in the field (L. Cai, S. Lu, V. Pike, Eur. J. Org. Chem 2008, 2853-2873; J. Fluorine Chem., 27 (1985):177-191; Coenen, Fluorine-18 Labeling Methods: Features and Possibilities of Basic Reactions, (2006), in: Schubiger P.A., Friebe M., Lehmann L., (eds), PET-Chemistry - The Driving Force in Molecular Imaging. Springer, Berlin Heidelberg, pp.15-50). Preferably, the solvents used in the ^{18}F -fluorination are DMF, DMSO, acetonitrile, DMA, or mixtures thereof, preferably the solvent is acetonitrile or DMSO.

Any suitable ^{18}F -fluorinating agent can be employed. Typical examples include H^{18}F , alkali or alkaline earth ^{18}F -fluorides (e.g., K^{18}F , Rb^{18}F , Cs^{18}F , and Na^{18}F). Optionally, the ^{18}F -fluorination agent can be used in combination with a chelating agent such as a cryptand (e.g.: 4,7,13,16,21,24-hexaoxa-1,10-

- diazabicyclo[8.8.8]-hexacosane - Kryptofix[®]) or a crown ether (e.g.: 18-crown-6). Alternatively, the ¹⁸F-fluorinating agent can be a tetraalkylammonium salt of ¹⁸F or a tetraalkylphosphonium salt of ¹⁸F; e.g., tetra(C₁₋₆ alkyl)ammonium salt of ¹⁸F or a tetra(C₁₋₆ alkyl)phosphonium salt of ¹⁸F. Preferably, the ¹⁸F-fluorination agent is K¹⁸F, H¹⁸F, Cs¹⁸F, Na¹⁸F tetra(C₁₋₆ alkyl) ammonium salt of ¹⁸F, Kryptofix[222]¹⁸F or tetrabutylammonium [¹⁸F]fluoride.

Although the reaction is shown above with respect to ¹⁸F as a radioactive label, other radioactive labels can be introduced following similar procedures.

- The invention is illustrated by the following examples which, however, should not be construed as limiting.

EXAMPLES

EXEMPLIFICATION OF THE INVENTION

- Compounds of the present disclosure may be prepared by methods known in the art of organic synthesis. In all of the methods it is understood that protecting groups for sensitive or reactive groups may be employed where necessary in accordance with general principles of chemistry. Protecting groups are manipulated according to standard methods of organic synthesis (T. W. Green and P. G. M. Wuts (2014) Protective Groups in Organic Synthesis, 5th edition, John Wiley & Sons). These groups are removed at a convenient stage of the compound synthesis using methods that are readily apparent to those skilled in the art.

Unless otherwise noted, all reagents and solvents were obtained from commercial sources and used without further purification.

The chemical names were generated using ChemBioDraw Ultra v20 from CambridgeSoft.

- Temperatures are given in degrees Celsius. If not mentioned otherwise, all evaporations are performed under reduced pressure, typically between about 15 mm Hg and 100 mm Hg (= 20 - 133 mbar). The structure of final products, intermediates and starting materials was confirmed by standard analytical methods, e.g., microanalysis and spectroscopic characteristics, e.g., MS, IR, NMR.

ABBREVIATIONS

Abbreviations used are those conventional in the art.

CAN	ammonium cerium(IV) nitrate
CsF	cesium fluoride
CyDMEDA	1-N,2-N-dimethylcyclohexane-1,2-diamine
DCM	dichloromethane

DMEDA	1,2-dimethylethylenediamine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
EtOAc	ethyl acetate
HATU	(1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate
HPLC	High Performance Liquid Chromatography
LCMS	Liquid Chromatography Mass Spectrometry
LG	leaving group
NMI	N-methyl imidazole
Pg	protecting group
RT	room temperature
SNAr	nucleophilic aromatic substitution
TCFH	chloro-N,N,N',N'-tetramethylformamidinium hexafluorophosphate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
ACN	acetonitrile

ANALYTICAL DETAILS, PREPARATIVE AND ANALYTICAL METHODS

NMR measurements were performed on a DRX-400 MHz NMR spectrometer, on a Bruker AV-400 MHz NMR spectrometer or Spinsolve 80MHz NMR spectrometer in deuterated solvents, using or not tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are reported in ppm downfield from TMS, spectra splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint), septet (sept), multiplet, unresolved or overlapping signals (m), or broad signal (br). Deuterated solvents are given in parentheses and have chemical shifts of dimethyl sulfoxide (δ 2.50 ppm), methanol (δ 3.31 ppm), chloroform (δ 7.26 ppm), or other solvent as indicated in NMR spectral data.

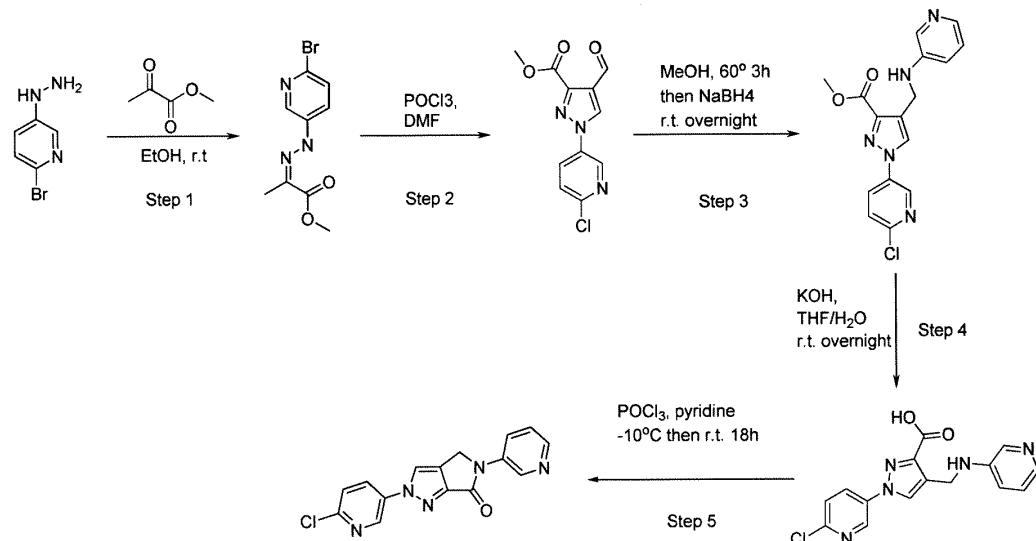
Mass spectra (MS) were recorded on an Advion CMS mass spectrometer or an UPLC H-Class Plus with Photodiode Array detector and Qda Mass spectrometer from Waters.

Column chromatography was performed using silica gel (Fluka: Silica gel 60, 0.063-0.2 mm) and suitable solvents as indicated in the specific examples.

Flash Column Chromatography System: flash purification was conducted with a Biotage Isolera One flash purification system using HP-Sil or KP-NH SNAP cartridges (Biotage) and the solvent gradient indicated in the specific examples.

Thin layer chromatography (TLC) was carried out on silica gel plates with UV detection.

PREPARATIVE EXAMPLES

Preparative Example 1

- 5 **Step 1:** A stirred solution of 2-bromo-5-hydrazinylpyridine (10 g, 0.053 mol) in ethanol (100 ml) was cooled to 0°C and a solution of commercially available methyl 2-oxopropanoate (6.24 g, 0.062mol) in ethanol (25 ml) was added drop wise at 0°C for 15 min. After the mixture had been allowed to stir at room temperature for 3h it was evaporated to obtain 14.4 g of product (92% by LCMS) LC/MS [M+H] 273.0.

10

- Step 2:** The Vilsmeier-Haack reagent was prepared by adding 11 mL (0.13 mol) POCl₃ to 55 mL DMF (0.7 mol) at 0°C in a round-bottomed flask in an ice-cold condition (0-5°C) under constant stirring. Methyl 2-(2-(6-bromopyridin-3-yl)hydrazono)propanoate of step 1 (11.3 g, 0.041mol) in 25 mL DMF was added to the Vilsmeier-Haack reagent and stirred for an hour, after that the reaction mixture was kept on a water bath at 70°C for 4 h. After the reaction, the mixture was poured into crushed ice under constant manual stirring. After neutralization with K₂CO₃ solution, ethyl acetate was added. The organic phase was separated and the aqueous phase was extracted with EtOAc. The combined organic solutions were washed with water (100 mL) and brine (100 mL), dried, and concentrated. The product was purified by recrystallization from ethanol (10 g, purity 95%) LC/MS [M+H] 266.0.

20

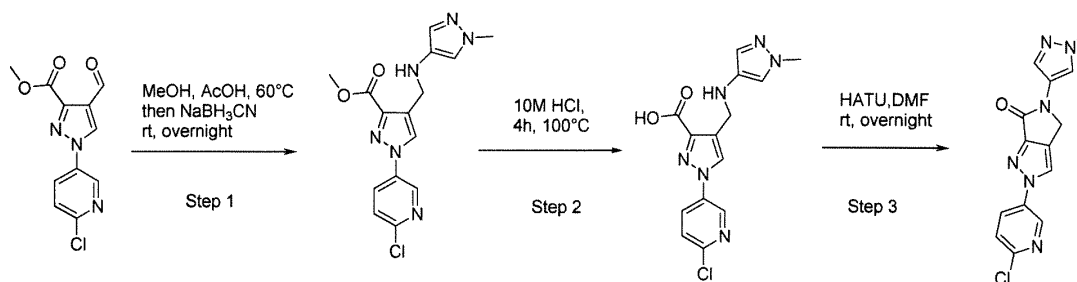
Step 3: A suspension of pyridin-3-amine (0.18 g, 0.0019mol) in MeOH (80 mL) was treated with methyl 1-(6-chloropyridin-3-yl)-4-formyl-1H-pyrazole-3-carboxylate of step 2 (0.5 g, 0.0019mol) and the resulting mixture was heated at reflux for 4 h. The mixture was cooled to room temperature and

NaBH₄ (0.078 g, 0.0021 mmol) was added portionwise. The reaction mixture was stirred at RT overnight. The solvent of the mixture was then evaporated, water and AcOH were added. The resulting mixture was extracted with CH₂Cl₂ 3 times, dried over MgSO₄ and the solvent was removed in vacuo to afford a crude product (0.5 g) which was used in the next step without purification. LC/MS [M+H]⁺ 344.0.

Step 4: Methyl 1-(6-chloropyridin-3-yl)-4-((pyridin-3-ylamino)methyl)-1H-pyrazole-3-carboxylate of step 3 (0.3g, 0.00087mol) was dissolved in THF and 1 mL of aqueous solution of KOH (0.098g, 0.00175mol) was added at RT and the mixture was stirred overnight. Solvent was evaporated; 1.5 ml of water was added. Acid was added to the reaction solution to adjust the pH to 6.0. Solvent was evaporated to obtain the product 0.28 g as a HCl salt. LC/MS [M+H]⁺ 330.2.

Step 5: To a suspension of the 1-(6-chloropyridin-3-yl)-4-((pyridin-3-ylamino)methyl)-1H-pyrazole-3-carboxylic acid of step 4 (0.1 mg, 0.0003 mol) in pyridine (Volume: 2 ml) at -10°C, phosphoryl trichloride (0.46g, 0.38ml, 0.003 mol) was added and the mixture was stirred at -10°C for 10 min, the reaction mixture was then stirred at RT for 16h. The reaction mixture was slowly quenched with water, a saturated solution of NaHCO₃ was added and the aqueous phase was extracted three times with a solution of DCM/MeOH (9:1). The organic phase was dried over Na₂SO₄, and the solvent was evaporated to obtain a crude product (0.13g) LC/MS [M+H]⁺ 312.0.

Preparative Example 2



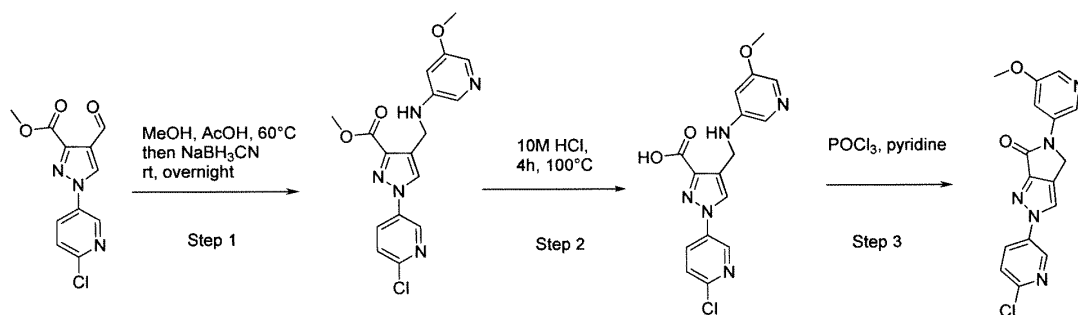
Step 1: Methyl 1-(6-chloropyridin-3-yl)-4-formyl-1H-pyrazole-3-carboxylate (2.0 g, 7.53 mmol) was dissolved in MeOH (50 ml) and 1-methyl-1H-pyrazol-4-amine (731.25 mg, 7.53 mmol), acetic acid (474.77 mg, 7.91 mmol, 460.0 μ l, 1.05 eq.) were added. The mixture was stirred for 18 h at 60°C, then cooled to 0°C and sodium cyanoborohydride (946.33 mg, 15.06 mmol) was added. The resulting mixture was stirred at RT overnight. The mixture was poured into aqueous Na₂CO₃ solution and extracted twice with DCM (50 mL). The combined organic layers were washed with brine and

concentrated under vacuum to afford the methyl 1-(6-chloropyridin-3-yl)-4-[(1-methyl-1H-pyrazol-4-yl)amino]methyl-1H-pyrazole-3-carboxylate (2.2 g, 6.34 mmol, 84.3% yield). LC/MS [M+H] 347.2

Step 2: Methyl 1-(6-chloropyridin-3-yl)-4-[(1-methyl-1H-pyrazol-4-yl)amino]methyl-1H-pyrazole-3-carboxylate of step 1 (2.2 g, 6.34 mmol) was dissolved in 10M HCl (50 ml), stirred for 4 h at 100°C and concentrated under vacuum to afford 1-(6-chloropyridin-3-yl)-4-[(1-methyl-1H-pyrazol-4-yl)amino]methyl-1H-pyrazole-3-carboxylic acid (2.2 g, 92.0% purity, 6.08 mmol, 95.9% yield). LC/MS [M+H] 333.0

Step 3: 1-(6-Chloropyridin-3-yl)-4-[(1-methyl-1H-pyrazol-4-yl)amino]methyl-1H-pyrazole-3-carboxylic acid of step 2 (1.5 g, 4.51 mmol) and 1-methyl-1H-imidazole (1.67 g, 20.32 mmol, 1.62 ml, 4.5 eq.) were dissolved in DMF (10mL) and chloro-N,N,N,N-tetramethylformamidinium hexafluorophosphate (1.52 g, 5.42 mmol) was added in a single portion. The reaction mixture was stirred overnight. Precipitate that formed was filtered off and dried to afford 2-(6-chloropyridin-3-yl)-5-(1-methyl-1H-pyrazol-4-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-6-one (200.0 mg, 635.47 μmol, 14.1% yield). LC/MS [M+H] 315.2.

Preparative Example 3

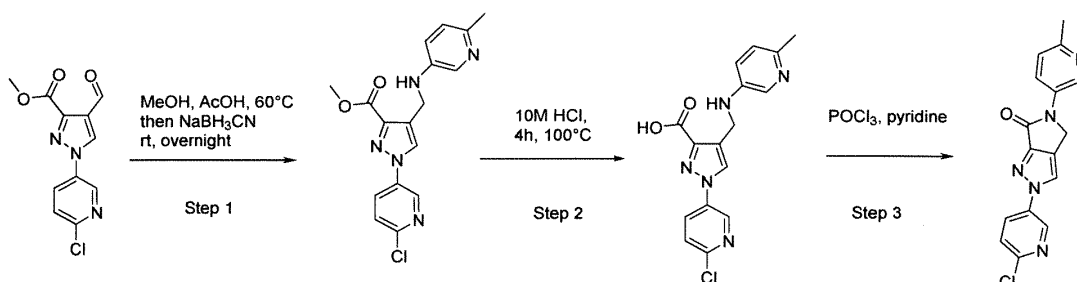


Step 1: Methyl 1-(6-chloropyridin-3-yl)-4-formyl-1H-pyrazole-3-carboxylate (2.19 g, 8.25 mmol) was dissolved in MeOH (50 ml) and 5-methoxypyridin-3-amine (1.02 g, 8.25 mmol), acetic acid (520.0 mg, 8.66 mmol, 500.0 μl, 1.05 eq.) were added. The mixture was stirred for 18 h at 60°C, then cooled to 0°C and sodium cyanoborohydride (1.04 g, 16.5 mmol) was added. The resulting mixture was stirred at RT overnight. The mixture was poured into aqueous Na₂CO₃ solution and was extracted twice with DCM (50 ml). The combined organic layers were washed with brine and concentrated under vacuum to afford methyl 1-(6-chloropyridin-3-yl)-4-[(5-methoxypyridin-3-yl)amino]methyl-1H-pyrazole-3-carboxylate (2.3 g, 95.0% purity, 5.85 mmol, 70.9% yield). LC/MS [M+H] 374.0.

Step 2: Methyl 1-(6-chloropyridin-3-yl)-4-[(5-methoxypyridin-3-yl)amino]methyl-1H-pyrazole-3-carboxylate of step 1 (2.1 g, 5.62 mmol) was dissolved in 10M HCl (50 ml), stirred for 4 h by at 100°C and concentrated under vacuum to afford 1-(6-chloropyridin-3-yl)-4-[(5-methoxypyridin-3-yl)amino]methyl-1H-pyrazole-3-carboxylic acid (2.2 g, 80.0% purity, 4.89 mmol, 87.1% yield) which
 5 was used in the next step without additional purification. LC/MS [M+H] 374.0.

Step 3: Phosphoryl trichloride (12.35 g, 80.57 mmol, 7.51 ml, 10.0 equiv) was cooled to -15°C and was added to 1-(6-chloropyridin-3-yl)-4-[(5-methoxypyridin-3-yl)amino]methyl-1H-pyrazole-3-carboxylic acid of step 2 (2.9 g, 8.06 mmol). The resulting mixture was stirred at RT overnight. The
 10 reation mixture was added to ice water, quenched with K₂CO₃ and extracted twice with CH₃Cl/MeOH(4/1). The combined organic layers were concentrated under vacuum. The crude product was purified by flash chromatography (interchim, 40 g SiO₂, CHCl₃/MeCN/methanol with MeCN from 50%, methanol - 18% flow rate = 40 mL/min) afford 2-(6-chloropyridin-3-yl)-5-(5-methoxypyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-6-one (180.0 mg, 97.5% purity, 513.53
 15 μmol, 6.4% yield) LC/MS [M+H] 374.0.

Preparative Example 4



Step 1: To a solution of methyl 1-(6-chloropyridin-3-yl)-4-formyl-1H-pyrazole-3-carboxylate (2.19 g, 8.25 mmol) in MeOH (50 mL) 6-methylpyridin-3-amine (891.92 mg, 8.25 mmol) and acetic acid (520.0 mg, 8.66 mmol, 500.0 μl, 1.05 equiv) were added. The mixture was stirred at 60 °C for 18 h, then cooled to 0 °C, and sodium cyanoborohydride (1.04 g, 16.5 mmol) was added. The resulting mixture was stirred at room temperature overnight. The mixture was poured into a saturated aqueous solution
 20 of Na₂CO₃, and extracted twice with DCM (50 mL). The combined organic layers were washed with brine and concentrated under vacuum to afford methyl 1-(6-chloropyridin-3-yl)-4-[(6-methylpyridin-3-yl)amino]methyl-1H-pyrazole-3-carboxylate (2.5 g, 90.0% purity, 6.29 mmol, 76.2% yield). LC/MS [M+H] 358.0.

Step 2: Methyl 1-(6-chloropyridin-3-yl)-4-[(6-methylpyridin-3-yl)amino]methyl-1H-pyrazole-3-

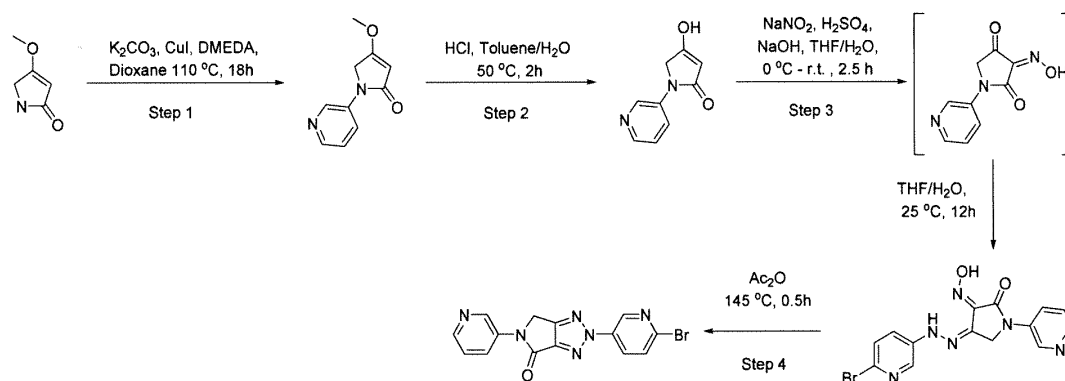
carboxylate of step 1 (2.3 g, 6.43 mmol) was dissolved in HCl (10 M, 50 mL), stirred at 100 °C for 4 h, then concentrated under vacuum to afford 1-(6-chloropyridin-3-yl)-4-[(6-methylpyridin-3-yl)amino]methyl-1H-pyrazole-3-carboxylic acid (2.3 g, 91.0% purity, 6.09 mmol, 94.7% yield). LC/MS [M+H] 344.0.

5

Step 3: Phosphoryl trichloride (9.82 g, 64.05 mmol, 5.97 ml, 10.0 eq.) was cooled to -15°C and added to 1-(6-chloropyridin-3-yl)-4-[(6-methylpyridin-3-yl)amino]methyl-1H-pyrazole-3-carboxylic acid of step 2 (2.2 g, 6.4 mmol). The resulting mixture was stirred at RT overnight. The reaction mixture was added to ice water, quenched with K₂CO₃ and extracted twice with CH₂Cl₂/MeOH(4/1). The combined organic layers were concentrated under vacuum to obtain a crude product which was purified by flash chromatography (Interchim, 40 g SiO₂, CHCl₃/MeCN/methanol with MeCN from 50%, methanol - 18% flow rate = 40 mL/min, RT 11min) to afford 2-(6-chloropyridin-3-yl)-5-(6-methylpyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-6-one (200.0 mg, 90.0% purity, 552.57 µmol, 8.6% yield) LC/MS [M+H] 326.0.

15

Preparative Example 5



Step 1: 4-Methoxy-1H-pyrrol-2(5H)-one (1 g, 8.84 mmol), 3-iodopyridine (3.625 g, 17.68 mmol), K₂CO₃ (2.44 g, 17.68 mmol), and CuI (673 mg, 3.536 mmol) were mixed in 35 ml of dry dioxane. Then tetramethylethylenediamine (TMEDA) (410 mg, 3.536 mmol) and proline (407 mg, 3.536 mmol) were added in one portion. The reaction mixture was sealed and heated with shaking for 48 hours at 110°C. The mixture was cooled, and the solvent was evaporated under a high vacuum. The residue was dissolved in H₂O and extracted twice with EtOAc, washed with brine and evaporated to give a crude product (1.2 g, 92% by LCMS). LC/MS [M+H] 191.2.

25

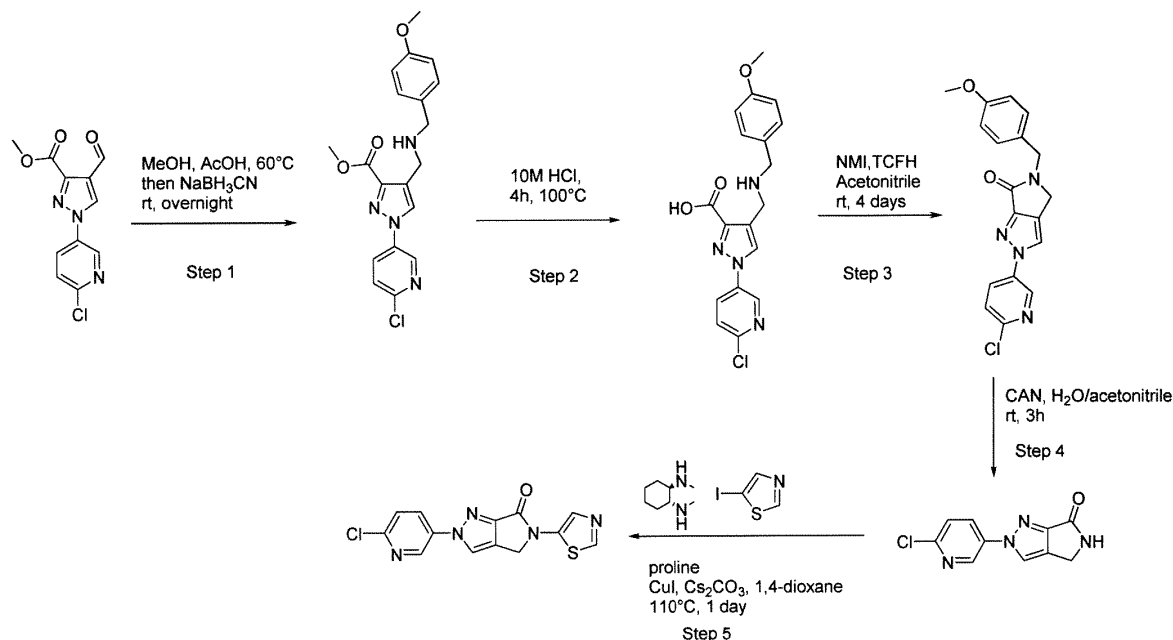
Step 2: 4-Methoxy-1-(pyridin-3-yl)-1H-pyrrol-2(5H)-one of step 1 (1 g, 5.25 mmol) and 37% HCl (1 ml, 10.5 mmol, 2 eq.) were mixed in toluene (52.5 ml). The reaction mixture was heated for 2 hours

at 50°C. Then the mixture was cooled and the solvent was evaporated under a high vacuum to give a crude product which was used in the next step without additional purification (0.9 g, 87% purity by LCMS) LC/MS [M+H] 177.2.

- 5 **Step 3:** To 1.5M aqueous sulfuric acid (0.83 ml, 1.245 mmol) at 0°C was added dropwise a solution of 4-hydroxy-1-(pyridin-3-yl)-1H-pyrrol-2(5H)-one of step 2 (155.73 mg, 0.884 mmol), sodium nitrite (95.0 mg, 1.336 mmol) and 1.0 M aqueous sodium hydroxide (0.96 ml, 0.960 mmol) in THF (7.0 ml). The mixture was stirred at 0°C for 30 min and was added to a solution of 2-bromo-5-hydrazinylpyridine (166.2 mg, 0.884 mmol) and NaHCO₃ (75 mg, 1 eq.) in water (2.4 ml)/ THF (7.5
- 10 ml) and the mixture was stirred at RT for 12 h. At this time, water (10 ml) was added and the resulting heterogeneous mixture was filtered. The collected solid was washed with water and taken up in DCM. The DCM solution was dried (Na₂SO₄) and evaporated to give a brown solid (170 mg, 92%, LCMS) LC/MS [M+H] 376.0 ¹H NMR (400 MHz, DMSO-d₆) δ 13.14 (s, 1H), 10.32 (s, 1H), 9.05 (s, 1H), 8.49 (s, 2H), 8.30 (d, J = 14.9 Hz, 2H), 7.63 (s, 2H), 7.55 (s, 2H), 4.67 (s, 2H).

15

- Step 4:** The 4-(2-(6-bromopyridin-3-yl)hydrazono)-3-(hydroxyimino)-1-(pyridin-3-yl)pyrrolidin-2-one of step 3 (0.7g, 1.865 mmol) was refluxed in acetic anhydride (5 ml) for 30 min. Subsequently, the dark brown solution was poured onto cold saturated sodium bicarbonate solution and the precipitated dark colored product was filtered, washed with water, and air dried to give a brown solid (0.456 g,
- 20 100%, LCMS) LC/MS [M+H] 358.8.

Preparative Example 6

Step 1: A suspension of (4-methoxyphenyl)methanamine (2.7 g, 0.0196 mol) in MeOH (300 mL) was treated with ethyl 1-(6-chloropyridin-3-yl)-4-formyl-1H-pyrazole-3-carboxylate (5 g, 0.018 mol) and AcOH (1.2 g, 0.019 mol) was added. The resulting mixture was heated at reflux for 4 h. The mixture was cooled to 0°C and sodium cyanoborohydride (2.36 g, 0.037 mmol) was added portionwise. The reaction mixture was stirred at RT overnight. NaHCO₃ aqueous solution was added, and solvent was then evaporated. The resulting mixture was extracted with DCM 3 times, dried over MgSO₄ and the solvent was removed in vacuo to afford a crude product (7.4 g mixture of CO₂Et and CO₂Me esters) which was used in the next step without purification.

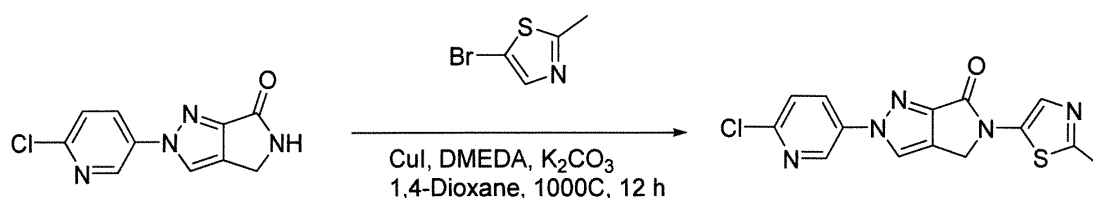
Step 2: Ethyl 1-(6-chloropyridin-3-yl)-4-(((4-methoxybenzyl)amino)methyl)-1H-pyrazole-3-carboxylate of step 1 (7.4 g, 0.019 mol) was dissolved in THF and 1 mL of aqueous solution of KOH (3.2 g, 0.057 mol) was added at rt. The mixture was stirred overnight. Solvent was evaporated, 70 ml of water was added. Acid was added to the reaction solution to adjust the pH to 5. The resulting mixture was extracted with CH₂Cl₂/MeOH (80/20) 3 times, dried over MgSO₄ and the solvent was removed in vacuo to afford a crude product (5.4 g, 92% purity) which was used in the next step. LC/MS [M+H]⁺ 373.0.

Step 3: 1-(6-Chloropyridin-3-yl)-4-(((4-methoxybenzyl)amino)methyl)-1H-pyrazole-3-carboxylic acid of step 2 (5.4 g, 0.014 mol) was dissolved in acetonitrile (200 mL) and DMF (50 mL). NMI (3.7 g, 0.045 mol, 3.1 eq.) was added, followed by TCFH (4.9 g, 0.017 mol, 1.2 eq.). The reaction mixture was stirred at room temperature for 3 days, then solvent was evaporated, and water was added. The resulting mixture was extracted with CH₂Cl₂/MeOH (80/20) 3 times, dried over MgSO₄ and the solvent was removed in vacuo to afford a residue that was washed by water and MeOH to give a product (1.5 g, 90% purity by LCMS) LC/MS [M+H] 355.0.

Step 4: To a solution of 2-(6-chloropyridin-3-yl)-5-(4-methoxybenzyl)-4,5-dihydropyrrolo[3,4-c]pyrazol-6(2H)-one of step 3 (1 g, 0.0028 mol) in acetonitrile: water (60mL:20 mL) was added CAN (6.18 g, 0.0011 mol) and the mixture was stirred at RT for 3 h. Acetonitrile was removed under vacuum and the reaction mixture was then extracted with ethyl acetate (5 x30 mL). The organic layer was washed with brine, dried over sodium sulfate, and concentrated to yield the crude product which was recrystallized from methanol to afford 0.6 g product as a yellow solid. LC/MS [M+H] 235.0.

Step 5: 2-(6-Chloropyridin-3-yl)-4,5-dihydropyrrolo[3,4-c]pyrazol-6(2H)-one of step 4 (0.3 g, 0.0013 mol), 5-iodothiazole (0.4 g, 0.0019 mol), Cs₂CO₃ (1.25 g, 0.0038 mol, 3 eq.), and CuI (0.25 g, 0.0013 mol, 1 eq.) were mixed in 15 ml of dry dioxane. Then, trans-N,N'-dimethylcyclohexane-1,2-diamine (CyDMEDA) (0.18 g, 0.0013 mol, 1 eq.) and proline (0.15 g, 0.0013 mol, 1 eq) were added in one portion. The reaction mixture was sealed and heated with shaking for 24 hours at 110 °C. The mixture was cooled, and the solvent was evaporated under a high vacuum. The residue was dissolved in H₂O. An aqueous solution of NaHSO₄ was added and the solid was filtered, washed with NH₃×H₂O, washed with IPS once, and dried under reduced pressure to give a crude product (300mg, 79% by LCMS). The product as a TFA salt was crystallized twice using THF to give a 150mg TFA salt (100% LCMS). LC/MS [M+H] 318.0.

Preparative Example 7



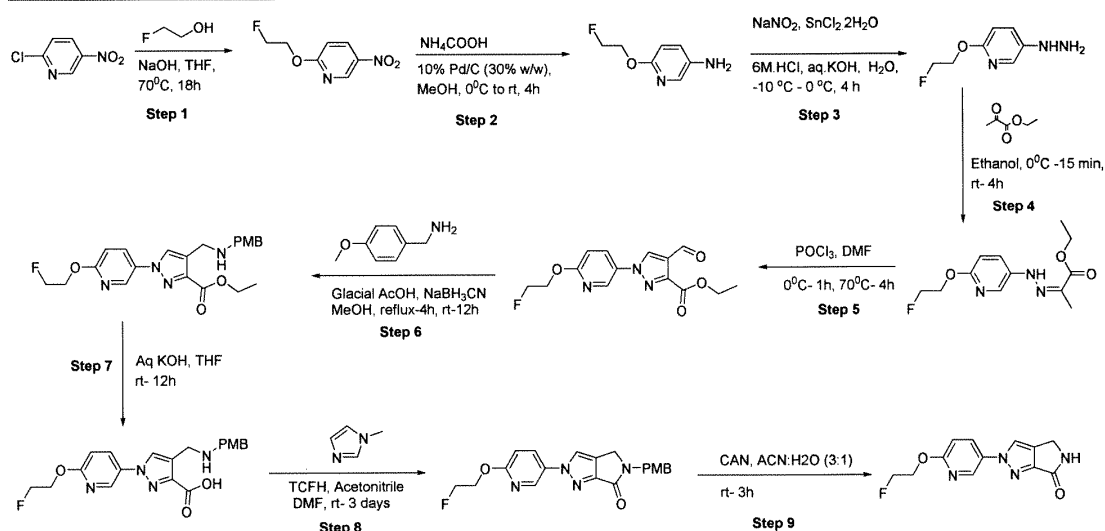
An oven-dried screw capped vial was charged with the product of step 4 of Preparative Example 6 (350 mg, 1.5 mmol), 5-bromo-2-methylthiazole (530 mg, 3.0 mmol), K₂CO₃ (415 mg, 3.0 mmol),

DMEDA (56 mg, 0.30 mmol), CuI (56 mg, 0.30 mmol) and 1,4-dioxane (17.5 mL) under argon. Then the mixture was degassed with argon for 15 min and allowed to heat to 100°C for 12 h. The progression of the reaction was monitored by TLC. After that the reaction mixture was quenched with water (15 mL) and the product was extracted with DCM three times (25 mL x3). The extract was dried over Na₂SO₄, concentrated and purified by chromatography over silica gel (60-120 mesh) eluted in 3% MeOH in DCM to afford 2-(6-chloropyridin-3-yl)-5-(2-methylthiazol-5-yl)-4,5-dihydropyrrolo[3,4-c]pyrazol-6(2H)-one as yellow solid (110 mg, 22%).

¹H NMR (DMSO-*d*₆) δ 9.04 (d, 1H), 8.80 (s, 1H), 8.42 (dd, 1H), 7.76 (d, 1H), 7.55 (s, 1H), 5.01 (s, 2H), 2.61 (s, 3H).

LCMS: 385.1 [M+H]⁺

Preparative Example 8



Step 1: To a stirred solution of fluoroethanol (9.5 mL, 164 mmol) and NaOH (6.04 g, 151 mmol) in THF (500 mL) under N₂ was added portion wise 2-chloro-5-nitropyridine (20 g, 126 mmol) and stirred at 70°C for 18 h. The progression of the reaction was monitored by TLC. Then, the reaction mixture was quenched with water (100 mL) and the product was extracted with EtOAc three times (100 mL x3). The combined organic layer was dried over Na₂SO₄, concentrated and purified by chromatography over silica gel (100-200 mesh) eluted in 7% EtOAc in hexane to afford 2-(2-fluoroethoxy)-5-nitropyridine as yellow solid (12.6 g, 54%).

¹H NMR (DMSO-*d*₆) δ 9.09 (d, 1H), 8.51 (dd, 1H), 7.11 (d, 1H), 4.84 (dd, 1H), δ 4.74 (dd, 1H), 4.69 (dd, 1H), 4.63 (t, 1H).

Step 2: To an oven dried single neck round bottom flask 2-(2-fluoroethoxy)-5-nitropyridine (7.5 g, 40.3 mmol), MeOH (300 mL, 40 vol.) and NH₄COOH (12.7 g, 202 mmol.) were added at 0°C. The reaction mixture was allowed to stir at 0°C for 30 min. Then, Pd/C (2.2 g, 30% w/w) (300 mg, 0.48 mmol.) was added portion wise to the above mixture and allowed to stir at RT for 4 h. Then, reaction mixture was filtered through a celite bed, washed with methanol twice (2 x 50 mL). Filtrate was evaporated to afford a crude 6-(2-fluoroethoxy) pyridin-3-amine (5.64 g, 90%) which was directly used in next step without further purification.

¹H NMR (DMSO-*d*₆) δ 7.48 (d, 1H), 7.02 (dd, 1H), 6.59 (d, 1H), 4.79 (s, 2H), δ 4.73 (t, 1H), 4.63 (t, 1H), 4.36 (t, 1H), 4.30 (t, 1H).

MS (ESI):156.86 [M+H]⁺

Step 3: A solution of sodium nitrite (0.97 g, 14.1 mmol) was added dropwise through addition funnel to a solution of 6-(2-fluoroethoxy) pyridin-3-amine (2.0 g, 156.25 mmol) in 6.0 M HCl (222 mL) at 0°C. The reaction mixture was allowed to stir for 0.5 h at -10°C to 0°C. Then, a solution of stannous chloride (6.9 g, 31 mmol) in 6.0 M HCl (22 mL) was added dropwise through addition funnel to the reaction mixture. The reaction mixture was allowed to stir for another 4h at -10°C to 0°C. The progression of the reaction was monitored by TLC. After completion, the reaction mixture is basified to pH 10 with 40% aq. KOH solution (60 mL) was added dropwise through addition funnel to the reaction mixture at -5.0°C to 0°C. To the crude reaction mixture was added DCM (80 mL) and allowed to stir the reaction mixture at -10°C for 15 min. Layers were separated. The organic layer is collected and this procedure is repeated twice. The organic layers are combined, dried over Na₂SO₄ and concentrated under vacuum. The obtained mass that was washed with hexane (20 mL) to give a product 2-(2-fluoroethoxy)-5-hydrazineylpyridine as brown solid (1.58 g, 75%).

¹H NMR (DMSO-*d*₆) δ 7.67 (d, 1H), 7.21 (dd, 1H), 6.66 (d, 1H), 6.46 (s, 1H), 4.74 (t, 1H), 4.64 (t, 1H), 4.39 (t, 1H), 4.33 (dd, 1H), 3.97 (s, 2H).

MS (ESI):171.94 [M]⁺

Step 4: A solution of 2-(2-fluoroethoxy)-5-hydrazineylpyridine (1.5 g, 8.8 mmol) in ethanol (15 mL) was cooled to 0°C and a solution of commercially available ethyl 2-oxopropanoate (1.16 mL, 10.5 mmol) in ethanol (3.0 mL) was added dropwise at 0°C for 15 min. Thereafter the mixture was allowed to stir at room temperature for 4h. After completion of the reaction, it was evaporated to obtain ethyl (Z)-2-(2-(6-(2-fluoroethoxy)pyridin-3-yl)hydrazineylidene) propanoate as white solid (1.8 g, 78%); which was directly used in next step.

¹H NMR (DMSO-*d*₆) δ 9.93 (s, 1H), 8.07 (d, 1H), 7.65 (dd, 1H), 6.85 (d, 1H), 4.77 (t, 1H), 4.68 (t, 1H), 4.47 (t, 1H), 4.40 (t, 1H), 4.18 (q, 2H), 2.04 (s, 3H), 1.26 (t, 3H).

MS (ESI): 270.15 [M+H]⁺

Step 5: POCl₃ (3.3 mL, 22 mmol) was added to DMF (15 mL) at 0°C in a round-bottomed flask in an ice-cold condition (0-5°C) under constant stirring. Intermediate ethyl (Z)-2-(2-(6-(2-fluoroethoxy) pyridin-3-yl) hydrazineylidene) propanoate (1.8 g, 6.6 mmol) in DMF (3.6 mL, 2.0 vol) was added to the Vilsmeier-Haack reagent and stirred for an hour and the reaction mixture was kept at 70°C for 4 h. After completion, the mixture was poured into crushed ice under constant manual stirring. After neutralization with K₂CO₃ solution (10 mL), ethyl acetate (50 mL) was added. The organic phase was separated, and the aqueous phase extracted with EtOAc (40 mL x3). The combined organic solutions were washed with water (25 mL) and brine (25 mL), dried, and concentrated. The product ethyl 1-(6-(2-fluoroethoxy)pyridin-3-yl)-4-formyl-1H-pyrazole-3-carboxylate was purified by recrystallization from ethanol to get title compound (1.4 g, 70%).

¹H NMR (DMSO-*d*₆) δ 10.29 (s, 1H), 9.20 (s, 1H), 8.74 (d, 1H), 8.28 (dd, 1H), 7.09 (d, 1H), 4.83 (t, 1H), 4.73 (t, 1H), 4.61 (t, 1H), 4.55 (dd, 1H), 4.42 (q, 2H), 1.36 (t, 3H).

MS (ESI): 308.11 [M+H]⁺

Step 6: A suspension of (4-methoxyphenyl) methanamine (0.4 g, 2.9 mmol) in MeOH (60 mL) was treated with ethyl 1-(6-(2-fluoroethoxy) pyridin-3-yl)-4-formyl-1H-pyrazole-3-carboxylate (1.0 g, 3.2 mmol) and glacial AcOH (0.9 mL) was added. The resulting mixture heated at reflux for 4 h. The mixture was cooled to 0°C and sodium cyanoborohydride (0.38 g, 6.0 mmol) was added portion-wise. The reaction mixture was stirred at RT for 12 h. The progression of the reaction was monitored by TLC. The reaction mixture was quenched with aqueous saturated NaHCO₃ (10 mL) solution and the product was extracted with DCM (40 mL x3). The combined organic layer was dried over Na₂SO₄ and concentrated under vacuum to afford a residue ethyl 1-(6-(2-fluoroethoxy) pyridin-3-yl)-4-(((4-methoxybenzyl) amino) methyl)-1H-pyrazole-3-carboxylate (1.3 g, Crude) mixture of CO₂Et and CO₂Me esters) which was used in the next step without purification.

¹H NMR (DMSO-*d*₆) δ 8.65 (t, 1H), 8.48 (d, 1H), 8.21 (td, 1H), 7.27 (m, 2H), 7.06 (d, 1H), 6.88 (dd, 3H), 4.82 (t, 1H), 4.73 (t, 1H), 4.59 (t, 1H), 4.53 (t, 1H), 4.29 (q, 3H), 3.82 (s, 2H), 3.79 (s, 2H), 3.73 (s, 4H), 3.70 (d, 1H), 3.68 (s, 2H), 1.29 (t, 1H).

MS (ESI): 429.33 [M+H]⁺

Step 7: Compound ethyl 1-(6-(2-fluoroethoxy) pyridin-3-yl)-4-(((4-methoxybenzyl) amino) methyl)-1H-pyrazole-3-carboxylate (0.5 g, 1.2 mmol) was dissolved in THF (15 mL) and 2.0 mL of aqueous solution KOH (0.2 g, 3.5 mmol) was added. The reaction mixture was allowed to stir at RT for 12 h. The progression of the reaction was monitored by TLC. Then, the mixture was cooled to 0°C and treated with 2M HCl (aq) solution until the pH reaches up to 3-4. The biphasic mixture was stirred for 5 min and the layers were separated. The aqueous layer was further extracted with 10% MeOH in

DCM (3 x 20 mL) and the combined organic layers were dried over Na₂SO₄ and concentrated under vacuum to afford 1-(6-(2-fluoroethoxy) pyridin-3-yl)-4-(((4-methoxybenzyl) amino) methyl)-1H-pyrazole-3-carboxylic acid as a yellow solid (330 mg, 70%); which was directly used in the next step
¹H NMR (DMSO-*d*₆) δ 10.97 (s, 1H), 8.60 (d, 1H), 8.52 (s, 1H), 8.16 (dd, 1H), 7.40 (d, 2H), 7.06 (d, 1H), 6.98 (d, 2H), 4.82 (t, 1H), 4.72 (t, 1H), 4.59 (t, 1H), 4.53 (t, 1H), 4.06 (d, 4H), 3.77 (s, 3H).
MS (ESI): 401.29 [M+H]⁺

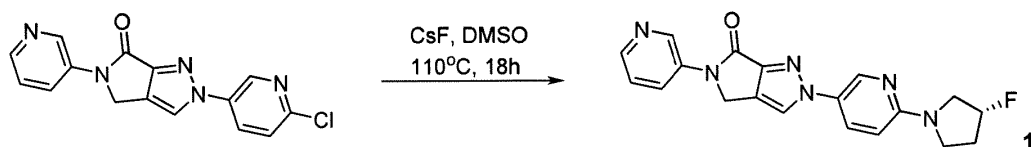
Step 8: Compound 1-(6-(2-fluoroethoxy)pyridin-3-yl)-4-(((4-methoxybenzyl)amino)methyl)-1H-pyrazole-3-carboxylic acid (0.3 g, 0.75 mmol) was dissolved in acetonitrile (10.5 mL). Then, DMF (3.0 mL) and NMI (0.19 g, 2.3 mmol) were added. The reaction mixture was allowed to stir at RT for 30 min. A clear solution was observed. Then, TCFH (0.25 g, 0.9 mmol) was added and the reaction was stirred at room temperature for 3 days. After completion of the reaction as monitored by TLC, the reaction mixture was quenched with ice cold water (5 mL). The crude reaction mass was filtered through Büchner funnel and the obtained mass was washed by water and MeOH (4:1, 3 mL) to get a product 2-(6-(2-fluoroethoxy)pyridin-3-yl)-5-(4-methoxybenzyl)-4,5-dihydropyrrolo [3,4-*c*]pyrazol-6(2H)-one as yellow solid (240 mg, Crude), which was directly used in next step without further purification.

¹H NMR (DMSO-*d*₆) δ 8.67 (d, 1H), 8.46 (s, 1H), 8.22 (q, 1H), 7.22 (d, 2H), 7.07 (d, 1H), 6.92 (m, 2H), 4.82 (t, 1H), 4.73 (t, 2H), 4.62 (s, 2H), 4.59 (t, 2H), 4.53 (d, 2H), 4.29 (s, 2H), 3.73 (s, 3H), 3.67 (s, 2H).
MS (ESI): 383.22 [M+H]⁺

Step 9: To a solution of intermediate 2-(6-(2-fluoroethoxy)pyridin-3-yl)-5-(4-methoxybenzyl)-4,5-dihydropyrrolo [3,4-*c*]pyrazol-6(2H)-one (0.2 g, 0.52 mmol) in acetonitrile:water (3:1, 16 mL) was added CAN (1.1 g, 4.0 mmol) and the mixture stirred at RT for 3 h. Acetonitrile was removed under vacuum and the reaction mixture was then extracted with ethyl acetate (5 X 10 mL). Organic layer was washed with brine (5 mL), dried over Na₂SO₄ and concentrated to yield the crude product which was recrystallized from methanol to afford product 2-(6-(2-fluoroethoxy) pyridin-3-yl)-4,5-dihydropyrrolo[3,4-*c*]pyrazol-6(2H) -one (45 mg, 27%) as a yellow solid.

¹H NMR (DMSO-*d*₆) δ 8.67 (d, 1H), 8.56 (s, 1H), 8.48 (s, 1H), 8.22 (dd, 1H), 7.07 (d, 1H), 4.83 (t, 1H), 4.73 (t, 1H), 4.60 (t, 2H), 4.54 (t, 1H), 4.31 (s, 2H).
MS (ESI): 263.10 [M+H]⁺

EXAMPLES

Example 1

5

To a solution of 2-(6-chloropyridin-3-yl)-5-(pyridin-3-yl)-4,5-dihydropyrrolo[3,4-c]pyrazol-6(2H)-one (0.3 g, 0.00032 mol) and (R)-3-fluoropyrrolidine as HCl salt (0.23 g, 0.0019 mol) in 3 ml DMSO was added of CsF (0.29 g, 0.0019 mol) and the resulting mixture was heated to 110 °C for 16 h. After monitoring the end of the reaction on TLC, the mixture was cooled to room temperature and diluted with water. The resulting mixture was extracted with ethyl acetate twice. The combined organic layers were washed with water twice, dried over MgSO₄ and the solvent was removed in vacuo to afford a residue (80 mg). The compound was purified by HPLC to obtain 4.2 mg 2-{6-[(3R)-3-fluoropyrrolidin-1-yl]pyridin-3-yl}-5-(pyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-6-one as a beige solid.

10

LC/MS [M+H]⁺ 365.0

15

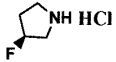
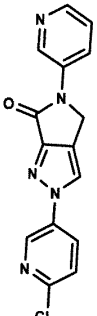
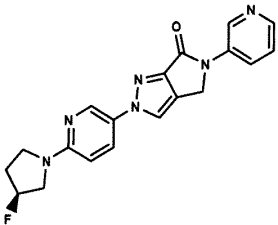
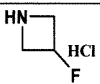
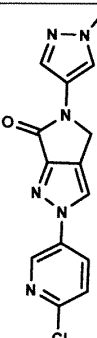
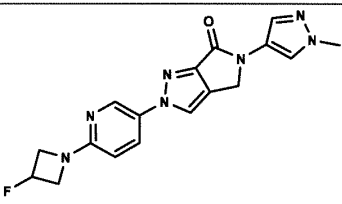
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.05 (d, 1H), 8.60 (d, 1H), 8.50 (s, 1H), 8.37 (dd, 1H), 8.36 – 8.26 (m, 1H), 8.01 (dd, 1H), 7.46 (dd, 1H), 6.67 (d, 1H), 5.46 (d, 1H), 5.00 (s, 2H), 3.87 – 3.58 (m, 3H), 3.47 (m, 1H), 2.35 – 2.08 (m, 2H).

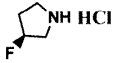
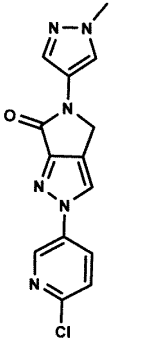
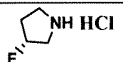
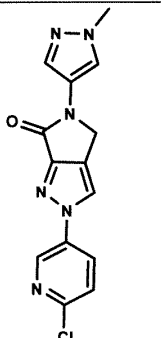
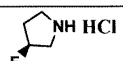
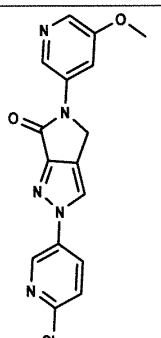
Examples 2 to 16

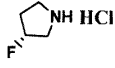
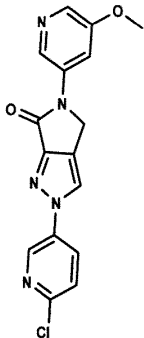
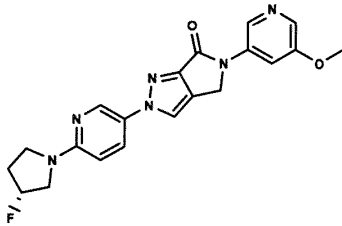
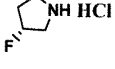
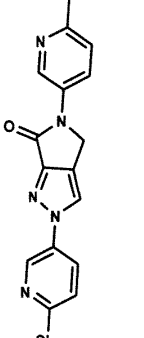
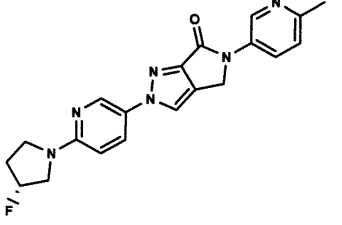
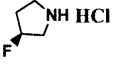
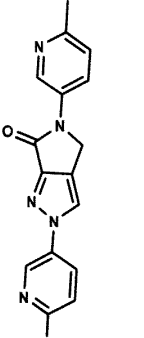
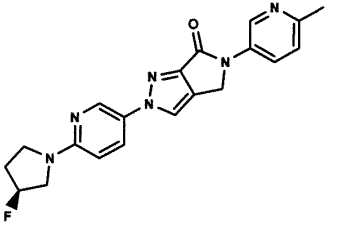
20

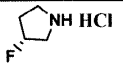
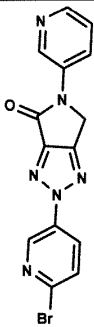
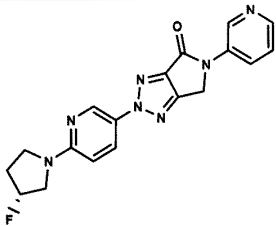
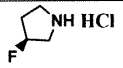
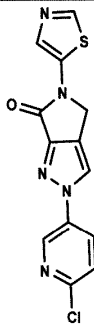
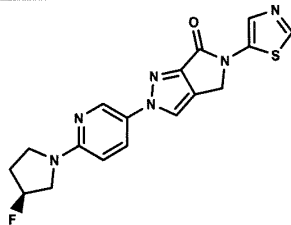
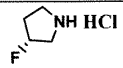
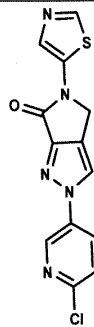
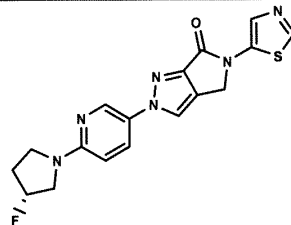
Following the S_NAr reaction procedure as described in **Example 1**, using the halogenated heteroaryl and the amine starting material indicated in Table 1 below, the following compounds were prepared.

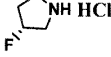
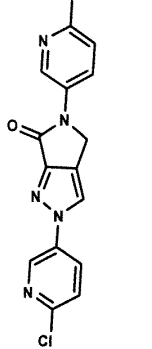
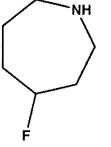
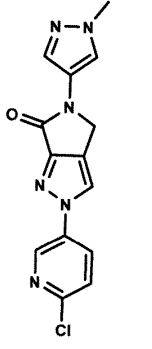
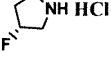
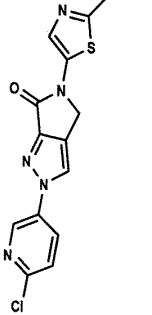
Table 1:

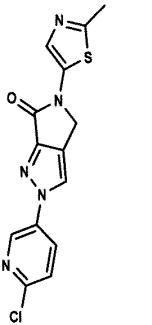
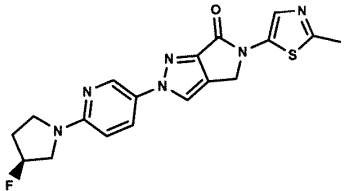
Amine	halogenated heteroaryl	Compound of example	1. Yield 2. ¹ H-NMR 3. MH ⁺ (ESI)
		 2 2-{6-[(3S)-3-fluoropyrrolidin-1-yl]pyridin-3-yl}-5-(pyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-6-one	1. 34 % 2. ¹ H NMR (600 MHz, DMSO- <i>d</i> ₆) δ 9.05 (d, 1H), 8.60 (d, 1H), 8.50 (s, 1H), 8.37 (dd, 1H), 8.36 – 8.26 (m, 1H), 8.01 (dd, 1H), 7.46 (dd, 1H), 6.67 (d, 1H), 5.46 (d, 1H), 5.00 (s, 2H), 3.87 – 3.58 (m, 3H), 3.47 (m, 1H), 2.35 – 2.08 (m, 2H). 3. 365.2
		 3 2-(6-(3-fluoroazetidin-1-yl)pyridin-3-yl)-5-(1-methyl-1H-pyrazol-4-yl)-4,5-dihydropyrrolo[3,4-c]pyrazol-6-one	1. 51 % 2. ¹ H NMR (600 MHz, DMSO- <i>d</i> ₆) δ 8.58 (d, 1H), 8.46 (s, 1H), 8.12 (s, 1H), 8.03 (dd, 1H), 7.70 (s, 1H), 6.61 (d, 1H), 5.51 (m, 1H), 4.75 (s, 2H), 4.33 (m, 2H), 4.06 (m, 2H), 3.84 (s, 3H). 3. 354.2

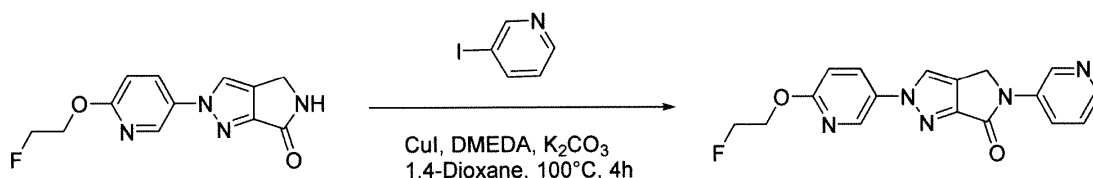
		4 2-{6-[(3S)-3-fluoropyrrolidin-1-yl]pyridin-3-yl}-5-(1-methyl-1H-pyrazol-4-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-6-one	1. 94 % 2. ¹H NMR (600 MHz, DMSO-<i>d</i>₆) δ 8.57 (d, 1H), 8.43 (s, 1H), 8.12 (s, 1H), 7.99 (dd, 1H), 7.70 (s, 1H), 6.65 (d, 1H), 5.45 (dt, 1H), 4.75 (s, 2H), 3.84 (s, 3H), 3.81 – 3.56 (m, 3H), 3.52 – 3.41 (m, 1H), 2.33 – 2.07 (m, 2H). 3. 368.2
		5 2-{6-[(3R)-3-fluoropyrrolidin-1-yl]pyridin-3-yl}-5-(1-methyl-1H-pyrazol-4-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-6-one	1. 17 % 2. ¹H NMR (600 MHz, DMSO-<i>d</i>₆) δ 8.57 (d, 1H), 8.43 (s, 1H), 8.12 (s, 1H), 7.99 (dd, 1H), 7.70 (s, 1H), 6.65 (d, 1H), 5.45 (dt, 1H), 4.74 (s, 2H), 3.84 (s, 3H), 3.82 – 3.55 (m, 3H), 3.46 (td, 1H), 2.34 – 2.07 (m, 2H). 3. 368.2
		6 2-{6-[(3S)-3-fluoropyrrolidin-1-yl]pyridin-3-yl}-5-(5-methoxypyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-6-one	1. 75 % 2. ¹H NMR (400 MHz, DMSO-<i>d</i>₆) δ 8.71 (s, 1H), 8.60 (s, 1H), 8.52 (s, 1H), 8.16 (s, 1H), 8.07 – 7.93 (m, 2H), 6.69 (d, 1H), 5.47 (d, 1H), 5.01 (s, 2H), 3.88 (s, 3H), 3.87 – 3.50 (m, 3H), 2.28 (m, 2H). 3. 395.4

		 7 2-{6-[(3R)-3-fluoropyrrolidin-1-yl]pyridin-3-yl}-5-(5-methoxypyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-6-one	1. 58 % 2. ¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.68 (s, 1H), 8.61 (s, 1H), 8.52 (s, 1H), 8.14 (s, 1H), 8.03 (d, 1H), 7.96 (s, 1H), 6.67 (d, 1H), 5.48 (d, 1H), 5.01 (s, 2H), 3.88 (s, 3H), 3.86 – 3.56 (m, 2H), 3.49 (m, 1H), 2.31 (m, 2H). 3. 395.2
		 8 2-{6-[(3R)-3-fluoropyrrolidin-1-yl]pyridin-3-yl}-5-(6-methylpyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-6-one	1. 58 % 2. ¹ H NMR (600 MHz, DMSO- <i>d</i> ₆) δ 8.90 (d, 1H), 8.61 (d, 1H), 8.49 (s, 1H), 8.17 (dd, 1H), 8.02 (dd, 1H), 7.33 (d, 1H), 6.68 (d, 1H), 5.56 – 5.40 (m, 1H), 4.97 (s, 2H), 3.83 – 3.51 (m, 4H), 2.29 – 2.19 (m, 2H). 3. 379.0
		 9 2-{6-[(3S)-3-fluoropyrrolidin-1-yl]pyridin-3-yl}-5-(6-methylpyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-6-one	1. 7 % 2. ¹ H NMR (600 MHz, DMSO- <i>d</i> ₆) δ 8.90 (d, 1H), 8.61 (d, 1H), 8.49 (s, 1H), 8.17 (dd, 1H), 8.02 (dd, 1H), 7.33 (d, 1H), 6.68 (d, 1H), 5.56 – 5.40 (m, 1H), 4.97 (s, 2H), 3.83 – 3.51 (m, 4H), 2.29 – 2.19 (m, 2H). 3. 379.2

		 10 2-{6-[(3R)-3-fluoropyrrolidin-1-yl]pyridin-3-yl}-5-(pyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-d][1,2,3]triazol-4-one	1. 6 % 2. ¹H NMR (600 MHz, DMSO-<i>d</i>₆) δ 9.05 (d, 1H), 8.80 (d, 1H), 8.45 – 8.38 (m, 1H), 8.27 (d, 1H), 8.19 (dd, 1H), 7.49 (dd, 1H), 6.72 (d, 1H), 5.47 (d, 1H), 5.24 (s, 2H), 3.90 – 3.39 (m, 4H), 2.33 – 2.07 (m, 2H). 3. 366.2
		 11 2-{6-[(3S)-3-fluoropyrrolidin-1-yl]pyridin-3-yl}-5-(1,3-thiazol-5-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-6-one	1. ND 2. ¹H NMR (600 MHz, DMSO-<i>d</i>₆) δ 8.90 (d, 1H), 8.61 (d, 1H), 8.49 (s, 1H), 8.17 (dd, 1H), 8.02 (dd, 1H), 7.33 (d, 1H), 6.68 (d, 1H), 5.56 – 5.40 (m, 1H), 4.97 (s, 2H), 3.83 – 3.51 (m, 4H), 2.29 – 2.19 (m, 2H). 3. 371.2
		 12 2-{6-[(3R)-3-fluoropyrrolidin-1-yl]pyridin-3-yl}-5-(1,3-thiazol-5-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-6-one	1. ND 2. ¹H NMR (600 MHz, DMSO-<i>d</i>₆) δ 8.73 (s, 1H), 8.60 (d, 1H), 8.52 (s, 1H), 8.01 (dd, 1H), 7.80 (s, 1H), 6.66 (d, 1H), 5.46 (d, 1H), 5.00 (s, 2H), 3.86 – 3.58 (m, 3H), 3.47 (td, 1H), 2.32 – 2.08 (m, 2H). 3. 371.2

		13 2-{6-[(3S)-3-fluoropyrrolidin-1-yl]pyridin-3-yl}-5-(6-methylpyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-6-one hydrochloride	1. 7 % 2. ¹H NMR (600 MHz, DMSO-d₆) δ 8.90 (d, 1H), 8.61 (d, 1H), 8.49 (s, 1H), 8.17 (dd, 1H), 8.02 (dd, 1H), 7.33 (d, 1H), 6.68 (d, 1H), 5.56 – 5.40 (m, 1H), 4.97 (s, 2H), 3.83 – 3.51 (m, 4H), 2.29 – 2.19 (m, 2H). 3. 379.2
		14 2-(6-(4-fluoroazepan-1-yl)pyridin-3-yl)-5-(1-methyl-1H-pyrazol-4-yl)-4,5-dihydropyrrolo[3,4-c]pyrazol-6(2H)-one hydrochloride	1. NA 2. NA 3. 396.2
		15 (R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-5-(2-methylthiazol-5-yl)-4,5-dihydropyrrolo[3,4-c]pyrazol-6(2H)-one	1. 77% 2. ¹H NMR (500 MHz, DMSO-d₆) δ 8.61 (d, 1H), 8.53 (s, 1H), 8.03 (dd, 1H), 7.52 (s, 1H), 6.68 (d, 1H), 5.47 (dd, 1H), 4.97 (s, 2H), 3.71 (m, 3H), 3.48 (td, 1H), 2.60 (s, 3H), 2.24 (m, 2H). 3. 385.0

		 16 (S)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-5-(2-methylthiazol-5-yl)-4,5-dihydropyrrolo[3,4-c]pyrazol-6(2H)-one	1. 40% 2. ¹ H NMR (500 MHz, DMSO-d ₆) δ 8.61 (d, 1H), 8.53 (s, 1H), 8.03 (dd, 1H), 7.52 (s, 1H), 6.68 (d, 1H), 5.48 (m, 1H), 4.97 (s, 2H), 3.71 (m, 3H), 3.48 (td, 1H), 2.60 (s, 3H), 2.23 (m, 2H). 3. 385.0
---	---	---	---

Example 17

5

17

Step 10: An oven-dried screw capped vial was charged with 2-(6-(2-fluoroethoxy) pyridin-3-yl)-4,5-dihydropyrrolo[3,4-c]pyrazol-6(2H)-one (40 mg, 0.15 mmol), 3-iodopyridine (62 mg, 0.3 mmol), K₂CO₃ (42 mg, 0.3 mmol), DMEDA (5.0 mg, 0.06 mmol), CuI (5.0 mg, 0.03 mmol) and 1,4-dioxane (2.0 mL) under argon. Then, the mixture was degassed with argon for 15 min and allowed to heat to 100°C for 12 h. The progression of the reaction was monitored by TLC. After that the reaction mixture was quenched with water (3 mL) and the product was extracted with DCM (10 mL x3). The extract was dried over Na₂SO₄, concentrated and purified by chromatography over silica gel (60-120 mesh) eluted in 3% MeOH in DCM to afford to afford 2-(6-(2-fluoroethoxy) pyridin-3-yl)-5-(pyridin-3-yl)-4,5-dihydropyrrolo [3,4-c]pyrazol-6(2H)-one as a yellow solid (16 mg, 31%).

¹H NMR (DMSO-D₆) δ 9.07 (d, 1H), 8.73 (dd, 1H), 8.66 (s, 1H), 8.40 (dd, 1H), 8.30 (m, 2H), 7.49 (m, 1H), 7.11 (dd, 1H), 5.05 (s, 2H), 4.85 (m, 1H), 4.73 (m, 1H), 4.62 (m, 1H), 4.54 (m, 1H).

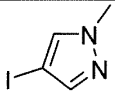
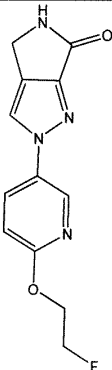
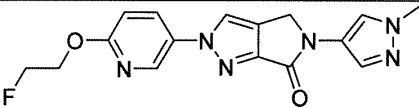
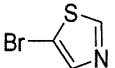
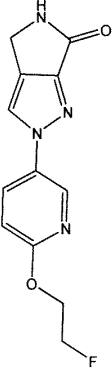
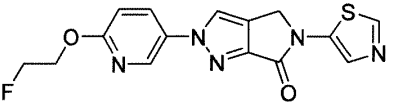
LCMS: 340.25 [M+H]⁺

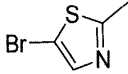
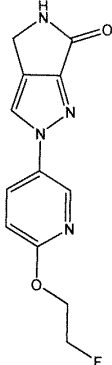
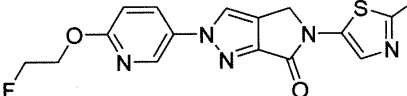
20

Examples 18 to 20

Following the coupling reaction procedure as described in **Example 17**, using the halogenated heteroaryl and the amide starting material indicated in Table 2 below, the following compounds were prepared.

Table 2:

halogenated heteroaryl	amide	Compound of example	1. Yield 2. ¹ H-NMR 3. MH ⁺ (ESI)
		 18 2-(6-(2-fluoroethoxy)pyridin-3-yl)-5-(1-methyl-1H-pyrazol-4-yl)-4,5-dihydropyrrolo[3,4-c]pyrazol-6(2H)-one	1. 33 % 2. ¹H NMR (500 MHz, DMSO-<i>d</i>₆) δ 8.71 (d, 1H), 8.60 (s, 1H), 8.26 (dd, 1H), 8.15 (s, 1H), 7.73 (s, 1H), 7.09 (d, 1H), 4.83 (t, 1H), 4.80 (s, 2H), 4.74 (dd, 1H), 4.60 (t, 1H), 4.54 (t, 1H), 3.86 (s, 3H). 3. 342.9
		 19 2-(6-(2-fluoroethoxy)pyridin-3-yl)-5-(thiazol-5-yl)-4,5-dihydropyrrolo [3,4-c]pyrazol-6(2H)-one	1. 34 % 2. ¹H NMR (500 MHz, DMSO-<i>d</i>₆) δ 8.76 (s, 1H), 8.73 (d, 1H), 8.68 (s, 1H), 8.28 (dd, 1H), 7.83 (s, 1H), 7.10 (d, 1H), 5.05 (s, 2H), 4.85 (t, 1H), 4.73 (t, 1H), 4.62 (t, 1H), 4.54 (t, 1H). 3. 345.1

		 20 2-(6-(2-fluoroethoxy) pyridin-3-yl)-5-(2-methylthiazol-5-yl)-4,5-dihydro pyrrolo[3,4-c]pyrazol-6(2H)-one	1. 22 % 2. ¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ 8.73 (d, 1H), 8.66 (s, 1H), 8.27 (dd, 1H), 7.53 (s, 1H), 7.10 (d, 1H), 4.99 (s, 2H), 4.84 (m, 1H), 4.73 (m, 1H), 4.62 (m, 1H), 4.54 (m, 1H), 2.61 (s, 3H). 3. 360.4
---	---	---	---

BIOLOGICAL ASSAY DESCRIPTION AND CORRESPONDING RESULTS

1. Preparation of human Parkinson's disease (PD) brain-derived alpha-synuclein (a-syn)

5 aggregates

The procedure was adapted from the protocol described in Spillantini et al., 1998. Frozen tissue blocks from PD donors were thawed on ice and homogenized using a glass dounce homogenizer. The homogenate was then centrifuged at 11,000 x g (12,700 RPM) in an ultracentrifuge (Beckman, XL100K) for 20 minutes at 4°C using a pre-cooled 70.1 rotor (Beckman, 342184). Pellets were resuspended in extraction buffer [10 mM Tris-HCl pH 7.4, 10% sucrose, 0.85 mM NaCl, 1% protease inhibitor (Calbiochem 539131), 1 mM EGTA, 1% phosphatase inhibitor (Sigma P5726 and P0044)] and centrifuged at 15,000 x g (14,800 RPM, a 70.1 Ti rotor) for 20 minutes at 4°C. Pellets were discarded and sarkosyl (20% stock solution, Sigma L7414) was added to the supernatants to a final concentration of 1% at room temperature for one hour. This solution was then centrifuged at 100,000 x g (38,000 RPM, 70.1 Ti rotor) for one hour at 4°C. Pellets containing enriched alpha-synuclein aggregates were resuspended in PBS and stored at -80°C until use.

2. Micro-radiobinding competition assay for the determination of binding affinity

PD brain-derived alpha-synuclein aggregates were spotted onto microarray slides. The slides were incubated with tritiated reference alpha-synuclein ligand at 6nM, 20nM or 30nM and the example compounds (non-radiolabelled) at 1μM and 100nM. In some cases, the non-radiolabelled example compounds were further assessed for a range of different concentrations, varying from 0.05nM to 2μM. After incubation, slides were washed and scanned by a real-time autoradiography system (BeaQuant, ai4R). Quantification of the signal was performed by using the Beamage image analysis

software (ai4R). Non-specific signal was determined with an excess of non-radiolabelled reference alpha-synuclein ligand (2 μ M) and specific binding was calculated by subtracting the non-specific signal from the total signal. Competition was calculated as percent, where 0% was defined as the specific binding in the presence of vehicle and 100% as the values obtained in the presence of excess of the non-radiolabelled reference alpha-synuclein ligand. K_i values were calculated in GraphPad Prism7 by applying a nonlinear regression curve fit using a one site, specific binding model. All measurements were performed with at least two technical replicates. For compounds tested in more than one experiment, the mean of the replicates or K_i values in independent experiments is reported.

- 10 Results: Example compounds were assessed for their potency to compete with the binding of a tritiated reference alpha-synuclein ligand to PD patient brain-derived alpha-synuclein aggregates. Results of the micro-radiobinding competition assay for the example compounds tested are shown in Table 2 as: % competition at 1 μ M and 100nM. The Table 2 also shows K_i values.

15 **Table 2**

Example Compound no.	Micro-radiobinding competition assay		
	Competition at 1 μ M (%)	Competition at 100nM (%)	K_i (nM)
1	86	58	84
2	80	34	115
3	89	38	54
4	75	28	
5	101	74	
6	60	26	
7	52	10	
8	71	37	226
9	87	37	
10	97	74	30
11	90	41	
12	94	64	52
13	87	37	

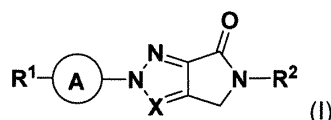
14	66	0	
15	92	71	
16	90	69	40
17	63	31	214
18	81	54	78
19	78	46	86
20	86	55	

Table 2: Assessment of binding affinity by micro-radiobinding competition assay on human PD brain-derived alpha-synuclein aggregates. Percent (%) competition over the tritiated reference alpha-synuclein ligand in the presence of 1 μ M and 100nM of example compounds 1-12. K_i values are also shown for selected example compounds. As shown in Table 2, example compounds 1-12 of the present invention show potent binding to PD brain-derived alpha-synuclein aggregates.

5

CLAIMS

1. A compound of formula (I):



10

or a detectably labelled compound, stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

(A) is a 6-membered heteroaryl;

X is CH or N;

R¹ is a 4- to 7-membered heterocyclyl which is optionally substituted with at least one halo; or

15

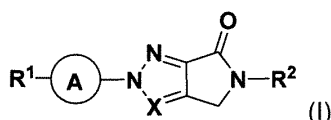
R¹ is -NH₂; -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl); wherein the C₁-C₄alkyl is optionally substituted with at least one halo or wherein at least one H which is attached to N in -NH₂ or -NH(C₁-C₄alkyl) is replaced by halo, or

R¹ is haloC₁-C₄alkoxy; and

20

R² is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl.

2. A compound of formula (I) according to claim 1.



or a detectably labelled compound, stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

25

(A) is a 6-membered heteroaryl;

X is CH or N;

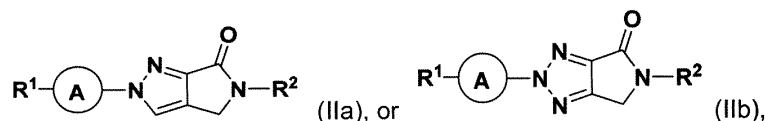
R¹ is a 4- to 6-membered heterocyclyl which is optionally substituted with at least one halo; or

R¹ is -NH₂; -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl), wherein the C₁-C₄alkyl is optionally substituted with at least one halo, and

30

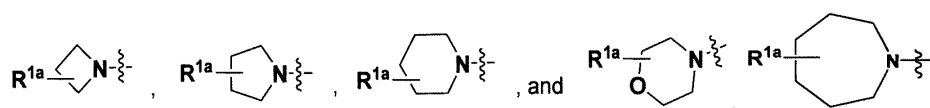
R² is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl.

3. The compound according to claim 1 or 2, having a formula (IIa), or (IIb):



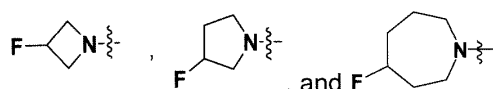
or a detectably labelled compound, stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof.

4. The compound according to any one of claims 1 to 3, wherein R^1 is a 4- to 7-membered heterocyclyl which is optionally substituted with at least one halo.
5. The compound according to claim 4, wherein R^1 is a 4- to 7-membered heterocyclyl selected from the following:

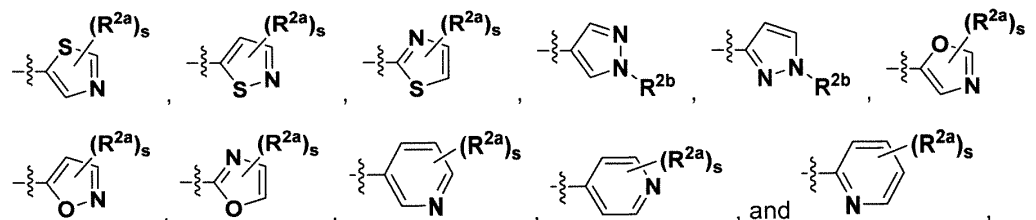


wherein R^{1a} is H or halo, preferably halo.

6. The compound according to claims 4 or 5, wherein R^1 is a 4- or 5- or 7 membered heterocyclyl selected from the following:



7. The compound according to claim 1, wherein R^1 is F- C_1 - C_4 alkoxy.
8. The compound according to any one of claims 1 to 7, wherein R^2 is a 5-membered or 6 membered heteroaryl selected from the following:

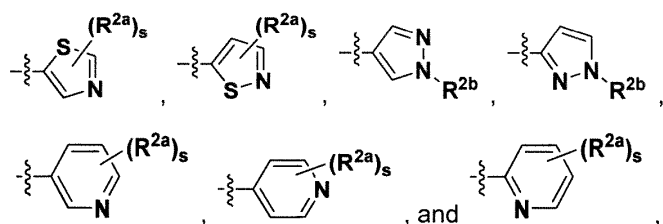


wherein

- R^{2a} is independently selected from -halo, -OH, -CN, - $N(C_1-C_4alkyl)_2$, - $NH(C_1-C_4alkyl)$, - $N(haloC_1-C_4alkyl)_2$, - $NH(haloC_1-C_4alkyl)$, halo C_1-C_4alkyl , halo $C_1-C_4alkoxy$, - $C_1-C_4alkoxy$, and - C_1-C_4alkyl ;

R^{2b} is selected from -H, halo C_1-C_4alkyl , halo $C_1-C_4alkoxy$, - $C_1-C_4alkoxy$, and - C_1-C_4alkyl ; and s is 0, 1 or 2.

9. The compound according to claim 8, wherein R^2 is a 5-membered or 6-membered heteroaryl selected from the following:



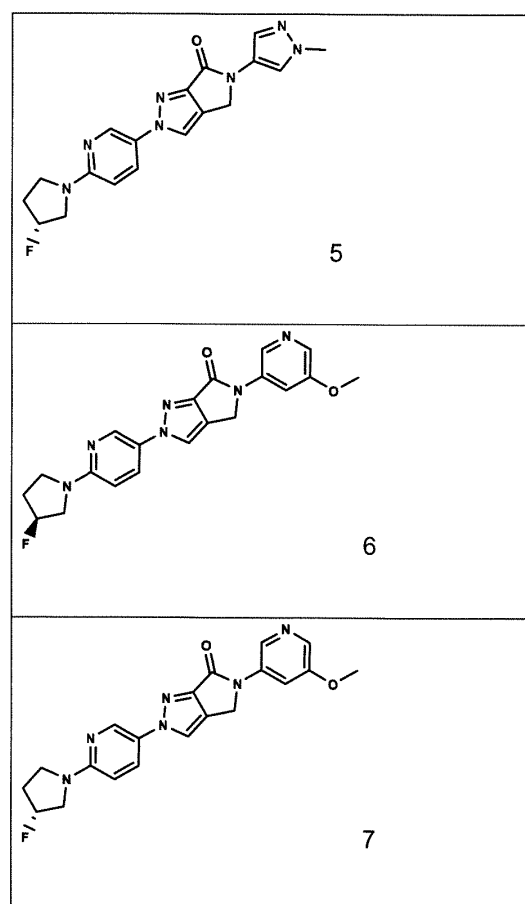
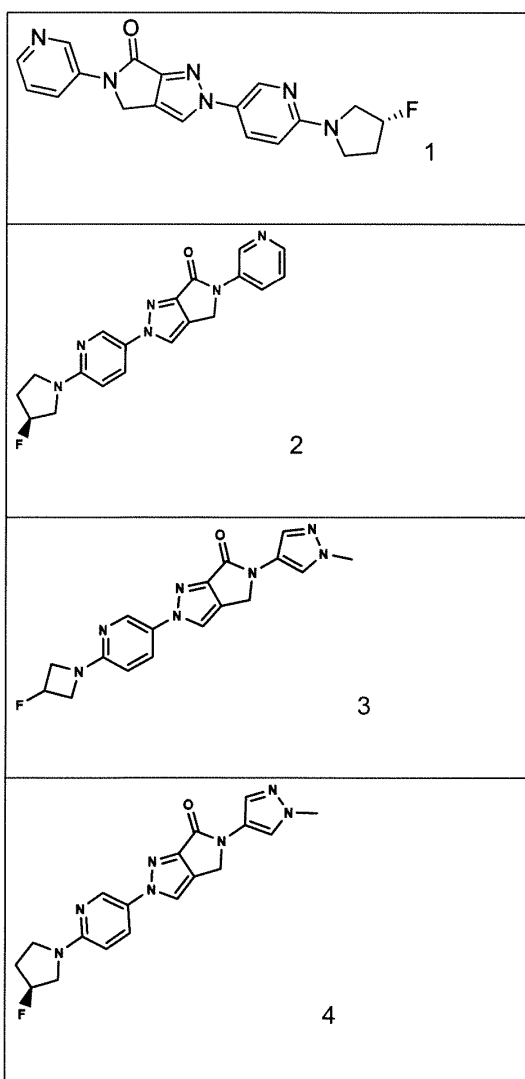
wherein

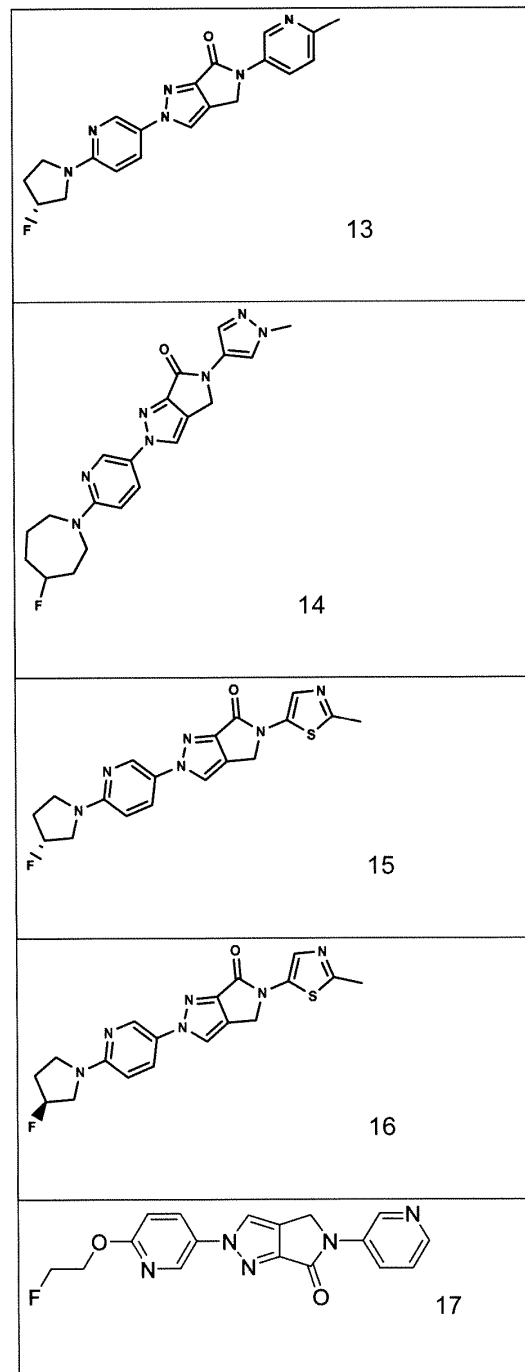
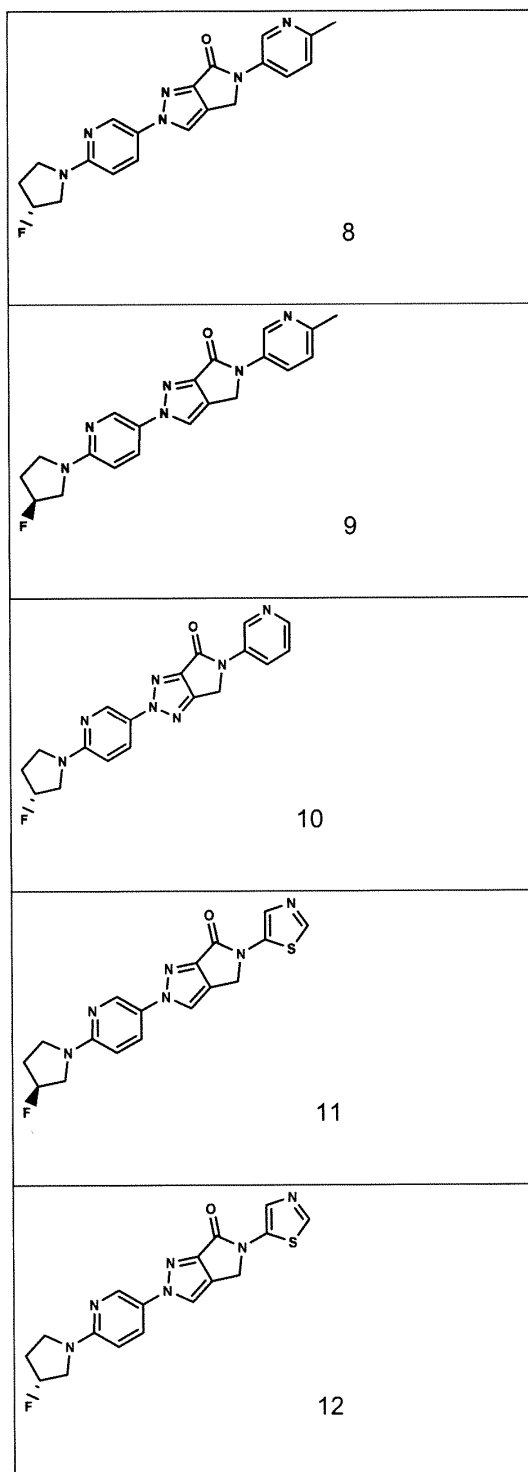
R^{2a} is independently selected from, $-C_1-C_4$ alkoxy, and $-C_1-C_4$ alkyl;

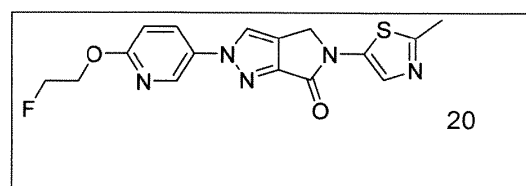
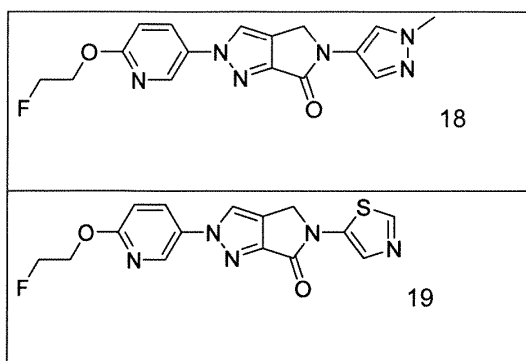
5 R^{2b} is selected from $-H$, and $-C_1-C_4$ alkyl; and

s is 0, 1 or 2.

10. The compound according to claim 1 wherein the compound is selected from:







or a detectably labelled compound, stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof.

11. The compound according to any one of the preceding claims, wherein the compound is a detectably labelled compound.
12. The compound according to claim 11, wherein the detectably labelled compound comprises a detectable label selected from a radioisotope, preferably ^2H , ^3H or ^{18}F .
13. A diagnostic composition comprising a compound according to any one of claims 1 to 12, and optionally at least one pharmaceutically acceptable excipient, carrier, diluent and/or adjuvant.
14. The compound according to claim 11 or 12, or the diagnostic composition according to claim 13, for use in the imaging of alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites.
15. The compound according to claim 11 or 12, or the diagnostic composition according to claim 13, for use in positron emission tomography imaging of alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites.
16. The compound for use or the diagnostic composition for use according to claim 14 or 15, wherein the use is for *in vitro* imaging, *ex vivo* imaging, or *in vivo* imaging, preferably the use is for *in vivo* imaging, more preferably the use is for brain imaging.
17. The compound according to claim 11 or 12, or the diagnostic composition according to claim 13, for use in diagnostics.
18. The compound for use or the diagnostic composition for use according to claim 17, wherein the diagnostics are the diagnostics of a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites or a predisposition therefor, wherein the disease, disorder or abnormality is optionally selected from Parkinson's disease (including sporadic, familial with alpha-synuclein mutations, familial with mutations other than alpha-synuclein, pure autonomic failure or Lewy body dysphagia), SNCA duplication carrier, Lewy Body dementia (LBD), dementia with Lewy bodies (DLB) (including "pure" Lewy body dementia), Parkinson's disease dementia (PDD), diffuse Lewy body disease

- (DLBD), Alzheimer's disease, sporadic Alzheimer's disease, familial Alzheimer's disease with APP mutations, familial Alzheimer's disease with PS-1, PS-2 or other mutations, familial British dementia, Lewy body variant of Alzheimer's disease, Down syndrome, multiple system atrophy (MSA) (including Shy-Drager syndrome, striatonigral degeneration or olivopontocerebellar atrophy), traumatic brain injury, chronic traumatic encephalopathy, dementia puglistica, tauopathies (including Pick's disease, frontotemporal dementia, progressive supranuclear palsy, corticobasal degeneration, Niemann-Pick type C1 disease, frontotemporal dementia with Parkinsonism linked to chromosome 17), Creutzfeldt-Jakob disease, Huntington's disease, motor neuron disease, amyotrophic lateral sclerosis (including sporadic, familial or ALS-dementia complex of Guam), neuroaxonal dystrophy, neurodegeneration with brain iron accumulation type 1 (including Hallervorden-Spatz syndrome), prion diseases, ataxia telangiectatica, Meige's syndrome, subacute sclerosing panencephalitis, Gerstmann-Straussler-Scheinker disease, inclusion-body myositis, Gaucher disease, Krabbe disease as well as other lysosomal storage disorders (including Kufor-Rakeb syndrome and Sanfilippo syndrome) and rapid eye movement (REM) sleep behavior disorder.
19. The compound for use or the diagnostic composition for use according to claim 18, wherein the disease is Parkinson's disease.
20. The compound for use or the diagnostic composition for use according to claim 18, wherein the disease is multiple system atrophy.
21. The compound for use or the diagnostic composition for use according to claim 18, wherein the disease is dementia with Lewy bodies.
22. The compound for use or the diagnostic composition for use according to claim 18, wherein the disease is Parkinson's disease dementia.
23. The compound for use or the diagnostic composition for use according to claim 18, wherein the disease is SNCA duplication carrier.
24. The compound for use or the diagnostic composition for use according to claim 18, wherein the disease is Alzheimer's disease.
25. The compound for use or the diagnostic composition for use according to any one of claims 14 to 24, wherein the use is in a human.
26. A method of diagnosing a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites, in a subject, the method comprising the steps:
- (a) Administering a compound according to any one of claims 1 to 12, or a diagnostic composition according to claim 13 which comprises a compound according to any one of claims 1 to 12, to the subject;

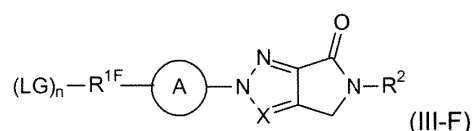
- (b) Allowing the compound to bind to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites; and
 - (c) Detecting the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites.
- 5 27. A method of diagnosing according to claim 26, the method further comprising the step of:
 - (d) Generating an image representative of the location and/or amount of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites.
- 10 28. A method of positron emission tomography (PET) imaging of alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites, in a tissue of a subject, the method comprising the steps:
 - (a) Administering a compound according to any one of claims 1 to 12, or a diagnostic composition according to claim 13 which comprises a compound according to any one of claims 1 to 12, to the subject;
 - 15 (b) Allowing the compound to bind to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites; and
 - (c) Detecting the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites by collecting a positron emission tomography (PET) image of the tissue of the subject.
- 20 29. The method of positron emission tomography (PET) imaging of the alpha-synuclein aggregates, including, but not limited to, Lewy bodies and/or Lewy neurites in a tissue of a subject according to claim 28, wherein the tissue is a tissue of the central nervous system (CNS), an eye tissue, tissue of a peripheral organ, or a brain tissue, preferably wherein the tissue is brain tissue.
- 25 30. A method for the detection and optionally quantification of alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites, in a tissue of a subject, the method comprising the steps:
 - (a) Bringing a sample or a specific body part or body area suspected to contain an alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites, into
30 contact with a compound according to any one of claims 1 to 12, or a diagnostic composition according to claim 13 which comprises a compound according to any one of claims 1 to 12;
 - (b) Allowing the compound to bind to the alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites;
 - 35 (c) Detecting the compound bound to the alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites using positron emission tomography; and

- (d) Optionally quantifying the amount of the compound bound to the alpha-synuclein aggregates, including but not limited to, Lewy bodies and/or Lewy neurites.
31. A method of collecting data for the diagnosis of a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites, the method comprising the steps:
- 5
- (a) Bringing a sample or a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with a compound according to any one of claims 1 to 12, or a diagnostic composition according to claim 13 which comprises a compound according to any one of
- 10
- claims 1 to 12;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites; and
- 15
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence or absence of the alpha-synuclein aggregates including, but not limited to,
32. A method of collecting data for determining a predisposition to a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy
- 20
- bodies and/or Lewy neurites, the method comprising the steps:
- (a) Bringing a sample or a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with a compound according to any one of claims 1 to 12, or a diagnostic composition according to claim 13 which comprises a compound according to any one of
- 25
- claims 1 to 12;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites; and
- 30
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area.
33. A method of collecting data for prognosing a disease, disorder or abnormality associated with
- 35
- alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites, wherein the method comprises the steps:

- 5 (a) Bringing a sample, a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with a compound according to any one of claims 1 to 12, or a diagnostic composition according to claim 13 which comprises a compound according to any one of claims 1 to 12;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- 10 (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area; and
- (e) Optionally repeating steps (a) to (c) and, if present, optional step (d) at least one time.
- 15 34. A method of collecting data for monitoring the progression of a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in a patient, the method comprising the steps:
- (a) Bringing a sample, a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with the compound according to any one of claims 1 to 12, or a diagnostic composition according to claim 13 which comprises a compound according to any one of claims 1 to 12;
- 20 (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- 25 (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area; and
- 30 (e) Optionally repeating steps (a) to (c) and, if present, optional step (d) at least one time.
- 35 35. A method of collecting data for predicting responsiveness of a patient suffering from a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites to a treatment with a medicament, method comprising the steps:

- 5 (a) Bringing a sample, a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites into contact with a compound according to any one of claims 1 to 12, or a diagnostic composition according to claim 13 which comprises a compound according to any one of claims 1 to 12;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- 10 (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area; and
- (e) Optionally repeating steps (a) to (c) and, if present, optional step (d) at least one time.
- 15 36. The method of any one of claims 31 to 35, wherein the step of optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area; comprises
- 20 – determining the amount of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites;
- correlating the amount of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites with the amount of the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area; and
- 25 – optionally comparing the amount of the compound bound with the alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites in the sample or specific body part or body area to a normal control value in a healthy control subject.

37. A compound of formula (III-F)



or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

(A) is a 6-membered heteroaryl;

X is CH or N;

R^{1F} is a 4- to 7-membered heterocycl; or

R^{1F} is -N(C₁-C₄alkyl)₂, or -NH(C₁-C₄alkyl); or

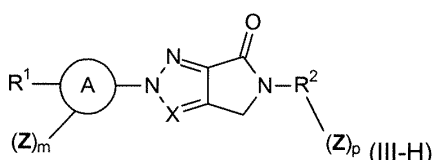
R^{1F} is C₁-C₄alkoxy;

- 5 **R²** is a 5-membered or 6-membered heteroaryl optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;
LG is a leaving group; and

n is at least 1.

- 10 38. The compound of formula (III-F) according to claim 37, wherein **LG** is selected from bromo, chloro, iodo, C₁-C₄ alkylsulfonate and C₆-C₁₀ arylsulfonate, wherein the C₆-C₁₀ arylsulfonate can be optionally substituted with -CH₃ or -NO₂.

39. A compound of formula (III-H)



- 15 or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

A is a 6-membered heteroaryl;

X is CH or N;

R¹ is a 4- to 7-membered heterocycl; which is optionally substituted with at least one halo; or

- 20 **R¹** is -NH₂; -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl); or

R¹ is fluoroC₁-C₄alkoxy;

R² is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from -halo, -OH, -CN, -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, and -C₁-C₄alkyl;

- 25 **m** is 0, 1, or 2;

p is 0, 1, or 2; and

Z is bromo, chloro or iodo;

wherein -N(C₁-C₄alkyl)₂, -NH(C₁-C₄alkyl), -N(haloC₁-C₄alkyl)₂, -NH(haloC₁-C₄alkyl), haloC₁-C₄alkyl, haloC₁-C₄alkoxy, -C₁-C₄alkoxy, or -C₁-C₄alkyl optionally comprise at least one **Z**,

- 30 with the proviso that the compound of formula (III-H) comprises at least one **Z**.

40. A method of preparing the compound according to claim 11 or 12 comprising reacting the compound of formula (III-F) according to claim 37 or 38 with a ^{18}F -fluorinating agent, so that LG is replaced by ^{18}F .
41. The method according to claim 40, wherein the ^{18}F -fluorinating agent is selected from K^{18}F , Rb^{18}F , Cs^{18}F , Na^{18}F , Kryptofix[222] K^{18}F , tetra($\text{C}_{1-6}\text{alkyl}$)ammonium salt of ^{18}F , and tetrabutylammonium [^{18}F]fluoride.
42. A method of preparing the compound according to claim 11 or 12, comprising reacting the compound of formula (III-H) according to claim 39 with a ^3H radiolabeling agent.
43. The compound according to any one of claims 1 to 12, for use as an *in vitro* analytical reference or an *in vitro* screening tool.
44. A test kit for the detection and/or diagnosis of a disease, disorder or abnormality associated with alpha-synuclein aggregates, wherein the test kit comprises at least one compound as defined in any one of claims 1 to 12.
45. A kit for preparing a radiopharmaceutical preparation, wherein the kit comprises a sealed vial containing at least one compound as defined in any one of claims 37 to 39.