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(54) Title: DIHYDROPYRROLO[3,4-C]PYRAZOLE DERIVATIVES AND THEIR USE IN DIAGNOSIS

(57) Abstract: 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 an alpha-synuclein aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions (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.



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

FIELD OF THE INVENTION

5 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)
10 aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions (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
15 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

20 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 A β).

25 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
30 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), Lewy Body dementia (LBD), dementia with Lewy bodies (DLB) ("pure" Lewy body dementia), Parkinson's disease dementia (PDD), Parkinson's disease with mild-cognitive impairment (PD-MCI), 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-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 pugilistica, 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 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 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; 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. 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 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 posttranslational 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.

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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 familiar 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

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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).

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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

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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 (¹²³I) (trade name DaTSCAN) and iometopane (Dopascan) for SPECT or fluorodeoxyglucose (¹⁸F) (¹⁸F-FDG) and dihydrotetrabenazine (¹¹C) (¹¹C-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).

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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 hypocrotin (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 and WO 2019/234243 refer to bicyclic compounds for diagnosing alpha-synuclein aggregates.

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

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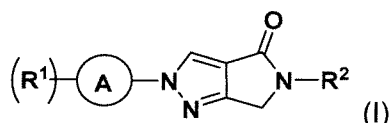
SUMMARY OF THE INVENTION

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,
30 Lewy neurites and/or cytoplasmic glial inclusions (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,
35 disorder or abnormality to the treatment with a certain medicament. Furthermore, the compounds

should be suitable for imaging a disease, disorder or abnormality associated with alpha-synuclein aggregates and / or detecting and optionally quantifying alpha-synuclein aggregates.

Various embodiments of the invention are described herein.

- 5 Within a certain aspect, provided herein is 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 comprising at least 2 heteroatoms;

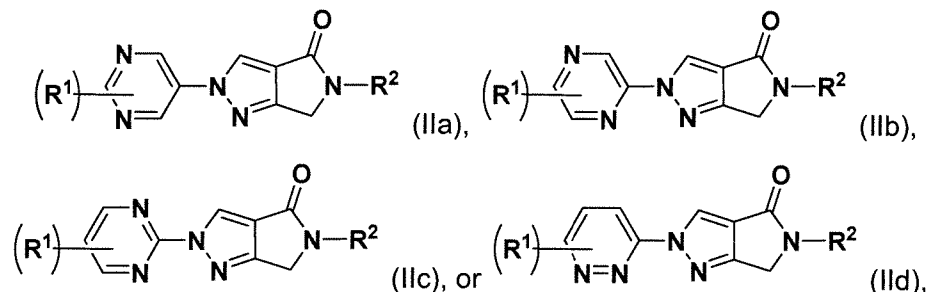
- 10 **R¹** is C₁-C₄alkyl, haloC₁-C₄alkyl, -NH₂; -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl), wherein the C₁-C₄alkyl is optionally substituted with at least one halo; or

R¹ is a heterocyclyl which 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 haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkoxy, and C₁-C₄alkyl.

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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.

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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, Lewy neurites and/or cytoplasmic glial inclusions. 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, Lewy neurites and/or cytoplasmic glial inclusions. In another aspect, the 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.

5 In yet another aspect, the compound of formula (I) or the diagnostic composition, as defined herein, can be use in diagnostics.

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

- (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, Lewy neurites and/or cytoplasmic glial inclusions; and
- 15 (c) Detecting the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions.

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, Lewy neurites
20 and/or cytoplasmic glial inclusions, in a tissue of a subject, the method comprising the steps:

- (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, Lewy neurites and/or cytoplasmic glial inclusions; and
- 25 (c) Detecting the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions by collecting a positron emission tomography (PET) image of the tissue of the subject.

In a further aspect, the present invention is directed to a method for the detection and optionally
30 quantification of alpha-synuclein aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions, 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 alpha-synuclein aggregates, including but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions, into contact with a compound of formula (I), or a diagnostic composition which
35 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, Lewy neurites and/or cytoplasmic glial inclusions;
- (c) Detecting the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions; and
- 5 (d) Optionally quantifying the amount of the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions.

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

25 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,, 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, Lewy neurites and/or cytoplasmic glial
30 inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to,
35 Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions; and

- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy neurites and/or cytoplasmic glial inclusions with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions, wherein the method comprises the steps:

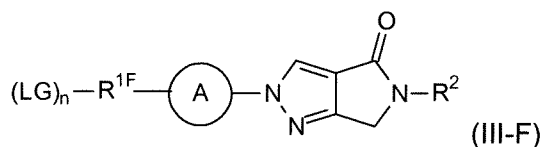
- (a) Bringing a sample, a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions into contact with a compound of the formula (I), or a diagnostic composition which comprises a compound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

- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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.
- 10 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, Lewy neurites and/or cytoplasmic glial inclusions to a medicament, 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, Lewy neurites and/or cytoplasmic glial inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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 comprising at least 2 heteroatoms;

R^{1F} is C_1 - C_4 alkyl, halo C_1 - C_4 alkyl, $-N(C_1$ - C_4 alkyl) $_2$; or $-NH(C_1$ - C_4 alkyl); or

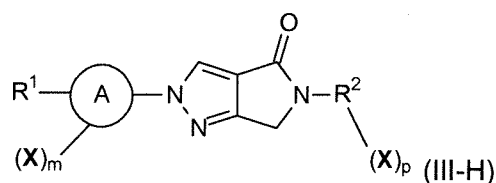
R^{1F} is a heterocyclyl; and

R^2 is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from halo C_1 - C_4 alkyl, halo C_1 - C_4 alkoxy, C_1 - C_4 alkoxy, and C_1 - C_4 alkyl;

5 **LG** is a leaving group; and

n is at least 1, preferably 1 or 2, more preferably 1.

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



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

(A) is a 6-membered heteroaryl comprising at least 2 heteroatoms;

R^1 is C_1 - C_4 alkyl, halo C_1 - C_4 alkyl, $-NH_2$, $-N(C_1$ - C_4 alkyl) $_2$; or $-NH(C_1$ - C_4 alkyl), wherein the C_1 - C_4 alkyl is optionally substituted with at least one halo; or

15 R^1 is a heterocyclyl which is optionally substituted with at least one halo;

R^2 is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from halo C_1 - C_4 alkyl, halo C_1 - C_4 alkoxy, C_1 - C_4 alkoxy, and C_1 - C_4 alkyl;

m is 0, 1, or 2;

p is 0, 1, or 2; and

20 **X** is bromo, chloro or iodo;

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

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 ^{18}F -fluorinating agent.

25

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.

In another aspect, the invention is further directed to the use of the compound according to compound
30 of formula (I) as an *in vitro* analytical reference or an *in vitro* screening tool.

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.

- 5 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

10 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.

15 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, 1-methylethyl, n-butyl, t-butyl and isobutyl.

20 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.

25 The term "halogenC₁-C₄alkyl" or "haloC₁-C₄alkyl" refers to a C₁-C₄alkyl radical as defined above, substituted with one or more (e.g., 1, 2 or 3) 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.

30 The term "halogenC₁-C₄alkoxy" refers to a C₁-C₄alkoxy radical as defined above, substituted with one or more (e.g. 1, 2 or 3) 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.

35 The term "heterocyclyl" refers to a stable 4- to 8-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, pyrrolidy, tetrahydrofuryl, tetrahydrothienyl, piperidy, piperaziny, tetrahydropyranly, morpholinyl, perhydroazepiny, azepanyl, or azocanyl, preferably azetidiny, pyrrolidiny, piperidy, azepanyl, or azocanyl.

The term "heteroaryl" refers to a 5 to 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, furyl, pyrroly, thienyl, pyrazoly, imidazolyl, indazolyl, thiazoly, isothiazoly, oxazolyl, isoxazolyl, triazolyl, tetrazoly, pyraziny, pyridaziny, pyrimidy 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., ¹⁹F and ¹⁸F) is particularly preferred.

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 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 the C₆-C₁₀arylsulfonate can be optionally substituted with -CH₃ or -NO₂.

Unless specified otherwise, the term "compound of the invention" refers to a compound of formula (I), or of subformulae thereof (e.g. (IIa), (IIb), (IIc), (IId), (IIa'), (IIb'), (IIc'), (IId'), (I-F), (I-H*), (I-H), or a detectably labelled compound, stereoisomer (including diastereomeric mixture and individual diastereomer, enantiomeric mixture and single enantiomer, mixture of conformer and single conformer), racemic 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), (IIc), (IId), (IIa'), (IIb'), (IIc'), (IId'), (I-F), (I-H*), (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.

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.

"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 of the pharmaceutically acceptable solvent 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.

5 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.
10 Moreover, alpha-synuclein aggregates are the major component of intracellular fibrillary inclusions 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,
15 nitration, and truncation.

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
20 brainstem or cortical type. Classic brainstem Lewy bodies are eosinophilic cytoplasmic inclusions 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.

25 Lewy neurites are abnormal neuronal processes in diseased neurons, containing granular material, 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.

30

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

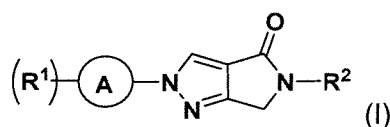
The compounds of formula (I) can bind to alpha-synuclein aggregates. The type of bonding with the compounds of formula (I) has not been elucidated and any type of bonding is covered by the present
35 invention. The wording "compound bound to the alpha-synuclein aggregates", and the like are used interchangeably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

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

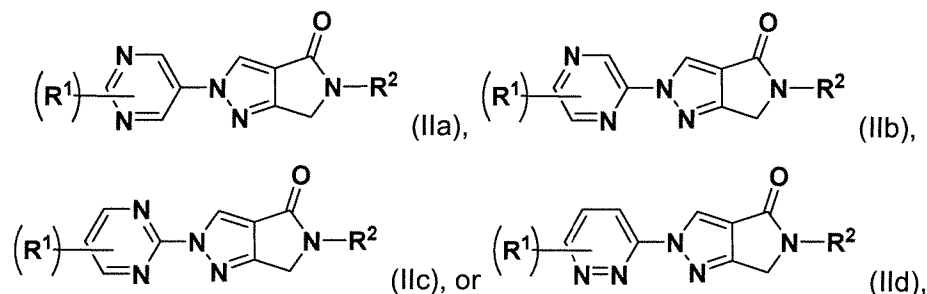
(A) is a 6-membered heteroaryl comprising at least 2 heteroatoms;

R^1 is C_1 - C_4 alkyl, halo C_1 - C_4 alkyl, $-NH_2$, $-N(C_1$ - C_4 alkyl) $_2$; or $-NH(C_1$ - C_4 alkyl), wherein the C_1 - C_4 alkyl is optionally substituted with at least one halo; or

R^1 is a heterocyclyl which is optionally substituted with at least one halo; and

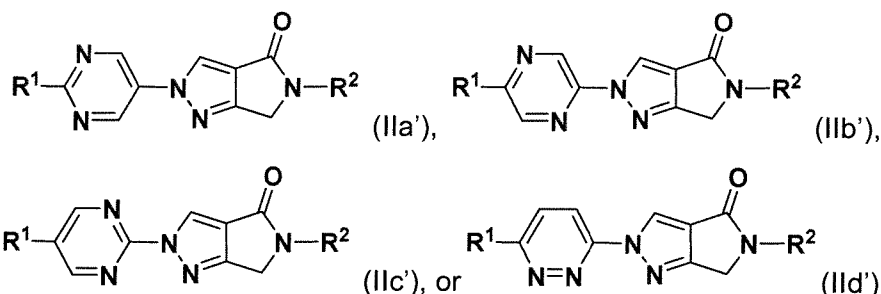
R^2 is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from halo C_1 - C_4 alkyl, halo C_1 - C_4 alkoxy, C_1 - C_4 alkoxy, and C_1 - C_4 alkyl.

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



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

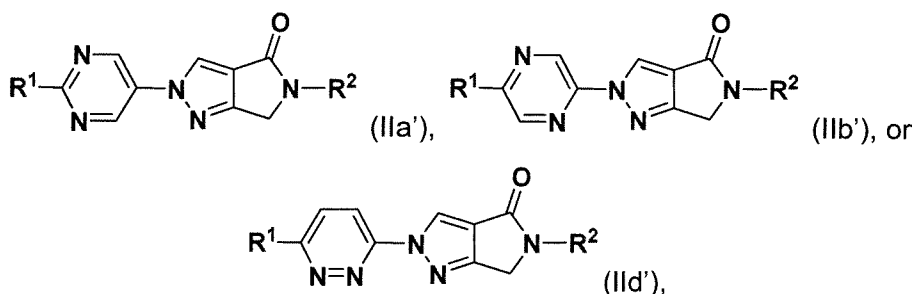
In another embodiment, the invention provides a compound of formula (I), having a formula (IIa'), (IIb'), (IIc'), or (IId'):



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

5

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



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

10

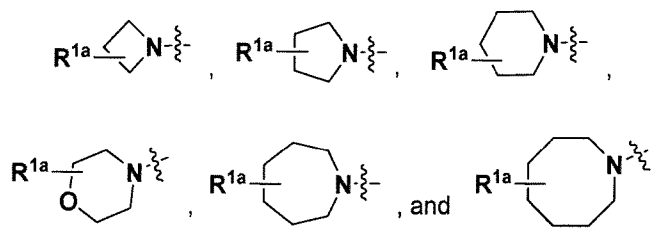
In one embodiment, **R¹** is C₁-C₄alkyl, haloC₁-C₄alkyl, -NH₂, -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl), wherein the C₁-C₄alkyl is optionally substituted with at least one halo. Preferably, **R¹** is C₁-C₄alkyl, or haloC₁-C₄alkyl. Even more preferably, **R¹** is -CH₃, or -CF₃.

15

In another embodiment, **R¹** is a heterocyclyl which is optionally substituted with at least one halo. Preferably, **R¹** is 4- to 8-membered heterocyclyl 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.

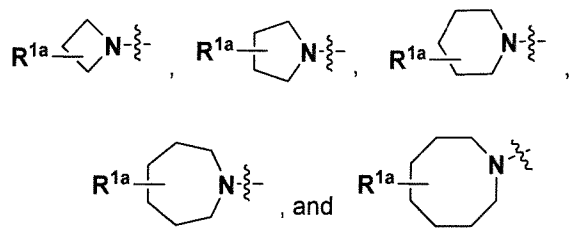
20

In another embodiment, **R¹** is a 4- to 8-membered heterocyclyl selected from the following:



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

Preferably, R^1 is a 4- to 8-membered heterocyclyl selected from the following:



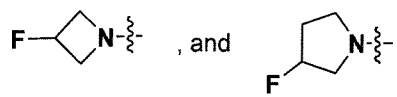
wherein R^{1a} is H or halo, preferably halo.

5

More preferably, R^{1a} is H or fluoro, preferably fluoro.

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

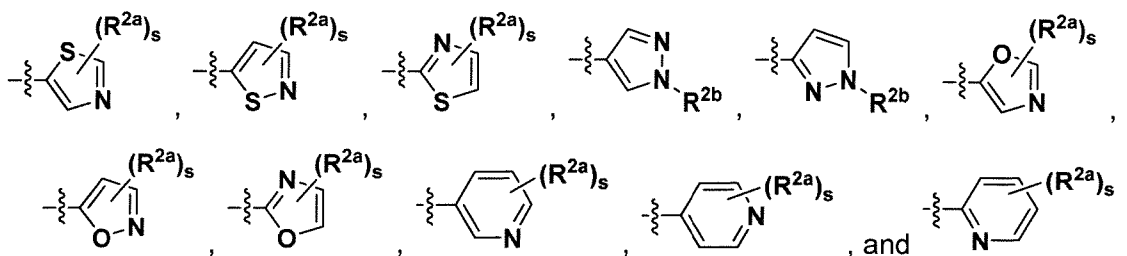
10 In yet another embodiment R^1 is a 4- or 5-membered heterocyclyl selected from the following:



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

15 R^2 is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkoxy, and C₁-C₄alkyl.

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



20 wherein

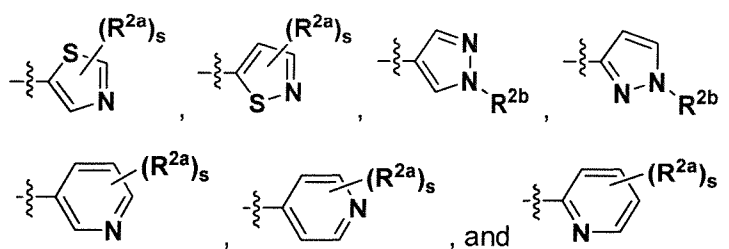
R^{2a} is independently selected from haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkoxy, and C₁-C₄alkyl;

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.

25

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

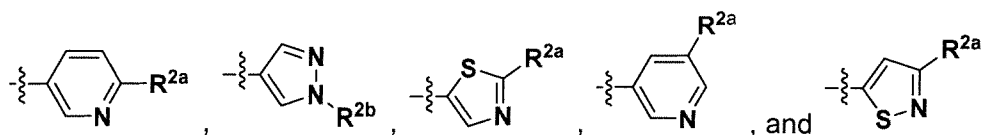


wherein

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

- 5 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.

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



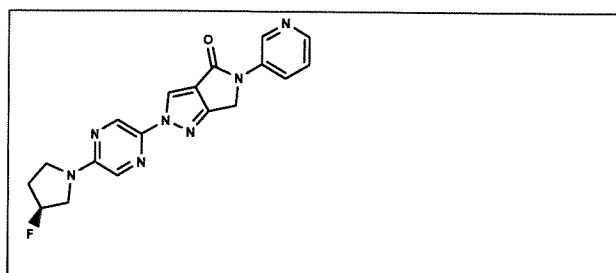
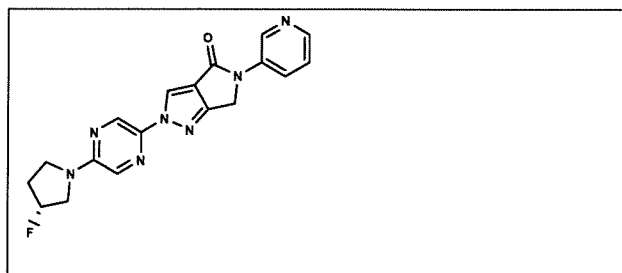
10 wherein

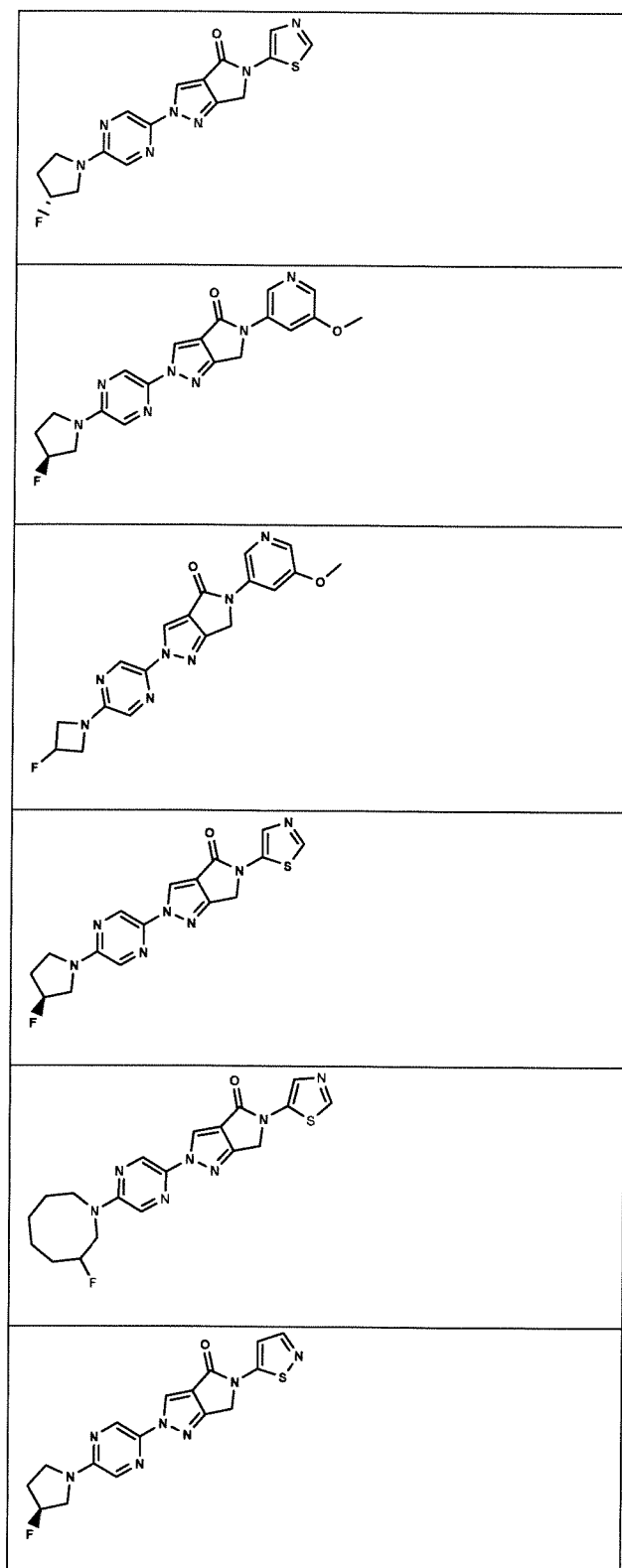
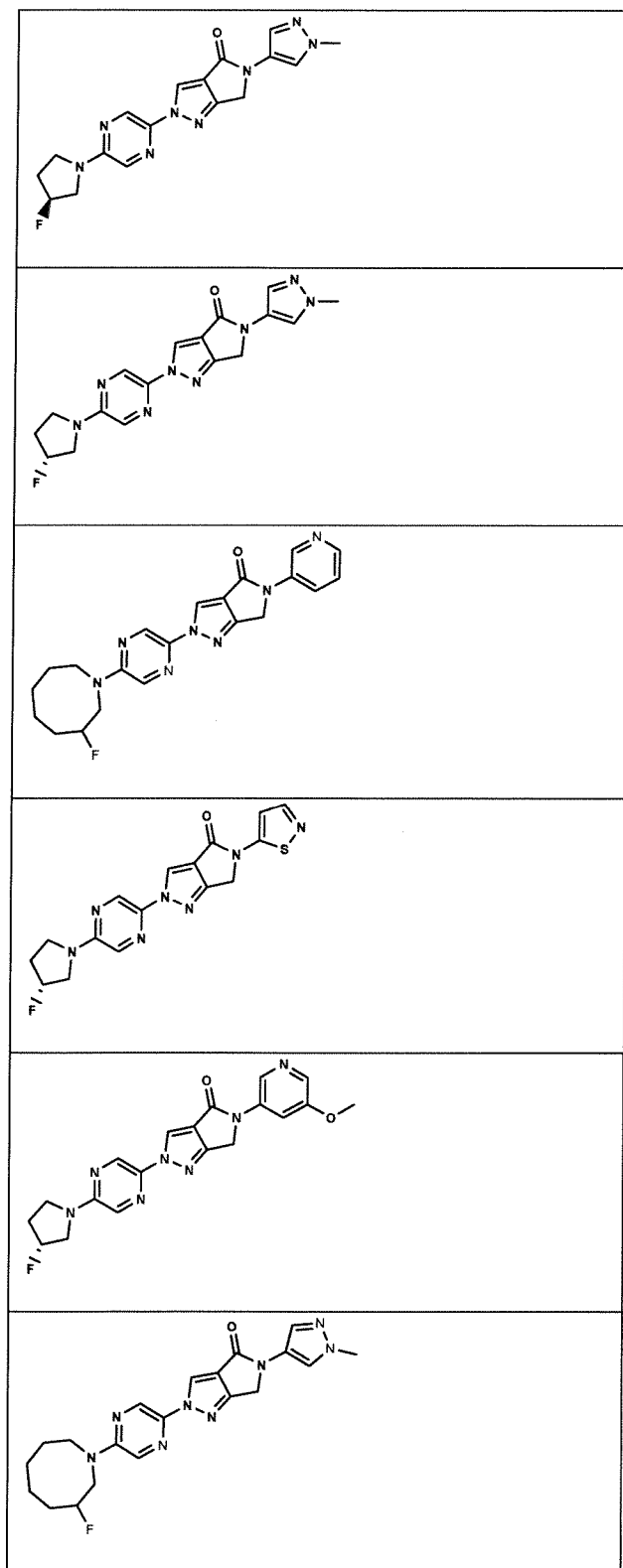
R^{2a} and R^{2b} are independently selected from H, haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkoxy, and C₁-C₄alkyl.

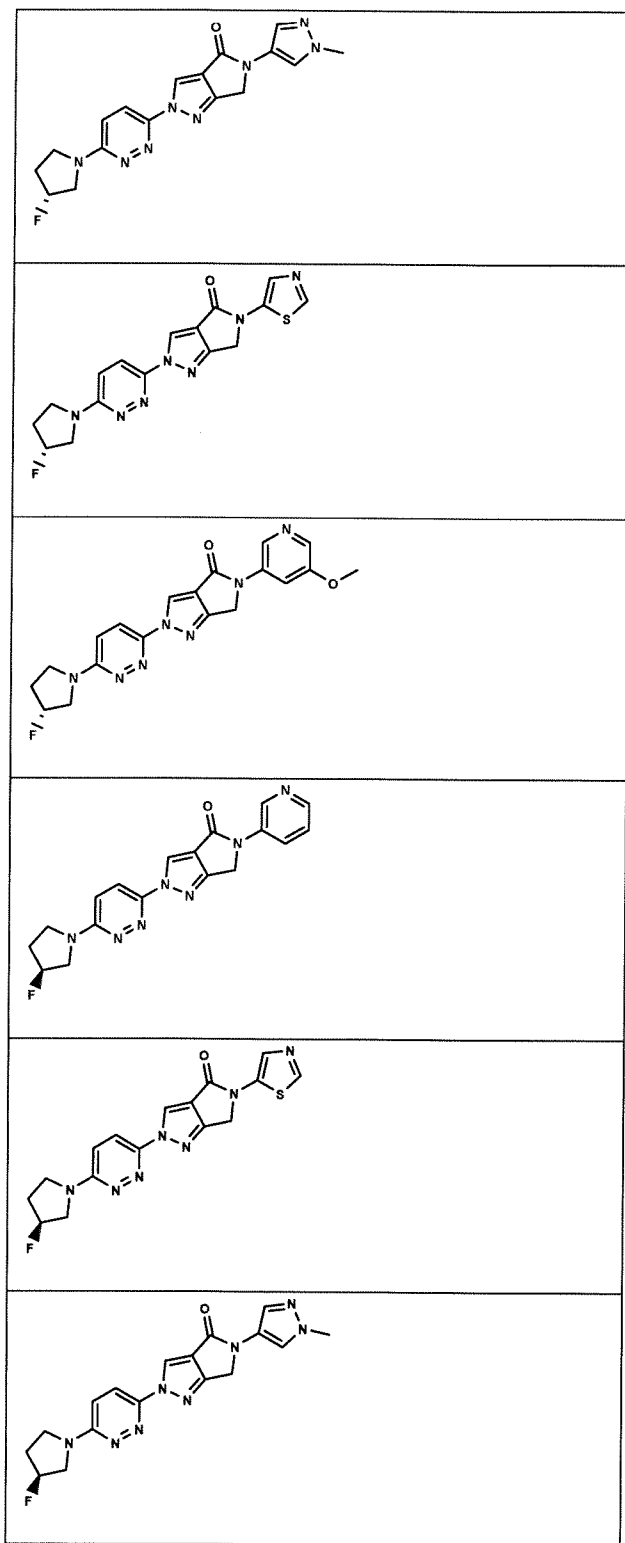
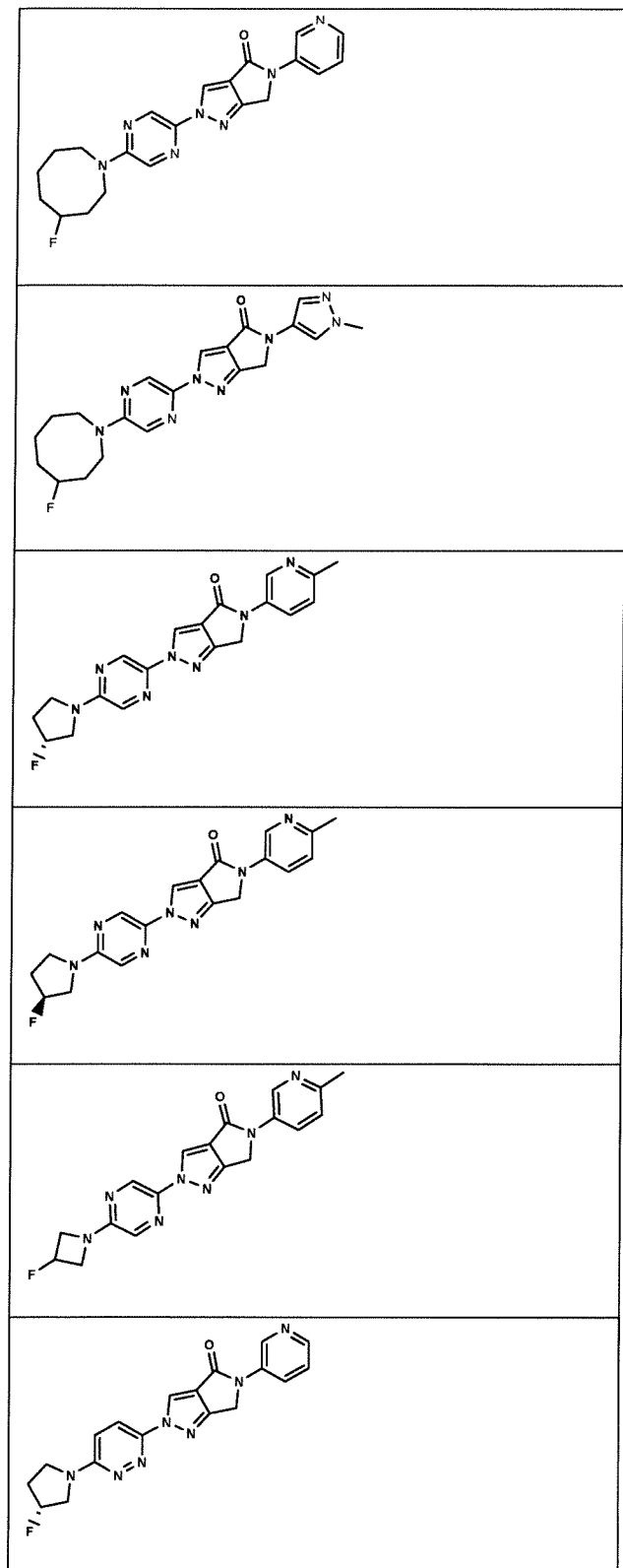
Preferably, R^{2a} and R^{2b} are independently selected from H, CH₃, and OCH₃.

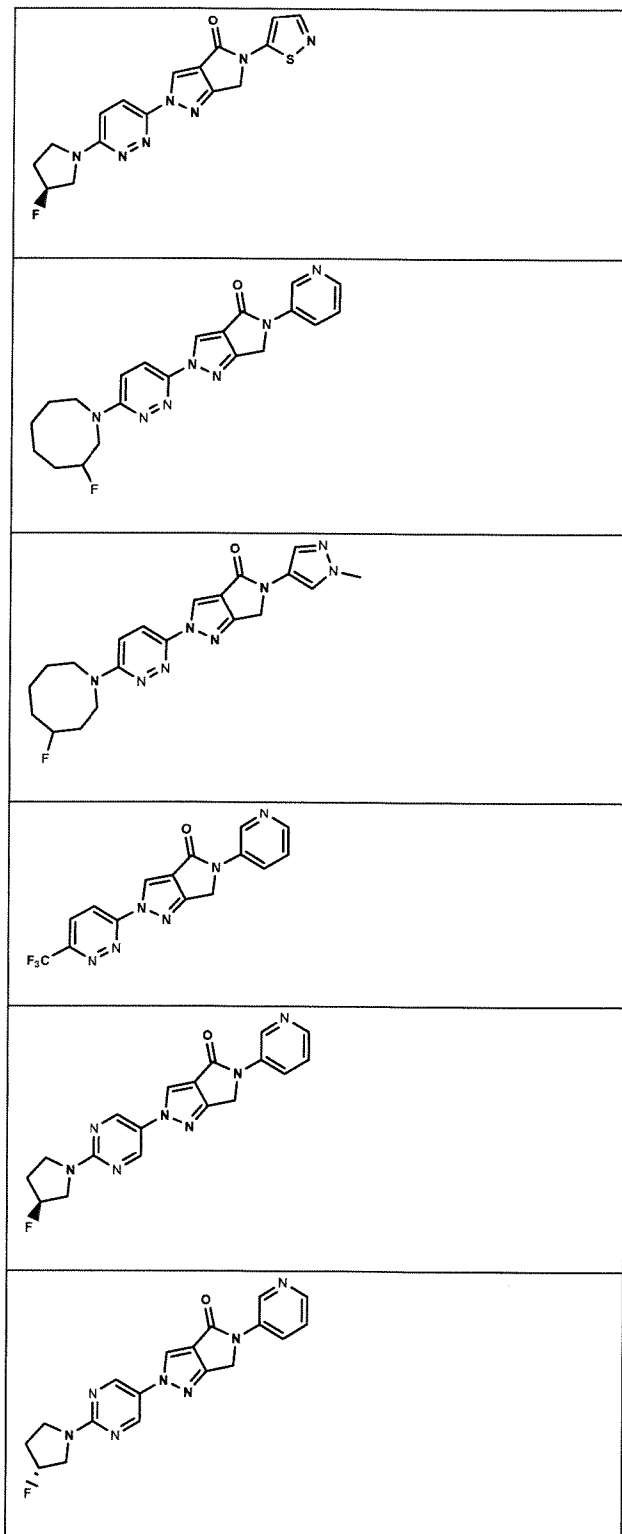
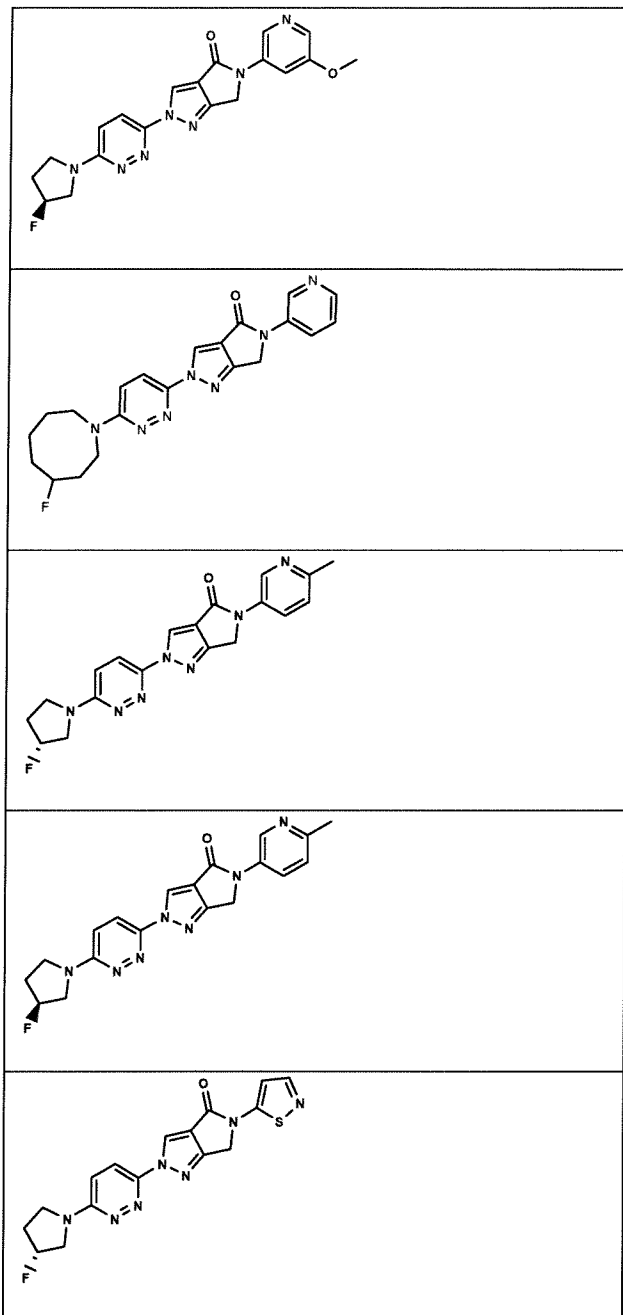
- 15 In all embodiments of R^2 , R^{2a} and R^{2b} in the present invention, the optional substituents are preferably C₁-C₄alkoxy, and C₁-C₄alkyl.

- 20 In one embodiment, the present invention provides a compound of formula (I), wherein the compound is selected from





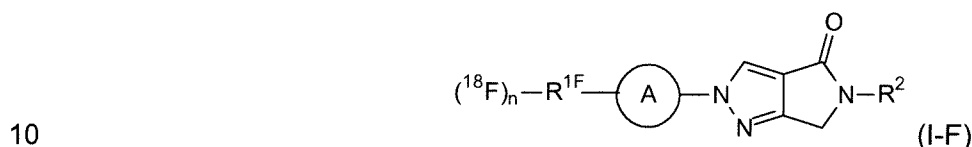




or a detectably labelled compound, stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof.

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

$\textcircled{\text{A}}$ is a 6-membered heteroaryl comprising at least 2 heteroatoms;

$\text{R}^{1\text{F}}$ is $\text{C}_1\text{-C}_4$ alkyl, $-\text{N}(\text{C}_1\text{-C}_4\text{alkyl})_2$; or $-\text{NH}(\text{C}_1\text{-C}_4\text{alkyl})$; or

15 $\text{R}^{1\text{F}}$ is a heterocyclyl; and

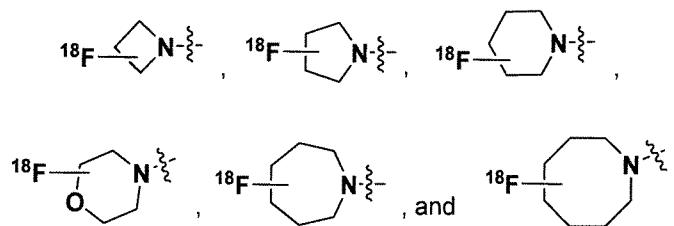
R^2 is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from halo $\text{C}_1\text{-C}_4$ alkyl, halo $\text{C}_1\text{-C}_4$ alkoxy, $\text{C}_1\text{-C}_4$ alkoxy, and $\text{C}_1\text{-C}_4$ alkyl; and

n is at least 1, preferably 1 or 2, more preferably 1.

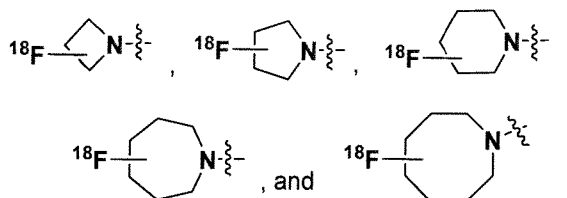
20 Preferably, $\text{R}^{1\text{F}}$ is $\text{C}_1\text{-C}_4$ alkyl. Even more preferably, $-\text{R}^{1\text{F}}-(^{18}\text{F})_n$ is $-\text{CH}_2^{18}\text{F}$, or $-\text{CF}_2^{18}\text{F}$.

In another preferred embodiment, $\text{R}^{1\text{F}}$ is a heterocyclyl. Preferably, $\text{R}^{1\text{F}}$ is 4- to 8-membered heterocyclyl.

25 In another embodiment, $-\text{R}^1-(^{18}\text{F})_n$ is selected from the following:

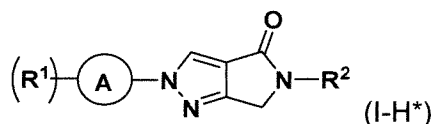


Preferably, $-\text{R}^1-(^{18}\text{F})_n$ is selected from the following:



5 The detectably labelled compound of formula (I-F) comprises at least one ^{18}F . Preferably, the detectably labelled compound of formula (I-F) comprises one or two ^{18}F . Even more preferably, one ^{18}F .

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



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

(A) is a 6-membered heteroaryl comprising at least 2 heteroatoms;

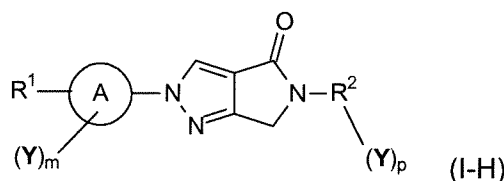
R¹ is C₁-C₄alkyl, haloC₁-C₄alkyl, -NH₂, -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl), wherein the C₁-C₄alkyl is optionally substituted with at least one halo; or

R¹ is a heterocyclyl which is optionally substituted with at least one halo;

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

20

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,

25 (A) is a 6-membered heteroaryl comprising at least 2 heteroatoms;

R^1 is C_1 - C_4 alkyl, halo C_1 - C_4 alkyl, $-NH_2$, $-N(C_1-C_4alkyl)_2$, or $-NH(C_1-C_4alkyl)$, wherein the C_1 - C_4 alkyl is optionally substituted with at least one halo; or

R^1 is a heterocyclyl which is optionally substituted with at least one halo;

R^2 is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from halo C_1 - C_4 alkyl, halo C_1 - C_4 alkoxy, C_1 - C_4 alkoxy, and C_1 - C_4 alkyl;

Y is T or CT_3 ;

m is 0, 1, 2 or 3;

p is 0, 1, 2 or 3;

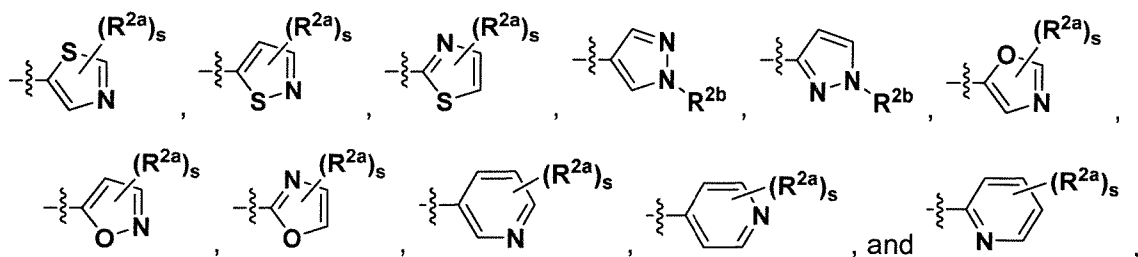
with the proviso that the compound of formula (I-H) comprises at least one T or CT_3 , wherein T is 3H (Tritium).

It is understood that the tritium can present at any available position at which a hydrogen is present. For instance, in the group R^2 tritium can be present either directly bound to the 5-membered or 6-membered heteroaryl (such as in the form of T) or can be present in the halo C_1 - C_4 alkyl, halo C_1 - C_4 alkoxy, C_1 - C_4 alkoxy, and C_1 - C_4 alkyl (such as in the form of CT_3). In the 4- to 6-membered heterocyclyl of R^1 tritium can be, e.g., directly bound to the 4- to 6-membered heterocyclyl.

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

In one embodiment, R^2 is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from halo C_1 - C_4 alkyl, halo C_1 - C_4 alkoxy, C_1 - C_4 alkoxy, and C_1 - C_4 alkyl, and p is 1, 2 or 3, e.g., 1.

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



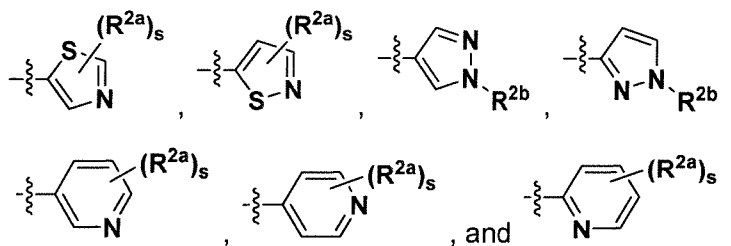
wherein

R^{2a} is independently selected from T , halo C_1 - C_4 alkyl, halo C_1 - C_4 alkoxy, C_1 - C_4 alkoxy, and C_1 - C_4 alkyl (e.g., CT_3);

R^{2b} is selected from H, T, haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkoxy, and C₁-C₄alkyl (e.g., CT₃); s is 0, 1 or 2 (preferably 0 or 1); and wherein haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkoxy, and C₁-C₄alkyl optionally comprise one or more T.

5

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



wherein

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

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

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

15 wherein haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkoxy, and C₁-C₄alkyl optionally comprises one or more T.

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

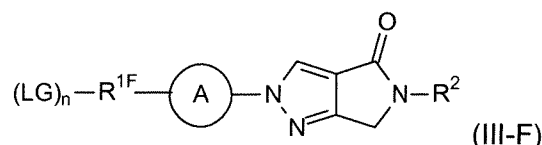
In another embodiment, the invention provides a detectably labelled compound of formula (I-H*) or (I-H) wherein ³H Tritium ("T") can be replaced by ²H Deuterium ("D"). The deuterated compound can be prepared by reacting a compound of formula (III-H) with a ²H radiolabelling agent.

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 , ^{13}N , ^{15}O , and ^{18}F , more preferably ^2H , ^3H and ^{18}F , even more preferably ^3H and ^{18}F .
- 5 ^{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 control, release, and clinical use of their ^{18}F -analogs.
- 15 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 the original compound efficacy.
- 20 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 reagents, which are commercially available or prepared by known synthetic techniques.
- 25 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 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.
- 30 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 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
- 35

at which H is present. If ^2H is employed as a detectable label it can be attached at any available position at which H is present.

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



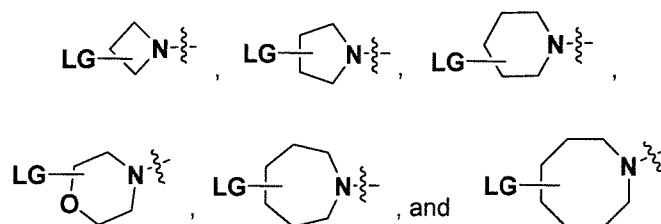
or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein

- (A) is a 6-membered heteroaryl comprising at least 2 heteroatoms;
- 10 $\text{R}^{1\text{F}}$ is $\text{C}_1\text{-C}_4$ alkyl, halo $\text{C}_1\text{-C}_4$ alkyl, $-\text{N}(\text{C}_1\text{-C}_4\text{alkyl})_2$; or $-\text{NH}(\text{C}_1\text{-C}_4\text{alkyl})$; or $\text{R}^{1\text{F}}$ is a heterocyclyl; and R^2 is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from halo $\text{C}_1\text{-C}_4$ alkyl, halo $\text{C}_1\text{-C}_4$ alkoxy, $\text{C}_1\text{-C}_4$ alkoxy, and $\text{C}_1\text{-C}_4$ alkyl; LG is a leaving group; and
- 15 n is at least 1, preferably 1 or 2, more preferably 1.

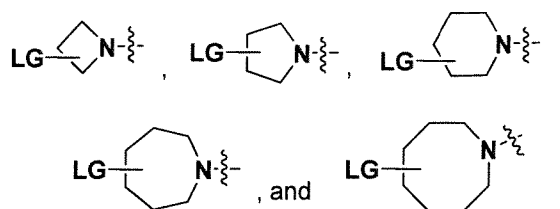
Preferably, $\text{R}^{1\text{F}}$ is $\text{C}_1\text{-C}_4$ alkyl. Even more preferably, $(\text{LG})_n\text{-R}^{1\text{F}}$ is $-\text{CH}_2\text{LG}$, or $-\text{CF}_2\text{LG}$.

In another preferred embodiment, $\text{R}^{1\text{F}}$ is a heterocyclyl. Preferably, $\text{R}^{1\text{F}}$ is a 4- to 8-membered
20 heterocyclyl.

In another embodiment, $(\text{LG})_n\text{-R}^{1\text{F}}$ is selected from the following:



25 Preferably, $(\text{LG})_n\text{-R}^{1\text{F}}$ is selected from the following:

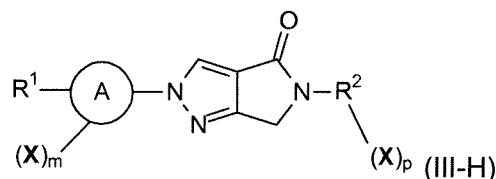


Preferably, the Leaving Group (LG) is halogen, C₁–C₄alkylsulfonate, C₁–C₄alkyl ammonium, or C₆–C₁₀arylsulfonate, wherein the C₆–C₁₀arylsulfonate can be optionally substituted with –CH₃ or –NO₂.

- 5 More preferably, the Leaving Group (LG) is bromo, chloro, iodo, C₁–C₄alkylsulfonate, or C₆–C₁₀arylsulfonate, wherein the C₆–C₁₀arylsulfonate can be optionally substituted with –CH₃ or –NO₂. Even more preferably, the Leaving Group (LG) is mesylate, tosylate or nosylate. Even more preferably, the Leaving Group (LG) is mesylate, or nosylate. Preferably the Leaving Group (LG) is mesylate.

10

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



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

(A) is a 6-membered heteroaryl comprising at least 2 heteroatoms;

R¹ is C₁–C₄alkyl, haloC₁–C₄alkyl, –NH₂, –N(C₁–C₄alkyl)₂; or –NH(C₁–C₄alkyl), wherein the C₁–C₄alkyl is optionally substituted with at least one halo; or

R¹ is a heterocyclyl which is optionally substituted with at least one halo;

- 20 R² is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from haloC₁–C₄alkyl, haloC₁–C₄alkoxy, C₁–C₄alkoxy, and C₁–C₄alkyl;

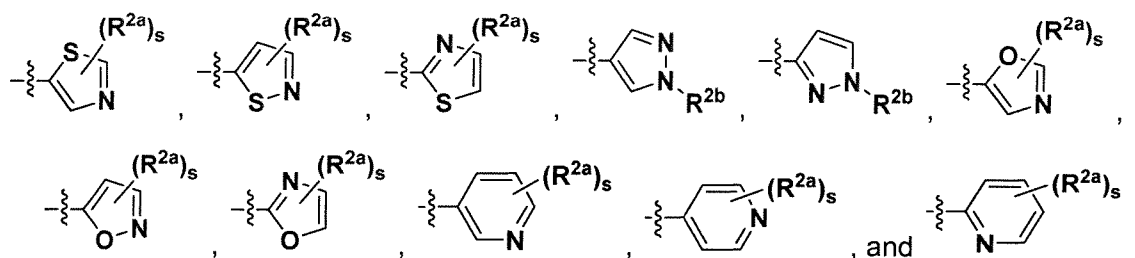
m is 0, 1, or 2;

p is 0, 1, or 2; and

X is bromo, chloro or iodo;

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

In a preferred embodiment, (X)_p–R² is selected from the following:



wherein

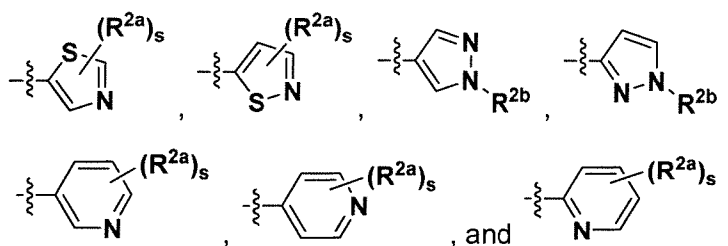
R^{2a} is independently selected from X, haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkoxy, and C₁-C₄alkyl;

5 R^{2b} is selected from H, X, haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkoxy, 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 comprises one or more X.

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



wherein

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

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

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

wherein haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkyl, or C₁-C₄alkoxy optionally comprises one or more X.

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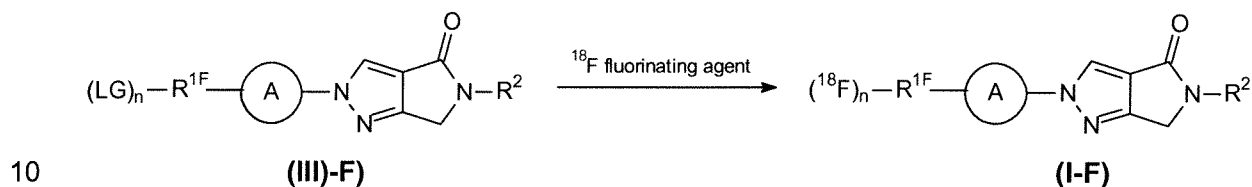
In a preferred embodiment, the detectably labelled compound of formula (III-H) comprises one, two or three X. In a preferred embodiment, the detectably labelled compound of formula (III-H) comprises one X. In another preferred embodiment, the detectably labelled compound of formula (III-H) comprises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

25 embodiment X is bromine.

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), (IIc), (IId), (IIa'), (IIb'), (IIc'), (IId'), (I-F), (I-H*), (I-H)), and in particular a compound of formula (III-F) or (III-H) comprising a detectable label.

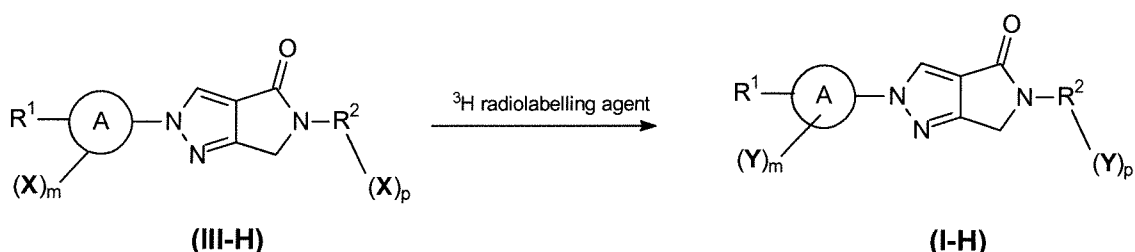
In one embodiment, the present invention relates to a method for preparing a compound of formula (I-F), by reacting a compound of formula (III-F) with a ^{18}F -fluorinating agent.



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

Suitable solvents for the ^{18}F -fluorination comprise DMF, DMSO, acetonitrile, DMA, or mixtures thereof, preferably acetonitrile or DMSO. Suitable agents for the ^{18}F -fluorination are selected from K^{18}F , Rb^{18}F , Cs^{18}F , Na^{18}F , tetra(C_{1-6} alkyl)ammonium salt of ^{18}F , Kryptofix[222] ^{18}F and tetrabutylammonium [^{18}F]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.



wherein A , R^1 , R^2 , X , Y , p , and m are as defined herein above.

25

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 CT₃ radiolabeling agent, wherein T is ³H. The CT₃ radiolabeling agent can be ICT₃ (derivative of iodomethane with ³H). The method can be conducted in the presence of a solvent such as dimethylformamide (DMF) and
5 a base such cesium carbonate or sodium hydride.

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
10 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 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 ¹⁸F, or ³H.

15 In one embodiment, the kit of parts 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 of formula (III-F) or (III-H)).

20 In another embodiment, the kit of parts 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

25 The compounds of the present invention are particularly suitable for imaging of alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions. 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,
30 Lewy neurites and/or cytoplasmic glial inclusions. 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.

5

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.

10 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-
15 synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions .

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
20 for brain imaging. In particular, the use is in humans.

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

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 (including
30 sporadic, familial with alpha-synuclein gene mutations or changes in copy number (e.g. SNCA duplication or triplication, familial with mutations other than alpha-synuclein, pure autonomic failure and Lewy body dysphagia), 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
35 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, Parkinson's disease with mild cognitive impairment, Parkinson's disease linked to the SNCA gene mutation and/or changes in copy number as duplication or triplication, 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, Lewy neurites and/or cytoplasmic glial inclusions, (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, Lewy neurites and/or cytoplasmic glial inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions .

The compounds of the present invention can be used for imaging of alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions. 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, Lewy neurites and/or cytoplasmic glial inclusions 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.

5 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.

15 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.

30 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 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.

5 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, 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
10 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 and will depend upon a variety of factors including the activity of the specific compound employed,
15 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.

The diagnostic compositions of the invention can be produced in a manner known per se to the skilled
20 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
25 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 according to the invention, a pharmaceutically acceptable excipient, carrier, diluent or adjuvant. The
30 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

In one embodiment, the invention provides a method of imaging a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions, in a subject, the method comprising the steps:

- 5 (a) Administering a compound of the invention, or a diagnostic composition which comprises a compound of the invention to the subject;
- (b) Allowing said compound to bind to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions; and
- 10 (c) Detecting the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions.

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, Lewy neurites and/or cytoplasmic glial inclusions.
- 15

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, Lewy neurites and/or cytoplasmic glial inclusions, 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, Lewy neurites and/or cytoplasmic glial inclusions; and
- (c) Detecting the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions by collecting a positron emission tomography (PET) image of the tissue of the subject.
- 25

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, Lewy neurites and/or cytoplasmic glial inclusions, 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, Lewy neurites and/or cytoplasmic glial inclusions, into contact with a compound of the invention, or a diagnostic composition which comprises a compound of the invention;
- 35

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

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, Lewy neurites and/or cytoplasmic glial inclusions, the method comprising the steps:

- 15 (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, Lewy neurites and/or cytoplasmic glial inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions; and
- 20 (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions in the sample or specific body part or body area.

25 If the amount of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions.

35 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, Lewy neurites and/or cytoplasmic glial inclusions, 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, Lewy neurites and/or cytoplasmic glial inclusions into contact with a compound according to the present invention, or a diagnostic composition which comprises a compound according to the present invention;
- 5 (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions; and
- 10 (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions in the sample or specific body part or body area.
- 15 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
- 20 associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions, it can be assumed that the patient has a disposition to a disease, disorder or abnormality associated with alpha-synuclein aggregates.

In a further aspect, the present invention relates to a method of collecting data for prognosing a

25 disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions, 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, Lewy neurites and/or cytoplasmic glial
- 30 inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to,
- 35 Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;

- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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 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) of a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions into contact with the 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, Lewy neurites and/or cytoplasmic glial inclusions;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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 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.

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.

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, Lewy neurites and/or cytoplasmic glial inclusions to a treatment with a medicament, the method comprising the steps of

- (a) Bringing a sample, a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;
- (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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 comprise steps (i) to (vi) before step (a):

- (i) bringing a sample or specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions ;
- (ii) allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;

- (iii) detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;
- (iv) correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions with
5 the presence or absence of alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions to
10 a normal control value; and
- (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,
15 but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions determined in step (iv) to the amount of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions determined in step (d).

20 In the method for predicting responsiveness 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. A change, especially a decrease, in the amount of the compound bound to the alpha-synuclein aggregates may indicate that the patient has a high potential of being responsive to the
25 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 the alpha-synuclein aggregates is essentially constant or increases over time, it can be assumed that
30 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 aggregates can be compared to a control value such as a normal control value, a preclinical control
35 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 or "response undetermined" to a certain therapy. Response to the therapy may be different for the respective patients.

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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 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).

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

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

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

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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, Lewy neurites and/or cytoplasmic glial inclusions.

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.

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

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.

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.

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.

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, Lewy neurites and/or cytoplasmic glial inclusions by any suitable method.

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

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, Lewy neurites and/or cytoplasmic glial inclusions 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.

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The compound is then allowed to bind to the alpha-synuclein aggregates, including but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions. The step of allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions includes allowing sufficient time for the compound of the
10 invention to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions . The amount of time required for 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-
15 synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions . The amount of time should not be too extended to avoid washout and/or metabolism of the compound of the invention.

The compound which has bound to the alpha-synuclein aggregates including, but not limited to, Lewy
20 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, Lewy neurites and/or cytoplasmic glial inclusions 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
25 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
30 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, Lewy neurites and/or cytoplasmic glial
35 inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions can be determined by visual or quantitative analysis, for example, using PET scan images.

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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, Lewy neurites and/or cytoplasmic glial inclusions. 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, Lewy neurites and/or cytoplasmic glial inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions .

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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 $\mu\text{g}/\text{kg}$ to 10 $\mu\text{g}/\text{kg}$, preferably 0.01 $\mu\text{g}/\text{kg}$ to 1.0 $\mu\text{g}/\text{kg}$. The radioactive dose can be, e.g., 100 to 600 MBq, more preferably 150 to 450 MBq.

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METHODS OF SYNTHESIZING THE COMPOUNDS OF THE INVENTION

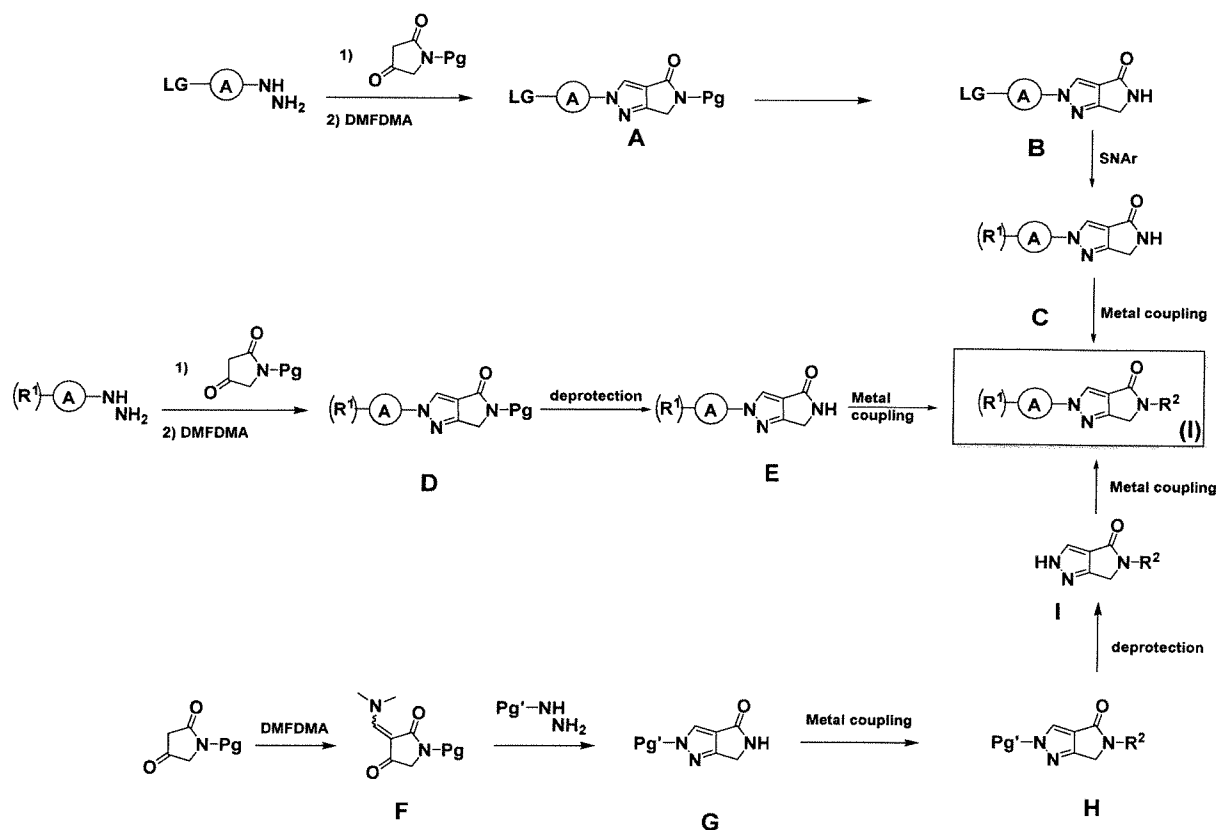
The compounds of the present invention may be prepared in accordance with the definition of compound of formula (I) by the routes described in the following Schemes or the Examples. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly 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 , and LG are as previously defined in the above embodiments, or limited to designations

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in the Schemes. Unless otherwise stated, starting materials are either commercially available or are prepared by known methods.

5 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 DMFDMA
 10 to give intermediate A. Deprotection with suitable conditions can afford intermediate B. $\text{S}_{\text{N}}\text{Ar}$ can be conducted with a suitable nucleophile in a suitable solvent and base to give intermediate C. Finally, intermediate C can be further functionalized using palladium catalyzed amidation or Ullmann reaction to give compounds of formula (I). An alternative approach can consist in purchasing hydrazine already functionalized with R^1 . In such a case, the synthetic route is similar to the previously
 15 described one with the exception of the deprotection step. A third synthetic pathway could comprise synthesizing the protected pyrazol ring G via the intermediate F. The R^2 group can be introduced by palladium catalyzed amidation or Ullmann reaction. Subsequent deprotection of the pyrazole ring can be achieved for example under acidic conditions. Finally, a metal coupling reaction can afford the compound of formula (I).

For example, a ^{18}F -precursor can be obtained by treating intermediate A with hydroxypyrrolidine under heating in a suitable solvent. For example, the ^3H -precursor can be obtained by introducing an appropriate R^2 group by palladium catalyzed amidation or Ullmann reaction into an intermediate C.

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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 .

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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.

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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 ^{18}F -fluorinating agent can be a tetraalkylammonium salt of ^{18}F or a tetraalkylphosphonium salt of ^{18}F ; e.g., tetra(C_{1-6} alkyl)ammonium salt of ^{18}F or a tetra(C_{1-6} alkyl)phosphonium salt of ^{18}F . Preferably, the ^{18}F -fluorination agent is K^{18}F , H^{18}F , Cs^{18}F , Na^{18}F , tetra(C_{1-6} alkyl) ammonium salt of ^{18}F , Kryptofix[222] ^{18}F or tetrabutylammonium [^{18}F]fluoride.

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Although the reaction is shown above with respect to ^{18}F 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.

EXAMPLESEXEMPLIFICATION 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 is 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.

CsF	cesium fluoride
CyDMEDA	1-N,2-N-dimethylcyclohexane-1,2-diamine
DCM	dichloromethane
DMEDA	1,2-dimethylethylenediamine
DMF	dimethylformamide
DMFDMA	N,N-dimethylformamide dimethyl acetal
DMSO	dimethylsulfoxide
EtOAc	ethyl acetate
HPLC	High Performance Liquid Chromatography
LCMS	Liquid Chromatography Mass Spectrometry
LG	leaving group
MTBE	methyl tert-butyl ether
Pg	protecting group
rt	room temperature
SNAr	nucleophilic aromatic substitution
TFA	trifluoroacetic acid
Xantphos	4,5-bis(diphenylphosphino)-9,9-dimethylxanthene

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 solvents as indicated in NMR spectral data.

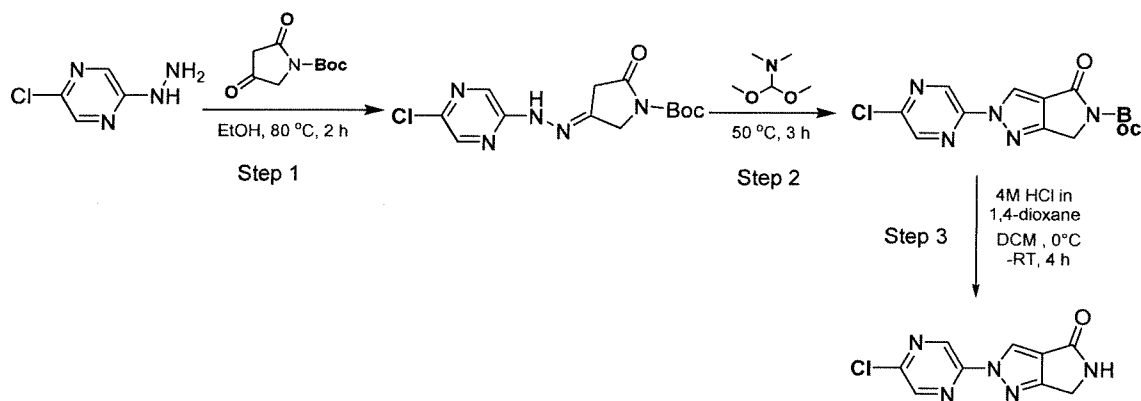
Mass spectra (MS) were recorded on an Advion CMS mass spectrometer or an UPLC H-Class Plus with Photodiode Array detector and Qda Mass spectrometer from Waters.

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.

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PREPARATIVE EXAMPLESPreparative Example 1

Step 1: To a stirred solution of hydrazine (500 mg, 3.5 mmol) in ethanol (4 mL, 25 vol) was added N-boc-dioxopyrrolidine carboxylate (689 mg, 3.5 mmol). Then the mixture was stirred at 80°C under N₂ atmosphere. After completion of the reaction, the solvent was removed completely under reduced pressure. The obtained crude product (1 g) was directly used in the next step without further purification. LCMS: 269.9 (M+H-^tBu)⁺

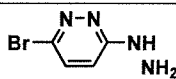
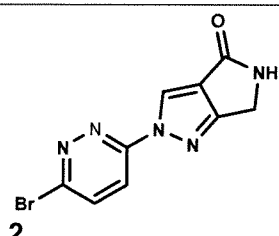
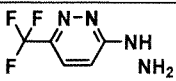
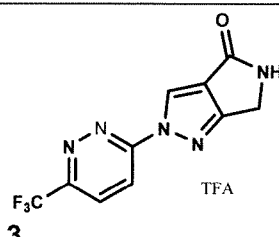
Step 2: A solution of hydrazone of **step 1** (1 g, 3.0 mmol) and DMFDMA (14 mL, 14 vol) was stirred for 15 minutes at room temperature, and then the solution was refluxed at 50°C for 3 hours under N₂ atmosphere. After completion of the reaction, the reaction mixture was cooled to room temperature, evaporated to remove DMFDMA. Ethanol was then added to the crude residue and the formed precipitate was filtered off. The solid was washed with 50% EtOH: hexane (10 mL x 2) to afford the title compound as a yellow solid (550 mg, 55% two steps). ¹H NMR -(500 MHz, DMSO-*d*6) δ 9.14 (s, 1H), 9.09 (d, 1H), 8.80 (d, 1H), 4.86 (s, 2H), 1.52 (s, 9H). MS (ESI): 280.14 (M+H-tBu)⁺.

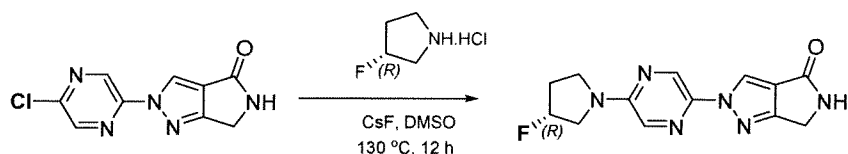
Step 3: To a stirred solution of the product of **step 2** (350 mg, 1.0 mmol) in DCM (11 mL) was added 4M HCl in 1,4-dioxane (1.7 mL) at 0°C under N₂ atmosphere. The mixture was stirred at room temperature for 4 hours. After completion of the reaction, the reaction mixture was cooled to 0°C. The crude residue was quenched with a saturated NaHCO₃ solution (5 mL) and was basified to pH 12 at 0°C. The crude residue was filtered off and the obtained solid was co-distilled with toluene (10 mL), dried under high vacuum to afford the product as a yellow solid (220 mg, 89%). ¹H NMR (400 MHz, DMSO-*d*6) δ 9.07 (d, 1H), 8.87 (s, 1H), 8.76 (d, 1H), 8.37 (s, 1H), 4.44 (d, 2H). MS (ESI): 236.14 (M+H)⁺.

Preparative Examples 2 and 3

The following preparative examples were prepared following the same procedure described in preparative example 1. TFA could be used as an alternative source of acid to deprotect the BOC group.

Table 1:

Hydrazine	Preparative example	1. ¹ H-NMR 2. MH ⁺ (ESI)
	 2	2. ¹ H NMR (400 MHz, DMSO- <i>d</i> 6) δ 8.23 (s, 1H), 7.57 (s, 1H), 7.47 (d, 1H), 7.31 (d, 1H), 3.61 (s, 2H). 3. 236.19
	 3	2. ¹ H NMR (500 MHz, DMSO- <i>d</i> 6) δ 9.16 (s, 1H), 8.45 (s, 2H), 8.39 (s, 1H), 4.45 (s, 2H). 3. 2701

Preparative Example 4


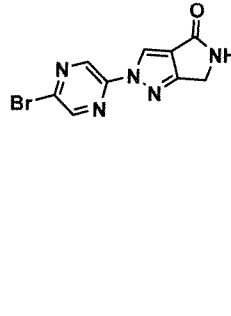
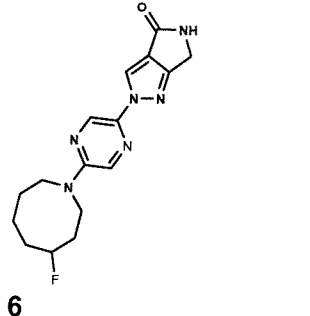
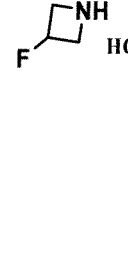
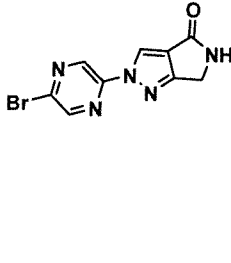
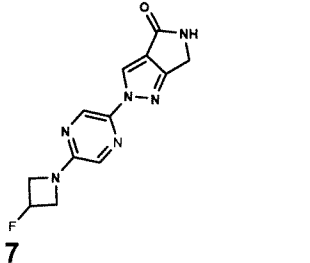
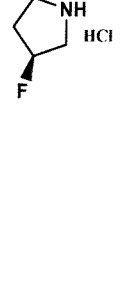
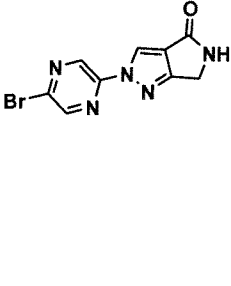
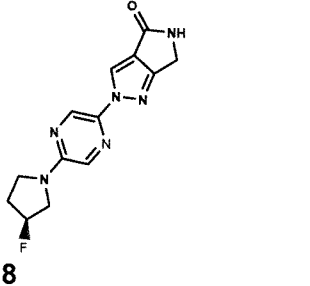
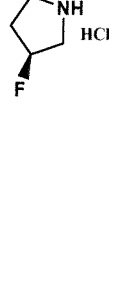
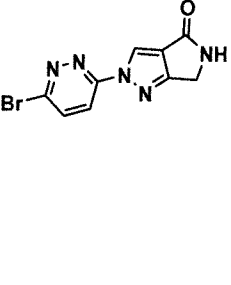
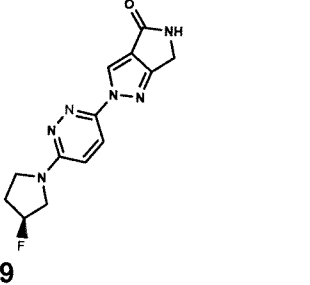
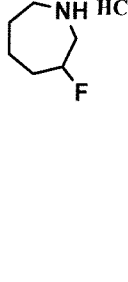
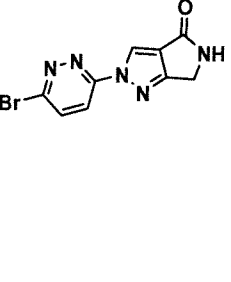
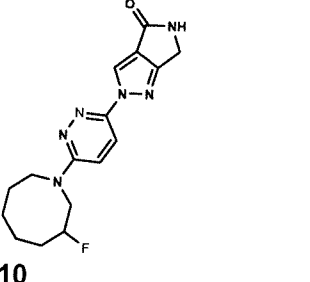
To a stirred solution of **preparative example 1** (50 mg, 0.2 mmol) in DMSO (0.5 mL) was added (*R*)-3-fluoropyrrolidine hydrochloride (160 mg, 1.3 mmol) and CsF (258 mg, 1.7 mmol) under N₂ atmosphere. The mixture was stirred for 16 hours at 130°C. After completion of the reaction, the reaction mixture was quenched with cold water (2 mL) and the crude residue was filtered. The obtained solid was co-distilled with toluene (5 mL x 2), and dried under high vacuum to afford the product as a white solid (30 mg, 49%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.65 (d, 1H), 8.61 (s, 1H), 8.17 (s, 1H), 7.93 (d, 1H), 5.64 – 5.38 (m, 1H), 4.38 (s, 2H), 3.90 – 3.60 (m, 3H), 3.53 (td, 1H), 2.40 – 2.10 (m, 2H). MS (ESI): 289.16 (M⁺+H)⁺.

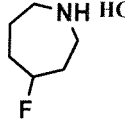
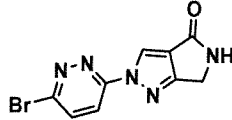
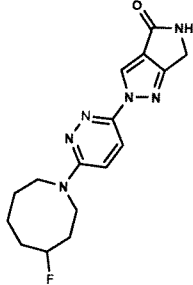
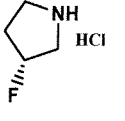
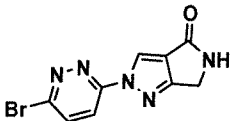
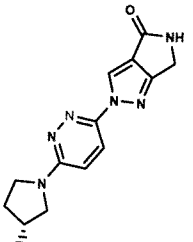
Preparative Examples 5 to 12

Following the S_NAr procedure as described in **preparative example 4**, using the amine starting material and the appropriate halogenated heteroaryl indicated in the **Table 2** below, the following compounds were prepared.

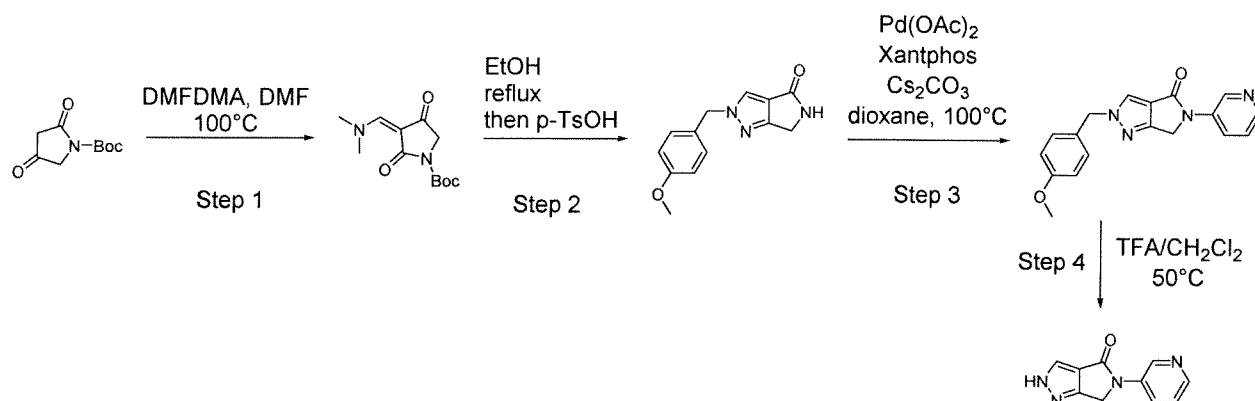
Table 2:

Amine	halogenated heteroaryl	Compound of example	1. Yield 2. ¹ H-NMR 3. MH ⁺ (ESI)
			1. 67 % 2. ¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ 8.61 (s, 1H), 8.59 (s, 1H), 8.17 (s, 1H), 8.11 (s, 1H), 4.98 (dt, 1H), 4.37 (s, 2H), 4.14 – 3.87 (m, 2H), 3.86 – 3.71 (m, 1H), 3.71 – 3.58 (m, 1H), 1.92 – 1.39 (m, 6H). 3. 317.2

		 <p>6</p>	<p>1. 80 % 2. ¹HNMR (400 MHz, DMSO-<i>d</i>₆) δ 8.62 (s, 1H), 8.59 (s, 1H), 8.18 (s, 1H), 8.06 (s, 1H), 4.97 – 4.74 (m, 1H), 4.37 (s, 2H), 3.81 – 3.56 (m, 4H), 2.19 – 1.67 (m, 6H). 3. 317.4</p>
		 <p>7</p>	<p>1. 75 % 2. ¹HNMR (500 MHz, DMSO-<i>d</i>₆) δ 8.66 (d, 1H), 8.63 (s, 1H), 8.19 (s, 1H), 7.88 (d, 1H), 5.55 (ddq, 1H), 4.52 – 4.32 (m, 4H), 4.17 (dddd, 2H). 3. 275.3</p>
		 <p>8</p>	<p>1. 57 % 2. ¹HNMR (500 MHz, DMSO-<i>d</i>₆) δ 8.65 (d, 1H), 8.61 (s, 1H), 8.17 (s, 1H), 7.93 (d, 1H), 5.50 (d, 1H), 4.38 (s, 2H), 3.93 – 3.61 (m, 3H), 3.53 (td, 1H), 2.40 – 2.11 (m, 2H). 3. 289.2</p>
		 <p>9</p>	<p>1. 85 % 2. ¹HNMR (500 MHz, DMSO-<i>d</i>₆) δ 8.82 (s, 1H), 8.23 (s, 1H), 7.95 (d, 1H), 7.22 (d, 1H), 5.62 – 5.42 (m, 1H), 4.39 (s, 2H), 3.97 – 3.61 (m, 3H), 3.55 (td, 1H), 2.42 – 2.11 (m, 2H). 3. 289.2</p>
		 <p>10</p>	<p>1. 44 % 2. ¹HNMR (500 MHz, DMSO-<i>d</i>₆) δ 8.00 (s, 1H), 7.41 (s, 1H), 7.09 (d, 1H), 6.61 (d, 1H), 4.19 (dt, 1H), 3.57 (d, 2H), 3.39 – 3.24 (m, 1H), 3.22 – 2.96 (m, 2H), 2.96 – 2.76 (m, 1H), 1.13 – 0.76 (m, 5H), 0.70 – 0.55 (m, 1H). 3. 317.0</p>

		 <p style="text-align: center;">11</p>	<p>1. 41 %</p> <p>2. ¹HNMR (500 MHz, DMSO-<i>d</i>₆) δ 8.81 (s, 1H), 8.22 (s, 1H), 7.91 (d, 1H), 7.37 (d, 1H), 4.96 – 4.71 (m, 1H), 4.39 (s, 2H), 3.83 – 3.59 (m, 4H), 2.19 – 1.68 (m, 6H).</p> <p>3. 317.3</p>
		 <p style="text-align: center;">12</p>	<p>1. 66 %</p> <p>2. ¹HNMR (400 MHz, DMSO-<i>d</i>₆) δ 8.81 (s, 1H), 8.23 (s, 1H), 7.95 (d, 1H), 7.22 (d, 1H), 5.51 (d, 1H), 4.39 (s, 2H), 3.98 – 3.63 (m, 4H), 3.63 – 3.49 (m, 1H), 2.42 – 2.07 (m, 2H).</p> <p>3. 289.2</p>

Preparative Example 13



- 5 **Step 1:** A mixture of tert-butyl 4-hydroxy-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxylate (16.0 g, 80.32 mmol), DMFDMA (9.57 g, 80.32 mmol) and ethanol (2 ml) was stirred while heating for 2 hours. The reaction mixture evaporated under vacuum to afford tert-butyl (3Z)-3-[(dimethylamino)methylidene]-2,4-dioxopyrrolidine-1-carboxylate (20.5 g, 92.0% purity, 74.17 mmol, 92.3% yield). LC/MS [M+H] 255.2.
- 10 **Step 2:** Tert-butyl (3Z)-3-[(dimethylamino)methylidene]-2,4-dioxopyrrolidine-1-carboxylate of **step 1** (20.4 g, 80.23 mmol) was dissolved in ethanol (300 ml), and then [(4-methoxyphenyl)methyl]hydrazine (12.21 g, 80.23 mmol) was added. The mixture was stirred at room temperature for 4 hours until complete formation of the intermediate. Then, the reaction mixture was stirred while heating at 80°C for 3 hours, and 4-methylbenzene-1-sulfonic acid hydrate (7.63 g, 40.11

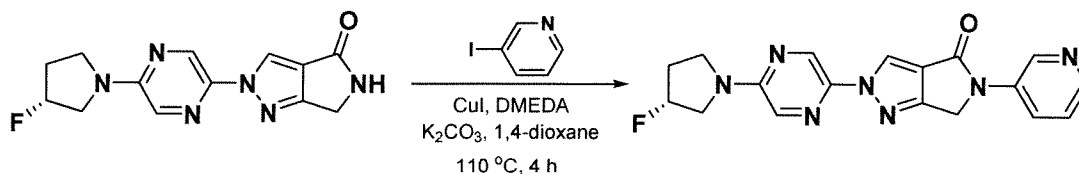
mmol) was added. The progress of the reaction was monitored by LCMS. Upon completion the reaction mixture was concentrated under reduced pressure. DCM was added to dissolve the mixture. A saturated solution of NaHCO₃ was added and the aqueous phase was extracted three times. The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and then evaporated under reduced pressure. The solid was then triturated with MTBE to get 2-[(4-methoxyphenyl)methyl]-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-4-one (10.5 g, 92.0% purity, 39.71 mmol, 50% yield). LC/MS [M+H] 244.2

Step 3: In a flask under argon, palladium(II) acetate (718.54 mg, 4.32 mmol) and XantPhos (7.49 g, 12.95 mmol) were mixed in dioxane (100 ml) and then heated at 100°C for a few seconds to form the Pd-Xphos complex. Then, 2-[(4-methoxyphenyl)methyl]-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-4-one of **step 2** (10.5 g, 43.16 mmol), 3-bromopyridine (13.64 g, 86.33 mmol) and cesium carbonate (42.19 g, 129.49 mmol) were added and the reaction mixture was stirred at 100°C for 18 hours. The progress of the reaction was monitored by LCMS. Upon completion the reaction mixture was concentrated under reduced pressure and then diluted with DCM. A solution of saturated NaHCO₃ was added, and the aqueous layer was extracted twice. The combined organic layers were dried over Na₂SO₄, filtered, and concentrated to dryness. The crude product was triturated in EtOAc to get 2-[(4-methoxyphenyl)methyl]-5-(pyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-4-one (7.0 g, 95.7% purity, 20.91 mmol, 48% yield). LC/MS [M+H] 321.0

Step 4: The mixture of 2-[(4-methoxyphenyl)methyl]-5-(pyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-4-one of **step 3** (7.0 g, 21.85 mmol) in TFA (70 ml) was heated to 60°C and stirred for 3 days. The progress of the reaction was monitored by LCMS. After completion, the reaction mixture was concentrated under reduced pressure and then a solution of saturated K₂CO₃ was added. The mixture was filtered to afford a crude material. Purification by flash chromatography afforded 5-(pyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-4-one (3.0 g, 95.0% purity, 14.24 mmol, 65% yield). LC/MS [M+H] 201.2.

EXAMPLES

Example 1: Synthesis of (R)-2-(5-(3-fluoropyrrolidin-1-yl)pyrazin-2-yl)-5-(pyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one



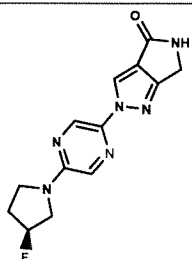
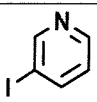
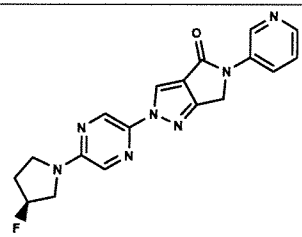
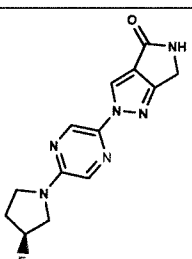
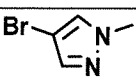
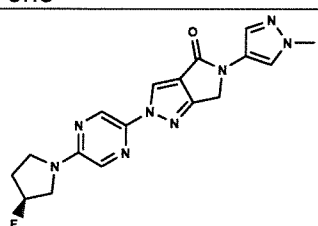
In a screw capped vial, to a stirred solution of **preparative example 1** (30 mg, 0.10 mmol) in 1,4-dioxane (1.5 mL), was added 3-iodo pyridine (43 mg, 0.20 mmol) and anhydrous K₂CO₃ (29 mg, 0.2 mmol) under N₂ atmosphere. The resulting mixture was degassed with N₂ and kept under N₂

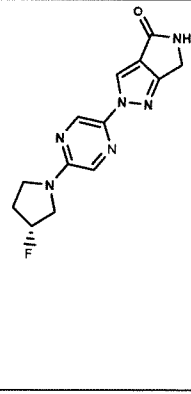

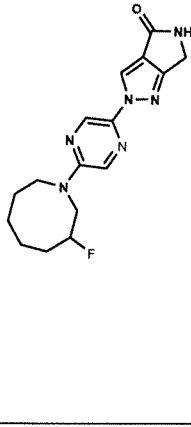

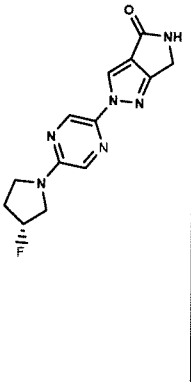

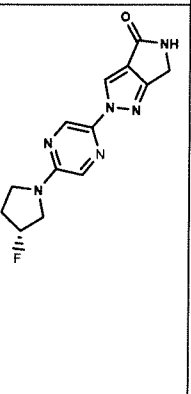
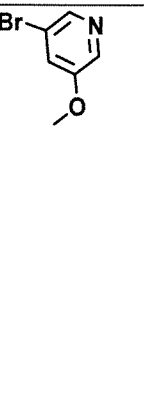
atmosphere. To this mixture was added CuI (4.0 mg, 2 mol %) and DMEDA (4 mg, 4 mol %). The reaction mixture was heated to 120°C for 16 hours. After completion, the reaction mixture was quenched with water (5 mL), and then extracted with 5% MeOH:DCM (10 mL x 3). The combined organic layers were dried over anhydrous Na₂SO₄, and the solvent was removed under reduced pressure to afford a crude material. The crude material was purified by column chromatography over silica gel (230-400 mesh), eluted in 3% MeOH/DCM to afford the desired product as a white solid (10 mg, 26%). ¹H-NMR (500 MHz, DMSO-*d*₆) δ 9.38 (s, 1H), 8.86 (s, 1H), 8.83 – 8.73 (m, 1H), 8.69 (s, 1H), 8.61 (s, 1H), 8.09 – 7.99 (m, 1H), 7.94 (s, 1H), 5.60 – 5.42 (m, 1H), 5.18 (s, 2H), 3.94 – 3.63 (m, 3H), 3.58 (d, 1H), 2.39 – 2.15 (m, 2H). MS (ESI): 366.20 (M⁺+H)⁺.

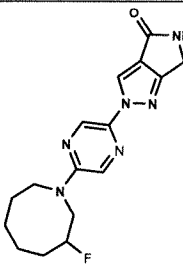
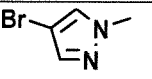
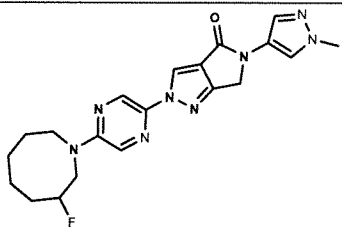
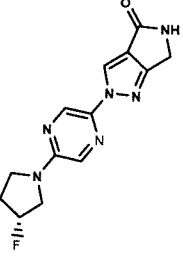
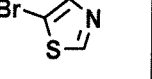
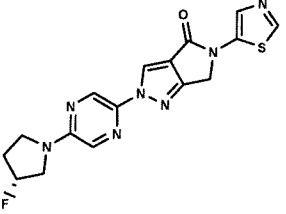
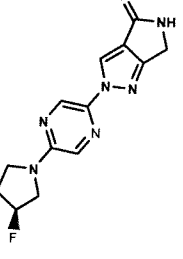
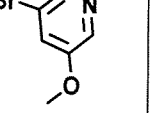
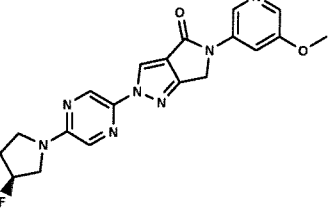
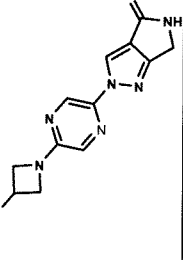
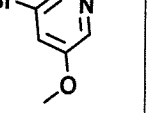
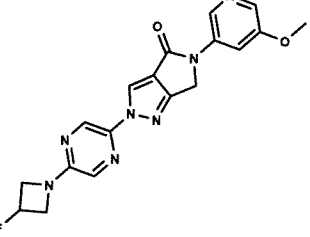
Examples 2 to 35

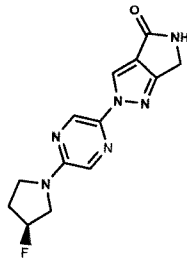
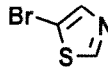
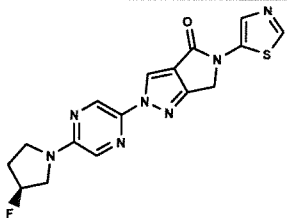
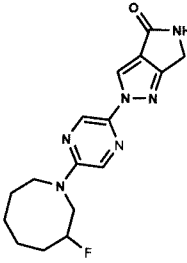
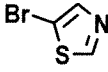
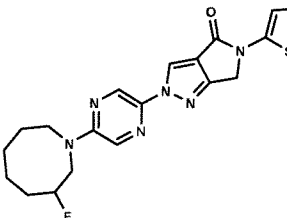
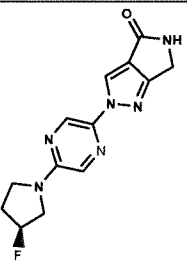
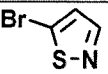
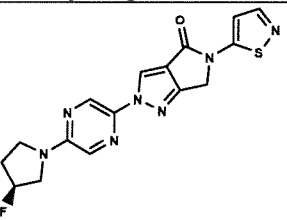
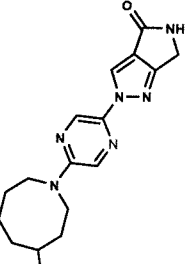
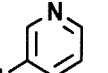
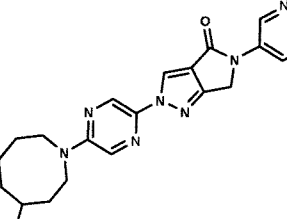
Following the copper coupling procedure described in **Example 1**, using the amide starting material and the appropriate halogenated heteroaryl indicated in the **Table 3** below, the following compounds were prepared.

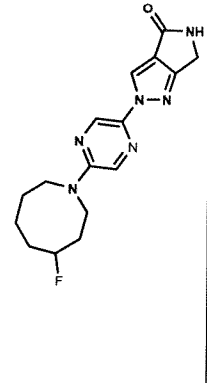
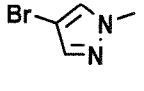
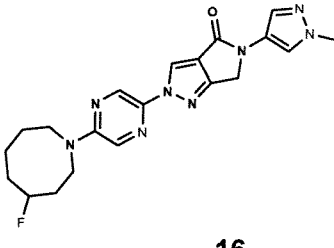
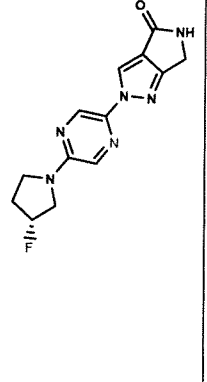
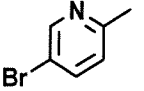
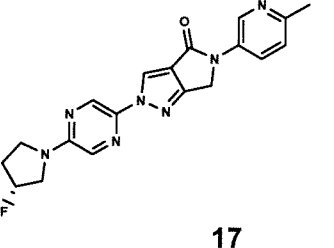
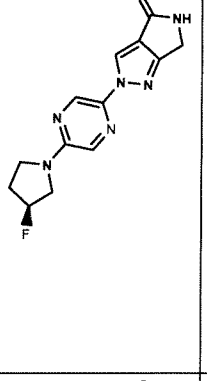
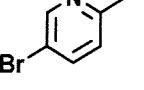
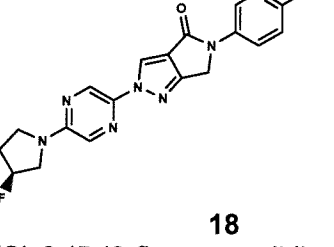
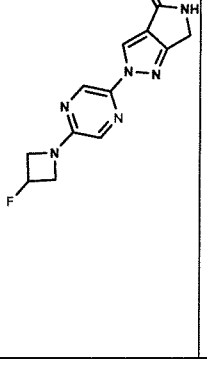
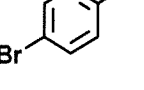
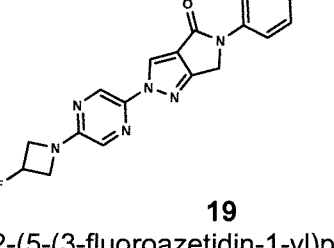
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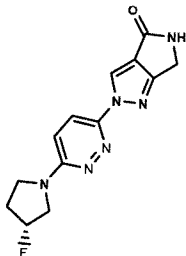
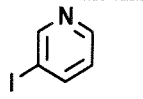
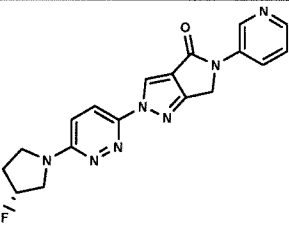
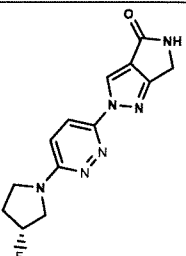
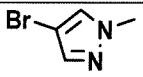
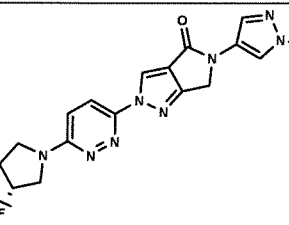
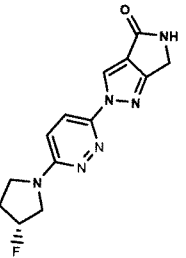
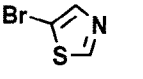
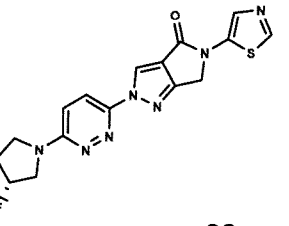
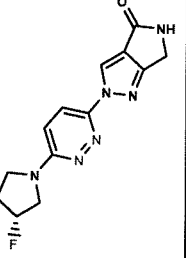
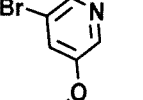
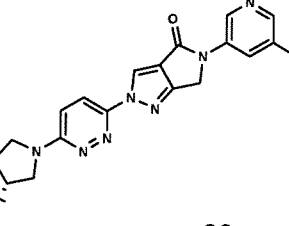
Amide	Halo-genated heteroaryl	Compound of example	1. Yield 2. ¹ H-NMR 3. MH ⁺ (ESI)
		 2 (S)-2-(5-(3-fluoropyrrolidin-1-yl)pyrazin-2-yl)-5-(pyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one	1. 23 % 2. ¹ H NMR (500 MHz, CF ₃ COOD) δ 9.93 (d, 1H), 8.92 (s, 1H), 8.90 – 8.82 (m, 1H), 8.70 (s, 1H), 8.62 (d, 1H), 8.55 (s, 1H), 8.17 (dd, 1H), 5.56 (d, 1H), 5.28 (s, 2H), 4.07 (d, 4H), 2.77 – 2.63 (m, 1H), 2.52 – 2.31 (m, 1H). 3. 366.3
		 3 (S)-2-(5-(3-fluoropyrrolidin-1-yl)pyrazin-2-yl)-5-(1-methyl-1H-pyrazol-4-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one	1. 44 % 2. ¹ H NMR (500 MHz, CF ₃ COOD) δ 8.85 (s, 1H), 8.74 (s, 1H), 8.68 (s, 1H), 8.60 (s, 1H), 8.51 (s, 1H), 5.55 (d, 1H), 5.10 (s, 2H), 4.29 (s, 3H), 4.25 – 3.76 (m, 4H), 2.81 – 2.58 (m, 1H), 2.36 (d, 1H). 3. 369.3

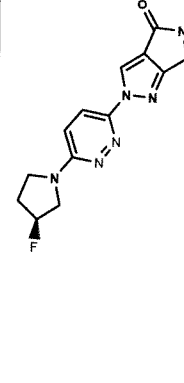

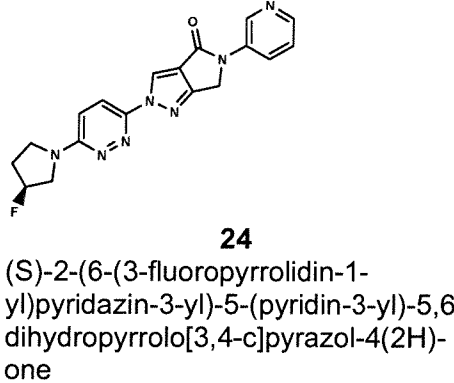
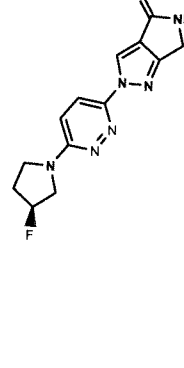

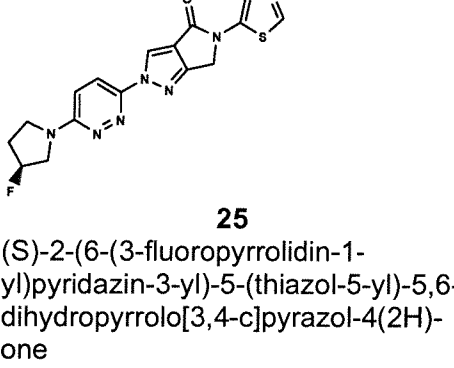
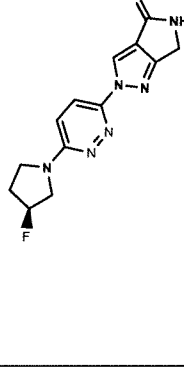

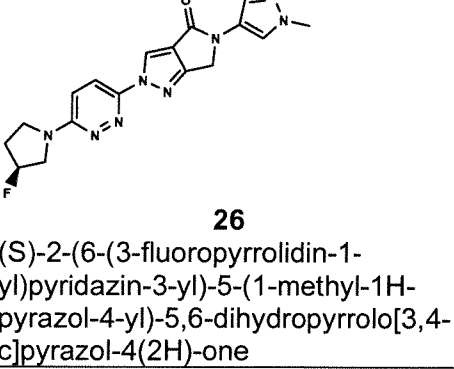
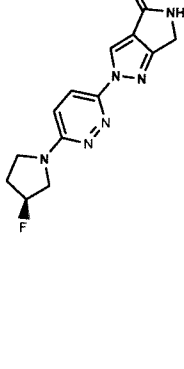

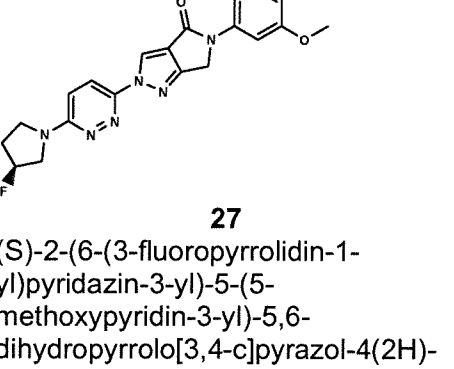
		<p style="text-align: center;">4</p> <p>(R)-2-(5-(3-fluoropyrrolidin-1-yl)pyrazin-2-yl)-5-(1-methyl-1H-pyrazol-4-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 39 %</p> <p>2. ¹H NMR (500 MHz, CF₃COOD) δ 8.94 (s, 1H), 8.83 (s, 1H), 8.78 (s, 1H), 8.69 (s, 1H), 8.60 (s, 1H), 5.64 (d, 1H), 5.19 (s, 2H), 4.39 (s, 3H), 4.34 – 3.98 (m, 4H), 2.76 (dd, 1H), 2.49 (d, 1H).</p> <p>3. 369.3</p>
		<p style="text-align: center;">5</p> <p>2-(5-(3-fluoroazepan-1-yl)pyrazin-2-yl)-5-(pyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 31 %</p> <p>2. ¹H NMR (500 MHz, CF₃COOD) δ 9.91 (d, 1H), 8.92 (s, 2H), 8.86 (d, 1H), 8.60 (d, 1H), 8.54 (s, 1H), 8.16 (dd, 1H), 5.41 – 5.08 (m, 3H), 4.80 – 4.14 (m, 2H), 3.88 (dd, 1H), 3.79 – 3.63 (m, 1H), 2.34 – 2.08 (m, 2H), 2.08 – 1.69 (m, 4H).</p> <p>3. 394.3</p>
		<p style="text-align: center;">6</p> <p>(R)-2-(5-(3-fluoropyrrolidin-1-yl)pyrazin-2-yl)-5-(1-methyl-1H-pyrazol-4-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 26 %</p> <p>2. ¹H NMR (400 MHz, CF₃COOD) δ 8.99 (s, 1H), 8.74 (s, 1H), 8.67 (s, 1H), 8.56 (s, 1H), 7.35 (s, 1H), 5.53 (d, 1H), 5.33 (s, 2H), 4.48 – 3.70 (m, 4H), 2.78 – 2.55 (m, 1H), 2.55 – 2.17 (m, 1H).</p> <p>3. 372.3</p>
		<p style="text-align: center;">7</p> <p>(R)-2-(5-(3-fluoropyrrolidin-1-yl)pyrazin-2-yl)-5-(5-methoxypyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 15 %</p> <p>2. ¹H NMR (500 MHz, CF₃COOD) δ 9.37 (d, 1H), 8.89 (d, 1H), 8.67 (s, 1H), 8.52 (s, 1H), 8.45 – 8.33 (m, 1H), 8.22 (d, 1H), 5.53 (d, 1H), 5.22 (s, 2H), 4.32 – 3.86 (m, 7H), 2.66 (td, 1H), 2.56 – 2.25 (m, 1H).</p> <p>3. 396.3</p>

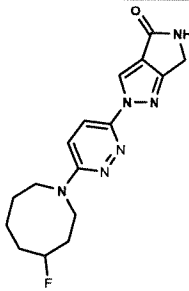

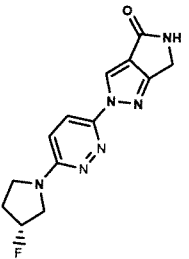
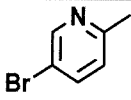
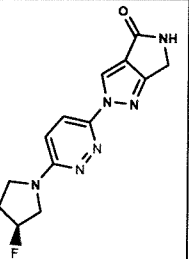
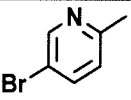
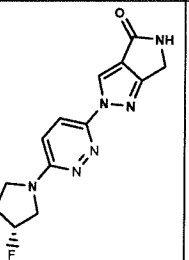

		 <p style="text-align: center;">8</p> <p>2-(5-(3-fluoroazepan-1-yl)pyrazin-2-yl)-5-(1-methyl-1H-pyrazol-4-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 32 %</p> <p>2. ¹H NMR (500 MHz, DMSO-<i>d</i>₆) δ 8.72 (s, 1H), 8.64 (s, 1H), 8.13 (s, 1H), 8.07 (d, 1H), 7.68 (d, 1H), 4.99 (dt, 1H), 4.86 (s, 2H), 4.16 – 3.89 (m, 2H), 3.85 (s, 3H), 3.83 – 3.73 (m, 1H), 3.73 – 3.57 (m, 1H), 1.90 – 1.57 (m, 5H), 1.52 – 1.35 (m, 1H).</p> <p>3. 397.4</p>
		 <p style="text-align: center;">9</p> <p>(R)-2-(5-(3-fluoropyrrolidin-1-yl)pyrazin-2-yl)-5-(thiazol-5-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 15 %</p> <p>2. ¹H NMR (500 MHz, CF₃COOD) δ 9.49 (s, 1H), 8.92 (s, 1H), 8.66 (s, 1H), 8.54 (s, 1H), 8.21 (s, 1H), 5.53 (d, 1H), 5.22 (s, 2H), 4.26 – 3.78 (m, 4H), 2.66 (s, 1H), 2.39 (d, 1H).</p> <p>3. 372.2</p>
		 <p style="text-align: center;">10</p> <p>(S)-2-(5-(3-fluoropyrrolidin-1-yl)pyrazin-2-yl)-5-(5-methoxypyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 10 %</p> <p>2. ¹H NMR (500 MHz, CF₃COOD) δ 9.39 (d, 1H), 8.90 (s, 1H), 8.68 (d, 1H), 8.60 – 8.45 (m, 1H), 8.39 (t, 1H), 8.23 (d, 1H), 5.54 (d, 1H), 5.23 (s, 2H), 4.42 – 3.71 (m, 7H), 2.67 (td, 1H), 2.54 – 2.18 (m, 1H).</p> <p>3. 396.2</p>
		 <p style="text-align: center;">11</p> <p>2-(5-(3-fluoroazetid-1-yl)pyrazin-2-yl)-5-(5-methoxypyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 9 %</p> <p>2. ¹H NMR (500 MHz, CF₃COOD) δ 9.47 – 9.30 (m, 1H), 8.87 (s, 1H), 8.48 (d, 1H), 8.43 – 8.40 (m, 1H), 8.38 (t, 1H), 8.23 (d, 1H), 5.76 – 5.41 (m, 1H), 5.22 (s, 2H), 5.05 – 4.81 (m, 2H), 4.73 (dd, 2H), 4.10 (s, 3H).</p> <p>3. 382.3</p>

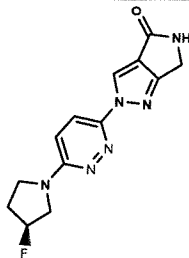
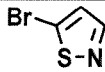
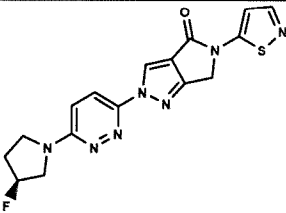
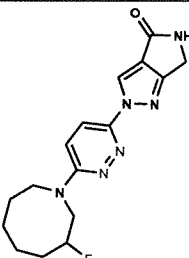

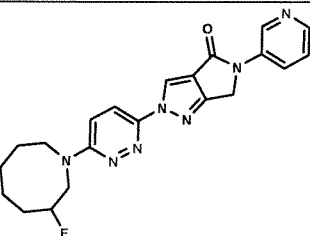
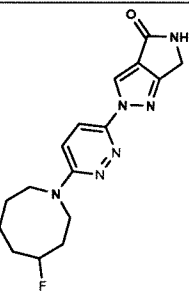
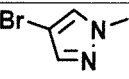
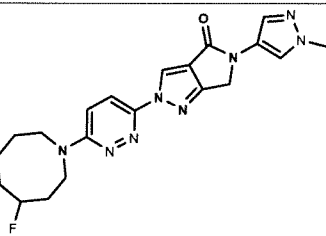
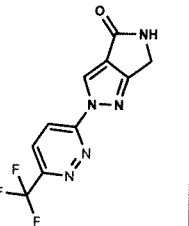

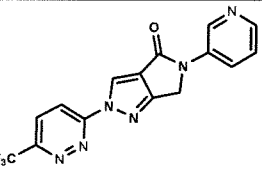
		 <p style="text-align: center;">12</p> <p>(S)-2-(5-(3-fluoropyrrolidin-1-yl)pyrazin-2-yl)-5-(thiazol-5-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 22 % 2. ¹H NMR (500 MHz, CF₃COOD) δ 9.50 (s, 1H), 8.93 (s, 1H), 8.79 – 8.61 (m, 1H), 8.55 (s, 1H), 8.21 (s, 1H), 5.53 (d, 1H), 5.23 (s, 2H), 4.35 – 3.68 (m, 4H), 2.67 (s, 1H), 2.39 (d, 1H). 3. 372.3</p>
		 <p style="text-align: center;">13</p> <p>2-(5-(3-fluoroazepan-1-yl)pyrazin-2-yl)-5-(thiazol-5-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one hydrogen chloride</p>	<p>1. 15 % 2. ¹H NMR (500 MHz, CF₃COOD) δ 9.64 (t, 1H), 9.06 (s, 2H), 8.67 (s, 1H), 8.34 (s, 1H), 5.55 – 5.18 (m, 3H), 5.00 – 4.24 (m, 2H), 4.02 (dd, 1H), 3.94 – 3.79 (m, 1H), 2.50 – 2.24 (m, 2H), 2.24 – 1.78 (m, 4H). 3. 400.0</p>
		 <p style="text-align: center;">14</p> <p>(S)-2-(5-(3-fluoropyrrolidin-1-yl)pyrazin-2-yl)-5-(1-methyl-1H-pyrazol-4-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 11 % 2. ¹H NMR (400 MHz, CF₃COOD) δ 8.97 (s, 1H), 8.73 (s, 1H), 8.66 (s, 1H), 8.55 (s, 1H), 7.34 (s, 1H), 5.51 (d, 1H), 5.32 (s, 2H), 4.52 – 3.70 (m, 4H), 2.65 (s, 1H), 2.53 – 2.17 (m, 1H). 3. 371.9</p>
		 <p style="text-align: center;">15</p> <p>2-(5-(4-fluoroazepan-1-yl)pyrazin-2-yl)-5-(pyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 28 % 2. ¹H NMR (500 MHz, DMSO-<i>d</i>₆) δ 9.04 (s, 1H), 8.82 (s, 1H), 8.68 (s, 1H), 8.37 (s, 1H), 8.27 (d, 1H), 8.10 (s, 1H), 7.47 (dd, 1H), 5.12 (s, 2H), 4.84 (d, 1H), 3.80 – 3.57 (m, 4H), 2.15 – 1.70 (m, 6H). 3. 394.3</p>

		 16 2-(5-(4-fluoroazepan-1-yl)pyrazin-2-yl)-5-(1-methyl-1H-pyrazol-4-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one	1.22 % 2. ¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ 8.71 (s, 1H), 8.65 (d, 1H), 8.14 – 8.02 (m, 2H), 7.69 (d, 1H), 4.86 (s, 3H), 3.86 (s, 3H), 3.79 – 3.55 (m, 4H), 2.17 – 1.64 (m, 6H). 3.397.4
		 17 (R)-2-(5-(3-fluoropyrrolidin-1-yl)pyrazin-2-yl)-5-(6-methylpyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one	1.66 % 2. ¹ H NMR (500 MHz, CF ₃ COOD) δ 9.71 (d, 1H), 8.90 (s, 1H), 8.69 (dd, 2H), 8.54 (s, 1H), 7.94 (d, 1H), 5.54 (d, 1H), 5.23 (s, 2H), 4.09 (s, 4H), 2.86 (s, 3H), 2.68 (td, 1H), 2.54 – 2.25 (m, 1H). 3. 380.0
		 18 (S)-2-(5-(3-fluoropyrrolidin-1-yl)pyrazin-2-yl)-5-(6-methylpyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one	1.66 % 2. ¹ H NMR (500 MHz, CF ₃ COOD) δ 9.70 (d, 1H), 8.89 (s, 1H), 8.75 – 8.61 (m, 2H), 8.52 (d, 1H), 7.93 (d, 1H), 5.65 – 5.42 (m, 1H), 5.23 (s, 2H), 4.34 – 3.87 (m, 4H), 2.86 (s, 3H), 2.68 (tt, 1H), 2.53 – 2.29 (m, 1H). 3.380.2
		 19 2-(5-(3-fluoroazetididin-1-yl)pyrazin-2-yl)-5-(6-methylpyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one	1.16 % 2. ¹ H NMR (500 MHz, CF ₃ COOD) δ 9.71 (d, 1H), 8.88 (s, 1H), 8.69 (dd, 1H), 8.52 (s, 1H), 8.44 (s, 1H), 7.95 (d, 1H), 5.60 (d, 1H), 5.24 (s, 2H), 5.03 – 4.85 (m, 2H), 4.85 – 4.62 (m, 2H), 2.88 (s, 3H). 3.366.0

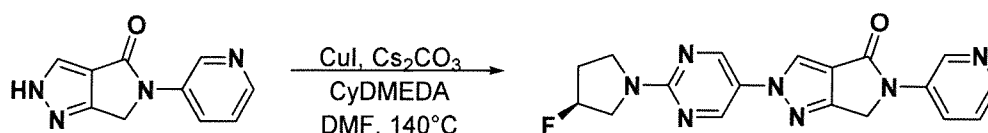
		 <p style="text-align: center;">20</p> <p>(R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridazin-3-yl)-5-(pyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 20 %</p> <p>2. ¹H NMR (500 MHz, DMSO-<i>d</i>₆) δ 9.05 (s, 2H), 8.37 (dd, 1H), 8.29 (ddd, 1H), 8.01 (d, 1H), 7.53 – 7.42 (m, 1H), 7.25 (d, 1H), 5.52 (d, 1H), 5.14 (s, 2H), 3.96 – 3.66 (m, 3H), 3.56 (td, 1H), 2.42 – 2.09 (m, 2H).</p> <p>3. 366.3</p>
		 <p style="text-align: center;">21</p> <p>(R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridazin-3-yl)-5-(1-methyl-1H-pyrazol-4-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 13 %</p> <p>2. ¹H NMR (500 MHz, CF₃COOD) δ 8.78 (s, 1H), 8.74 (s, 1H), 8.64 – 8.49 (m, 2H), 7.83 (dd, 1H), 5.53 (dd, 1H), 5.08 (s, 2H), 4.29 (s, 3H), 4.22 – 3.85 (m, 4H), 2.79 – 2.55 (m, 1H), 2.39 (ddd, 1H).</p> <p>3. 369.3</p>
		 <p style="text-align: center;">22</p> <p>(R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridazin-3-yl)-5-(thiazol-5-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 12 %</p> <p>2. ¹H NMR (500 MHz, CF₃COOD) δ 9.50 (d, 1H), 8.89 (s, 1H), 8.68 – 8.53 (m, 1H), 8.21 (d, 1H), 7.83 (dd, 1H), 5.53 (dd, 1H), 5.23 (s, 3H), 4.29 – 3.85 (m, 6H), 2.81 – 2.56 (m, 1H), 2.56 – 2.21 (m, 1H).</p> <p>3. 372.0</p>
		 <p style="text-align: center;">23</p> <p>(R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridazin-3-yl)-5-(5-methoxypyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 6 %</p> <p>2. ¹H NMR (500 MHz, CF₃COOD) δ 9.35 (d, 1H), 8.85 (s, 1H), 8.59 (d, 1H), 8.42 (s, 1H), 8.24 (d, 1H), 7.84 (dd, 1H), 5.53 (dd, 1H), 5.24 (s, 2H), 4.26 – 3.87 (m, 7H), 2.83 – 2.54 (m, 1H), 2.54 – 2.25 (m, 1H).</p> <p>3. 396.1</p>

		 <p style="text-align: center;">24</p> <p>(S)-2-(6-(3-fluoropyrrolidin-1-yl)pyridazin-3-yl)-5-(pyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 23 %</p> <p>2. ¹H NMR (400 MHz, DMSO-<i>d</i>₆) δ 9.11 – 9.00 (m, 2H), 8.37 (dd, 1H), 8.29 (dt, 1H), 8.01 (d, 1H), 7.47 (dd, 1H), 7.25 (d, 1H), 5.52 (d, 1H), 5.14 (s, 2H), 3.99 – 3.63 (m, 3H), 3.56 (td, 1H), 2.42 – 2.15 (m, 2H).</p> <p>3. 366.3</p>
		 <p style="text-align: center;">25</p> <p>(S)-2-(6-(3-fluoropyrrolidin-1-yl)pyridazin-3-yl)-5-(thiazol-5-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 38 %</p> <p>2. ¹H NMR (500 MHz, CF₃COOD) δ 9.71 (t, 1H), 9.09 (s, 1H), 8.80 (d, 1H), 8.40 (d, 1H), 8.04 (dd, 1H), 5.89 – 5.55 (m, 1H), 5.43 (s, 2H), 4.48 – 4.07 (m, 4H), 3.11 – 2.76 (m, 1H), 2.76 – 2.33 (m, 1H).</p> <p>3. 372.3</p>
		 <p style="text-align: center;">26</p> <p>(S)-2-(6-(3-fluoropyrrolidin-1-yl)pyridazin-3-yl)-5-(1-methyl-1H-pyrazol-4-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 39 %</p> <p>2. ¹H NMR (500 MHz, CF₃COOD) δ 9.02 – 8.94 (m, 1H), 8.91 (s, 1H), 8.82 – 8.69 (m, 2H), 8.01 (dd, 1H), 5.71 (dd, 1H), 5.26 (s, 2H), 4.47 (s, 3H), 4.40 – 4.05 (m, 4H), 2.85 (d, 1H), 2.73 – 2.48 (m, 1H).</p> <p>3. 369.3</p>
		 <p style="text-align: center;">27</p> <p>(S)-2-(6-(3-fluoropyrrolidin-1-yl)pyridazin-3-yl)-5-(5-methoxypyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 36 %</p> <p>2. ¹H (500 MHz, CF₃COOD) δ 9.34 (d, 1H), 8.83 (s, 1H), 8.57 (d, 1H), 8.40 (d, 1H), 8.22 (d, 1H), 7.82 (dd, 1H), 5.51 (dd, 1H), 5.22 (s, 2H), 4.25 – 3.87 (m, 7H), 2.81 – 2.54 (m, 1H), 2.54 – 2.24 (m, 1H).</p> <p>3. 396.1</p>

		<p style="text-align: center;">28</p> <p>2-(6-(4-fluoroazepan-1-yl)pyridazin-3-yl)-5-(pyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 27 %</p> <p>2. ¹H NMR (500 MHz, DMSO-<i>d</i>₆) δ 9.17 – 8.94 (m, 2H), 8.37 (dd, 1H), 8.28 (ddd, 1H), 7.96 (d, 1H), 7.47 (dd, 1H), 7.39 (d, 1H), 5.13 (s, 2H), 4.84 (d, 1H), 3.88 – 3.59 (m, 4H), 2.20 – 1.66 (m, 6H).</p> <p>3. 394.4</p>
		<p style="text-align: center;">29</p> <p>(<i>R</i>)-2-(6-(3-fluoropyrrolidin-1-yl)pyridazin-3-yl)-5-(6-methylpyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 21 %</p> <p>2. ¹H NMR (400 MHz, CF₃COOD) δ 9.66 (d, 1H), 8.84 (s, 1H), 8.69 (dd, 1H), 8.57 (d, 1H), 7.93 (d, 1H), 7.83 (dd, 1H), 5.52 (dd, 1H), 5.23 (s, 2H), 4.25 – 3.85 (m, 4H), 2.85 (s, 3H), 2.77 – 2.53 (m, 1H), 2.53 – 2.21 (m, 1H).</p> <p>3. 380.0</p>
		<p style="text-align: center;">30</p> <p>(<i>S</i>)-2-(6-(3-fluoropyrrolidin-1-yl)pyridazin-3-yl)-5-(6-methylpyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 51 %</p> <p>2. ¹H NMR (400 MHz, CF₃COOD) δ 9.66 (d, 1H), 8.85 (s, 1H), 8.69 (d, 1H), 8.57 (d, 1H), 7.93 (d, 1H), 7.83 (dd, 1H), 5.52 (dd, 1H), 5.23 (s, 2H), 4.28 – 3.83 (m, 4H), 2.85 (s, 3H), 2.75 – 2.53 (m, 1H), 2.44 (s, 1H).</p> <p>3. 380.0</p>
		<p style="text-align: center;">31</p> <p>(<i>R</i>)-2-(6-(3-fluoropyrrolidin-1-yl)pyridazin-3-yl)-5-(isothiazol-5-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 58 %</p> <p>2. ¹H NMR (500 MHz, CF₃COOD) δ 8.97 (s, 1H), 8.77 (d, 1H), 8.61 (d, 1H), 7.86 (dd, 1H), 7.37 (d, 1H), 5.54 (dd, 1H), 5.36 (s, 2H), 4.36 – 3.84 (m, 4H), 2.85 – 2.56 (m, 1H), 2.56 – 2.21 (m, 1H).</p> <p>3. 371.8</p>

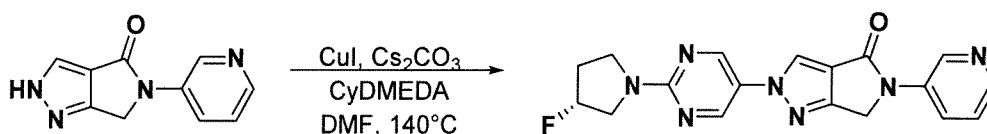
		 <p style="text-align: center;">32</p> <p>(S)-2-(6-(3-fluoropyrrolidin-1-yl)pyridazin-3-yl)-5-(isothiazol-5-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 29 % 2. ¹H NMR (400 MHz, CF₃COOD) δ 9.00 (s, 1H), 8.86 – 8.73 (m, 1H), 8.64 (dd, 1H), 7.88 (dd, 1H), 7.39 (d, 1H), 5.56 (dd, 1H), 5.38 (s, 2H), 4.29 – 3.88 (m, 4H), 2.82 – 2.59 (m, 1H), 2.59 – 2.24 (m, 1H). 3. 371.8</p>
		 <p style="text-align: center;">33</p> <p>2-(6-(3-fluoroazepan-1-yl)pyridazin-3-yl)-5-(pyridin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 24 % 2. ¹H NMR (500 MHz, CF₃COOD) δ 9.88 (d, 1H), 8.95 – 8.81 (m, 2H), 8.72 – 8.47 (m, 2H), 8.26 – 7.97 (m, 2H), 5.47 – 4.95 (m, 3H), 4.52 (s, 1H), 4.15 (d, 1H), 4.00 – 3.79 (m, 1H), 3.73 (s, 1H), 2.38 – 1.67 (m, 6H). 3. 393.9</p>
		 <p style="text-align: center;">34</p> <p>2-(6-(4-fluoroazepan-1-yl)pyridazin-3-yl)-5-(1-methyl-1H-pyrazol-4-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 25 % 2. ¹H NMR (500 MHz, DMSO-<i>d</i>₆) δ 9.17 – 8.94 (m, 2H), 8.37 (dd, 1H), 8.28 (ddd, 1H), 7.96 (d, 1H), 7.47 (dd, 1H), 7.39 (d, 1H), 5.13 (s, 2H), 4.84 (d, 1H), 3.88 – 3.59 (m, 4H), 2.20 – 1.66 (m, 6H). 3. 396.4</p>
		 <p style="text-align: center;">35</p> <p>5-(pyridin-3-yl)-2-(6-(trifluoromethyl)pyridazin-3-yl)-5,6-dihydropyrrolo[3,4-c]pyrazol-4(2H)-one</p>	<p>1. 24 % 2. ¹H NMR (600 MHz, DMSO-<i>d</i>₆) δ 9.41 (s, 1H), 9.05 (s, 1H), 8.57 – 8.46 (m, 2H), 8.39 (s, 1H), 8.28 (d, 1H), 7.48 (dd, 1H), 5.20 (s, 2H). 3. 347.0</p>

Example 36: Synthesis of 2-2-[(3S)-3-fluoropyrrolidin-1-yl]pyrimidin-5-yl-5-(pyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-4-one



The mixture of **preparative example 13** (276.82 mg, 1.12 mmol), copper (I) iodide (142.83 mg, 749.94 μ mol), cesium carbonate (488.69 mg, 1.5 mmol), and 1-N,2-N-dimethylcyclohexane-1,2-diamine (106.67 mg, 749.94 μ mol) in DMF (5 ml) was bubbled with argon for 1 minute. The tube was sealed, and the mixture was heated to 140°C and stirred overnight. Upon completion the reaction mixture was concentrated under reduced pressure, then diluted with water and NH₃, filtered, and then washed twice with water. The precipitate was crystallized from EtOH-DMF and purified by HPLC to obtain 2-2-[(3S)-3-fluoropyrrolidin-1-yl]pyrimidin-5-yl-5-(pyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-4-one (2.3 mg, 95.0% purity, 5.98 μ mol, 8% yield). ¹H NMR (500 MHz, CD₃CN) δ 9.40 (s, 1H), 8.70 (s, 2H), 8.60 (d, 1H), 8.43 (s, 2H), 7.93 (s, 1H), 5.56 – 5.25 (m, 1H), 5.01 (s, 2H), 4.00 – 3.48 (m, 4H), 2.45 – 2.11 (m, 2H). MS (ESI): 366.20 (M⁺+H)⁺.

Example 37: Synthesis of 2-2-[(3R)-3-fluoropyrrolidin-1-yl]pyrimidin-5-yl-5-(pyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-4-one hydrochloride



A mixture of **preparative example 13** (400.0 mg, 2.0 mmol), 5-bromo-2-[(3R)-3-fluoropyrrolidin-1-yl]pyrimidine (737.64 mg, 3.0 mmol), copper(I)iodide (380.59 mg, 2.0 mmol), cesium carbonate (1.3 g, 4.0 mmol), and 1-N,2-N-dimethylcyclohexane-1,2-diamine (284.25 mg, 2.0 mmol) in DMF (5 ml) was bubbled with argon for 1 minute. The tube was sealed, and the mixture was heated to 140°C and stirred overnight. Upon completion the reaction mixture was concentrated under reduced pressure, diluted with water and NH₃, filtered, and then the precipitate was washed twice with water. The precipitate was crystallized from EtOH-DMF and purified by HPLC (water-acetonitrile+HCl) to obtain 2-2-[(3R)-3-fluoropyrrolidin-1-yl]pyrimidin-5-yl-5-(pyridin-3-yl)-2H,4H,5H,6H-pyrrolo[3,4-c]pyrazol-4-one hydrochloride (25.3 mg, 90.0% purity, 56.67 μ mol, 3% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.43 (s, 1H), 9.00 (s, 1H), 8.89 (s, 2H), 8.81 (d, 1H), 8.76 – 8.60 (m, 1H), 8.12 – 8.01 (m, 1H), 5.58 – 5.37 (m, 1H), 5.19 (s, 2H), 3.97 – 3.62 (m, 3H), 3.56 (d, 1H), 2.35 – 2.16 (m, 2H). MS (ESI): 366.20 (M⁺+H)⁺.

BIOLOGICAL ASSAY DESCRIPTION AND CORRESPONDING RESULTS**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 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 (2µM) 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.

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 4 as: % competition at 1µM and 100nM. Table 4 also shows K_i values.

Table 4

Example Compound no.	Micro-radiobinding competition assay		
	Competition at 1µM (%)	Competition at 100nM (%)	K_i (nM)
1	95	89	
2	98	74	
3	102	81	25
4	105	69	
5	68	39	
6	20	3	
7	69	48	
8	78	45	33
9		106	10
10	82	59	
11	69	41	

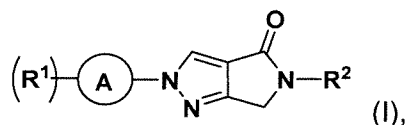
12		50	
13	51	42	
14		63	
15	67	39	
16	73	37	
17	91	46	
18	82	38	
19	74	44	
20	74	38	422
21	86	65	
22		30	
23	76	65	182
24	77	30	236
25	73	17	222
26	76	8	
27	57	50	
28	51	17	
29	42	15	
30	39	4	
31	86	71	
32	77	61	
33	35	6	
34	40	4	
35	34	29	
36	64	37	
37	78	32	356

5 Table 4: 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 1-37. K_i values are also shown for selected example compounds. As shown in Table 1, example compounds 1-37 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

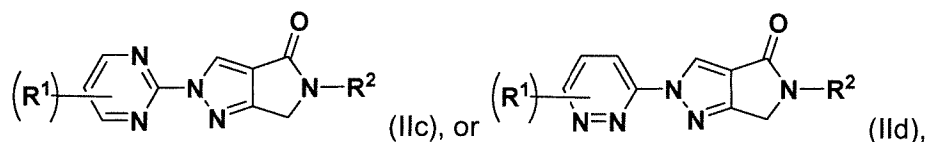
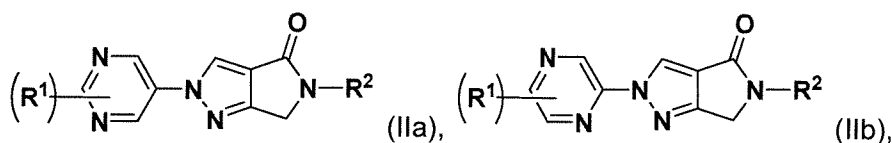
(A) is a 6-membered heteroaryl comprising at least 2 heteroatoms;

R¹ is C₁-C₄alkyl, haloC₁-C₄alkyl, -NH₂, -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl), wherein the C₁-C₄alkyl is optionally substituted with at least one halo; or

15 R¹ is a heterocyclyl which is optionally substituted with at least one halo; and

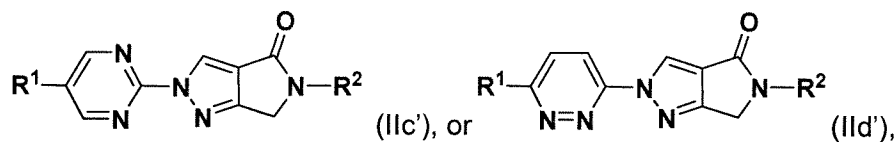
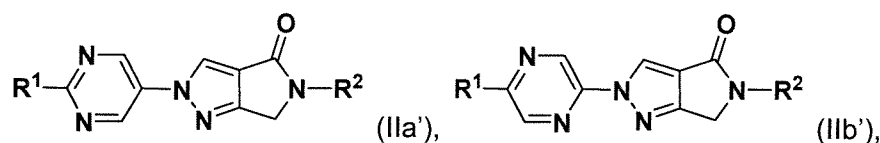
R² is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkoxy, and C₁-C₄alkyl.

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



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

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

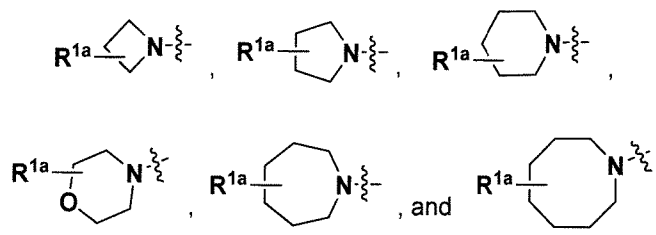


25

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

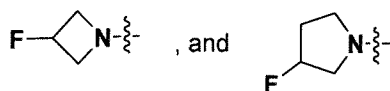
4. The compound according to any one of claims 1 to 3, wherein **R**¹ is C₁-C₄alkyl, haloC₁-C₄alkyl, or a 4- to 8-membered heterocyclyl optionally substituted with at least one halo.

5. The compound according to claim 4, wherein **R**¹ is a 4- to 8-membered heterocyclyl selected from the following:

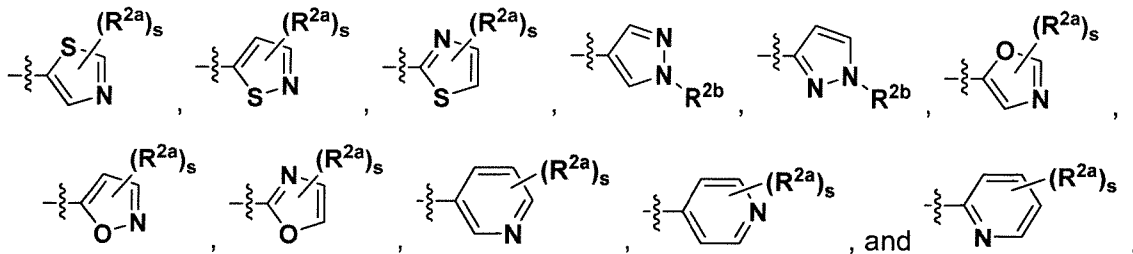


wherein **R**^{1a} is H or halo.

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



7. The compound according to any one of claims 1 to 3, wherein **R**² is a 5-membered or 6-membered heteroaryl selected from the following:



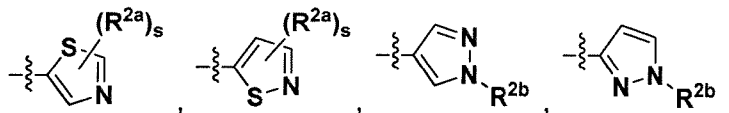
wherein

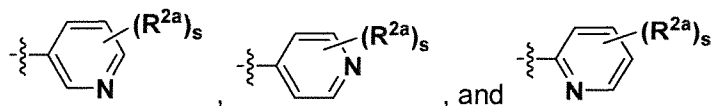
R^{2a} is independently selected from haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkoxy, and C₁-C₄alkyl;

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

s is 0, 1 or 2.

8. The compound according to claim 7, wherein **R**² is a 5-membered or 6-membered heteroaryl selected from the following:



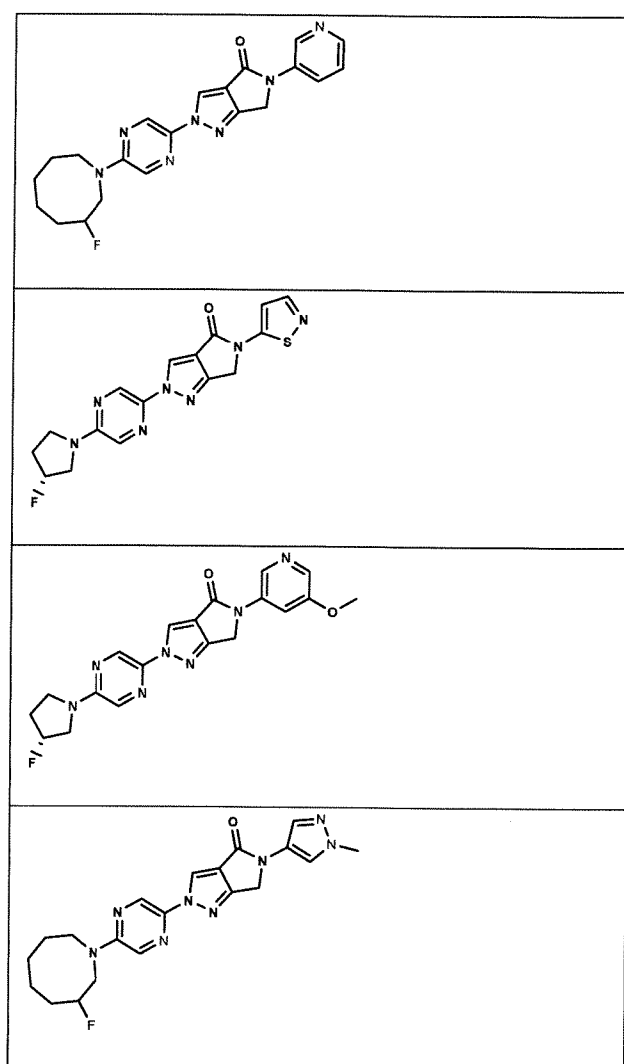
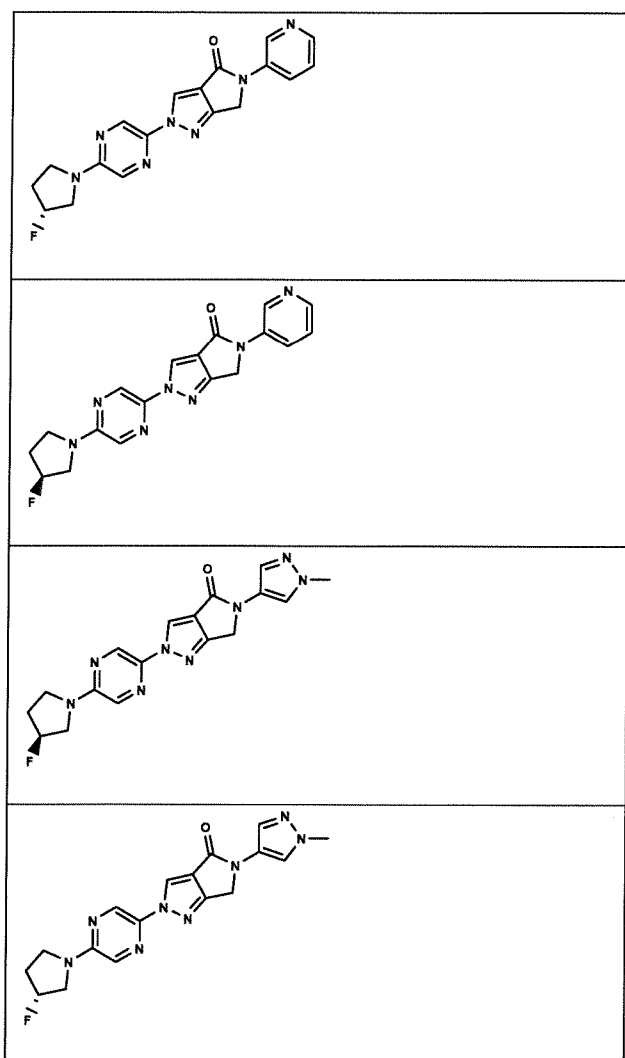


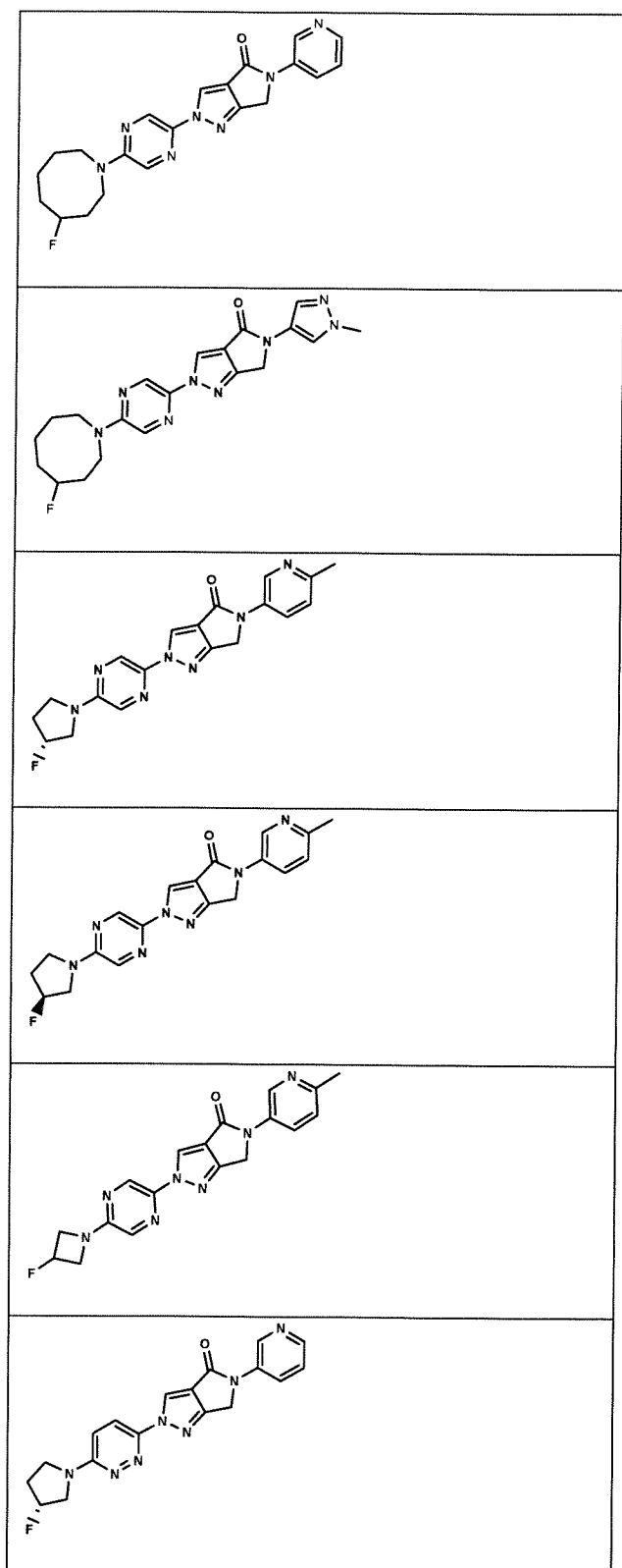
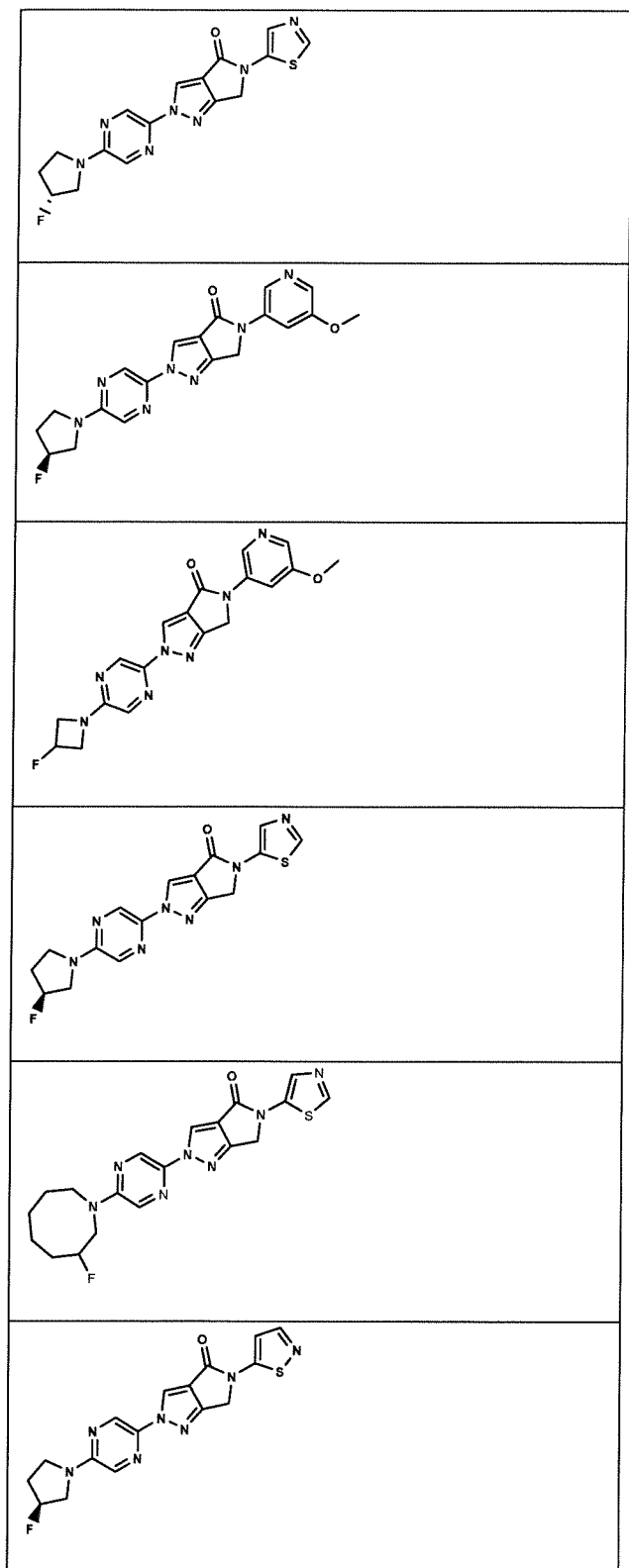
wherein

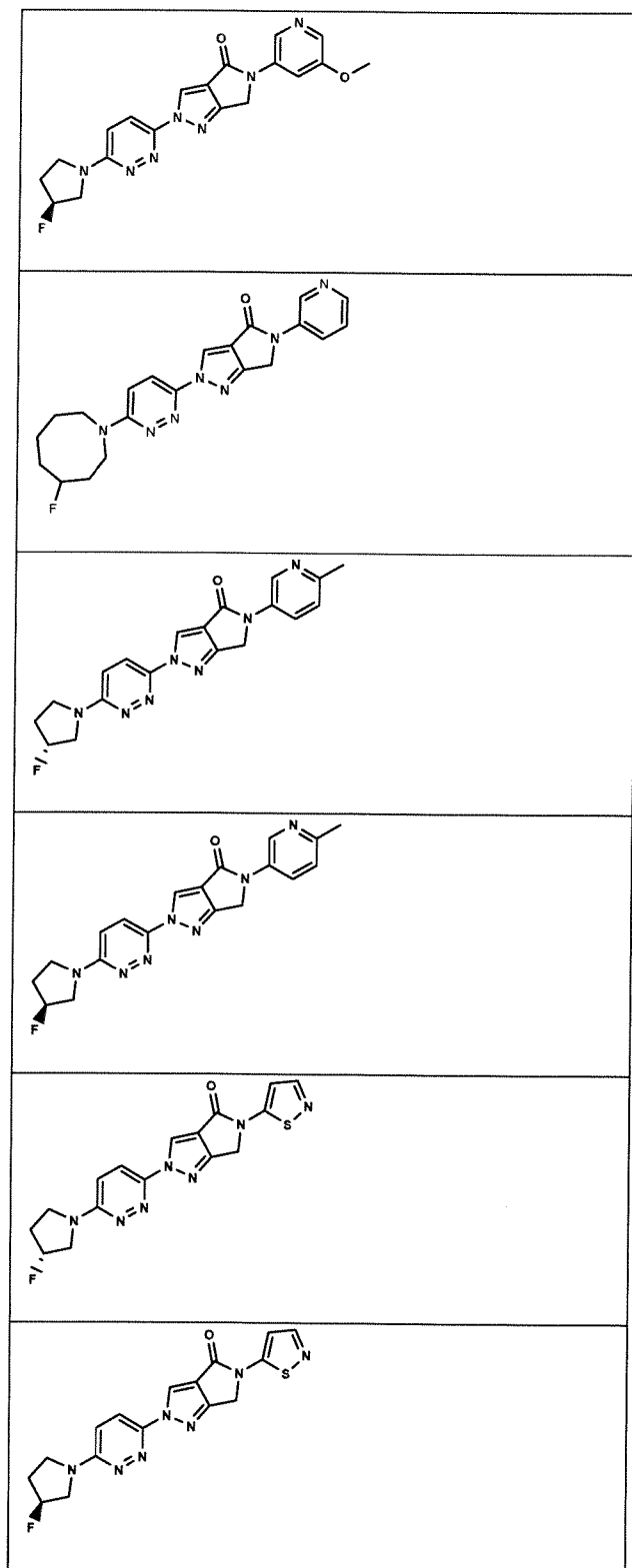
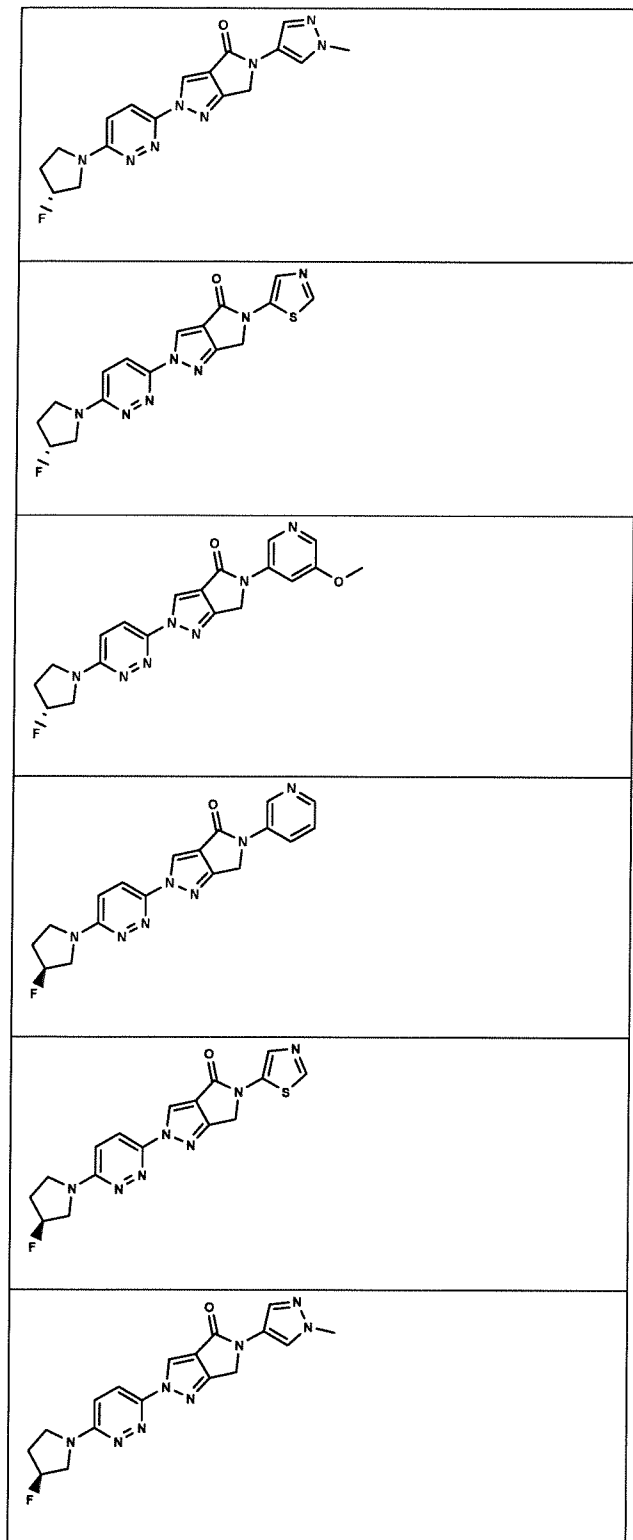
R^{2a} is independently selected from haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkoxy, and C₁-C₄alkyl;

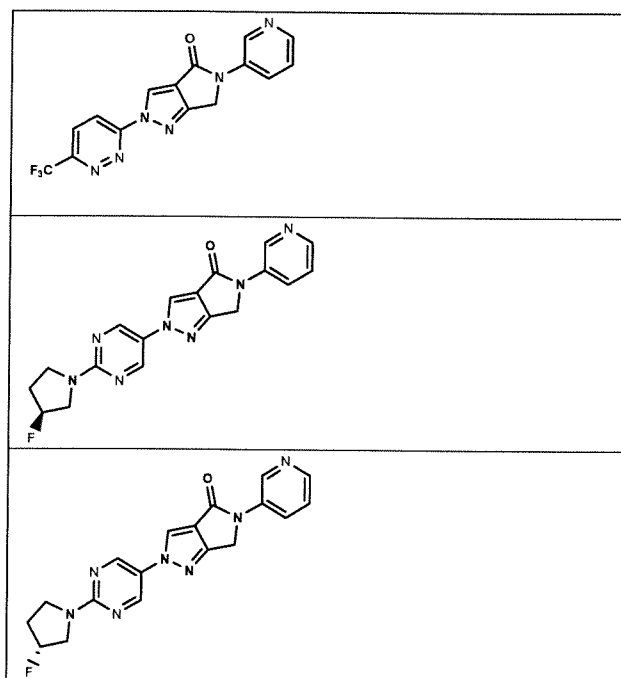
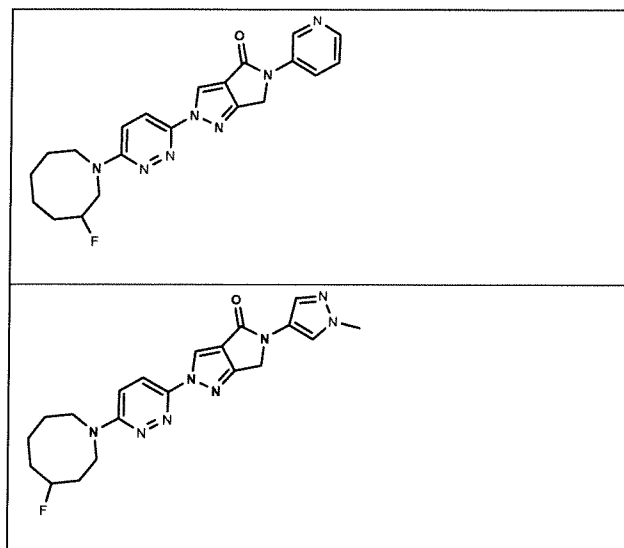
5 **R^{2b}** is selected from H, haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkoxy, and C₁-C₄alkyl; and **s** is 0, 1 or 2.

9. 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.
- 5 10. The compound according to any one of the preceding claims, wherein the compound is a detectably labelled compound.
11. The compound according to claim 10, wherein the detectably labelled compound comprises a detectable label selected from a radioisotope, preferably ^2H , ^3H or ^{18}F .
12. A diagnostic composition comprising a compound according to any one of claims 1 to 11, and optionally at least one pharmaceutically acceptable excipient, carrier, diluent and/or adjuvant.
- 10 13. The compound according to claim 10 or 11, or the diagnostic composition according to claim 12, for use in the imaging of alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions.
14. The compound according to claim 10 or 11, or the diagnostic composition according to claim 12, for use in positron emission tomography imaging of alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions.
- 15 15. The compound for use or the diagnostic composition for use according to claim 13 or 14, 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.
- 20 16. The compound according to claim 10 or 11, or the diagnostic composition according to claim 12, for use in diagnostics.

17. The compound for use or the diagnostic composition for use according to claim 16, wherein the diagnostics are the diagnostics of a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytosolic glial inclusions or a predisposition therefor, wherein the disease, disorder or abnormality is optionally selected from Parkinson's disease (including sporadic, familial with alpha-synuclein gene mutations or changes in copy number (e.g. SNCA duplication or triplication), familial with mutations other than alpha-synuclein, pure autonomic failure or Lewy body dysphagia), Lewy Body dementia (LBD), dementia with Lewy bodies (DLB) (including "pure" Lewy body dementia), Parkinson's disease with mild-cognitive impairment (PD-MCI) or Parkinson's disease with 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.
18. The compound for use or the diagnostic composition for use according to claim 17, wherein the disease is Parkinson's disease.
19. The compound for use or the diagnostic composition for use according to claim 17, wherein the disease is multiple system atrophy.
20. The compound for use or the diagnostic composition for use according to claim 17, wherein the disease is dementia with Lewy bodies.
21. The compound for use or the diagnostic composition for use according to claim 17, wherein the disease is Parkinson's disease with mild cognitive impairment (PD-MCI) or Parkinson's disease with dementia (PDD).

22. The compound for use or the diagnostic composition for use according to claim 17, wherein the disease is a familial for Parkinson's disease linked to the SNCA gene mutation and/or changes in copy number as duplication or triplication
23. The compound for use or the diagnostic composition for use according to claim 17, wherein the disease is Alzheimer's disease.
24. The compound for use or the diagnostic composition for use according to any one of claims 13 to 23, wherein the use is in a human.
25. A method of imaging a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions, in a subject, the method comprising the steps:
- (a) Administering a compound according to any one of claims 1 to 11, or a diagnostic composition according to claim 12 which comprises a compound according to any one of claims 1 to 11, to the subject;
 - (b) Allowing the compound to bind to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions; and
 - (c) Detecting the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions.
26. A method of imaging according to claim 25, 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, Lewy neurites and/or cytoplasmic glial inclusions.
27. A method of positron emission tomography (PET) imaging of alpha-synuclein aggregates, including but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions, in a tissue of a subject, the method comprising the steps:
- (a) Administering a compound according to any one of claims 1 to 11, or a diagnostic composition according to claim 12 which comprises a compound according to any one of claims 1 to 11, to the subject;
 - (b) Allowing the compound to bind to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions; and
 - (c) Detecting the compound bound to the alpha-synuclein aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions by collecting a positron emission tomography (PET) image of the tissue of the subject.
28. The method of positron emission tomography (PET) imaging of the alpha-synuclein aggregates, including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions in a tissue of a subject according to claim 27, 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.

29. A method for the detection and optionally quantification of alpha-synuclein aggregates, including but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions, 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, Lewy neurites and/or cytoplasmic glial inclusion, into contact with a compound according to any one of claims 1 to 11, or a diagnostic composition according to claim 12 which comprises a compound according to any one of claims 1 to 11;

(b) Allowing the compound to bind to the alpha-synuclein aggregates, including but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;

(c) Detecting the compound bound to the alpha-synuclein aggregates, including but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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, Lewy neurites and/or cytoplasmic glial inclusions.

30. 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, Lewy neurites and/or cytoplasmic glial inclusions, 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 neurites and/or cytoplasmic glial inclusions into contact with a compound according to any one of claims 1 to 11, or a diagnostic composition according to claim 12 which comprises a compound according to any one of claims 1 to 11;

(b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;

(c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions; and

(d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions in the sample or specific body part or body area.

31. 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, Lewy neurites and/or cytoplasmic glial inclusions, 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, Lewy neurites and/or cytoplasmic glial inclusions into contact with a compound according to any one of claims 1 to 11, or a diagnostic composition according to claim 12 which comprises a compound according to any one of claims 1 to 11;
- 10 (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions; 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, Lewy neurites and/or cytoplasmic glial inclusions with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions in the sample or specific body part or body area.
32. A method of collecting data for prognosing a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or
- 20 cytoplasmic glial inclusions, 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, Lewy neurites and/or cytoplasmic glial inclusions into contact with a compound according to any one of claims 1 to 11, or a diagnostic composition according to claim 12 which comprises a compound according to
- 25 any one of claims 1 to 11;
- (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;
- 30 (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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.

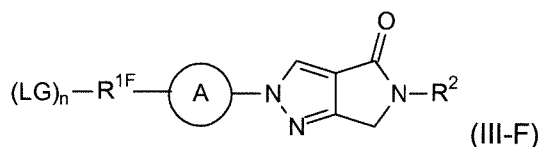
33. A method of collecting data for monitoring the progression of a disease, disorder or abnormality associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions in a patient, the method comprising the steps:
- 5 (a) Bringing a sample, a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions into contact with the compound according to any one of claims 1 to 11, or a diagnostic composition according to claim 12 which comprises a compound according to any one of claims 1 to 11;
- 10 (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;
- (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;
- 15 (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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.
34. 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, Lewy neurites and/or cytoplasmic glial inclusions to a treatment with a medicament, method comprising the steps:
- 20 (a) Bringing a sample, a specific body part or body area suspected to contain alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions into contact with a compound according to any one of claims 1 to 11, or a diagnostic composition according to claim 12 which comprises a compound according to any one of claims 1 to 11;
- 25 (b) Allowing the compound to bind to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;
- 30 (c) Detecting the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions;
- 35 (d) Optionally correlating the presence or absence of the compound bound to the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions 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.

35. The method of any one of claims 30 to 34, 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, Lewy neurites and/or cytoplasmic glial inclusions with the presence or absence of the alpha-synuclein aggregates including, but not limited to, Lewy bodies, Lewy neurites and/or cytoplasmic glial inclusions in the sample or specific body part or body area; comprises

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

36. 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 comprising at least 2 heteroatoms;

R^{1F} is C₁-C₄alkyl, haloC₁-C₄alkyl, -N(C₁-C₄alkyl)₂; or -NH(C₁-C₄alkyl); or

R^{1F} is a heterocyclyl;

R² is a 5-membered or 6-membered heteroaryl, optionally substituted with 1 or 2 substituents independently selected from haloC₁-C₄alkyl, haloC₁-C₄alkoxy, C₁-C₄alkoxy, and C₁-C₄alkyl;

LG is a leaving group; and

n is at least 1.

37. The compound of formula (III-F) according to claim 36, wherein **LG** is selected from bromo, chloro, iodo, C₁₋₄ alkyl sulfonate and C₆₋₁₀ aryl sulfonate, wherein the C₆₋₁₀ aryl sulfonate can be optionally substituted with -CH₃ or -NO₂.

38. A compound of formula (III-H)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/081468

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D487/04 A61K31/4162 A61K51/00
ADD.

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

B. FIELDS SEARCHED

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

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	WO 2021/224489 A1 (AC IMMUNE SA [CH]) 11 November 2021 (2021-11-11) examples	1-44
A	----- KOTZBAUER PAUL T. ET AL: "Current status of the development of PET radiotracers for imaging alpha synuclein aggregates in Lewy bodies and Lewy neurites", CLINICAL AND TRANSLATIONAL IMAGING, vol. 5, no. 1, 2017, pages 3-14, XP055816408, IT DOI: 10.1007/s40336-016-0217-4 Retrieved from the Internet: URL: https://link.springer.com/article/10.1007/s40336-016-0217-4 figure 2 ----- -/--	1-44

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- "&" document member of the same patent family

Date of the actual completion of the international search

30 January 2023

Date of mailing of the international search report

07/02/2023

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/081468

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>XU MING-MING ET AL: "Advances in the development of imaging probes and aggregation inhibitors for alpha-synuclein", ACTA PHARMACOLOGICA SINICA, NATURE PUBLISHING GROUP, GB, vol. 41, no. 4, 4 October 2019 (2019-10-04), pages 483-498, XP037079648, ISSN: 1671-4083, DOI: 10.1038/S41401-019-0304-Y [retrieved on 2019-10-04] figure 2</p> <p>-----</p>	1-44
A	<p>WATANABE HIROYUKI ET AL: "Synthesis and biological evaluation of novel radioiodinated benzimidazole derivatives for imaging [alpha]-synuclein aggregates", BIOORGANIC & MEDICINAL CHEMISTRY, vol. 25, no. 24, 14 October 2017 (2017-10-14), pages 6398-6403, XP085254616, ISSN: 0968-0896, DOI: 10.1016/J.BMC.2017.10.010 page 6399</p> <p>-----</p>	1-44

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2022/081468

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		BR 112022021774 A2	13-12-2022
		CA 3175602 A1	11-11-2021
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		WO 2021224489 A1	11-11-2021
