

NOVEL COMPOUNDS FOR THE TREATMENT OF DISEASE ASSOCIATED
WITH ALPHA-SYNUCLEIN AGGREGATES

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FIELD OF THE INVENTION

The present invention relates to novel compounds of formula (I), or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, that can be employed in the treatment, alleviation or prevention of diseases, disorders and abnormalities associated with alpha-synuclein (α -synuclein, A-synuclein, aSynuclein, A-syn, α -syn, aSyn, a-syn) aggregates. The present invention also relates to processes for the preparation of said compounds, pharmaceutical compositions comprising said compounds, methods using said compounds, combinations comprising said compounds, medicaments containing them, and their uses in the treatment, alleviation or prevention of diseases, disorders and/or abnormalities associated with alpha-synuclein aggregates.

BACKGROUND OF THE INVENTION

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

Amyloid-like proteins that form mainly intracellular aggregates include, but are not limited to, tau, alpha-synuclein, and huntingtin (HTT). Diseases involving alpha-synuclein aggregates are generally listed as synucleinopathies (or alpha-synucleinopathies) and these include, but are not limited to, Parkinson's disease (PD). Synucleinopathies with primarily neuronal aggregates include, but are not limited to, Parkinson's disease (sporadic, familial with SNCA (the gene encoding for the alpha-synuclein protein) mutations or SNCA gene duplication or triplication, familial with mutations in other genes than SNCA, pure autonomic failure and Lewy body dysphagia), 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 puglistica, tauopathies (Pick's disease, frontotemporal dementia, progressive supranuclear palsy, corticobasal degeneration and Niemann-Pick type C1 disease, frontotemporal dementia with Parkinsonism linked to chromosome 17), motor neuron 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 long, cytosolic protein abundantly and predominantly expressed in the CNS and localized in pre-synaptic terminals (Burré J., *J Parkinsons Dis.* 2015;5(4):699-713). Alpha-synuclein is a natively unfolded protein but adopts secondary structure of mostly helical nature upon association with lipid vesicles or membranes (Iwai et al., *Biochemistry* 1995, 34(32), 10139-10145). The physiological function of alpha-synuclein still remains elusive. Because of the association of alpha-synuclein to synaptic vesicles and its presynaptic localization it is suggested that it regulates synaptic activity and plasticity, neurotransmitter release, dopamine production and

metabolism, vesicle trafficking, synaptic vesicle pool maintenance and chaperone-like activity (Cabin et al., J Neurosci. 2002;22:8797–8807; Chandra et al., Cell. 2005;123:383–396).

The sequence of alpha-synuclein can be divided into three main domains: 1) the N-terminal region comprising of residues 1-60, which contains 11-mer amphipathic imperfect repeat residues with highly conserved hexamer (KTKEGV). This region has been implicated in regulating alpha-synuclein association to lipid 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, has no distinct structural propensity.

Alpha-synuclein has been shown to undergo several post translational modifications, including truncations, phosphorylation, ubiquitination, sumoylation, oxidation, nitration, acetylation, glycation, glycosylation, 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; Dorval et al., J Biol Chem. 2006, 281(15):9919-24; Ruzafa et al., PlosOne 2017 12(5):e0178576; Ischiropoulos et al. Ann N Y Acad Sci. 2003, 991, 93-100; Munch et al., J Chem Neuroanat. 2000;20:253–257; Marotta et al., Chembiochem. 2012;13:2665–2670). The majority of these modifications involve residues within the C-terminal region.

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). 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);
5 although a clear role in the disease process remains to be defined. In *in vitro* models, alpha-synuclein (or some of its truncated forms) readily assembles into filaments resembling those isolated from the brain of patients with Lewy Body (LB) dementia and familial PD (Crowther et al., FEBS Lett. 1998, 436(3), 309-312). Alpha-synuclein and its mutated forms (A53T and A30P) have a random coil conformation and do not form significant secondary structures in aqueous solution at low
10 concentrations; however, at higher concentrations they are prone to self-aggregate, producing amyloid fibrils (Wood et al., J. Biol. Chem. 1999, 274(28), 19509-19512). Several differences in the aggregation behaviour 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).

15 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
20 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
25 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
30 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 sub-classified in parkinsonian (MSA-P) or cerebellar (MSA-C) variant, depending on the predominant motor phenotype (Fanciulli et al., *N. Engl. J. Med.* 2015; 372, 249-63). It is characterized by the aggregation of alpha-synuclein in the cytoplasm of oligodendrocytes, forming glial cytoplasmic inclusions (GCIs). GCIs, consisting primarily of fibrillary forms of alpha-synuclein, are the neuropathological hallmark of MSA and are found throughout the neocortex, hippocampus, brainstem, spinal cord and dorsal root ganglia (Galvin et al., *Arch Neurol.* 2001, 58,186-90). GCIs are considered a central player in the pathogenesis of MSA. A correlation between the GCI load and the degree of neuronal loss has been reported in both the striatonigral and the olivopontocerebellar regions (Stefanova et al., *Neuropathol. Appl. Neurobiol.* 2016, 42, 20-32). Furthermore, a causative link between GCIs and the induction of neuronal loss has been shown in transgenic mice overexpressing human alpha-synuclein in oligodendrocytes under various oligodendroglia-specific promoters. A key event in the pathophysiological cascade is considered to be the permissive templating ('prion-like' propagation) of misfolded alpha-synuclein.

Alpha-synuclein pathological inclusions detected in all neurodegenerative diseases are almost exclusively intracellular deposits detected in the cytoplasm or processes of neurons and glia cells. Alpha-synuclein aggregates isolated from brains or biofluids of Parkinson's disease or other Synucleinopathies have the capacity to promote the assembly of monomeric alpha-synuclein into fibrillar structures via a prion-like propagation mechanism (Candelise et al., *Ann Neurol*, 2019, 85: 691-703, Prusiner et al., *Proc Natl Acad Sci USA* 2015, 112(38):E5308-17; Siderowf et al., *Lancet Neurol*, 2023, 22(5):407-417). Recent evidence from cellular and animal models suggests that

pathological or aggregated alpha-synuclein can spread from one neuron to another. Once inside the new cell alpha-synuclein aggregates act as seeds, recruiting endogenous alpha-synuclein and advancing protein aggregation (Luk et al., Science. 2012, 338(6109):949-5; Tran et al., Cell Rep. 2014, 7(6):2054-65). Moreover, the transsynaptic spreading of pathological or aggregated alpha-synuclein could explain the progressive advancing of Lewy pathology through defined anatomical connected brain areas in PD that was first described by Braak and colleagues (Braak et al., Neurobiol. Aging. 2003; 24:197–211).

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. Alpha-synuclein is a priority target for drug development given not only its contribution to neurodegeneration but also because it can offer the possibility to treat the disease while still in the asymptomatic or prodromal stages.

Therefore, alpha-synuclein is a compelling target for therapeutic approaches aiming to treat, alleviate, prevent, retard, or halt the progression of PD and other synucleinopathies via orally active, brain and cell penetrant compounds that can inhibit and/or delay the accumulation of pathological alpha-synuclein intracellularly as well as the transsynaptic transmission, seeding and spreading of alpha-synuclein aggregated species in the brain.

WO 2011/128455 refers to specific compounds which are suitable for treating disorders associated with amyloid proteins or amyloid-like proteins.

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.

WO 2017/153601 discloses compounds that can be employed in the diagnosis, monitoring of disease progression or monitoring of drug activity, of a group of disorders and abnormalities associated with alpha-synuclein aggregates including, but not limited to, Lewy bodies and/or Lewy neurites, such as Parkinson's disease.

Different physiochemical and biological properties (e.g. pharmacokinetic profile, efficacy in inhibition/reduction of protein aggregation, reversibility of target binding) are desired in compounds for therapeutic use in treatment, prevention or alleviation of neurodegenerative diseases characterized by pathological protein accumulation (proteinopathies) compared to compounds for use in imaging of such diseases.

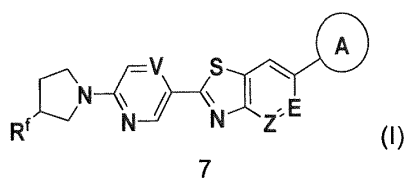
The development of disease-modifying drug therapies for PD and other synucleinopathies would address an unmet medical need since currently only symptomatic treatments are available (McFarthing et al., J Parkinsons Dis. 2022;12(4):1073-1082). Therefore, there remains a need for identifying novel therapeutic agents that can inhibit the accumulation and spreading of alpha-synuclein.

SUMMARY OF THE INVENTION

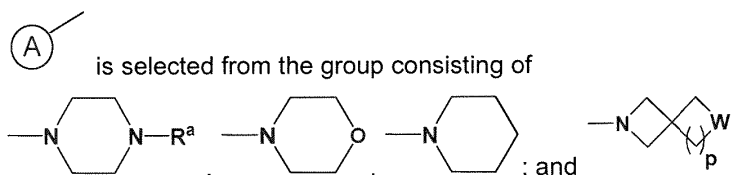
The present invention provides compounds that can be employed in the treatment, alleviation or prevention of a disease, disorder or abnormality associated with alpha-synuclein aggregates (such as Parkinson's disease). The invention further provides methods of treating, alleviation, or preventing diseases, disorders and abnormalities associated with alpha-synuclein aggregates.

Compounds according to the invention described herein can bind specifically to aggregated alpha-synuclein species acting as "seeds" and inhibit and/or delay the accumulation of pathological alpha-synuclein intracellularly as well as the transsynaptic transmission, seeding, and spreading of alpha-synuclein aggregated species in the brain. Due to their unique design features, these compounds display properties such as appropriate lipophilicity, molecular weight, solubility, permeability and metabolic stability, which result in cell penetration, oral bioavailability, and brain uptake, adequate to be a successful medicament for the treatment, alleviation or prevention of diseases, disorders or abnormalities associated with alpha-synuclein aggregates.

Various embodiments of the invention are described herein. Within a certain aspect, provided herein is a compound of formula (I):



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



- 5 Optionally (A) can be substituted at any available position by one to three substituents R^A , wherein each R^A is independently selected from the group consisting of -OH, halogen, -C₁-C₃alkyl, -OC₁-C₃alkyl, -NR^bR^c, spiro-cycloalkyl including 3 to 6 ring atoms, and spiro-heterocycloalkyl including 3 to 6 ring atoms wherein 1 ring atom is O.

W is selected from NR^a and CR^dR^e

- 10 **p** is 1 or 2.

For each occurrence **R^a** is selected from hydrogen and C₁-C₃ alkyl.

For each occurrence **R^b** is selected from hydrogen and C₁-C₃ alkyl.

For each occurrence **R^c** is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring atom is selected from O, S and NR^a.

- 15 For each occurrence **R^d** is selected from hydrogen, -OH, and C₁-C₃ alkyl.

For each occurrence **R^e** is selected from hydrogen and C₁-C₃ alkyl.

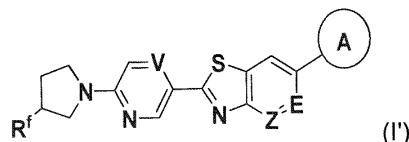
V is selected from CH or N.

Z is selected from CH or N.

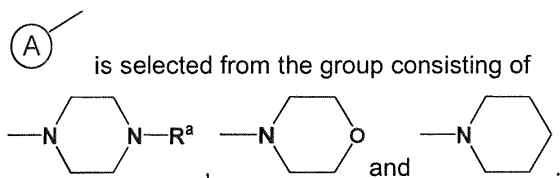
E is selected from CH or N; wherein at least one of **Z** and **E** is CH.

- 20 **R^f** is selected from hydrogen, -OC₁-C₃alkyl and halogen.

In another aspect, the invention is also directed to a compound having the following subformula (I'):



- 25 or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof.



Optionally (A) can be substituted at any available position by one to three substituents **R^A**, wherein each **R^A** is independently selected from the group consisting of -OH, halogen, -**NR^bR^c**, spiro-cycloalkyl including 3 to 6 ring atoms and spiro-heterocycloalkyl including 3 to 6 ring atoms wherein 1 ring atom is O.

For each occurrence **R^a** is selected from hydrogen and C₁-C₃ alkyl.

For each occurrence **R^b** is selected from hydrogen and C₁-C₃ alkyl.

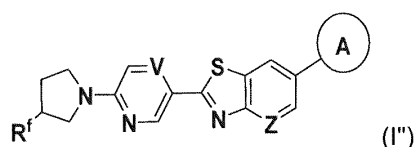
For each occurrence **R^c** is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring atom is selected from O, S and **NR^a**.

Z is selected from CH or N and **E** is selected from CH or N; wherein at least one of **Z** and **E** is CH;

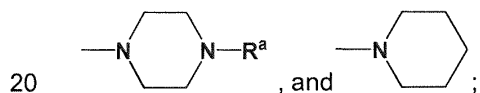
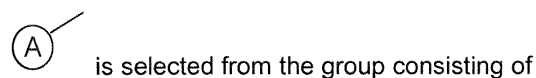
V is selected from CH or N.

R^f is selected from hydrogen and halogen.

15 In another aspect, the invention is also directed to a compound having the following subformula (I''):



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



wherein (A) is optionally substituted at any available position by one to three substituents **R^A**, wherein each **R^A** is independently selected from the group consisting of -OH, halogen, -**NR^bR^c**, and spiro-heterocycloalkyl including 3 to 6 ring atoms wherein 1 ring atom is O;

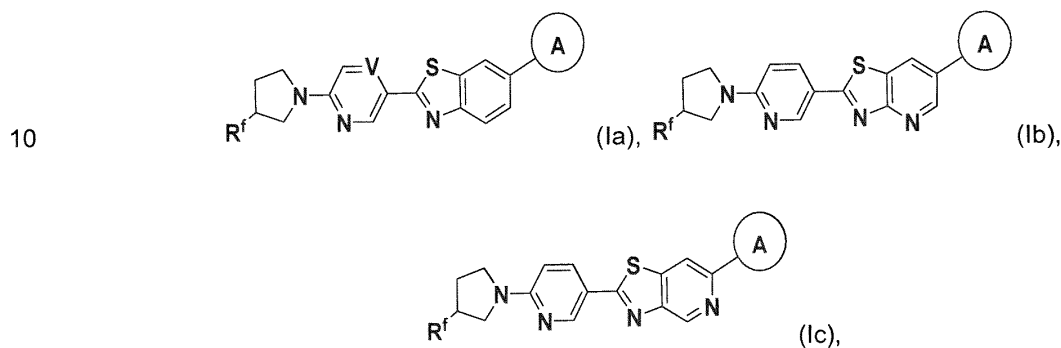
for each occurrence R^a is selected from hydrogen and C_1 - C_3 alkyl;
 for each occurrence R^b is selected from hydrogen and C_1 - C_3 alkyl;
 for each occurrence R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring atom is selected from O, S and NR^a ;

5 Z is selected from CH or N;

V is selected from CH or N;

and R^f is selected from hydrogen and halogen.

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



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

wherein , V and R^f are as defined above.

In another aspect, the invention provides a pharmaceutical composition comprising a compound according to the definition of compound of formula (I), and optionally a pharmaceutically acceptable
 20 carrier, diluent, adjuvant and/or excipient.

In another aspect, the invention provides a compound of formula (I), or a combination, in particular a pharmaceutical composition, as disclosed herein, for use as a medicament.

In another aspect, the invention provides a compound of formula (I), as disclosed herein, or a pharmaceutical composition, for use in the treatment, alleviation or prevention of a disease, disorder or abnormality associated alpha-synuclein aggregates.

- 5 In another aspect, the invention provides a method of treating, alleviating or preventing a disease, disorder or abnormality associated with alpha-synuclein aggregates comprising administering a compound of formula (I), as disclosed herein, or a pharmaceutical composition, to a subject in need thereof.
- 10 In another aspect, the invention provides the use of a compound of formula (I), as disclosed herein, for the manufacture of a medicament for treating, alleviating or preventing a disease, disorder or abnormality associated with alpha-synuclein aggregates.

In some aspects, the disease, disorder or abnormality is selected from Parkinson's disease (including sporadic, familial with alpha-synuclein mutations, familial with mutations other than alpha-synuclein, pure autonomic failure or Lewy body dysphagia), SNCA duplication carrier, Lewy Body dementia (LBD), dementia with Lewy bodies (DLB) (including "pure" Lewy body dementia), Parkinson's disease dementia (PDD), diffuse Lewy body disease (DLBD), Alzheimer's disease, sporadic Alzheimer's disease, familial Alzheimer's disease with APP mutations, familial Alzheimer's disease with PS-1, PS-2 or other mutations, familial British dementia, Lewy body variant of Alzheimer's disease, Down syndrome, multiple system atrophy (MSA) (including Shy-Drager syndrome, striatonigral degeneration or olivopontocerebellar atrophy), traumatic brain injury, chronic traumatic encephalopathy, dementia puglistica, tauopathies (including Pick's disease, frontotemporal dementia, progressive supranuclear palsy, corticobasal degeneration, Niemann-Pick type C1 disease, frontotemporal dementia with Parkinsonism linked to chromosome 17), Creutzfeldt-Jakob disease, Huntington's disease, motor neuron disease, amyotrophic lateral sclerosis (including sporadic, familial or ALS-dementia complex of Guam), neuroaxonal dystrophy, neurodegeneration with brain iron accumulation type 1 (including Hallervorden-Spatz syndrome), prion diseases, ataxia telangiectatica, Meige's syndrome, subacute sclerosing panencephalitis, Gerstmann-Straussler-Scheinker disease, inclusion-body myositis, Gaucher disease, Krabbe disease as well as other lysosomal storage disorders (including Kufor-Rakeb syndrome and Sanfilippo syndrome) and rapid eye movement (REM) sleep behavior disorder.

In another aspect, the invention provides a compound of formula (I), as defined herein, for use as an *in vitro* analytical reference or an *in vitro* screening tool.

- 5 In another aspect, the invention provides a combination comprising a compound of formula (I), as defined herein, and one or more therapeutic agents different from the compound of formula (I).

In another aspect, the invention provides a mixture comprising a compound of formula (I), as defined herein, and one or more therapeutic agents different from the compound of formula (I).

10

DEFINITIONS

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",
15 "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.

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The term "C₁-C₃ alkyl" refers to a saturated straight or branched hydrocarbon chain consisting solely of carbon and hydrogen atoms containing no unsaturation, having from one to three carbon atoms, and which is attached to the rest of the molecule by a single bond. Examples of suitable alkyl groups having 1 to 3 carbon atoms include, but are not limited to, methyl, ethyl, propyl, isopropyl, 1-methylethyl.

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The term "heterocycloalkyl" refers to a stable 3- to 8-membered saturated monocyclic ring radical which comprises 1 heteroatom which is selected from N, O or S. The heterocycloalkyl group may be bonded via a carbon atom or a heteroatom, preferably by a carbon atom. Examples include, but are not limited to, aziridinyl, oxiranyl, thiiranyl, azetidiny, oxetanyl, thietanyl, pyrrolidinyl, tetrahydrofuryl, tetrahydrothienyl, piperidyl, tetrahydropyranyl, perhydroazepinyl, azepanyl, or azocanyl, preferably azetidiny, oxetanyl, thietanyl, pyrrolidinyl, tetrahydrofuryl, and piperidyl, more preferably oxetanyl.

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The term "spiro-heterocycloalkyl" refers to a heterocycloalkyl moiety which is attached in a spiro manner, i.e., the heterocycloalkyl ring and the ring to which it is attached have a common ring atom.

The term "spiro-cycloalkyl" refers to a cycloalkyl moiety which is attached in a spiro manner, i.e., the cycloalkyl ring and the ring to which it is attached have a common ring atom.

- 5 The term "Hal" or "halogen" or "Halo" refers to F, Cl, Br, and I. With respect to pharmaceutical applications, F is particularly preferred.

Unless specified otherwise, the term "compound of the invention" refers to a compound of formula (I), or subformula (I') or (I''), or of subformulae (Ia), (Ib), (Ic) thereof, or a stereoisomer (including
10 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. (I'), (I''), (Ia), (Ib), (Ic)).

- 15 Compounds of the present invention 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.

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"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
25 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,
30 glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, 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
5 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.

10 "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.

15 "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
20 particularly humans.

Alpha-synuclein aggregates are multimeric beta-sheet rich assemblies of alpha-synuclein monomers that can form either soluble oligomers or soluble/insoluble protofibrils or mature fibrils which coalesce into intracellular deposits detected as a range of Lewy pathologies in Parkinson's disease and other
25 synucleinopathies. Alpha-synuclein aggregates that are composing Lewy pathologies can be detected as having the following morphologies: Lewy bodies, Lewy neurites, premature Lewy bodies or pale bodies, perikaryal deposits with diffuse, granular, punctate or pleomorphic patterns. Moreover, alpha-synuclein aggregates are the major component of intracellular fibrillary inclusions detected in oligodendrocytes (also referred to as glial cytoplasmic inclusions) and in neuronal
30 somata, axons and nuclei (referred to as neuronal cytoplasmic inclusions) that are the histological hallmarks of multiple system atrophy. Alpha-synuclein aggregates in Lewy pathologies often display

substantial increase in post-translational modifications such as phosphorylation, ubiquitination, nitration, and truncation.

5 Lewy bodies are abnormal aggregates of protein that develop inside nerve cells in Parkinson's disease (PD), Lewy body dementia and other synucleinopathies. Lewy bodies appear as spherical masses that displace other cell components. Morphologically, Lewy bodies can be classified as being brainstem or cortical type. Classic brainstem Lewy bodies are eosinophilic cytoplasmic inclusions 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
10 presence of Lewy bodies is a hallmark of Parkinson's disease.

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 -
15 synucleinopathies such as dementia with Lewy bodies, Parkinson's disease, and multiple system atrophy.

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

20 "Therapeutically effective amount" means an amount of compound of the invention that is sufficient, when administered to a subject suffering from a disease, disorder, and/or abnormality to treat, reduce the incidence and/or severity of, and/or delay onset of, one or more symptoms of this disease, disorder, and/or this abnormality.

25 The term "subject" refers to mammals, such as primates (e.g., humans, male or female), dogs, rabbits, guinea pigs, pigs, rats and mice. Preferably, the subject is a human or an animal. More preferably, the subject is a human.

As defined herein, a subject is "in need of" a treatment if such subject would benefit biologically,
30 medically or in quality of life from such treatment.

The term "pharmaceutical combination" or "combination" refers to a product that results from the mixing or combining of more than one therapeutic agent and includes both a fixed combination into one dosage unit form, and a non-fixed combination of the therapeutic agents, or a kit of parts for the combined administration, or a combined administration where a compound of the present invention and a combination partner (e.g. another drug as explained below, also referred to as "therapeutic agent") may be administered independently at the same time or separately within time intervals, especially where these time intervals allow that the combination partners show a cooperative, e.g. synergistic, effect. The single components may be packaged in a kit or separately. One or both of the components (e.g. powders or liquids) may be reconstituted or diluted to a desired dose prior to administration. The term "fixed combination" means that the therapeutic agents, e.g. a compound of the present invention and a combination partner, are both administered to a patient simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the therapeutic agents, e.g. a compound of the present invention and a combination partner, are both administered to a patient as separate entities either simultaneously, concurrently or sequentially, wherein such administration provides therapeutically effective levels of the two compounds in the body of the patient. The latter also applies to cocktail therapy, e.g. the administration of three or more therapeutic agents.

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

The compounds of the present invention can be used as an analytical reference or an *in vitro* screening tool.

For example, the compounds of formula (I) according to of the present invention can be used as an analytical reference in *in vitro* assays for testing compounds targeting alpha-synuclein aggregates.

The compounds of the present invention can be used as an *in vitro* screening tool for characterization of tissue with alpha-synuclein pathology and for testing of compounds targeting alpha-synuclein pathology on such tissue.

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.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Graphical representation of delayed compound treatment effects of Compound 9 in primary neuron seeding assay. Data are expressed as the mean $\pm$ SD.

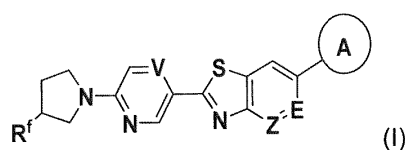
Figure 2: Graphical representation of the pharmacokinetic profiles of Compound 4 (Figure 2A) and Compound 9 (Figure 2B). Unbound plasma, unbound brain and CSF concentration of each compound plotted over time.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a novel class of compounds that are useful in the treatment, alleviation or prevention of a group of diseases, disorders and/or abnormalities associated with alpha-synuclein aggregates.

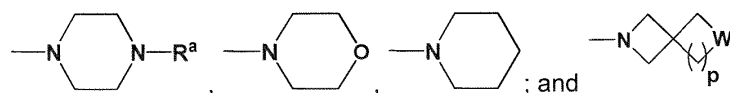
Various embodiments of the invention are described herein, it will be recognized that features specified in each embodiment may be combined with other specified features to provide further embodiments of the present invention.

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



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

 is selected from the group consisting of



wherein $\textcircled{A}$ is optionally substituted at any available position by one to three substituents R^A , wherein each R^A is independently selected from the group consisting of -OH, halogen, $\text{—C}_1\text{—C}_3$ alkyl, $\text{—OC}_1\text{—C}_3$ alkyl, $\text{—NR}^b\text{R}^c$, spiro-cycloalkyl including 3 to 6 ring atoms, and spiro-heterocycloalkyl

5 including 3 to 6 ring atoms wherein 1 ring atom is O;

W is selected from NR^a and CR^dR^e ;

p is 1 or 2;

for each occurrence R^a is selected from hydrogen and $\text{C}_1\text{—C}_3$ alkyl;

for each occurrence R^b is selected from hydrogen and $\text{C}_1\text{—C}_3$ alkyl;

10 for each occurrence R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring atom is selected from O, S and NR^a ;

for each occurrence R^d is selected from hydrogen, -OH, and $\text{C}_1\text{—C}_3$ alkyl;

for each occurrence R^e is selected from hydrogen and $\text{C}_1\text{—C}_3$ alkyl;

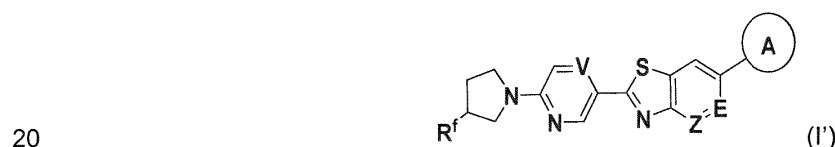
V is selected from CH or N;

15 Z is selected from CH or N;

E is selected from CH or N; wherein at least one of Z and E is CH; and

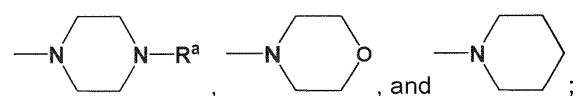
R^f is selected from hydrogen, $\text{—OC}_1\text{—C}_3$ alkyl and halogen.

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



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

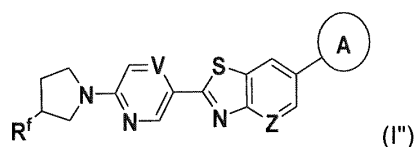
$\textcircled{A}$ is selected from the group consisting of



wherein  is optionally substituted at any available position by one to three substituents R^A , wherein each R^A is independently selected from the group consisting of -OH, halogen, $-NR^bR^c$, spiro-cycloalkyl including 3 to 6 ring atoms, and spiro-heterocycloalkyl including 3 to 6 ring atoms wherein 1 ring atom is O;

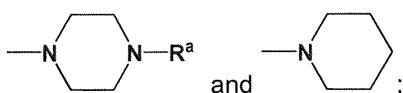
- 5 for each occurrence R^a is selected from hydrogen and C_1 - C_3 alkyl;
 for each occurrence R^b is selected from hydrogen and C_1 - C_3 alkyl;
 for each occurrence R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring atom is selected from O, S and NR^a ;
 V is selected from CH or N;
 10 Z is selected from CH or N; E is selected from CH or N; wherein at least one of Z and E is CH;
 and R^f is selected from hydrogen and halogen.

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



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

 is selected from the group consisting of



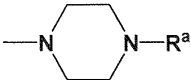
- optionally  can be substituted at any available position by one to three substituents R^A , wherein each R^A is independently selected from the group consisting of -OH, halogen, $-NR^bR^c$; and spiro-heterocycloalkyl including 3 to 6 ring atoms wherein 1 ring atom is O;
 20 for each occurrence R^a is selected from hydrogen and C_1 - C_3 alkyl;
 for each occurrence R^b is selected from hydrogen and C_1 - C_3 alkyl;
 for each occurrence R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring atom is selected from O, S and NR^a ;
 25

Z is selected from CH or N;

V is selected from CH or N; and

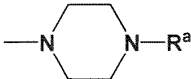
R^f is selected from hydrogen and halogen.

- 5 In one embodiment, the invention provides for a compound of formula (I) or (I'), wherein

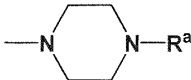
(A) is selected from , wherein **R^a** is selected from hydrogen and C₁-C₃ alkyl, and may optionally be substituted at any available position by one to three, preferably one or two, substituent(s) **R^A**, wherein each **R^A** is independently selected from the group consisting of -OH, halogen, -**NR^bR^c**, spiro-cycloalkyl including 3 to 6 ring atoms, and spiro-heterocycloalkyl including 3 to 6 ring atoms wherein 1 ring atom is O.

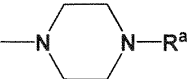
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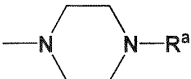
In one embodiment, the invention provides for a compound of formula (I), (I') or (I''), wherein

(A) is selected from , wherein **R^a** is selected from hydrogen and C₁-C₃ alkyl, and may optionally be substituted at any available position by one to three, preferably one or two, substituent(s) **R^A**, wherein each **R^A** is independently selected from the group consisting of -OH, halogen, -**NR^bR^c**, and spiro-heterocycloalkyl including 3 to 6 ring atoms wherein 1 ring atom is O.

15

In a preferred embodiment,  in formula (I), (I') or (I'') is not substituted.

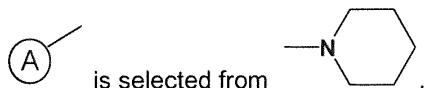
- 20 In another embodiment,  in formula (I), (I') or (I'') is substituted by spiro-heterocycloalkyl including 3 to 6 ring atoms, preferably 4 ring atoms, wherein 1 ring atom is O.

In another embodiment,  in formula (I) or (I') is substituted by spiro-cycloalkyl including 3 to 6 ring atoms, preferably 3 ring atoms.

25

R^a may be hydrogen or C_1 - C_3 alkyl. In some embodiments, R^a is hydrogen. In other embodiments, R^a is C_1 - C_3 alkyl, for example, methyl, ethyl, isopropyl, propyl, preferably methyl or ethyl, more preferably methyl.

5 In another embodiment, the invention provides for a compound of formula (I) or (I'), wherein



optionally substituted at any available position by one to three, preferably one or two, substituents R^A , wherein each R^A is independently selected from the group consisting of -OH, halogen (preferably F), $-NR^bR^c$, spiro-cycloalkyl including 3 to 6 ring atoms, and spiro-heterocycloalkyl including 3 to 6
10 ring atoms wherein 1 ring atom is O;

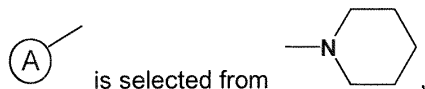
wherein

R^b is selected from hydrogen and C_1 - C_3 alkyl; and

R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring atom is selected from O, S and NR^a , preferably O.

15

In another embodiment, the invention provides for a compound of formula (I), wherein



optionally substituted at any available position by one to three, preferably one or two, substituents R^A , wherein each R^A is independently selected from the group consisting of -OH, $-C_1$ - C_3 alkyl (preferably CH_3), $-OC_1$ - C_3 alkyl (preferably $-OCH_3$), halogen (preferably F), $-NR^bR^c$, and spiro-
20 heterocycloalkyl including 3 to 6 ring atoms wherein 1 ring atom is O;

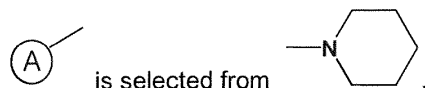
wherein

R^b is selected from hydrogen and C_1 - C_3 alkyl; and

R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring atom is selected from O, S and NR^a , preferably O.

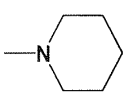
25

In one embodiment, the invention provides for a compound of formula (I), (I') or (I''), wherein

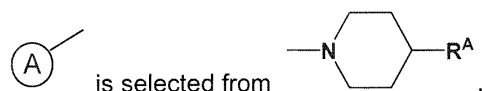


optionally substituted at any available position by one to three, preferably one or two, substituents R^A , wherein each R^A is independently selected from the group consisting of -OH, halogen (preferably F), $-NR^bR^c$, and spiro-heterocycloalkyl including 3 to 6 ring atoms wherein 1 ring atom is O; wherein

- 5 R^b is selected from hydrogen and C_1 - C_3 alkyl; and
 R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring atom is selected from O, S and NR^a , preferably O.

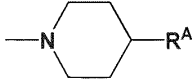
10 In one embodiment  in formula (I), (I') or (I'') is not substituted.

In one embodiment, the invention provides for a compound of formula (I), (I') or (I''), wherein

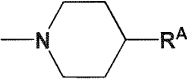


wherein R^A is independently selected from the group consisting of -OH and $-NR^bR^c$; wherein
 R^b is selected from hydrogen and C_1 - C_3 alkyl; and

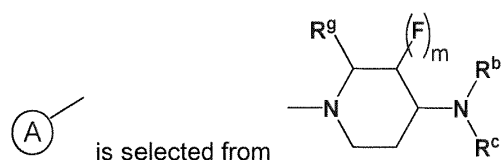
- 15 R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms, preferably 3 to 6 ring atoms, more preferably 4 ring atoms, wherein one ring atom is selected from O.

In one preferred embodiment,  in formula (I), (I') or (I'') is not further substituted.

20

In another embodiment,  in formula (I), (I') or (I'') is substituted by R^A which is independently selected from the group consisting of -OH, halogen, and $-NR^bR^c$.

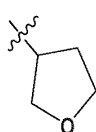
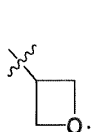
In another preferred embodiment, the invention provides for a compound of formula (I), wherein



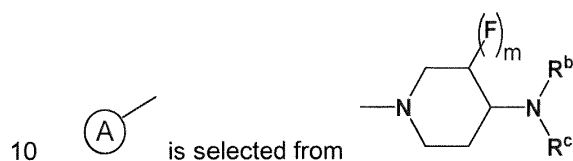
wherein

R^b is as defined above, and

R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring

- 5 atom is O, preferably R^c is selected from hydrogen,  and ; R^g is selected from hydrogen and C_1 - C_3 alkyl; and m is 0, 1 or 2.

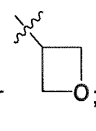
In one preferred embodiment, the invention provides for a compound of formula (I), (I') or (I''), wherein



wherein

R^b is selected from hydrogen and C_1 - C_3 alkyl, and

R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring

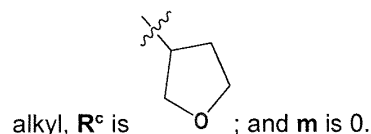
- 15 atom is selected from O, preferably R^c is selected from hydrogen or ; and m is 0, 1 or 2.

In one preferred embodiment, in formula (I), (I') or (I'') R^b is selected from hydrogen and C_1 - C_3 alkyl,

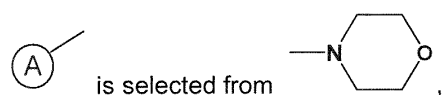
R^c is ; and m is 0.

In another preferred embodiment, in formula (I), (I') or (I'') R^b is selected from hydrogen and C_1 - C_3 alkyl, R^c is hydrogen and m is 0, 1 or 2. In one preferred embodiment, R^b is hydrogen, R^c is hydrogen and m is 1.

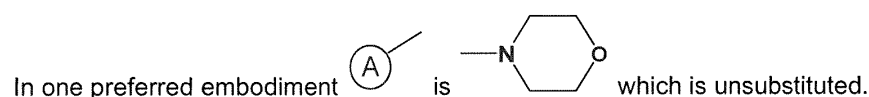
- 5 In another preferred embodiment, in formula (I), (I') or (I'') R^b is selected from hydrogen and C_1 - C_3



In one embodiment, the invention provides for a compound of formula (I) or (I'), wherein



- 10 wherein  is optionally substituted at any available position by one to three substituents R^A , wherein each R^A is independently selected from the group consisting of -OH, halogen, - NR^bR^c , spiro-cycloalkyl including 3 to 6 ring atoms, and spiro-heterocycloalkyl including 3 to 6 ring atoms wherein 1 ring atom is O; wherein
- R^b is selected from hydrogen and C_1 - C_3 alkyl; and
- 15 R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring atom is selected from O, S and NR^a with R^a being selected from hydrogen and C_1 - C_3 alkyl.



- 20 In one embodiment, the present invention provides for a compound of formula (I) or (I'), wherein



In another embodiment, the present invention provides for a compound of formula (I), wherein



W is selected from NR^a and CR^dR^e ;

p is 1 or 2;

R^a is selected from hydrogen and $\text{C}_1\text{-C}_3$ alkyl, preferably hydrogen;

5 R^d is selected from hydrogen, -OH, and $\text{C}_1\text{-C}_3$ alkyl, preferably -OH; and

R^e is selected from hydrogen and $\text{C}_1\text{-C}_3$ alkyl, preferably hydrogen.

In one particular embodiment **W** is selected from CR^dR^e ; wherein R^e is hydrogen and R^d is selected from hydrogen, -OH, and $\text{C}_1\text{-C}_3$ alkyl, R^d is preferably -OH; and **p** is 1.

10

In another particular embodiment **W** is selected from NR^a wherein R^a is selected from hydrogen and $\text{C}_1\text{-C}_3$ alkyl, preferably hydrogen, and **p** is 1 or 2.

As regards **Z** and **E**, each is selected from CH and N but at least one thereof is CH:

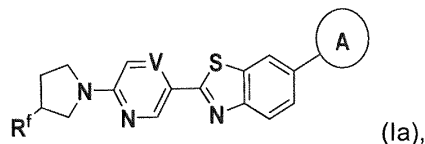
15 In some embodiments, **Z** is CH and **E** is N. In other embodiments, **Z** is N and **E** is CH. In some embodiments, **Z** is CH and **E** is CH.

In some preferred embodiments, in formula (I), (I') or (I'') **V** is CH. In other embodiments, in formula (I), (I') or (I'') **V** is N.

20

In some embodiments, in formula (I), (I') or (I'') R^f is hydrogen. In other embodiments, in formula (I), (I') or (I'') R^f is halogen, preferably F. In yet another embodiment, in formula (I) R^f is $-\text{OC}_1\text{-C}_3$ alkyl, preferably $-\text{OCH}_3$.

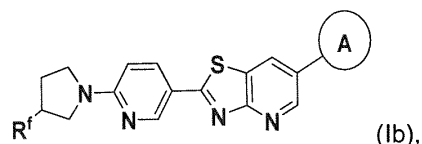
25 In one aspect, the invention provides a compound having the subformula



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

wherein , **V** and **R^f** are as defined above.

In another aspect, the invention provides a compound having the subformula (Ib)

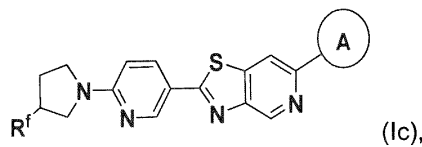


5

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

wherein  and **R^f** are as defined above.

In another aspect, the invention provides a compound having the subformula (Ic)



10

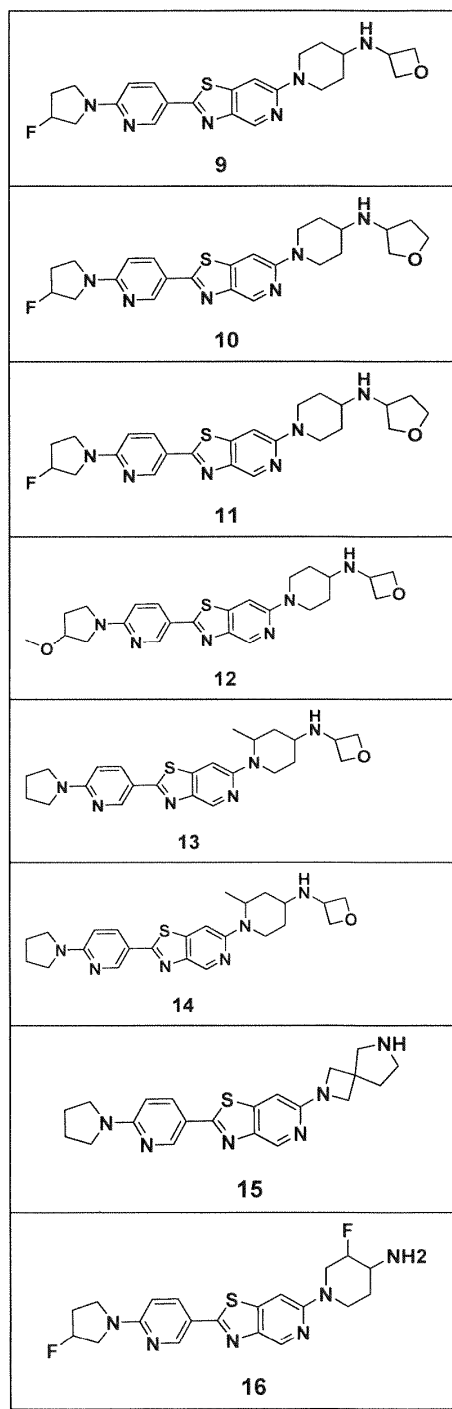
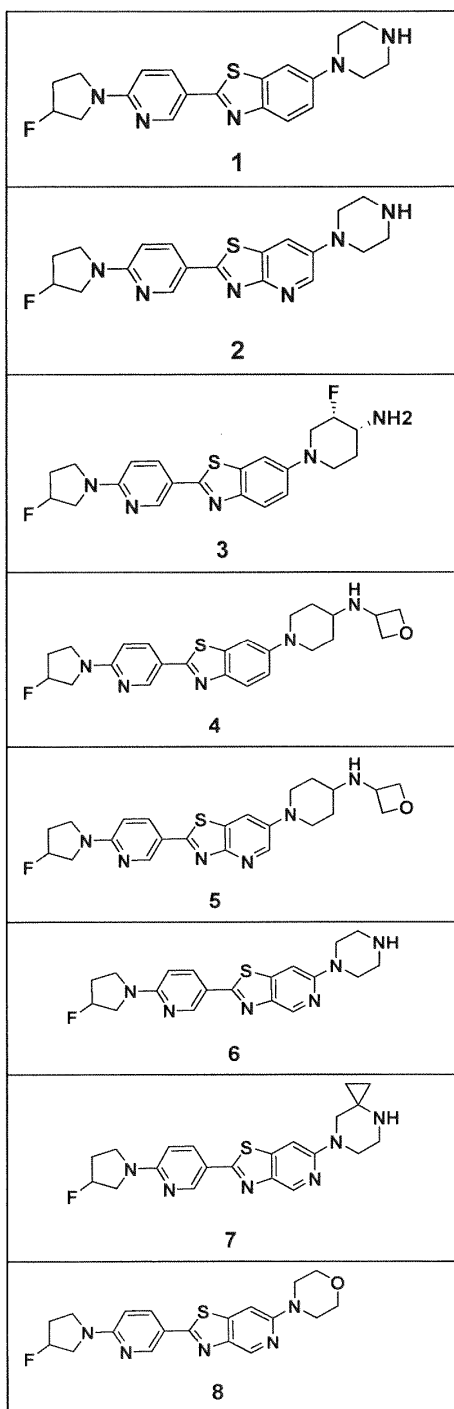
or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof,

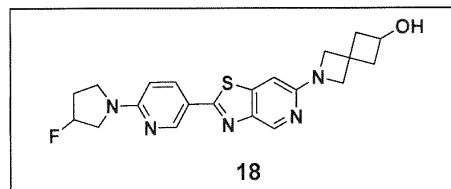
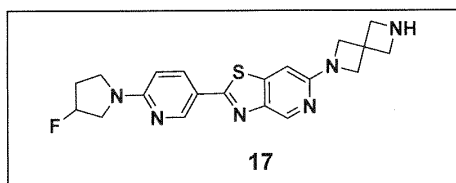
wherein  and **R^f** are as defined above.

In some embodiments, **R^f** is hydrogen in formula (I), (I'), (I''), (Ia), (Ib), or (Ic). In other embodiments,
 15 **R^f** is halogen, preferably F, in formula (I), (I'), (I''), (Ia), (Ib), or (Ic). In other embodiments, **R^f** is
 -OC₁₋₃alkyl, preferably -OCH₃, in formula (I), (Ia), (Ib), or (Ic).

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

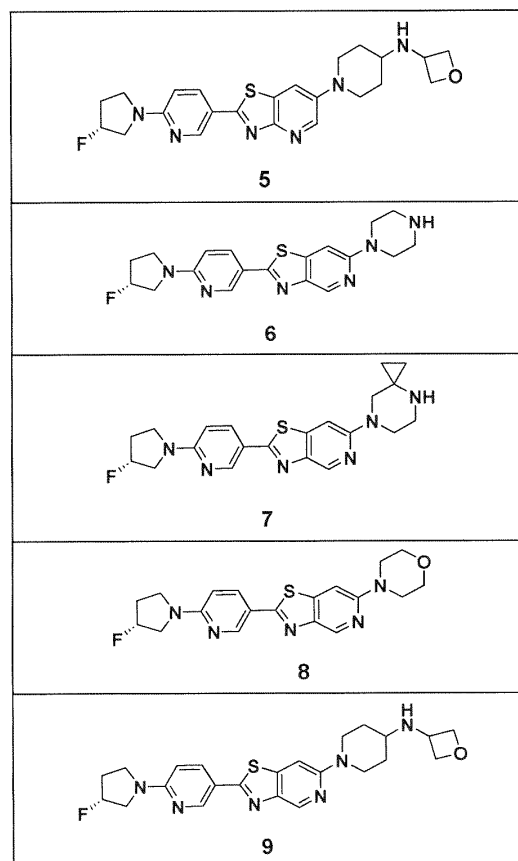
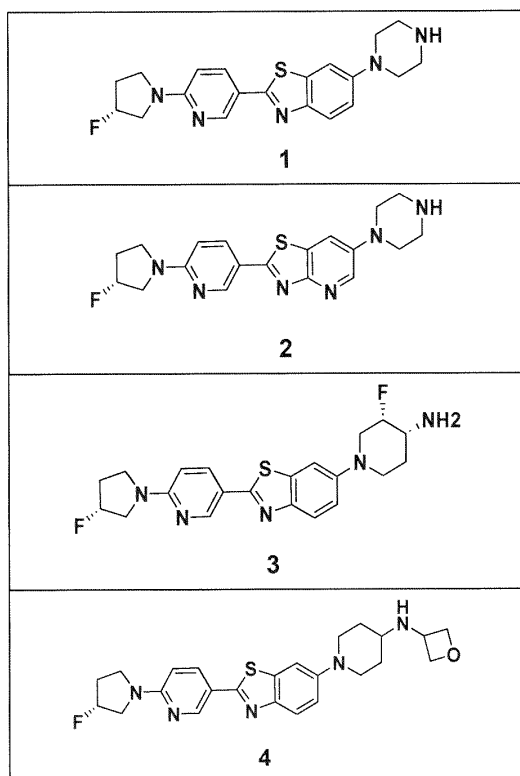
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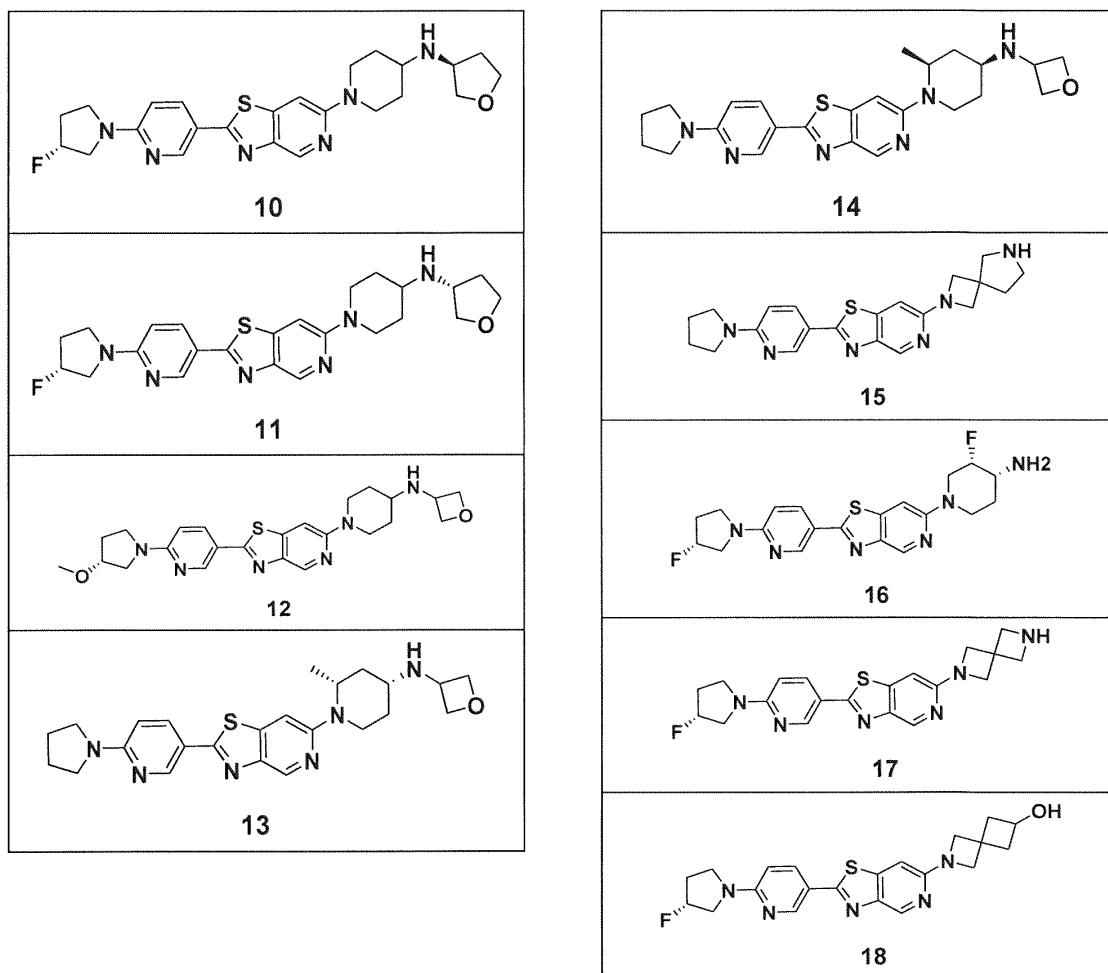




or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof.

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





or a pharmaceutically acceptable salt, hydrate, or solvate thereof.

5 PHARMACEUTICAL COMPOSITIONS

The compounds of the present invention are particularly suitable for the prevention, alleviation or treatment of diseases, disorders and abnormalities associated with alpha-synuclein aggregates. The prevention, alleviation or treatment of diseases, disorders and abnormalities associated with alpha-synuclein aggregates can be conducted in mammals, preferably in humans.

Due to their unique design features, the compounds of the invention display properties such as binding specifically to aggregated alpha-synuclein species and inhibiting and/or delaying the accumulation of pathological alpha-synuclein intracellularly, as well as preventing cell uptake, seeding and the spreading of alpha-synuclein aggregated species in the brain, making them particularly suitable for the treatment, alleviation or prevention of diseases, disorders or abnormalities associated with alpha-synuclein aggregates.

10 In one embodiment, compounds of the invention inhibit and/or delay aggregation of alpha-synuclein protein.

In one embodiment, compounds of the invention inhibit and/or delay the formation of alpha-synuclein fibrils.

15 In one embodiment, compounds of the invention inhibit and/or delay the formation of alpha-synuclein aggregates, including but not limited to fibrils, Lewy Bodies, Lewy Neurites, and/or glial cytoplasmic inclusions.

20 In one embodiment, compounds of the invention inhibit and/or delay the accumulation of pathological alpha-synuclein intracellularly.

In one embodiment, compounds of the invention inhibit and/or delay cell uptake, seeding and the spreading of alpha-synuclein aggregated species in the brain.

25 In one embodiment, compounds of the invention selectively bind aggregated alpha-synuclein and/or pathological in preference to monomeric alpha-synuclein.

In one embodiment there are provided compounds of formula (I), (I'), (I''), (Ia), (Ib), or (Ic) according to the invention for use in inhibiting or preventing alpha-synuclein aggregation.

30 In one embodiment there are provided compounds of formula (I), (I'), (I''), (Ia), (Ib), or (Ic) according to the invention for use in reducing the concentration of alpha-synuclein aggregates.

In one embodiment there are provided compounds of formula (I), (I'), (I''), (Ia), (Ib), or (Ic) according to the invention for use in inhibiting *de novo* alpha-synuclein aggregate formation.

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

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 prevention, alleviation or treatment of diseases, disorders or abnormalities including, but not limited to, 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 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 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 prevention, alleviation

or treatment 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).

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In therapeutic applications, the compounds of the present invention are preferably administered in the form of a pharmaceutical composition comprising the compound of the invention. A "pharmaceutical 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 treatment, alleviation or prevention of the specific disease, disorder or abnormality at issue. Preferably a pharmaceutical composition further comprises a pharmaceutically acceptable excipient, carrier, diluent or adjuvant. Administration is preferably carried out as defined below. More preferably by oral administration. 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 pharmaceutical composition in accordance with standard pharmaceutical practice. Thus, the invention also provides a pharmaceutical composition which comprises a compound of the present invention in admixture with, optionally, at least one pharmaceutically acceptable excipient, carrier, diluent and/or adjuvant. The compound of the present invention is preferably employed in a therapeutically effective amount.

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 pharmaceutical 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,
- 5 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.
- 10 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,
- 15 ophthalmic (including intravitreal or intracameral), transdermal, rectal, buccal, epidural and sublingual. Preferably, the route of administration (delivery) of the compounds of the invention is oral.

- 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-,
- 20 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
- 25 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
- 30 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.

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

Alternatively, the compounds of the present invention can be administered in the form of a suppository or pessary, or it may be applied topically in the form of a gel, hydrogel, lotion, solution, cream, ointment or dusting powder. The compounds of the present invention may also be dermally or transdermally administered, for example, by the use of a skin patch.

They may also be administered by the pulmonary or rectal routes. They may also be administered 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.

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 alcohol, 2-octyldodecanol, benzyl alcohol and water.

5 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
10 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.

Typically, a physician will determine the actual dosage which will be most suitable for an individual
15 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 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 treatment.

20 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 therapeutic agent different from the compound according to the invention, a pharmaceutically acceptable excipient, carrier, diluent or adjuvant. The therapeutic agent different from the compound according to the invention is preferably present in a
25 therapeutically effective amount.

The compounds of the invention may be used in combination with other therapeutic agents. When a compound of the invention is used in combination with a second therapeutic agent active against the same disease, the dose of each compound may differ from that when the compound is used alone.
30 Thus, the invention also relates to a combination comprising a compound of the present invention, and one or more therapeutic agents. The compound of the present invention is preferably employed in a therapeutically effective amount.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation. The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations by any convenient route. When administration is sequential, either the compound of the invention or the second therapeutic agent may be administered first. When administration is simultaneous, the combination may be administered either in the same or different pharmaceutical composition. When combined in the same formulation it will be appreciated that the two compounds must be stable and compatible with each other and the other components of the formulation. When formulated separately they may be provided in any convenient formulation, conveniently in such manner as are known for such compounds in the art.

In another embodiment, the invention provides a method for treating, alleviating or preventing a disease, disorder or an abnormality associated with alpha-synuclein aggregates comprising the step of administering a compound of the present invention, as defined above, or a pharmaceutical composition thereof, said pharmaceutical composition comprising a compound of the present invention.

In one embodiment, the invention relates to a compound of the present invention, or a pharmaceutical composition comprising a compound of the present invention for use as a medicament. In particular the invention relates to a compound of the present invention for use as a medicament for treating, alleviating or preventing of a disease, disorder or an abnormality associated with alpha-synuclein aggregates. In yet another embodiment, the invention relates to a combination, as defined herein, for use in the treatment, alleviation, or prevention of a disease, disorder, or abnormality associated with alpha-synuclein aggregates. In yet another embodiment, the invention relates to a pharmaceutical composition, comprising a compound of the present invention, as defined herein, for use in the treatment, alleviation, or prevention of a disease, disorder, or abnormality associated alpha-synuclein aggregates.

In an embodiment, the disease, disorder or abnormality is selected from Parkinson's disease (including sporadic, familial with alpha-synuclein mutations, familial with mutations other than alpha-synuclein, pure autonomic failure or Lewy body dysphagia), SNCA duplication carrier, Lewy Body

dementia (LBD), dementia with Lewy bodies (DLB) (including "pure" Lewy body dementia), Parkinson's disease dementia (PDD), diffuse Lewy body disease (DLBD), Alzheimer's disease, sporadic Alzheimer's disease, familial Alzheimer's disease with APP mutations, familial Alzheimer's disease with PS-1, PS-2 or other mutations, familial British dementia, Lewy body variant of Alzheimer's disease, Down syndrome, multiple system atrophy (MSA) (including Shy-Drager syndrome, striatonigral degeneration or olivopontocerebellar atrophy), traumatic brain injury, chronic traumatic encephalopathy, dementia pugilistica, 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.

In one embodiment, the disease, disorder or abnormality is preferably selected from Parkinson's disease, multiple system atrophy, dementia with Lewy bodies, Parkinson's disease dementia, SNCA duplication carrier and Alzheimer's disease. In one preferred embodiment, the disease is Parkinson's disease. In another preferred embodiment, the disease is multiple system atrophy.

Depending on the choice of the starting materials and procedures, the compounds can be present in the form of one of the possible stereoisomers or as mixtures thereof, for example as pure optical isomers, or as stereoisomer mixtures, such as racemates and diastereoisomer mixtures, depending on the number of asymmetric carbon atoms. The present invention is meant to include all such possible stereoisomers, including racemic mixtures, diastereoisomeric mixtures and optically pure forms. Optically active (R)- and (S)- stereoisomers may be prepared using chiral synthons or chiral reagents or may be resolved using conventional techniques. If the compound contains a double bond, the substituent may be E or Z configuration. If the compound contains a disubstituted cycloalkyl, the cycloalkyl substituent may have a cis- or trans-configuration. All tautomeric forms are also intended to be included. The invention is also meant to include any pseudo-asymmetric carbon

atom, represented herein as (r)- and (s)-, and which are invariant on reflection in a mirror but are reversed by exchange of any two entities, (PAC 1996, 68, 2193, Basic terminology of stereochemistry IUPAC recommendations 1996).

5 According to the present invention a compound as defined herein can be in the form of one of the possible stereoisomers, rotamers, atropoisomers, tautomers or mixtures thereof, for example, as substantially pure geometric (cis or trans) stereoisomers, diastereomers, optical isomers (antipodes), racemates, or mixtures thereof. Any resulting mixtures of stereoisomers can be separated on the basis of the physicochemical differences of the constituents into the pure or
10 substantially pure geometric or optical isomers, diastereomers, racemates, for example, by chromatography and/or fractional crystallization. Any resulting racemates of final products or intermediates can be resolved into the optical antipodes by known methods, e.g., by separation of the diastereomeric salts thereof, obtained with an optically active acid or base, and liberating the optically active acidic or basic compound. Racemic products can also be resolved by chiral
15 chromatography (e.g., high performance liquid chromatography (HPLC)) using a chiral adsorbent.

Pharmaceutically acceptable acid addition salts can be formed with organic acids and inorganic acids. For example, organic acids from which salts can be derived include, sulfosalicylic acid, acetic acid, propionic acid, glycolic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid,
20 tartaric acid, citric acid, benzoic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, toluenesulfonic acid, and the like. Inorganic acids from which salts can be derived include, for example, sulfuric acid, hydrochloric acid, hydrobromic acid, nitric acid, phosphoric acid, and the like.

In the same way, pharmaceutically acceptable base addition salts can be formed with organic and
25 inorganic bases. For example, organic bases from which salts can be derived include, primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, basic ion exchange resins, and the like (e.g. *isopropylamine*, *benzathine*, *choline*, *diethanolamine*, *diethylamine*, *lysine*, *meclumine*, *piperazine*, and *tromethamine*). Examples of inorganic bases from which salts can be derived include, for example, ammonium salts and metals
30 from columns I to XII of the periodic table. Said salts are derived from, for example, sodium, potassium, ammonium, calcium, magnesium, iron, silver, zinc, and copper.

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), (I'), (I''), (Ia), (Ib) or (Ic) 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. Unless otherwise stated, starting materials are either commercially available or are prepared by known methods.

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

EXAMPLES

EXEMPLIFICATION OF THE INVENTION

Compounds of the present invention may be prepared by methods known in the art of organic synthesis such as the methods disclosed in WO 2017/153601. 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

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Abbreviations used are those conventional in the art.

CH ₃ CN	Acetonitrile
(COCl) ₂	Oxalyl chloride
Cs ₂ CO ₃	Cesium carbonate
CuI	Copper iodide
DCE	Dichloroethane
DCM	Dichloromethane
DIEA	<i>N,N</i> -Diisopropylethylamine
DIV	Day <i>in vitro</i>
DMEDA	1,2-Dimethylethylenediamine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
EDTA	Ethylenediaminetetraacetic acid
EtOAc	Ethyl acetate
EtOH	Ethanol
HCl	Hydrochloric acid
K ₂ CO ₃	Potassium carbonate
LCMS	Liquid chromatography mass spectrometry
MAP2	Microtubule-associated protein 2
MeOH	Methanol
MSA	Multiple System Atrophy
NaCl	Sodium chloride
Na ₂ CO ₃	Sodium carbonate
Na ₂ SO ₄	Sodium sulfate
NMR	Nuclear magnetic resonance
NBS	N-bromosuccinimide

PD	Parkinson's disease
Pd ₂ (dba) ₃	Tris(dibenzylideneacetone)dipalladium(0)
Pd(PPh ₃) ₄	Palladium tetrakis(triphenylphosphine)
Pd(dppf)Cl ₂ .DCM	[1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II); dichloromethane
prep-HPLC	Preparative high pressure liquid chromatography
P ₂ S ₅	Phosphorus pentasulfide
RT	Room temperature (approx. 25 °C)
SPR	Surface Plasmon Resonance
^t BuONa	Sodium tertbutoxide
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
ThT	Thioflavin T
Tris	Tris(hydroxymethyl)aminomethane
Xphos	2-Dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl

ANALYTICAL DETAILS, PREPARATIVE AND ANALYTICAL METHODS

NMR measurements were performed on a DPX-400 MHz NMR spectrometer, or on a Bruker AV-400
 5 MHz 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),
 10 chloroform (δ 7.26 ppm), or other solvents as indicated in NMR spectral data.

Mass spectra (MS): Analytical HPLC-MS analyses were conducted using an UPLC HClass Plus with a Photodiode Array detector and a QDa Mass spectrometer from Waters.

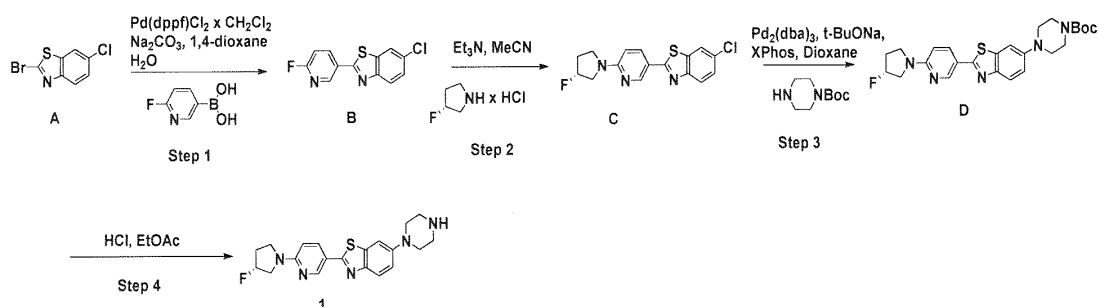
Column chromatography was performed using silica gel (Fluka: Silica gel 60, 0.063-0.2 mm) and suitable solvents as indicated in the specific examples.

15 **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.

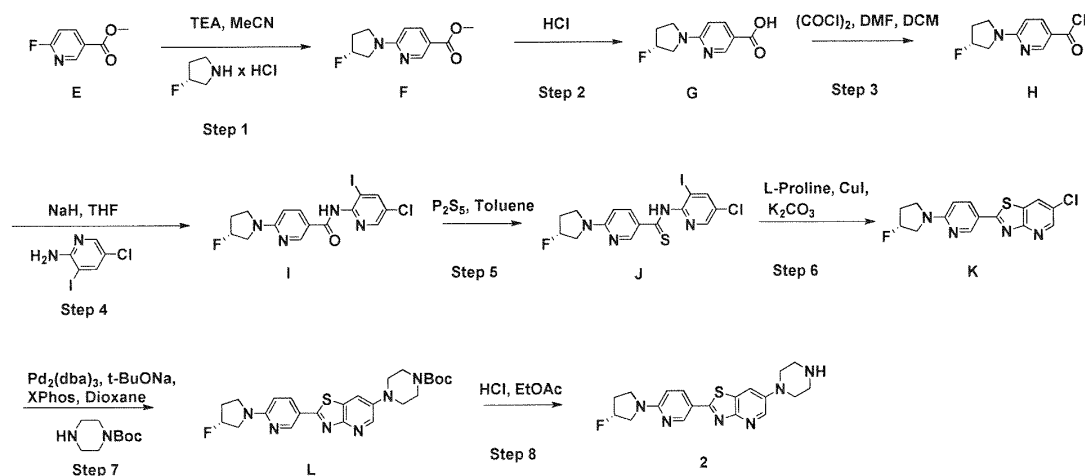
Thin layer chromatography (TLC) was carried out on silica gel plates with UV detection.

GENERAL SYNTHETIC SCHEMES:

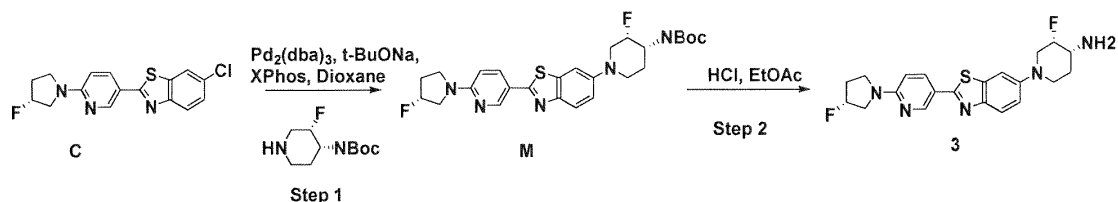
- 5 Synthetic scheme for the preparation of (R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-6-(piperazin-1-yl)benzo[d]thiazole (Compound 1)



- 10 Synthetic scheme for the preparation of (R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-6-(piperazin-1-yl)thiazolo[4,5-b]pyridine (Compound 2)

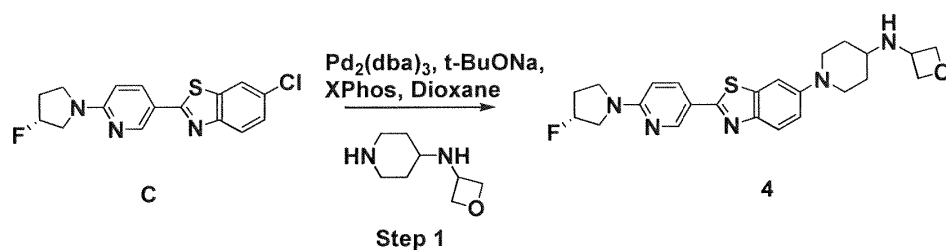


- 15 Synthetic scheme for the preparation of (R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-6-(piperazin-1-yl)thiazolo[4,5-b]pyridine (Compound 3)



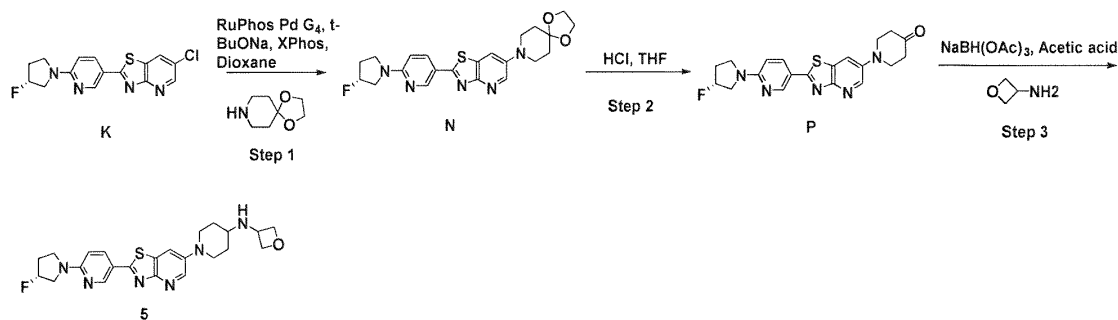
Synthetic scheme for the preparation of (R)-1-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)benzo[d]thiazol-6-yl)-N-(oxetan-3-yl)piperidin-4-amine (Compound 4)

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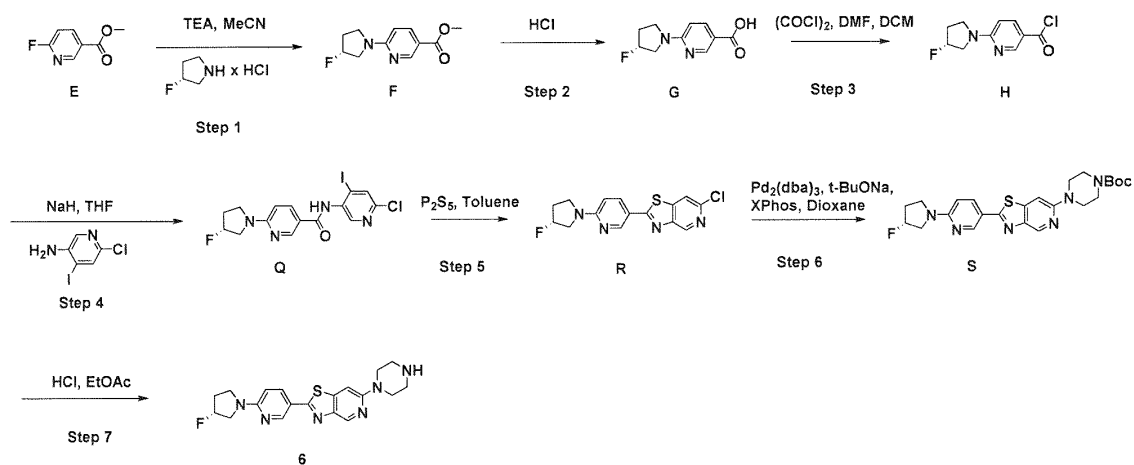
Synthetic scheme for the preparation of (R)-1-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)benzo[d]thiazol-6-yl)-N-(oxetan-3-yl)piperidin-4-amine (Compound 5)

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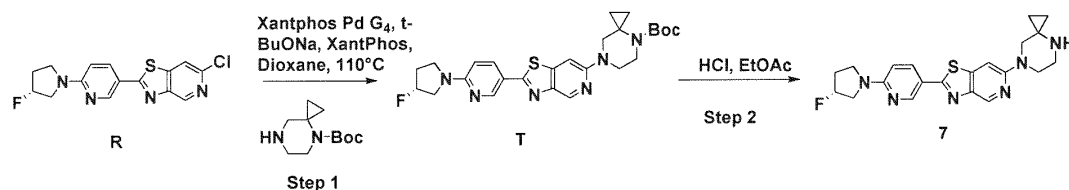
Synthetic scheme for the preparation of (R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-6-(piperazin-1-yl)thiazolo[4,5-c]pyridine (Compound 6)

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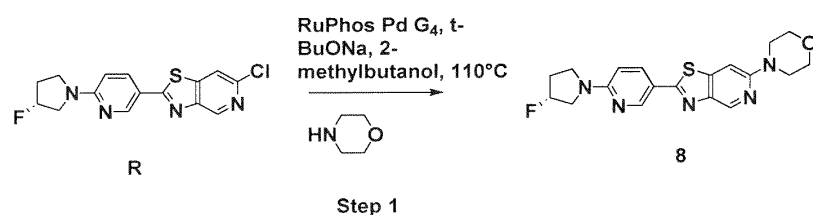
Synthetic scheme for the preparation of (R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-6-(4,7-diazaspiro[2.5]octan-7-yl)thiazolo[4,5-c]pyridine (Compound 7)

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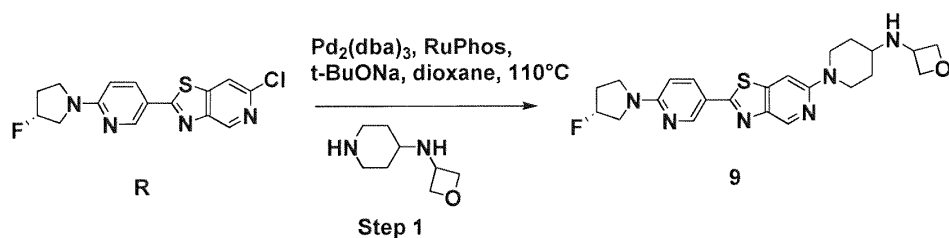


Synthetic scheme for the preparation of (R)-4-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)morpholine (Compound 8)

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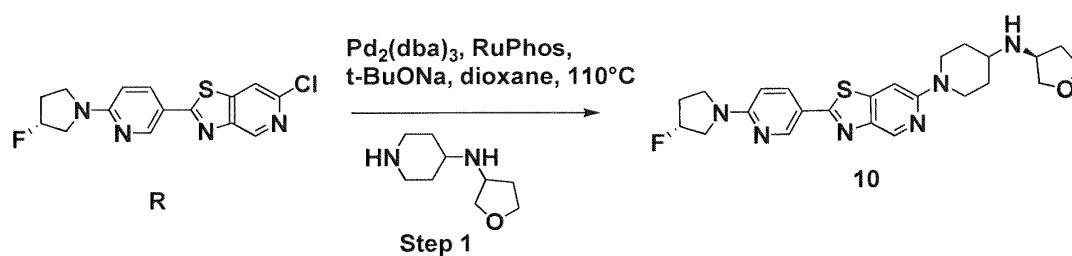


Synthetic scheme for the preparation of (R)-1-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-N-(oxetan-3-yl)piperidin-4-amine (Compound 9)



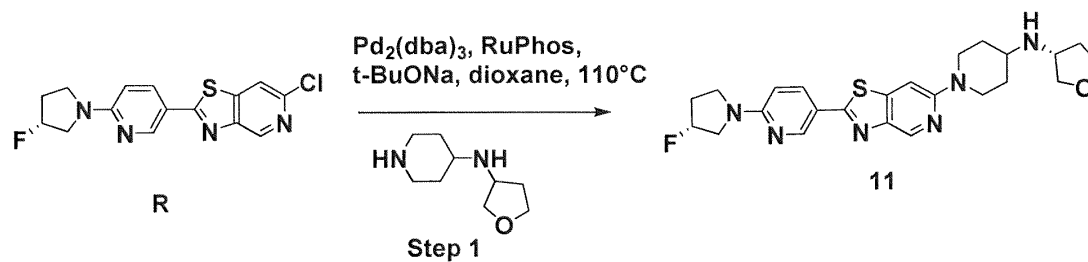
Synthetic scheme for the preparation of 1-(2-(6-((R)-3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-N-((R*)-tetrahydrofuran-3-yl)piperidin-4-amine (Compound 10)

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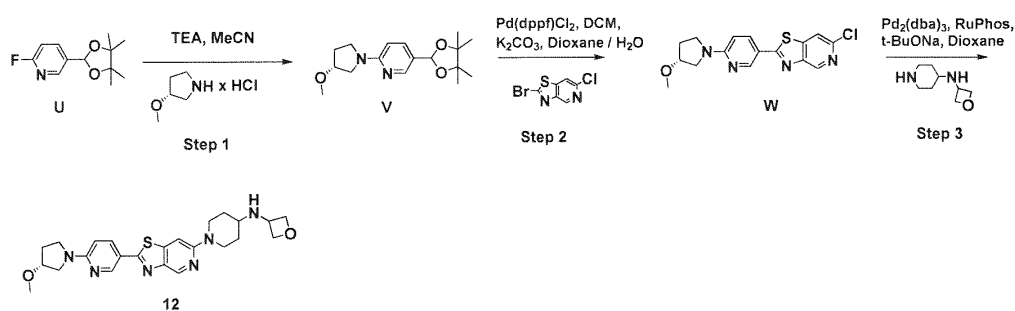


Synthetic scheme for the preparation of 1-(2-(6-((R)-3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-N-((R*)-tetrahydrofuran-3-yl)piperidin-4-amine (Compound 11)

10

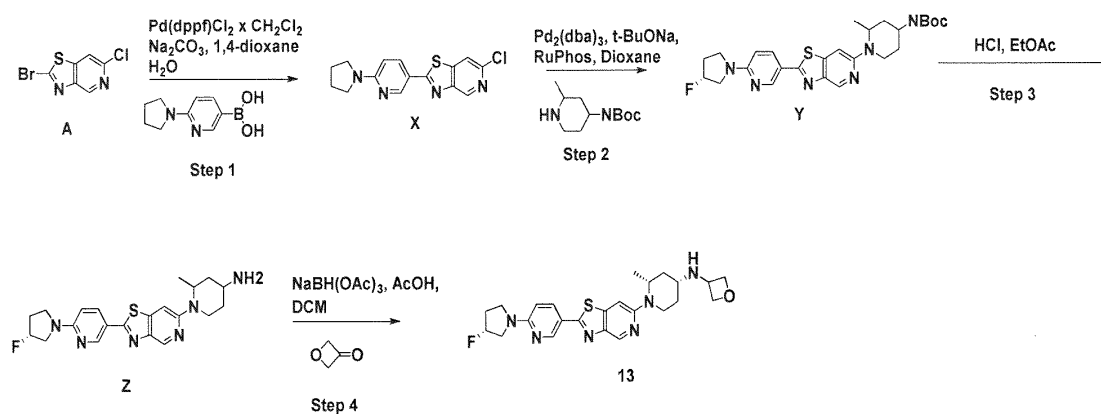


Synthetic scheme for the preparation of (R)-1-(2-(6-(3-methoxypyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-N-(oxetan-3-yl)piperidin-4-amine (Compound 12)



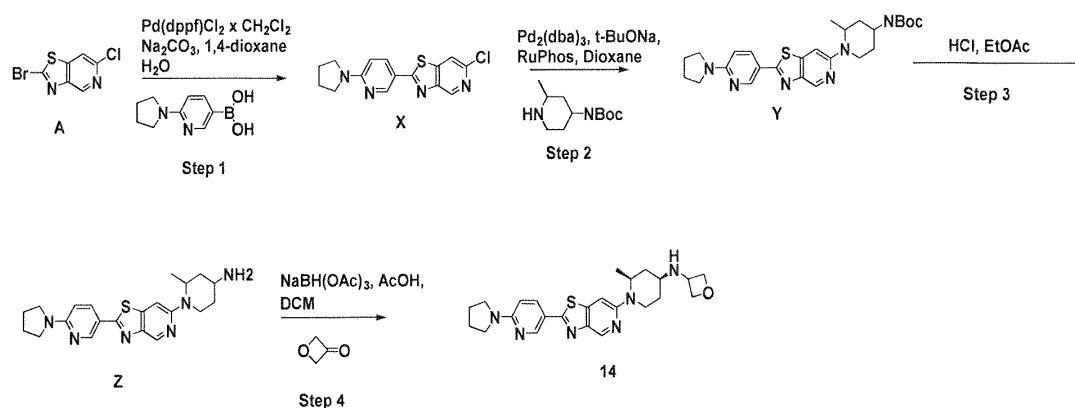
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Synthetic scheme for the preparation of (2R*,4R*)-1-(2-(6-((R)-3-fluoropyrrolidin-1-yl)pyridin-3-yl)benzo[d]thiazol-6-yl)-2-methyl-N-(oxetan-3-yl)piperidin-4-amine (Compound 13)



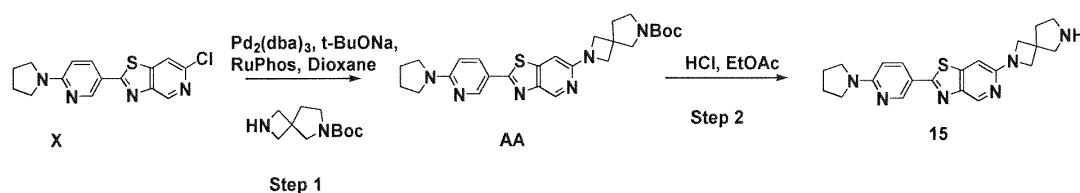
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Synthetic scheme for the preparation of ((2S*,4S*)-1-(2-(6-((R)-3-fluoropyrrolidin-1-yl)pyridin-3-yl)benzo[d]thiazol-6-yl)-2-methyl-N-(oxetan-3-yl)piperidin-4-amine (Compound 14)



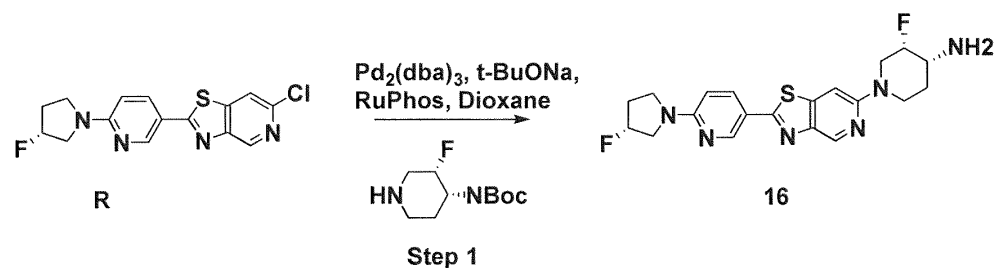
5

Synthetic scheme for the preparation of (2-(6-(pyrrolidin-1-yl)pyridin-3-yl)-6-(2,6-diazaspiro[3.4]octan-2-yl)thiazolo[4,5-c]pyridine (Compound 15)

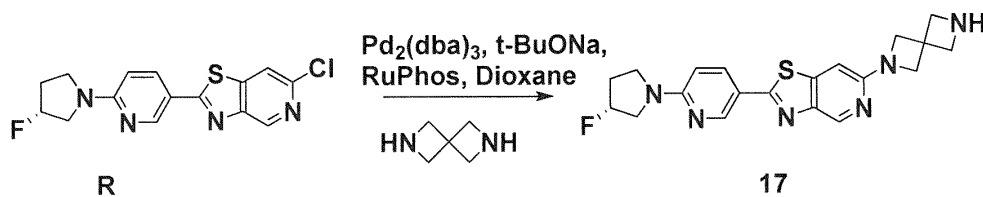


10

Synthetic scheme for the preparation of (3S,4R)-3-fluoro-1-(2-(6-((R)-3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)piperidin-4-amine (Compound 16)



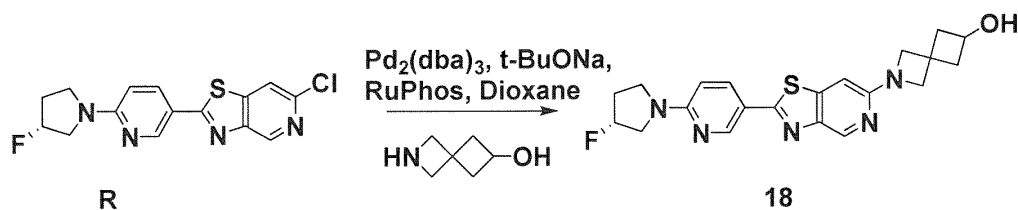
Synthetic scheme for the preparation of (R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-6-(2,6-diazaspiro[3.3]heptan-2-yl)thiazolo[4,5-c]pyridine (Compound 17)



5

Step 1

Synthetic scheme for the preparation of (R)-2-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-2-azaspiro[3.3]heptan-6-ol (Compound 18)

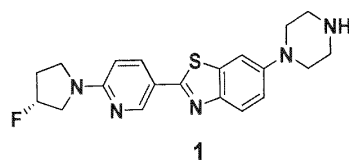


10

Step 1

EXAMPLES

- 15 **Example 1** Synthesis of (R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-6-(piperazin-1-yl)benzo[d]thiazole Compound 1



Step-1: Synthesis of 6-chloro-2-(6-fluoropyridin-3-yl)benzo[d]thiazole (B)

A mixture of 2-bromo-6-chlorobenzo[d]thiazole **A** (250 g, 1.01 mol), (6-fluoropyridin-3-yl)boronic acid (283.49 g, 2.01 mol), Pd(dppf)Cl₂.DCM (41.07 g, 50.30 mmol) and Na₂CO₃ (383.83 g, 3.62 mol) in THF (2000 mL) and H₂O (200 mL) was heated at 70 °C for 16 h. The reaction mixture was cooled to 25 °C. Saturated aqueous EDTA (1000 mL) and EtOAc (500 mL) were added and the mixture was stirred for 1 h. The mixture was extracted with EtOAc (3 x 2000 mL), the combined organic phases were washed with brine (2 x 1000 mL), dried over Na₂SO₄ filtered, and the filtrate concentrated under reduced pressure. The residue was triturated with petroleum ether/ethyl acetate (10/1, 1000 mL). The mixture was filtered and the filter cake was dried to afford title compound **B** (200 g, 75%) as a yellow solid.

¹H NMR (400 MHz, CHLOROFORM-d) δ 8.89 (s, 1 H) 8.48-8.51 (m, 1 H) 7.98-8.00 (d, *J* = 8.8 Hz, 1 H) 7.91 (s, 1 H) 7.48-7.51 (m, 1 H) 7.09-7.11 (m, 1 H)

LCMS: 265.1 [M + H]⁺

Step-2: Synthesis of (R)-6-chloro-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)benzo[d]thiazole (C)

To a mixture of title compound **B** (200 g, 755.56 mmol) and (R)-3-fluoropyrrolidine hydrochloride (104.36 g, 831.11 mmol) in CH₃CN (2000 mL) was added Et₃N (152.91 g, 1.51 mol, 210.33 mL) and the reaction mixture was stirred at 80 °C for 16 h. The reaction mixture was filtered and the filter cake was washed with CH₃CN (2 x 300 mL) to afford title compound **C** (200 g, 79%) as a yellow solid.

¹H NMR (400 MHz, CHLOROFORM-d) δ 12.15-12.20 (s, 1 H) 8.80 (s, 1 H) 8.16-8.18 (m, 1 H) 7.82-7.90 (m, 2 H) 7.40-7.42 (m, 1 H) 6.48-6.50 (m, 1 H) 5.36-5.49 (m, 1 H) 3.91-4.00 (m, 4 H) 2.16-2.48 (m, 2 H)

¹H NMR (400 MHz, CHLOROFORM-d/D₂O) δ 8.81 (s, 1 H) 8.15 - 8.17 (d, *J* = 8.4 Hz, 1 H) 7.88-7.90 (d, *J* = 8.4 Hz, 1 H) 7.83 (s, 1 H) 7.40-7.43 (m, *J* = 8.8 Hz, 1 H) 6.47-6.50 (d, *J* = 8.8 Hz, 1 H) 5.36-5.49 (m, 1 H) 3.64-4.00 (m, 4 H) 2.15-2.45 (m, 2 H)

LCMS: 334.1 [M + H]⁺

Step-3: Synthesis of *tert*-butyl (*R*)-4-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)benzo[d]thiazol-6-yl)piperazine-1-carboxylate (D**)**

To a mixture of title compound **C** (200 g, 599.14 mmol) and *tert*-butyl piperazine-1-carboxylate (122.75 g, 659.06 mmol) in 1,4-dioxane (2000 mL) was added Pd₂(dba)₃ (54.86 g, 59.91 mmol),
5 XPhos (85.69 g, 179.74 mmol), and a 2 M solution of *t*-BuONa (2 M, 898.71 mL) in THF in one portion under N₂. The reaction mixture was then heated to 110 °C and stirred for 3 h. The reaction mixture was cooled to 25 °C, saturated aqueous EDTA (1000 mL) and EtOAc (500 mL) were added and the reaction mixture was stirred for 1 h. The mixture was extracted with EtOAc (3 x 3000 mL), the combined organic phases were washed with saturated aqueous brine (1000 mL), dried over Na₂SO₄,
10 filtered, and the filtrate concentrated under reduced pressure. The residue was purified by silica gel chromatography (petroleum ether/ethyl acetate 80/20 -> 0/100) to afford title compound **D** (140 g, 48.32%) as a yellow solid.

¹H NMR (400 MHz, CHLOROFORM-*d*) δ 8.77-8.78 (m, 1 H) 8.15-8.18 (m, 1 H) 7.86-7.88 (d, *J* = 9.2 Hz, 1 H) 7.32-7.33 (m, 1 H) 7.11-7.14 (m, 1 H) 6.47-6.49 (d, *J* = 8.8 Hz, 1 H) 5.31-5.49 (m, 1 H) 3.71-
15 3.81 (m, 1 H) 3.61-3.68 (m, 7 H) 3.18-3.21 (m, 4 H) 2.36-2.53 (m, 1 H) 2.08-2.31 (m, 1 H) 1.50 (s, 9 H)

LCMS: 484.2 [M + H]⁺

Step-4: Synthesis of 2-{6-[(3*R*)-3-fluoropyrrolidin-1-yl]pyridin-3-yl}-6-(piperazin-1-yl)-1,3-benzo[d]thiazole (Compound **1)**
20

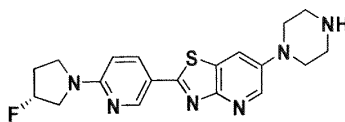
A mixture of title compound **D** (120 g, 248.14 mmol) in HCl / EtOAc (4 M, 1.20 L) was stirred at 25 °C for 1 h. The reaction mixture was filtered, and the filter cake was washed with EtOAc (2 x 100 mL) and dried. The crude product was purified by prep-HPLC (column: Phenomenex Luna c18 250 mm x 100 mm, 10 μm; mobile phase: [A: water (TFA, 0.1%); B:ACN]; B%: 0%-23% over 20 min). The
25 fractions containing the desired compound were collected, the pH was adjusted to pH = 8 by saturated aqueous NaHCO₃ and extracted with DCM (3 x 500 mL). The combined organic phases were washed with brine (2 x 300 mL), dried over Na₂SO₄, filtered, and the filtrate concentrated under reduced pressure. The product was dissolved in CH₃CN and H₂O. 1 M HCl was added, followed by lyophilization to afford title compound **1** as a yellow solid (56.94 g, 53.32%).

¹H NMR (400 MHz, DMSO-d₆) δ 9.65 (s, 2 H) 8.43-8.49 (m, 2 H) 7.90-7.92 (d, *J* = 9.2 Hz, 1 H) 7.71-7.72 (m, 1 H) 7.19-7.31 (m, 2 H) 5.52-5.65 (m, 1 H) 3.92-3.99 (m, 3 H) 3.71-3.72 (m, 1 H) 3.50-3.53 (m, 4 H) 3.16-3.22 (m, 4 H) 2.16-2.41 (m, 2 H)

¹H NMR (400 MHz, D₂O) δ 7.90-7.96 (m, 2 H) 7.54-7.57 (d, *J* = 8.4 Hz 1 H) 7.43 (s, 1 H) 7.14-7.16 (d, *J* = 8.8 Hz 1 H) 6.70-6.72 (d, *J* = 9.6 Hz 1 H) 5.45-5.58 (m, 1 H) 3.36-3.77 (m, 12 H) 2.13-2.50 (m, 2 H)

LCMS: 384.1 [M + H]⁺

Example 2 Synthesis of (*R*)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-6-(piperazin-1-yl)thiazolo[4,5-*b*]pyridine (Compound 2)



2

Step-1: Synthesis of methyl (*R*)-6-(3-fluoropyrrolidin-1-yl)nicotinate (F)

To a mixture of methyl 6-fluoronicotinate **E** (15 g, 96.70 mmol) and (*R*)-3-fluoropyrrolidine hydrochloride (13.36 g, 106.37 mmol) in CH₃CN (250 mL) was added TEA (29.35 g, 290.09 mmol) at 25 °C. The reaction mixture was stirred at 80 °C for 2 h. The mixture was cooled to 25 °C, filtered and the filter cake was dried to afford title compound **F** (13 g, 60%) as a white solid.

¹H NMR (400 MHz, DMSO-d₆) δ 8.65 (s, 1H), 8.02-7.85 (m, 1H), 6.53 (d, *J* = 9.2 Hz, 1H), 5.56-5.35 (m, 1H), 3.83-3.74 (m, 4H), 3.73-3.67 (m, 1H), 3.66-3.53 (m, 1H), 3.53-3.41 (m, 1H), 2.37-2.08 (m, 2H)

LCMS: 225.1 [M + H]⁺

Step-2: (*R*)-6-(3-fluoropyrrolidin-1-yl)nicotinic acid (G)

A mixture of title compound **F** (13 g, 57.98 mmol) and HCl (12 M, 130.00 mL) was heated to 80 °C and stirred for 16 h. The reaction mixture was concentrated in vacuum to afford title compound **G** (15g) as a white solid.

¹H NMR (400 MHz, DMSO-d₆) δ 12.37 (s, 1H), 8.41 (s, 1H), 8.21-8.16 (m, 1H), 7.07 (d, J = 9.2 Hz, 1H), 5.66-5.45 (m, 1H), 4.11-3.58 (m, 4H), 2.45-2.13 (m, 2H)

LCMS: 211.1 [M + H]⁺

5 **Step-3: Synthesis of (R)-6-(3-fluoropyrrolidin-1-yl)nicotinoyl chloride (H)**

To a mixture of title compound **G** (10 g, 47.57 mmol) in DCM (300 mL) was added (COCl)₂ (14.49 g, 114.17 mmol) and DMF (347.73 mg, 4.76 mmol) at 0°C. The reaction mixture was stirred at 25 °C for 1 h. The reaction mixture was concentrated in vacuum to afford title compound **H** (10 g) as a white solid.

10 LCMS: 225.1 [M + H]⁺

Step-4: Synthesis of (R)-N-(5-chloro-3-iodopyridin-2-yl)-6-(3-fluoropyrrolidin-1-yl)nicotinamide (I)

15 To a solution of title compound **H** (10.02 g, 39.36 mmol) in THF (250 mL) was added NaH (5.25 g, 131.20 mmol, 60% purity) at 0 °C and the reaction mixture was stirred at 0 °C for 1 h. Then 5-chloro-3-iodopyridin-2-amine (10 g, 43.73 mmol) was added at 0 °C. The reaction mixture was stirred at 25 °C for 11 h. The mixture was poured into ice-water (300 mL). The aqueous phase was extracted with DCM (3 x 300 mL). The combined organic phases were washed with brine (300 mL), dried over anhydrous Na₂SO₄, filtered, and the filtrate concentrated in vacuum. The residue was purified by
20 silica gel chromatography (petroleum ether / ethyl acetate 100/0 → 0/100) to afford title compound **I** (18 g, 92% yield) as a white solid.

¹H NMR (400 MHz, DMSO-d₆) δ 10.49 (s, 1H), 8.74 (s, 1H), 8.58-8.48 (m, 2H), 8.12-8.03 (m, 1H), 6.60 (d, J = 8.8 Hz, 1H), 5.57-5.37 (m, 1H), 3.89-3.42 (m, 4H), 2.38-2.05 (m, 2H)

LCMS: 447.0 [M + H]⁺

25

Step-5: Synthesis of (*R*)-*N*-(5-chloro-3-iodopyridin-2-yl)-6-(3-fluoropyrrolidin-1-yl)pyridine-3-carbothioamide (J)

To a mixture of title compound I (18 g, 40.30 mmol) in toluene (200 mL) was added P₂S₅ (17.92 g, 80.60 mmol) at 25 °C under N₂. The mixture was degassed with N₂ three times and stirred at 110 °C for 0.5 h. The mixture was cooled to 25 °C and adjusted to pH= 8 with saturated aqueous Na₂CO₃ (300 mL). The aqueous phase was extracted with DCM 3 x (300 mL). The combined organic phases were washed with brine (300 mL), dried over anhydrous Na₂SO₄, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by silica gel chromatography (petroleum ether/ethyl acetate 100/0 -> 0/100) to afford title compound J (5 g, 27% yield) as a yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ 11.74 (s, 1H), 8.81 (s, 1H), 8.66-8.52 (m, 2H), 8.22-8.12 (m, 1H), 6.60 (d, J = 8.8 Hz, 1H), 5.58-5.37 (m, 1H), 3.86-3.42 (m, 4H), 2.30-2.18 (m, 2H)

LCMS: 462.9 [M + H]⁺

Step-6: Synthesis of (*R*)-6-chloro-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-*b*]pyridine (K)

To a mixture of title compound J (5 g, 10.81 mmol) in 1,4-dioxane (50 mL) was added CuI (411.60 mg, 2.16 mmol), K₂CO₃ (4.48 g, 32.42 mmol) and L-proline (497.63 mg, 4.32 mmol) under N₂. The mixture was degassed with N₂ three times and stirred at 80 °C for 6 h. The mixture was cooled to 25 °C, diluted with DCM (100 mL), poured into saturated aqueous EDTA (100 mL) and stirred for 1 hour. The solid was precipitated and collected by filtration. The filter cake was dried to afford title compound K (3.2 g, 88% yield) as a yellow solid.

¹H NMR (400 MHz, CDCl₃) δ 8.89 (s, 1H), 8.60 (s, 1H), 8.30-8.24 (m, 1H), 8.18-8.15 (m, 1H), 6.51 (d, J = 8.8 Hz, 1H), 5.52-5.33 (m, 1H), 3.99-3.68 (m, 4H), 2.53-2.10 (m, 2H)

LCMS: 335.0 [M + H]⁺

Step-7: Synthesis of *tert*-butyl (*R*)-4-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-*b*]pyridin-6-yl)piperazine-1-carboxylate (L)

To a mixture of title compound K (1 g, 2.99 mmol) and *tert*-butyl piperazine-1-carboxylate (611.94 mg, 3.29 mmol) in 1,4-dioxane (10 mL) was added Pd₂(dba)₃ (0.274 g, 0.299 mmol), XPhos (0.428 g,

0.896 mmol), t-BuONa (2 M dissolved in THF, 4.48 mL) under N₂. The mixture was degassed, purged with N₂ three times and stirred at 110 °C for 3 h. The mixture was cooled to 25 °C, diluted with DCM (100 mL), poured into saturated aqueous EDTA (120 mL) and stirred for 1 h. The aqueous phase was extracted with DCM (3 x 40 mL). The combined organic phases were washed with brine (3 x 100 mL), dried with anhydrous Na₂SO₄, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by silica gel chromatography (petroleum ether / ethyl acetate 1/000 -> 0/100) to afford title compound **L** (0.75 g, 1.55 mmol, 52% yield) as a yellow solid.

¹H NMR (400 MHz, CDCl₃) δ 8.88-8.83 (m, 1H), 8.43-8.42 (m, 1H), 8.30-8.25 (m, 1H), 7.80-7.64 (m, 1H), 6.52-6.48 (m, 1H), 5.53-5.32 (m, 1H), 3.93-3.62 (m, 4H), 3.60-3.64 (m, 4H), 3.27-3.20 (m, 4H), 2.38-2.09 (m, 4H), 1.51 (s, 9H)

LCMS: 485.3 [M + H]⁺

Step-8: Synthesis of (R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-6-(piperazin-1-yl)thiazolo[4,5-b]pyridine (Compound 2)

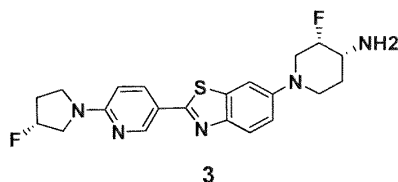
To a mixture of title compound **L** (750 mg, 1.55 mmol) was added HCl / EtOAc (4 M, 10 mL) in one portion at 25 °C under N₂. The mixture was stirred at 25 °C for 1 h. The reaction mixture was concentrated in vacuum. The residue was purified by prep-HPLC (column: Phenomenex Luna C18 (250 mm x 70 mm, 15 μm); mobile phase: [A: water (HCl 0.2%); B: ACN]; B%: 1%-30% over 20 min). Fractions containing the desired compound were collected. Then lyophilization followed to afford title compound **2** (0.317 g, 53% yield) as a yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ 9.07 (s, 2H), 8.81-8.72 (m, 1H), 8.57-8.45 (m, 1H), 8.26-8.11 (m, 2H), 6.75 (d, J = 9.2 Hz, 1H), 5.63-5.38 (m, 1H), 3.93-3.53 (m, 8H), 3.30-3.24 (m, 4H), 2.35-2.15 (m, 2H)

¹H NMR (400 MHz, METHANOL-d₄) δ 8.68-8.65 (m, 1H), 8.61-8.56 (m, 2H), 8.28-8.21 (m, 1H), 7.29(d, J = 9.6 Hz 1H), 5.66-5.45 (m, 1H), 4.09-3.78 (m, 4H), 3.65-3.60 (m, 4H), 3.50-3.43 (m, 4H), 2.65-2.23 (m, 2H)

LCMS: 385.2 [M + H]⁺

Example 3 Synthesis of (3*S*,4*R*)-3-fluoro-1-(2-(6-((*R*)-3-fluoropyrrolidin-1-yl)pyridin-3-yl)benzo[*d*]thiazol-6-yl)piperidin-4-amine Compound 3



Step-1: Synthesis of *tert*-butyl ((3*S*,4*R*)-3-fluoro-1-(2-(6-((*R*)-3-fluoropyrrolidin-1-yl)pyridin-3-yl)benzo[*d*]thiazol-6-yl)piperidin-4-yl)carbamate (M**)**

A mixture of title compound **C** (0.5 g, 1.50 mmol), *tert*-butyl ((3*S*,4*R*)-3-fluoropiperidin-4-yl)carbamate (0.360 g, 1.65 mmol), Pd₂(dba)₃ (0.137 g, 0.15 mmol), XPhos (0.214 g, 0.45 mmol) and Cs₂CO₃ (1.46 g, 4.49 mmol) in 1,4-dioxane (5 mL) was degassed and purged with N₂ for 3 times. The reaction mixture was stirred at 110 °C for 16 h under N₂ atmosphere. The reaction mixture was cooled to 25°C, saturated aqueous EDTA (20 mL) and DCM (20 mL) were added. The mixture was stirred for 1 h and extracted with DCM (3 x 20 mL). The combined organic layers were washed with brine (2 x 40 mL), dried over anhydrous Na₂SO₄, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by silica gel chromatography (petroleum ether / ethyl acetate 80/20 -> 0/100) to afford title compound **M** (0.5 g, 65% yield) as a yellow solid.

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.73-8.68 (m, 1H), 8.12-8.04 (m, 1H), 7.76 (d, *J* = 9.2 Hz, 1H), 7.54-7.53 (m, 1H), 7.19-7.13 (m, 1H), 7.08-7.06 (m, 1H), 6.65 (d, *J* = 8.8 Hz, 1H), 5.60-5.37 (m, 1H), 4.90-4.71 (m, 1H), 3.90-4.01 (m, 1H), 3.87-3.67 (m, 4H), 3.66-3.60 (m, 1H), 3.53-3.51 (m, 1H), 3.15-2.97 (m, 1H), 2.95-2.85 (m, 1H), 2.33-2.10 (m, 2H), 1.95-1.80 (m, 1H), 1.75-1.65 (m, 1H), 1.41 (s, 9H)

LCMS: 516.1 [M + H]⁺

Step-2: Synthesis of (3*S*,4*R*)-3-fluoro-1-(2-(6-((*R*)-3-fluoropyrrolidin-1-yl)pyridin-3-yl)benzo[*d*]thiazol-6-yl)piperidin-4-amine (Compound 3**)**

A mixture of title compound **M** (0.5 g, 0.97 mmol) in HCl / EtOAc (4 M, 10 mL) was stirred at 25 °C for 1 h. The reaction mixture was concentrated under reduced pressure. The crude product was purified by prep-HPLC (column: Phenomenex Luna C18 (250 mm x 70 mm, 15 μm); mobile phase:

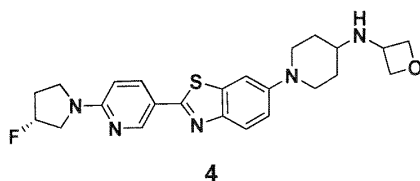
[A: water (0.04% HCl); B:ACN]; gradient: 10%-50% B over 20 min). Fractions containing the desired compound were collected. The lyophilization followed to afford the title compound **3** (0.2 g, 46% yield) as a yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ 8.67-8.66 (m, 1H), 8.45-8.36 (m, 3H), 8.21-8.13 (m, 1H), 7.80 (d, J=8.8 Hz, 1H), 7.60 (d, J = 2.4 Hz, 1H), 7.20 (br d, J = 8.8 Hz, 1H), 6.78 (d, J=9.2 Hz, 1H), 5.61-5.41 (m, 1H), 5.15-4.95 (m, 1H), 4.19-4.15 (m, 1H), 3.82-3.64 (m, 4H), 3.59-3.48 (m, 2H), 3.25-3.08 (m, 1H), 2.99-2.89 (m, 1H), 2.32-2.14 (m, 2H), 1.98-1.88 (m, 2H)

¹H NMR (400 MHz, DMSO-d₆/D₂O) δ 8.66-8.60 (m, 1H), 8.23-8.14 (m, 1H), 7.81 (d, J = 9.2 Hz, 1H), 7.62-7.55 (m, 1H), 7.24-7.16 (m, 1H), 6.83 (d, J = 9.2 Hz, 1H), 5.60-5.40 (m, 1H), 5.13-4.93 (m, 1H), 4.23-4.11 (m, 1H), 3.93-3.51 (m, 6H), 3.25-3.07 (m, 1H), 3.00-2.88 (m, 1H), 2.35-2.14 (m, 2H), 1.99-1.87 (m, 2H)

LCMS: 416.1 [M + H]⁺

Example 4 Synthesis of (R)-1-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)benzo[d]thiazol-6-yl)-N-(oxetan-3-yl)piperidin-4-amine Compound 4



Step-1: Synthesis of (R)-1-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)benzo[d]thiazol-6-yl)-N-(oxetan-3-yl)piperidin-4-amine (Compound 4)

To a mixture of title compound **C** (1 g, 3.00 mmol) and *N*-(oxetan-3-yl)piperidin-4-amine (0.51 g, 3.30 mmol) in 1,4-dioxane (10 mL) was added Pd₂(dba)₃ (0.274 g, 0.3 mmol), XPhos (0.286 g, 0.6 mmol), and *t*-BuONa (2 M, 4.49 mL) in THF at 25 °C under N₂. The mixture was degassed and purged with N₂ three times and stirred at 110 °C for 3 h. The reaction mixture was cooled to 25 °C, saturated aqueous EDTA (200 mL) and DCM (100 mL) were added. The reaction mixture was stirred for 1 h, extracted with DCM (3 x 100 mL), the combined organic phases were washed with brine (200 mL), dried over Na₂SO₄, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by prep-HPLC (column: Phenomenex Luna C18 100 mm x 40 mm, 3 μm; mobile phase:

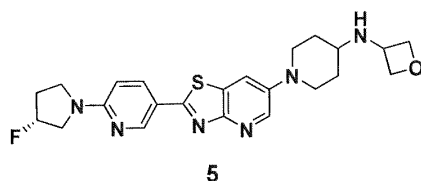
[A: water (0.2% formic acid); B: ACN]; gradient: 5%-30% B over 8.0 min). Fractions containing the desired compound were collected, the pH was adjusted to pH = 8 by saturated aqueous NaHCO₃, and extracted with DCM (3 x 30 mL). The combined organic phases were washed with brine (2 x 30 mL) dried over Na₂SO₄, filtered, and the filtrate concentrated under reduced pressure. The product was dissolved in CH₃CN and H₂O, followed by lyophilization to afford title compound **4** (0.303 g, 21% yield) as a yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ 8.70 (s, 1H), 8.11-8.04 (m, 1H), 7.75 (d, J = 9.2 Hz, 1H), 7.53-7.47 (m, 1H), 7.16-7.13 (m, 1H), 6.64 (d, J = 8.8 Hz, 1H), 5.57-5.38 (m, 1H), 4.68-4.60 (m, 2H), 4.36-4.29 (m, 2H), 4.03-3.92 (m, 1H), 3.87-3.59 (m, 5H), 3.55-3.45 (m, 1H), 2.83-2.71 (m, 2H), 2.57-2.52 (m, 1H), 2.37-2.10 (m, 2H), 1.82-1.69 (m, 2H), 1.40-1.28 (m, 2H)

¹H NMR (400 MHz, CDCl₃) δ 8.77 (s, 1H), 8.20-8.13 (m, 1H), 7.84 (d, J = 8.8 Hz, 1H), 7.33-7.30 (m, 1H), 7.14-7.09 (m, 1H), 6.47 (d, J = 8.8 Hz, 1H), 5.50-5.33 (m, 1H), 4.86 (t, J = 6.4 Hz, 2H), 4.47 (t, J = 6.4 Hz, 2H), 4.19-4.09 (m, 1H), 4.02-3.86 (m, 1H), 3.82-3.61 (m, 5H), 2.91-2.77 (m, 2H), 2.71-2.58 (m, 1H), 2.52-2.38 (m, 1H), 2.30-2.07 (m, 1H), 1.97-1.85 (m, 2H), 1.56-1.50 (m, 2H)

LCMS: 454.2 [M + H]⁺

Example 5 Synthesis of (R)-1-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-b]pyridin-6-yl)-N-(oxetan-3-yl)piperidin-4-amine Compound 5



Step-1: Synthesis of (R)-8-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-b]pyridin-6-yl)-1,4-dioxo-8-azaspiro[4.5]decane (N)

To a mixture of title compound **K** (0.8 g, 2.39 mmol) and 1,4-dioxo-8-azaspiro[4.5]decane (0.376 g, 2.63 mmol) in 1,4-dioxane (10 mL) was added Ruphos Pd G₄ (0.203 g, 0.239 mmol), and *t*-BuONa (2 M in THF, 2.39 mL, 4.78 mmol). The reaction mixture was degassed and purged with N₂ three times and stirred at 110 °C for 16 h. The reaction mixture was cooled to 25°C, saturated aqueous EDTA (80 mL) and EtOAc (80 mL) were added and the reaction mixture was stirred for 1 h. The

reaction mixture was extracted with EtOAc (2 x 80 mL), the combined organic phases were washed with brine (3 x 80 mL), dried with anhydrous Na₂SO₄, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by silica gel chromatography (petroleum ether/ethyl acetate 100/0 -> 0/100) to afford compound **N** (0.5 g, 48% yield) as a yellow solid.

- 5 ¹H NMR (400 MHz, CDCl₃) δ = 8.86-8.81 (m, 1H), 8.45-8.43 (m, 1H), 8.28-8.24 (m, 1H), 7.66-7.64 (m, 1H), 6.51-6.46 (m, 1H), 5.53-5.31 (m, 1H), 4.03-4.02 (m, 4H), 3.96-3.56 (m, 4H), 3.43-3.41 (m, 4H), 2.52-2.20 (m, 2H), 1.91-1.89 (m, 4H)

LCMS: 442.3 [M + H]⁺

10 **Step-2: Synthesis of (R)-1-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-b]pyridin-6-yl)piperidin-4-one (P)**

To a mixture of title compound **N** (0.45 g, 1.02 mmol) in THF (6 mL) was added HCl (3 M, 1.29 mL, 3.86 mmol). The mixture was stirred at 60 °C for 2 h. The reaction mixture was poured into saturated aqueous Na₂CO₃ (30 mL). The aqueous phase was extracted with ethyl acetate (3 x 40 mL). The
15 combined organic phases were washed with brine (3 x 50 mL), dried with anhydrous Na₂SO₄, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by silica gel chromatography (petroleum ether / ethyl acetate 100/0 -> 0/100) to afford compound **P** (0.24 g, 59%) as a yellow solid.

- 20 ¹H NMR (400 MHz, CDCl₃) δ 8.86-8.82 (m, 1H), 8.47 (s, 1H), 8.24 (d, J = 9.2 Hz, 1H), 7.71-7.64 (m, 1H), 6.49 (d, J = 8.8 Hz, 1H), 5.50-5.33 (m, 1H), 4.07-3.76 (m, 4H), 3.69-3.66 (m, 4H), 2.67-2.61 (m, 4H), 2.47-2.14 (m, 2H)

LCMS: 398.2 [M + H]⁺

25 **Step-3: Synthesis of (R)-1-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-b]pyridin-6-yl)-N-(oxetan-3-yl)piperidin-4-amine (Compound 5)**

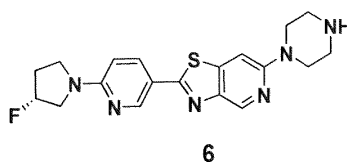
To a mixture of title compound **P** (0.22 g, 0.553 mmol) and oxetan-3-amine (0.0405 g, 0.553 mmol) in DCE (3 mL) were added NaBH(OAc)₃ (0.117 g, 0.553 mmol) and acetic acid (0.033 g, 0.553 mmol). The mixture was stirred at 25 °C for 2 h. The reaction mixture was poured into water (50 mL). The aqueous phase was extracted with DCM (3 x 50 mL). The combined organic phases were washed

with brine (3 x 50 mL), dried with anhydrous Na₂SO₄, filtered, and the filtrate concentrated under reduced pressure. The residue was purified by prep-HPLC (column: Waters Xbridge Prep OBD C18 150 mm x 4.0 mm, 10 µm; mobile phase: [A: water (0.05% NH₃H₂O + 10 mM NH₄HCO₃); B: ACN]; gradient: 10%-45% B over 8.0 min). Fractions containing the desired compound were collected, followed by lyophilization to afford compound **5** (0.43 g, 17% yield) as a yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ 8.77 (s, 1H), 8.41 (s, 1H), 8.15-8.09 (m, 1H), 8.03-7.98 (m, 1H), 6.67 (d, J = 8.8 Hz, 1H), 5.60-5.36 (m, 1H), 4.64 (t, J = 6.4 Hz, 2H), 4.32 (t, J = 6.4 Hz, 2H), 4.03-3.94 (m, 1H), 3.89-3.62 (m, 5H), 3.56-3.46 (m, 1H), 2.88-2.78 (m, 2H), 2.59-2.53 (m, 1H), 2.34-2.15 (m, 2H), 1.82-1.73 (m, 2H), 1.43-1.29 (m, 2H)

LCMS: 455.3 [M + H]⁺

Example 6 Synthesis of (R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-6-(piperazin-1-yl)thiazolo[4,5-c]pyridine Compound 6



15 Step-1: Synthesis of methyl (R)-6-(3-fluoropyrrolidin-1-yl)nicotinate (F)

To a mixture of methyl 6-fluoronicotinate **E** (10 g, 64.46 mmol) and (R)-3-fluoropyrrolidine hydrochloride (8.50 g, 67.69 mmol) in CH₃CN (100 mL) was added TEA (19.57 g, 193.39 mmol) at 25 °C. The reaction mixture was stirred at 80 °C for 2 h. The mixture was cooled to 25 °C, filtered and the filter cake was dried to afford title compound **F** (7.5 g, 60%) as a white solid.

¹H NMR (400 MHz, DMSO-d₆) δ = 8.65 (s, 1H), 7.98 - 7.93 (m, 1H), 6.56 (d, J = 8.8 Hz, 1H), 5.55 - 5.37 (m, 1H), 3.78 (s, 3H), 3.74 - 3.43 (m, 4H), 2.21 (m, 2H)

LCMS: 225.1 [M + H]⁺

Step-2: Synthesis of (R)-6-(3-fluoropyrrolidin-1-yl)nicotinic acid (G)

A mixture of title compound **F** (6.8 g, 30.33 mmol) and HCl (12 M, 68 mL) was heated to 80 °C and stirred for 16 h. The reaction was concentrated in vacuum to afford title compound **G** (6 g, crude) as a white solid.

- 5 ¹H NMR (400 MHz, DMSO-d₆) δ = 10.66 (s, 1H), 8.43 (s, 1H), 8.25 - 8.08 (m, 1H), 7.11 - 6.92 (m, 1H), 5.69 - 5.43 (m, 1H), 4.08 - 3.59 (m, 4H), 2.43 - 2.12 (m, 2H)

LCMS: 211.1 [M + H]⁺

Step-3: Synthesis of (R)-6-(3-fluoropyrrolidin-1-yl)nicotinoyl chloride (H)

- 10 To a mixture of title compound **G** (3 g, 14.27 mmol) in DCM (30 mL) was added (COCl)₂ (4.35 g, 34.25 mmol) and DMF (104.32 mg, 1.43 mmol) at 0 °C. The reaction mixture was stirred at 25 °C for 1 h. The reaction was concentrated in vacuum to afford title compound **H** (3 g, crude) as a white solid.

- 15 LCMS: 225.3 (quenched by MeOH)

Step-4: Synthesis of (R)-N-(6-chloro-4-iodopyridin-3-yl)-6-(3-fluoropyrrolidin-1-yl)nicotinamide (Q)

- 20 To a solution of 6-chloro-4-iodopyridin-3-amine (3.00 g, 11.81 mmol) in THF (30 mL) was added NaH (1.57 g, 39.36 mmol, 60% purity) at 0 °C and the reaction mixture was stirred at 0 °C for 1 h. Then title compound **H** (3 g, 13.12 mmol) was added at 0 °C. The reaction mixture was stirred at 25 °C for 11 h. The mixture was poured into saturated aqueous NH₄Cl (60 mL). The aqueous phase was extracted with DCM (3 x 30 mL). The combined organic phase was washed with brine (30 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuum. The residue was purified by silica gel chromatography (Petroleum ether / Ethyl acetate = 1/1) to afford title compound **Q** (2 g, 34% yield) as a yellow solid.

25 ¹H NMR (400 MHz, DMSO-d₆) δ = 8.78 (s, 1H), 8.36 (s, 1H), 8.15 (s, 1H), 8.12 - 8.03 (m, 1H), 6.62 (d, J = 8.8 Hz, 1H), 5.64 - 5.26 (m, 1H), 3.88 - 3.45 (m, 4H), 2.35 - 2.06 (m, 2H)

LCMS: 447.0 [M + H]⁺

Step-5: Synthesis of (R)-6-chloro-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridine (R)

- 5 To a mixture of title compound **Q** (1.7 g, 3.81 mmol) in toluene (20 mL) was added P₂S₅ (1.69 g, 7.61 mmol) at 25 °C under N₂. The mixture was degassed and purged with N₂ three times and stirred at 110 °C for 0.5 h. The mixture was cooled to 25 °C and adjusted to pH= 8 with saturated aqueous Na₂CO₃ (100 mL). The aqueous phase was extracted with DCM (3 x 30 mL). The combined organic phase was washed with brine (30 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuum. The residue was purified by silica gel chromatography (Petroleum ether / Ethyl acetate =1/2)
- 10 to afford title compound **R** (800 mg, 62% yield) as a yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ = 9.01 (s, 1H), 8.83 (s, 1H), 8.33 (s, 1H), 8.20 - 8.15 (m, 1H), 6.72 - 6.67 (m, 1H), 5.57 - 5.40 (m, 1H), 3.82 - 3.47 (m, 4H), 2.34 - 2.16 (m, 2H)

LCMS: 335.1 [M + H]

15

Step-6: Synthesis of tert-butyl (R)-4-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)piperazine-1-carboxylate (S)

- To a mixture of title compound **R** (700 mg, 2.09 mmol) and tert-butyl piperazine-1-carboxylate (428.36 mg, 2.30 mmol) in 1,4-dioxane (7 mL) was added Pd₂(dba)₃ (191.46 mg, 209.08 μmol),
- 20 XPhos (299.02 mg, 627.24 μmol) and tBuONa (2 M in THF, 2.09 mL, 2 eq). The reaction mixture was degassed and purged with N₂ three times and stirred at 110 °C for 3 h. The reaction mixture was cooled to 25°C, saturated aqueous EDTA (50 mL) and the reaction mixture was stirred for 1 h. The reaction mixture was extracted with DCM (2 x 30 mL), the combined organic phase was washed with brine (3 x 30 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure.
- 25 The residue was purified by silica gel chromatography (Petroleum ether/Ethyl acetate 1/2) to afford compound **S** (500 mg, 49% yield) as yellow solid.

¹H NMR (400 MHz, CDCl₃) δ = 8.87 (s, 1H), 8.75 (s, 1H), 8.20 - 8.09 (m, 1H), 7.04 (s, 1H), 6.48 (d, J = 8.8 Hz 1H), 5.51 - 5.33 (m, 1H), 3.99 - 3.49 (m, 12H), 2.46 - 2.05 (m, 2H), 1.51 (s, 9H)

LCMS: 485.3 [M + H]⁺

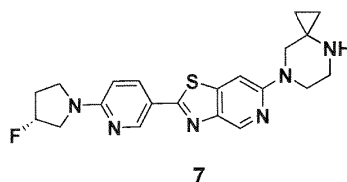
Step-7: Synthesis of (R)-1-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-b]pyridin-6-yl)piperidin-4-one (Compound 6)

A mixture of title compound **S** (450 mg) in HCl/EtOAc (4 M, 5 mL) was stirred at 25 °C for 1 h. The reaction mixture was concentrated in vacuum. The residue was purified by prep-HPLC (column: Phenomenex luna C18 80*40mm*3um; mobile phase: [water(HCl)-ACN]; B%: 1%-40%, 7min). Fractions containing the desired compound were collected, followed by lyophilization to afford compound **6** (88.35 mg, 22%) as a yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ = 9.55 - 9.40 (m, 2H), 8.87 (s, 1H), 8.63 (s, 1H), 8.34 - 8.24 (m, 1H), 7.69 (s, 1H), 6.95 (d, J = 9.2 Hz, 1H), 5.62 - 5.45 (m, 1H), 4.01 - 3.59 (m, 8H), 3.29 - 3.13 (m, 4H), 2.39 - 2.11 (m, 2H)

LCMS: 385.1 [M + H]⁺

Example 7 Synthesis of (R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-6-(4,7-diazaspiro[2.5]octan-7-yl)thiazolo[4,5-c]pyridine Compound 7



Step-1: Synthesis of tert-butyl (R)-7-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate (T)

A mixture of 6-chloro-2-[6-[(3R)-3-fluoropyrrolidin-1-yl]-3-pyridyl]thiazolo[4,5-c]pyridine **1** (500 mg, 1.49 mmol) and tert-butyl 4,7-diazaspiro[2.5]octane-4-carboxylate **R** (348.74 mg, 1.64 mmol), t-BuONa (2 M in THF, 1.49 mL), Xantphos Pd G₄ (143.72 mg, 149.34 μmol), Xantphos (864.13 mg, 1.49 mmol) in dioxane (5 mL) was degassed and purged with N₂ 3 times, and then the mixture was stirred at 110 °C for 16 hrs under N₂ atmosphere. The reaction mixture was cooled to 25 °C, saturated aqueous EDTA (100 mL) and EtOAc (50 mL) were added, and the mixture was stirred for 1 hr. The mixture was extracted with EtOAc (3 x 50 mL), the combined organic phase was washed with brine (2 x 50 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was

purified by column chromatography (Petroleum ether / Ethyl acetate=1/0 to 1/1). Compound **T** (300 mg, 39.34% yield) was obtained as a yellow solid.

¹H NMR (400 MHz, CDCl₃) δ = 8.30 (s, 1H), 8.73 (s, 1H), 8.13-8.11 (m, 1H), 6.98 (s, 1H), 6.49 - 6.44 (m, 1H), 5.49 - 5.34 (m, 1H), 4.01 - 3.87 (m, 1H), 3.74 - 3.67 (m, 3H), 3.58 - 3.53 (m, 2H), 3.50 - 3.47 (m, 2H), 2.49 - 2.38 (m, 1H), 2.26 - 2.12 (m, 1H), 1.74 - 1.57 (m, 2H), 1.52 - 1.46 (m, 9H), 1.08 - 1.02 (m, 2H), 0.94 - 0.86 (m, 2H)

LCMS: 511.3 [M + H]⁺

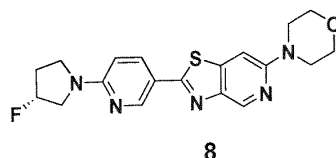
Step-2: Synthesis of (R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-6-(4,7-diazaspiro[2.5]octan-7-yl)thiazolo[4,5-c]pyridine (Compound 7)

A mixture of compound tert-butyl (R)-7-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-4,7-diazaspiro[2.5]octane-4-carboxylate **T** (300 mg, 587.51 μmol), and TFA (0.5 mL, 6.73 mmol) in DCM (2.5 mL) was stirred at 25 °C for 1 hr under N₂ atmosphere. The reaction mixture was quenched by addition saturated aqueous NaHCO₃ (50 mL) at 25 °C and extracted with DCM (3 x 50 mL). The combined organic layers were washed with brine (2 x 50 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (basic condition; column: Waters Xbridge Prep OBD C18 150*40mm*10μm; mobile phase: [H₂O (0.05% NH₃H₂O+10mM NH₄HCO₃)-ACN]; gradient: 25%-55% B over 8.0 min). Compound **7** (62.56 mg, 24.18% yield) was obtained as a yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ = 8.80 - 8.64 (m, 2H), 8.15 - 8.03 (m, 1H), 7.39 (s, 1H), 6.66 (d, J = 8.8 Hz, 1H), 5.59 - 5.36 (m, 1H), 3.88 - 3.60 (m, 4H), 3.55 - 3.45 (m, 4H), 2.91 - 2.83 (m, 2H), 2.31 - 2.08 (m, 2H), 0.57 - 0.42 (m, 4H)

LCMS: 411.1 [M + H]⁺

Example 8 Synthesis of (R)-4-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)morpholine Compound 8



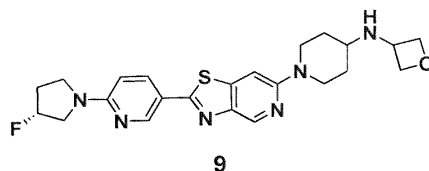
Step-1: Synthesis of (R)-4-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)morpholine (Compound 8)

A mixture of (R)-6-chloro-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridine **R** (500 mg, 1.49 mmol), morpholine (260.22 mg, 2.99 mmol, 262.84 μ L), RuPhos Pd G₄ (254.00 mg, 298.69 μ mol), t-BuONa (2 M in THF, 1.49 mL) in 2-methylbutan-2-ol (10 mL) was degassed and purged with N₂ 3 times, and then the mixture was stirred at 110 °C for 12 h under N₂ atmosphere. The mixture was cooled to 25 °C, then reaction mixture was quenched by addition saturated aqueous EDTA (50 mL) and stirred for 1 h, and then extracted with DCM (3 x 30 mL). The combined organic layers were washed with brine (2 x 20 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Phenomenex luna C18 250*50mm*10 μ m; mobile phase: [H₂O(0.05% HCl) - ACN]; gradient: 10%-50% B over 10.0 min). Fractions containing the desired compound were collected, followed by lyophilization to afford compound **8** (109.14 mg, 19% yield) as a yellow solid.

¹H NMR (400 MHz, MeOD) δ = 8.37 (s, 1H), 8.65 (s, 1H), 8.61 (d, J = 9.6 Hz, 1H), 8.03 (s, 1H), 7.33 (d, J = 9.6 Hz, 1H), 5.63 - 5.50 (m, 1H), 4.12 - 3.94 (m, 3H), 3.93 - 3.89 (m, 4H), 3.88 - 3.81 (m, 1H), 3.72 - 3.66 (m, 4H), 2.63 - 2.29 (m, 2H)

LCMS: 386.1 [M + H]⁺

Example 9 Synthesis of (R)-1-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-N-(oxetan-3-yl)piperidin-4-amine Compound 9



Step-1: Synthesis of (R)-1-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-N-(oxetan-3-yl)piperidin-4-amine (Compound 9)

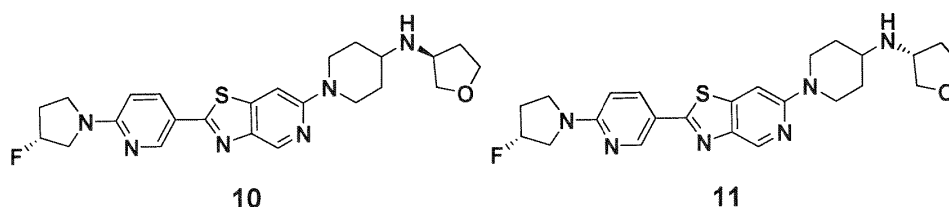
To a solution of title compound **R** (2 g, 5.97 mmol) and N-(oxetan-3-yl)piperidin-4-amine (1.12 g, 7.17 mmol) in 1,4-dioxane (20 mL) were added Pd₂(dba)₃ (547.03 mg, 597.37 μmol), RuPhos (557.51 mg, 1.19 mmol) and t-BuONa (2 M in THF, 8.96 mL). The mixture was degassed and purged with N₂ three times and stirred at 110 °C for 1 h under N₂ atmosphere. The reaction mixture was quenched by the addition saturated EDTA (50 mL) at 25 °C, stirred for 1 h, and then extracted with DCM (3 x 30 mL). The combined organic layers were washed with brine (2 x 20 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by preparative HPLC (column: Phenomenex Luna C18 (250 x 70mm, 15 μm); mobile phase: [A: H₂O (0.1% TFA); B: CH₃CN]; gradient: 1% to 30% B over 20.0 min). Fractions containing the desired compound were collected, the pH adjusted to pH = 8 by saturated aqueous NaHCO₃ and extracted with DCM (3 x 30 mL). The combined organic layers were washed with brine (2 x 20 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to afford compound **9** (0.4 g, 14% yield) as a yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ 8.74 (s, 1H), 8.71 (s, 1H), 8.09 (d, J = 8.8 Hz, 1H), 7.45 (s, 1H), 6.66 (d, J = 8.8 Hz, 1H), 5.54 - 5.41 (m, 1H), 4.68 - 4.58 (m, 2H), 4.35 - 4.27 (m, 2H), 4.26 - 4.17 (m, 2H), 4.03 - 3.94 (m, 1H), 3.89 - 3.60 (m, 3H), 3.57 - 3.46 (m, 1H), 2.97 - 2.87 (m, 2H), 2.65 - 2.56 (m, 1H), 2.46 - 2.38 (m, 1H), 2.35 - 2.11 (m, 2H), 1.79 - 1.68 (m, 2H), 1.30 - 1.16 (m, 2H)

¹H NMR (400 MHz, CDCl₃) δ 8.86 (s, 1H), 8.74 (s, 1H), 8.15 (d, J = 8.8 Hz, 1H), 7.05 (s, 1H), 6.48 (d, J = 8.8 Hz, 1H), 5.48 - 5.35 (m, 1H), 4.90 - 4.82 (m, 2H), 4.51 - 4.43 (m, 2H), 4.33 - 4.24 (m, 2H), 4.19 - 4.10 (m, 1H), 4.02 - 3.88 (m, 1H), 3.83 - 3.62 (m, 3H), 3.02 - 2.91 (m, 2H), 2.80 - 2.69 (m, 1H), 2.52 - 2.39 (m, 1H), 2.31 - 2.08 (m, 1H), 1.95 - 1.83 (m, 2H), 1.51 - 1.40 (m, 2H)

LCMS: 455.2 [M + H]⁺

Examples 10 and 11 Synthesis of 1-(2-(6-((R)-3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-N-((R*)-tetrahydrofuran-3-yl)piperidin-4-amine Compounds 10 and 11



5 To a mixture of (R)-6-chloro-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridine **R** (1 g, 2.99 mmol) and N-(tetrahydrofuran-3-yl)piperidin-4-amine (881.43 mg, 3.88 mmol) in dioxane (10 mL) was added Pd₂(dba)₃ (273.51 mg, 298.69 μmol), Xphos (427.17 mg, 896.06 μmol) and t-BuONa (2 M in THF, 2.99 mL). The mixture was degassed and purged with N₂ 3 times. The mixture was stirred at 110 °C for 2 h. The mixture was cooled to 25 °C, and then poured into saturated EDTA (50 mL) and stirred for 1 h. The aqueous phase was extracted with DCM (30 mL). The combined organic phases were washed with brine (30 mL), dried with anhydrous Na₂SO₄, filtered, and concentrated in vacuum. The residue was purified by prep-HPLC (column: 3_Phenomenex Luna C18 75mm x 30mm 3μm; mobile phase: [H₂O (0.04% HCl)- CH₃CN] gradient: 1%-26% B over 8.0 min) to afford a yellow solid (205.52 mg, 14% yield).

15 ¹H NMR (400 MHz, DMSO-d₆) δ = 9.80 - 9.35 (m, 2H), 8.89 - 8.76 (m, 1H), 8.68 - 8.55 (m, 1H), 8.38 - 8.20 (m, 1H), 7.89 - 7.69 (m, 1H), 7.08 - 6.88 (m, 1H), 5.59 - 5.50 (m, 1H), 4.47 - 4.38 (m, 2H), 4.01 - 3.53 (m, 9H), 3.47 - 3.29 (m, 1H), 3.16 - 2.94 (m, 2H), 2.44 - 1.99 (m, 6H), 1.83 - 1.59 (m, 2H)

LCMS: 469.2 [M + H]⁺

The mixture was separated by SFC (condition: DAICEL CHIRALCEL OJ (250mm*30mm,10μm); mobile phase: [CO₂-IPA(0.1%NH₃H₂O)];B%:60%, isocratic elution mode). Fractions containing the desired compounds were collected, then was salify by HCl (1 M, 100 μL), followed by lyophilization to afford compound **10** (40.63 mg, 2.7% yield) as a yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ = 9.68 - 9.42 (m, 2H), 8.81 (s, 1H), 8.64 (s, 1H), 8.28 (d, J= 8.8 Hz, 1H), 7.80 (s, 1H), 6.95 (d, J= 9.2 Hz, 1H), 5.65 - 5.43 (m, 1H), 4.51 - 4.36 (m, 2H), 4.07 - 3.55 (m, 9H), 3.49 - 3.33 (m, 1H), 3.12 - 2.96 (m, 2H), 2.42 - 1.99 (m, 6H), 1.77 - 1.62 (m, 2H)

¹H NMR (400 MHz, D₂O) δ = 8.68 (s, 1H), 8.53 (s, 1H), 8.44 (d, J= 9.6 Hz, 1H), 7.86 (s, 1H), 7.20 (d, J= 8.4 Hz, 1H), 5.69 - 5.46 (m, 1H), 4.28 - 3.61 (m, 12H), 3.43 - 3.29 (m, 2H), 2.68 - 2.20 (m, 5H), 2.15 - 2.01 (m, 1H), 1.90 - 1.69 (m, 2H)

LCMS: 469.2 [M + H]⁺

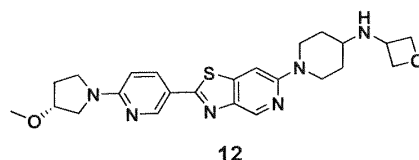
5 and compound **11** as a yellow solid (40.47 mg, 2.7% yield).

¹H NMR (400 MHz, DMSO-d₆) δ = 9.64 - 9.37 (m, 2H), 8.81 (s, 1H), 8.65 (s, 1H), 8.27 (d, J= 8.8 Hz, 1H), 7.77 (s, 1H), 6.92 (d, J= 9.2 Hz, 1H), 5.63 - 5.42 (m, 1H), 4.49 - 4.37 (m, 2H), 4.06 - 3.55 (m, 9H), 3.49 - 3.33 (m, 1H), 3.14 - 2.98 (m, 2H), 2.41 - 2.01 (m, 6H), 1.79 - 1.59 (m, 2H)

¹H NMR (400 MHz, D₂O) δ = 8.69 (s, 1H), 8.54 (s, 1H), 8.44 (d, J= 9.6 Hz, 1H), 7.85 (s, 1H), 7.20 (d, J= 9.2 Hz, 1H), 5.68 - 5.48 (m, 1H), 4.32 - 3.72 (m, 12H), 3.42 - 3.26 (m, 2H), 2.63 - 2.22 (m, 5H), 2.14 - 2.00 (m, 1H), 1.88 - 1.71 (m, 2H)

LCMS: 469.2 [M + H]⁺

Example 12 Synthesis of (R)-1-(2-(6-(3-methoxypyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-N-(oxetan-3-yl)piperidin-4-amine Compound 12



Step-1: Synthesis of (R)-2-(3-methoxypyrrolidin-1-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (V)

To a mixture of (R)-3-methoxypyrrolidine (1.36 g, 9.86 mmol, HCl) and 2-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine **U** (2 g, 8.97 mmol) in MeCN (20 mL) was added TEA (2.50 mL, 17.93 mmol). The mixture was degassed and purged with N₂ 3 times. It was stirred at 80 °C for 16 h under N₂ atmosphere. The mixture was cooled to 25 °C, then poured into ice-water (w/w = 1/1) (60 mL). The aqueous phase was extracted with DCM (20 mL*3). The combined organic phases were washed with brine (20 mL*3), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The crude product was triturated with petroleum ether (20 mL) at 25 °C for 16 h to afford the title compound **V** (1.3 g, 48% yield) as a yellow solid.

¹H NMR: (400 MHz, CDCl₃) δ = 8.51 (s, 1H), 7.79 (d, J = 8.4 Hz, 1H), 6.43 (d, J = 8.8 Hz, 1H), 4.15 - 4.04 (m, 1H), 3.70 - 3.54 (m, 4H), 3.37 (s, 3H), 2.24 - 2.05 (m, 2H), 1.32 (s, 12H)

LCMS: 305.3 [M + H]⁺

5 **Step-2: Synthesis of (R)-6-chloro-2-(6-(3-methoxypyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridine (W)**

To a mixture of compound **V** (1.3 g, 4.27 mmol) and 2-bromo-6-chlorothiazolo[4,5-c]pyridine (1 g, 4.01 mmol) in dioxane (10 mL) and H₂O (1 mL) was added Pd(dppf)Cl₂.DCM (327.29 mg, 400.78 μmol), K₂CO₃ (1.11 g, 8.02 mmol). The mixture was degassed and purged with N₂ 3 times.
10 The mixture was stirred at 75 °C for 2 h under N₂ atmosphere. The mixture was cooled to 25 °C, then poured into saturated EDTA (60 mL) and stirred for 1 h. The aqueous phase was extracted with DCM (20 mL*3). The combined organic phases were washed with brine (20 mL*3), dried with anhydrous Na₂SO₄, filtered, and concentrated in vacuum. The crude product was purified by silica gel chromatography (petroleum ether / ethyl acetate=1/1) to afford compound **5** (0.5 g, 48% yield) as
15 a yellow solid.

¹H NMR: (400 MHz, CDCl₃) δ = 8.99 (s, 1H), 8.80 (s, 1H), 8.13 (d, J = 9.2 Hz, 1H), 7.80 (s, 1H), 6.47 (d, J = 8.8 Hz, 1H), 4.19 - 4.13 (m, 1H), 3.76 - 3.57 (m, 4H), 3.40 (s, 3H), 2.32 - 2.08 (m, 2H)

LCMS: 347.1 [M + H]⁺

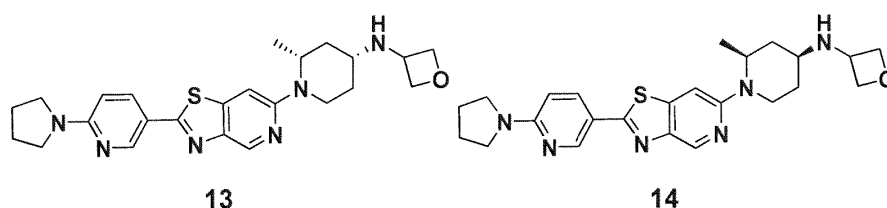
20 **Step-3: (R)-1-(2-(6-(3-methoxypyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-N-(oxetan-3-yl)piperidin-4-amine (compound 12)**

To a mixture of compound **W** (0.45 g, 1.30 mmol) and N-(oxetan-3-yl)piperidin-4-amine (304.04 mg, 1.95 mmol) in dioxane (5 mL) was added Pd₂(dba)₃ (74.60 mg, 129.75 μmol), RuPhos (181.63 mg, 389.24 μmol) t-BuONa (2 M in THF, 1.30 mL). The mixture was degassed and purged with N₂ 3
25 times. The mixture was stirred at 110 °C for 16 h under N₂ atmosphere, then poured into saturated EDTA (60 mL) and stirred for 1 h. The aqueous phase was extracted with DCM (20 mL*3). The combined organic phases were washed with brine (20 mL*3), dried with anhydrous Na₂SO₄, filtered, and concentrated in vacuum. The crude product was purified by silica gel chromatography (DCM/ MeOH= 10/ 1) to afford compound **12** (0.2 g, 30% yield) as a yellow solid.

¹H NMR: (400 MHz, DMSO-d₆) δ = 8.73 (s, 1H), 8.68 (s, 1H), 8.06 (d, J = 8.8 Hz 1H), 7.45 (s, 1H), 6.61 (d, J = 8.8 Hz, 1H), 4.70 - 4.56 (m, 2H), 4.39 - 4.28 (m, 2H), 4.27 - 4.18 (m, 2H), 4.14 - 4.07 (m, 1H), 4.05 - 3.92 (m, 1H), 3.64 - 3.53 (m, 3H), 3.48 - 3.43 (m, 1H), 3.28 (s, 3H), 2.97 - 2.85 (m, 2H), 2.63 - 2.60 (m, 1H), 2.14 - 2.05 (m, 2H), 1.78 - 1.69 (m, 2H), 1.27 - 1.17 (m, 2H).

5 LCMS: 467.2 [M + H]⁺

Examples 13 and 14 Synthesis of (2R*,4R*)-1-(2-(6-((R)-3-fluoropyrrolidin-1-yl)pyridin-3-yl)benzo[d]thiazol-6-yl)-2-methyl-N-(oxetan-3-yl)piperidin-4-amine Compounds 13 and 14



Step-1: Synthesis of 6-chloro-2-(6-(pyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridine (X)

10 To a solution of 2-bromo-6-chlorothiazolo[4,5-c]pyridine **A** (5 g, 20.04 mmol), (6-(pyrrolidin-1-yl)pyridin-3-yl)boronic acid (3.85 g, 20.04 mmol) in dioxane (50 mL), and H₂O (15 mL) was added Pd(dppf)Cl₂ (1.47 g, 2.00 mmol) and K₂CO₃ (5.54 g, 40.08 mmol). The mixture was degassed and purged with N₂ 3 times. The mixture was stirred at 75 °C for 2 h. The mixture was cooled to 25 °C, then poured into saturated EDTA (50 mL) and stirred for 1 h. The aqueous phase was extracted with
 15 DCM (30 mL*3). The combined organic phases were washed with brine (30 mL*3), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by silica gel chromatography (DCM : MeOH = 1: 1) to afford compound **X** (3 g, 47% yield) as a yellow solid.

¹H NMR (400 MHz, CDCl₃) δ = 8.97 (s, 1H), 8.79 (s, 1H), 8.11 (d, J = 9.2 Hz, 1H), 7.79 (s, 1H), 6.45 (d, J = 8.8 Hz, 1H), 3.70 - 3.41 (m, 4H), 2.13 - 2.03 (m, 4H)

20 LCMS: 317.1 [M + H]⁺

Step-2: Synthesis of tert-butyl (2-methyl-1-(2-(6-(pyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)piperidin-4-yl)carbamate (Y)

A mixture of compound **X** (1.2 g, 3.79 mmol), tert-butyl (2-methylpiperidin-4-yl)carbamate (730.56 mg, 3.41 mmol), Pd₂(dba)₃ (485.59 mg, 530.29 μmol), RuPhos (512.58 mg, 1.10 mmol) and t-BuONa

(2 M, 3.79 mL) in dioxane (10 mL) was degassed and purged with N₂ 3 times, and then the mixture was stirred at 110 °C for 1 h under a N₂ atmosphere. The mixture was cooled to 25 °C, then poured into saturated EDTA (20 mL) and stirred for 1 h. The aqueous phase was extracted with DCM (30 mL*3). The combined organic phases were washed with brine (30 mL*3), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by silica gel chromatography (DCM: MeOH= 30: 1) to afford the title compound **Y** (800 mg, 42.70% yield) as a yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ = 8.78 - 8.72 (m, 1H), 8.71 - 8.65 (m, 1H), 8.08 - 8.01 (m, 1H), 7.39 - 7.37 (m, 1H), 6.66 - 6.63 (m, 1H), 4.43 - 4.37 (m, 1H), 4.14 - 4.06 (m, 1H), 3.54 - 3.43 (m, 6H), 3.19 - 3.15 (m, 1H), 2.01 - 1.93 (m, 4H), 1.39 (s, 9H), 1.15 - 1.05 (m, 4H), 0.95 - 0.93 (m, 3H)

LCMS: 495.3 [M + H]⁺

Step-3: Synthesis of 2-methyl-1-(2-(6-(pyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)piperidin-4-amine (Z)

A solution of the title compound **Y** (750 mg, 1.62 mmol) in HCl / EtOAc (4 M, 8.00 mL) was stirred at 25 °C for 1 h. The reaction mixture was concentrated in vacuum to afford title compound **Z** (550 mg, 86.20% yield) as a yellow solid

¹H NMR (400 MHz, CDCl₃) δ = 8.84 (s, 1H), 8.76 - 8.65 (m, 1H), 8.18 - 8.05 (m, 1H), 6.99 (s, 1H), 6.44 (d, J = 8.8 Hz, 1H), 4.05 - 3.92 (m, 1H), 3.72 - 3.61 (m, 1H), 3.62 (s, 4H), 3.47 - 3.37 (m, 1H), 3.25 - 2.97 (m, 2H), 2.89 - 2.68 (m, 1H), 2.06 (s, 4H), 1.64 - 1.50 (m, 2H), 1.26 - 1.17 (m, 5H)

LCMS: 395.2 [M + H]⁺

Step-4: Synthesis of (2S,4S)-2-methyl-N-(oxetan-3-yl)-1-(2-(6-(pyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)piperidin-4-amine (13)

To a mixture of the title compound **Z** (500 mg, 1.27 mmol) and oxetan-3-one (136.99 mg, 1.90 mmol) in DCM (5 mL) was added NaBH(OAc)₃ (1.61 g, 7.60 mmol) and AcOH (435.30 μL, 7.60 mmol). The mixture was stirred at 25 °C for 4 h. The reaction mixture was poured into saturated NaHCO₃ (60 mL). The aqueous phase was extracted with DCM (30 mL*3). The combined organic phases were washed with brine (20 mL*2), dried with anhydrous Na₂SO₄, filtered, and concentrated in vacuum. The crude product was purified by prep-HPLC column: Phenomenex luna C18 100mm*40mm*3 μm;

mobile phase: [H₂O(0.2% FA) - CH₃CN] gradient: 1%-17% B over 8.0 min. Fractions containing the desired compound were collected, followed by lyophilization to afford a yellow solid (90 mg, 14% yield).

¹H NMR (400 MHz, DMSO-d₆) δ = 8.68 (s, 1H), 8.67 (s, 1H), 8.03 (d, J= 8.8 Hz, 1H), 7.33 (s, 1H),
5 6.58 (d, J = 8.8 Hz, 1H), 4.72 - 4.58 (m, 2H), 4.43 - 4.31 (m, 2H), 4.31 - 4.21 (m, 1H), 4.04 - 3.82 (m,
2H), 3.47 (s, 4H), 3.32 - 3.21 (m, 1H), 2.78 - 2.70 (m, 1H), 1.97 (s, 4H), 1.87 - 1.65 (m, 2H), 1.59 -
1.42 (m, 2H), 1.16 - 1.23 (m, 3H)

¹H NMR (400 MHz, MeOD) δ = 8.75 (s, 1H), 8.67 (s, 1H), 8.09 (d, J= 9.2 Hz, 1H), 7.38 (s, 1H), 6.62
10 (d, J = 9.2 Hz, 1H), 4.84 - 4.78 (m, 2H), 4.60 - 4.46 (m, 2H), 4.19 - 4.05 (m, 1H), 3.91 - 3.75 (m, 1H),
3.60 - 3.47 (m, 5H), 3.47 - 3.34 (m, 1H), 2.79 - 2.64 (m, 1H), 2.14 - 2.03 (m, 4H), 2.02 - 1.92 (m, 2H),
1.20 - 1.16 (m, 3H), 0.91 - 0.85 (m, 2H)

LCMS: 451.4 [M + H]⁺

The product was purified by SFC (condition: ID_EtOH_ACN_IPAm_50_4_35). Fractions containing
15 the desired compound were collected, followed by lyophilization to afford compound **13** (15 mg, 2.5%
yield) as a yellow solid.

¹H (400 MHz, DMSO-d₆) δ = 8.72 (s, 1H), 8.67 (s, 1H), 8.03 (d, J= 9.2 Hz, 1H), 7.33 (s, 1H), 6.58 (d,
J = 9.2 Hz, 1H), 4.71 - 4.58 (m, 2H), 4.42 - 4.32 (m, 2H), 4.31 - 4.19 (m, 1H), 4.05 - 3.82 (m, 2H),
3.58 - 3.47 (m, 4H), 3.30 - 3.20 (m, 1H), 2.79 - 2.70 (m, 1H), 1.97 (m, 4H), 1.88 - 1.66 (m, 2H), 1.64
20 - 1.44 (m, 2H), 1.24 - 1.22 (m, 3H)

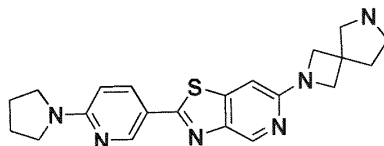
LCMS: 451.2 [M + H]⁺

and compound **14** (9mg, 1.4% yield) as a yellow solid

¹H (400 MHz, DMSO-d₆) δ = 8.72 (s, 1H), 8.67 (s, 1H), 8.04 (d, J= 8.8 Hz, 1H), 7.33 (s, 1H), 6.58 (d,
J = 9.2 Hz, 1H), 4.71 - 4.59 (m, 2H), 4.41 - 4.32 (m, 2H), 4.31 - 4.20 (m, 1H), 4.04 - 3.81 (m, 2H),
25 3.49 - 3.47 (m, 4H), 3.29 - 3.18 (m, 1H), 2.80 - 2.69 (m, 1H), 2.07 - 1.90 (m, 4H), 1.87 - 1.65 (m, 2H),
1.61 - 1.43 (m, 2H), 1.25 - 1.20 (m, 3H)

LCMS: 451.2 [M + H]⁺

Example 15 Synthesis of 2-(6-(pyrrolidin-1-yl)pyridin-3-yl)-6-(2,6-diazaspiro[3.4]octan-2-yl)thiazolo[4,5-c]pyridine Compound 15



15

Step-1: Synthesis of tert-butyl 2-(2-(6-(pyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-2,6-diazaspiro[3.4]octane-6-carboxylate (AA)

A mixture of compound **X** (500 mg, 1.58 mmol), tert-butyl 2,6-diazaspiro[3.4]octane-6-carboxylate (431.84 mg, 1.74 mmol, HCl), t-BuONa (2 M, 1.58 mL), RuPhos PdG₃ (132.00 mg, 157.82 μmol) in dioxane (5 mL) was degassed and purged with N₂ 3 times, and then the mixture was stirred at 110 °C for 16h under N₂ atmosphere. The mixture was cooled to 25 °C, then poured into saturated EDTA (20 mL) and stirred for 1 h. The aqueous phase was extracted with DCM (50 mL*3). The combined organic phases were washed with brine (50 mL*3), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by column chromatography (PE/ EA= 1/0 to 0/1). Compound **AA** (400 mg, 50.42% yield) was obtained as a white solid.

¹H NMR (400 MHz, CDCl₃) δ = 8.82 (s, 1H), 8.71 (s, 1H), 8.12 (d, J= 8.8 Hz, 1H), 6.69 (s, 1H), 6.46 (d, J= 9.2 Hz, 1H), 4.11 - 3.93 (m, 4H), 3.62 - 3.39 (m, 8H), 2.21 - 2.02 (m, 6H), 1.50 - 1.46 (m, 9H).
LCMS: 493.4 [M + H]⁺

Step-2: Synthesis of 2-(6-(pyrrolidin-1-yl)pyridin-3-yl)-6-(2,6-diazaspiro[3.4]octan-2-yl)thiazolo[4,5-c]pyridine (compound 15)

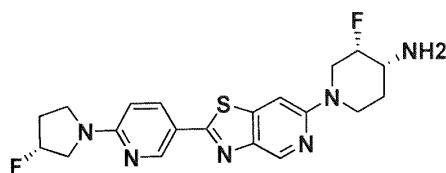
A mixture of compound **AA** (400 mg, 811.96 μmol) and TFA (1.50 mL, 20.19 mmol) in DCM (2.5 mL) was stirred at 25 °C for 1h under N₂ atmosphere. The residue was poured into saturated Na₂CO₃ (50 mL). The aqueous phase was extracted with DCM (50 mL*3). The combined organic phases were washed with brine (50 mL*3), dried with anhydrous Na₂SO₄, filtered, and concentrated in vacuum. The residue was purified by prep-HPLC (basic condition; column: Waters Xbridge Prep OBD

C18 150*40mm*10 μ m; mobile phase: [H₂O (0.05% NH₃H₂O + 10mM NH₄HCO₃)-ACN]; gradient: 20%-60% B over 8.0 min). Compound **15** (67.81 mg, 21.28% yield) was obtained as a yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ = 8.76 - 8.63 (m, 2H), 8.04 (d, J= 9.2 Hz, 1H), 7.05 - 6.95 (m, 1H), 6.59 (d, J= 9.2 Hz, 1H), 3.98 - 3.81 (m, 4H), 3.50 - 3.44 (m, 5H), 3.04 - 2.75 (m, 3H), 2.11 - 1.90 (m, 6H).

LCMS: 393.2 [M + H]⁺

Example 16 Synthesis of (3S,4R)-3-fluoro-1-(2-(6-((R)-3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)piperidin-4-amine Compound 16



16

Step-1: Synthesis of (3S,4R)-3-fluoro-1-(2-(6-((R)-3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)piperidin-4-amine (compound 16)

A mixture of (R)-6-chloro-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridine **R** (800 mg, 2.39 mmol), tert-butyl ((3S,4R)-3-fluoropiperidin-4-yl)carbamate (608.67 mg, 2.39 mmol, HCl), Pd₂(dba)₃ (218.81 mg, 238.95 μ mol), RuPhos (223.00 mg, 477.90 μ mol) and t-BuONa (2 M, 2.39 mL) in dioxane (10 mL) was degassed and purged with N₂ 3 times, and then the mixture was stirred at 110 °C for 1h under N₂ atmosphere. The mixture was cooled to 25 °C, then poured into saturated EDTA (20 mL) and stirred for 1 h, and then extracted with DCM (10mL *3). The combined organic layers were washed with brine (20mL *2), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Phenomenex luna C18 (250*70mm, 15 μ m); mobile phase: [H₂O(0.04%HCl)-ACN]; gradient: 5%-35% B over 20.0 min). Compound **16** (106.11 mg, 9.8% yield, HCl) was obtained as a yellow solid.

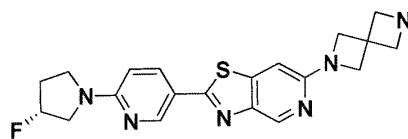
¹H NMR (400 MHz, DMSO-d₆) δ = 8.80 (s, 1H), 8.66 - 8.55 (m, 4H), 8.28 (d, J= 8.8 Hz, 1H), 7.69 (s, 1H), 6.94 (d, J= 9.2 Hz, 1H), 5.60 - 5.47 (m, 1H), 5.20 - 5.16 (m, 1H), 4.85 - 4.76 (m, 1H), 4.48 - 4.39

(m, 1H), 4.00 - 3.53 (m, 5H), 3.41 - 3.23 (m, 1H), 3.15 - 3.03 (m, 1H), 2.40 - 2.15 (m, 2H), 2.00 - 1.78 (m, 2H)

¹H NMR (400 MHz, D₂O) δ = 8.67 (s, 1H), 8.47 (s, 1H), 8.38 (d, J= 9.6 Hz, 1H), 7.79 (s, 1H), 7.14 (d, J= 9.6 Hz, 1H), 5.66 - 5.47 (m, 1H), 5.30 - 5.12 (m, 1H), 4.64 - 4.52 (m, 1H), 4.31 - 4.21 (m, 1H), 4.04 - 3.73 (m, 5H), 3.64 - 3.47 (m, 1H), 3.41 - 3.30 (m, 1H), 2.62 - 2.01 (m, 4H)

LCMS: 417.1 [M + H]⁺

Example 17 Synthesis of (R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-6-(2,6-diazaspiro[3.3]heptan-2-yl)thiazolo[4,5-c]pyridine Compound 17



17

Step-1: Synthesis of (R)-tert-butyl 6-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-2,6-diazaspiro[3.3]heptane-2-carboxylate (AB)

To a solution of (R)-6-chloro-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridine **R** (700 mg, 2.09 mmol), tert-butyl 2,6-diazaspiro[3.3]heptane-2-carboxylate hemioxalate (455.98 mg, 2.30 mmol) in dioxane (10 mL) was added Ruphos PdG₄ (177.80 mg, 209.08 μmol) and t-BuONa (2 M in THF, 2.09 mL). The mixture was degassed and purged three times with N₂. The mixture was stirred at 110 °C for 16 h. The mixture was cooled to 25 °C, then poured into sat. EDTA (30 mL) and stirred for 1 h. The aqueous phase was extracted with DCM (20 mL*3). The combined organic phases were washed with brine (30 mL*3), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by silica gel chromatography (PE/ EA=1/ 0 to 0/ 1). To afford compound **AB** (350 mg, 33.71% yield) as a yellow solid.

¹H NMR (400 MHz, CDCl₃) δ = 8.80 (s, 1H), 8.72 (s, 1H), 8.17 (d, J= 7.6 Hz, 1H), 6.72 (s, 1H), 6.52 (d, J= 8.4 Hz, 1H), 5.52 - 5.34 (m, 1H), 4.37 - 3.64 (m, 12H), 2.55 - 2.12 (m, 2H), 1.49 - 1.44 (m, 9H)

LCMS: 497.2 [M + H]⁺

Step-2: Synthesis of (R)-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)-6-(2,6-diazaspiro[3.3]heptan-2-yl)thiazolo[4,5-c]pyridine (compound 17)

To a solution of compound **AB** (350 mg, 704.79 μ mol) in DCM (3 mL) was added TFA (1 mL, 13.46 mmol). The mixture was stirred at 25 °C for 1h. The residue was poured into sat. Na_2CO_3 (50 mL).

5 The aqueous phase was extracted with DCM (50 mL*3). The combined organic phases were washed with brine (50 mL*3), dried with anhydrous Na_2SO_4 , filtered, and concentrated in vacuum. The residue was purified by prep-HPLC (basic condition; column: Waters Xbridge Prep OBD C18 150*40mm*10 μ m; mobile phase: [H_2O (0.05% $\text{NH}_3\text{H}_2\text{O}$ +10mM NH_4HCO_3)-ACN]; gradient: 20%-50% B over 8.0 min). Fractions containing the desired compound were collected, followed by lyophilization

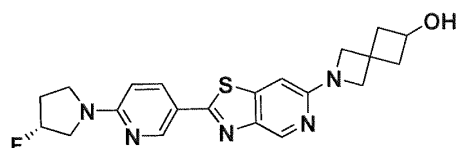
10 to afford compound **17** (60.64 mg, 21.70% yield) as a yellow solid.

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ = 8.82 - 8.65 (m, 2H), 8.08 (d, J = 8.8 Hz, 1H), 7.00 (s, 1H), 6.6 (d, J = 9.2 Hz 1H), 5.65 - 5.37 (m, 1H), 4.04 (s, 4H), 3.91 - 3.68 (m, 3H), 3.62 (s, 4H), 3.55 - 3.47 (m, 1H), 2.39 - 2.08 (m, 2H)

LCMS: 397.1 $[\text{M} + \text{H}]^+$

15

Example 18 Synthesis of (R)-2-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-2-azaspiro[3.3]heptan-6-ol Compound 18



18

Step-1: Synthesis of (R)-2-(2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridin-6-yl)-2-azaspiro[3.3]heptan-6-ol (compound 18)

A mixture of (R)-6-chloro-2-(6-(3-fluoropyrrolidin-1-yl)pyridin-3-yl)thiazolo[4,5-c]pyridine **R** (500 mg, 1.49 mmol), 2-azaspiro[3.3]heptan-6-ol (268.13 mg, 1.79 mmol, HCl), $\text{Pd}_2(\text{dba})_3$ (136.76 mg, 149.34 μ mol), RuPhos (139.38 mg, 298.69 μ mol) and t-BuONa (2 M, 1.12 mL) in dioxane (1 mL) was degassed and purged with N_2 3 times, and then the mixture was stirred at 110 °C for 1 h under N_2

25 atmosphere. The mixture was cooled to 25 °C, then poured into sat. EDTA (20 mL) and stirred for 1 h. The aqueous phase was extracted with DCM (20 mL*3). The combined organic phases were

washed with brine (20 mL*3), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum. The crude product was purified by prep-HPLC column: Phenomenex luna C18 100*40mm*3 µm; mobile phase: [H₂O (0.2% FA)-ACN]; gradient: 1%-30% B over 8.0 min. compound **18** (76 mg, 12% yield) was obtained as a yellow solid.

- 5 ¹H NMR (400 MHz, DMSO-d₆) δ = 8.71 - 8.69 (m, 2H), 8.08 (d, J= 8.8 Hz, 1H), 6.95 (s, 1H), 8.65 (d, J= 8.8 Hz, 1H), 5.60 - 5.35 (m, 1H), 5.08 (s, 1H), 4.09 - 3.97 (m, 1H), 3.95 (s, 2H), 3.90 (s, 2H), 3.87 - 3.73 (m, 3H), 3.52 - 3.49 (m, 1H), 2.45 - 2.42 (m, 2H), 2.37 - 2.10 (m, 2H), 2.08 - 1.97 (m, 2H)

LCMS: 412.2 [M + H]⁺

10 BIOLOGICAL ASSAY DESCRIPTION AND CORRESPONDING RESULTS

Seeded aggregation assay monitored by Thioflavin T for the evaluation of effects on alpha-synuclein aggregation kinetics

- Effects of compounds of the invention on alpha-synuclein aggregation kinetics was evaluated in an
15 *in vitro* assay monitored by Thioflavin T (ThT). Thioflavin T is a rotatory type fluorophore that binds to beta-sheet-rich structures, such as those found in alpha-synuclein fibrils. Upon binding, the emission spectrum of the dye shifts to red and the fluorescence intensity is increased. The assay is based on the seeded aggregation principle, where the alpha-synuclein aggregation process is catalyzed by the presence of short, preformed alpha-synuclein fibrils, referred to as seeds. This
20 approach improves the reproducibility of the aggregation kinetics and accelerates the kinetics *in vitro* (Buell et. al., 2014).

Method

- Alpha-synuclein protein (17.5 µM) was mixed with alpha-synuclein seeds DMSO, ThT and optionally
25 compounds of the present invention at increasing concentrations. The alpha-synuclein seeds consist of pre-formed, sonicated fibrils (2.5 µg/mL). The aggregation reactions were setup into 96-well plates, in three replicates per compound per dose. The ThT signal was then monitored over 18 hours using the assay parameters shown in Table 1.

30

Table 1: Seeded aggregation monitored by Thioflavin T assay parameters

Parameter	Value
Volume per well	70 μ L
Buffer composition	50 mM Tris 150 mM NaCl pH 7.4 at 37 °C
Percent DMSO	3 %
Temperature	37 °C
Measurement interval	10 min
Kinetic duration	18 h
Excitation (bandwidth)	440 nm (9 nm)
Emission (bandwidth)	485 nm (20 nm)

Data processing

For each well, the baseline value was determined by the value obtained at t_0 of the experiment in this well. The baseline subtraction was therefore computed as $t-t_0$ for each timepoint (t). The baseline-subtracted curves were fitted using a four-parameter or five-parameter fitting method.

Maximum signal inhibition determination

The maximum signal (S_{max}) was calculated for each replicate and each dose. The normalized maximum signal inhibition S_{inhib_norm} is expressed in percentage of signal reduction compared to the average maximum signal of the negative control (normalization), using the following formula, with C = compound concentration:

$$S_{inhib_norm}(C) = - [S_{max}(C) - S_{max}(\text{negative control})] / S_{max}(\text{negative control})$$

The logarithmic value of the concentration was computed, and the data were fitted using a non-linear four-parameter method, with the constrain: highest value <100.

Lag phase delay determination

The lag phase ($T_{1/2}$) was determined for each baseline-subtracted triplicate-averaged curve as follows:

- $T_{1/2}(C) = t(S_{max}/2)$
- If $S_{max}(\text{compound}) < 10\%$ of $S_{max}(\text{negative control})$: $T_{1/2} = 20$ h

with C = compound concentration

The normalized lag phase delays were then computed using the following formula, with C = compound concentration:

$$T_{1/2\text{delay_norm}}(C) = [T_{1/2}(C) - T_{1/2}(\text{negative control})] / [20 - T_{1/2}(\text{negative control})]$$

- 5 Then, the logarithmic value of the concentration was computed, and the data were fitted using a non-linear four- or five-parameter method, with the constrain: highest value <100.001. Results are shown in Table 2 below:

Table 2 Compound effects on aggregation kinetics monitored by ThT

Compound	ThT signal reduction, IC ₅₀ (μM)	ThT signal reduction at 100 μM (%)	Lag phase delay (μM), IC ₅₀	Lag phase delay at 100 μM (%)
Compound 1	15	98.1 ± 0.8	40	100
Compound 2	19.6	87.9 ± 0.8	79.7	-31 ± 50*
Compound 3	16.6	99.2 ± 0.4	80.4	100
Compound 4	< 5	101.6 ± 1.6	15.07	100
Compound 5	< 5	88.1 ± 7.9	47.56	67 ± 33
Compound 6	7.9	90 ± 0.7	80.6	65 ± 49
Compound 7	11.7	97.1 ± 0.9	22.8	100
Compound 8	7.75	85.8 ± 1.7	not calculated	-20.3 ± 4.2*
Compound 9	<5	77.8 ± 4.8	not calculated	-22.4 ± 4.8*
Compound 10	6.3	95.1 ± 2.4	22.3	100
Compound 11	6.2	95.6 ± 1.7	27.7	100
Compound 12	8.3	6.8 ± 0.1	40.8	100
Compound 13	< 5	98.3 ± 0.6	13.9	100
Compound 14	<5	98.4 ± 0.2	13.8	100
Compound 15	18.7	97.1 ± 0.3	60.6	100
Compound 16	17.4	75 ± 4.1	not calculated	-85.5 ± 5.5
Compound 17	38.1	90.4 ± 0.2	85.1	100
Compound 18	13.8	89.9 ± 0.2	90	100

10

*Negative values computed when $T_{1/2}$ at 100μM < $T_{1/2}$ of negative control according to above equation

As seen from Table 2, all tested compounds reduced the ThT signal at 100 μ M, with IC₅₀ values below 20 μ M. This data demonstrates that the compounds interact with the target and alter and/or prevent alpha-synuclein aggregation. This data thereby indicates good potential of the compounds for therapeutic use in diseases, disorders or abnormalities associated with aggregated alpha-synuclein. Moreover, some compounds are also capable of delaying the lag phase of the aggregation kinetics, further indicating that they interfere with the onset of the alpha-synuclein aggregation process at these concentrations.

Assessment of compound binding to recombinant alpha-synuclein by SPR

Binding specificity of test compounds toward aggregated versus monomeric alpha-synuclein was evaluated using surface plasmon resonance (SPR). Monomeric or recombinant aggregated alpha-synuclein was immobilized on a sensor chip surface by amine coupling. Test compounds of the present invention were flowed over the surface and the change of mass at the interface was measured by SPR.

Method

Alpha-synuclein monomer and aggregates were immobilized onto a CM3, CM4 or a CM5 chip by amine coupling: the flow cells were activated with EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) /NHS (N-hydroxysuccinimide). Alpha-synuclein monomers and aggregates diluted in 10 mM sodium acetate pH 4.0 were injected in separate flow cells. All flow cells were saturated with an injection of 1 M methanolamine-hydrochloric acid. Test compounds at 15 μ M or 150 nM (as indicated in Table 3) in TBS + P+ (Tris buffer saline + surfactant Polysorbate 20) 1.0 x and 3% DMSO were flowed over the immobilized alpha-synuclein monomers and aggregates (running buffer: 50 mM Tris, 300 mM NaCl, 0,05% P20 pH 7.4).

Data processing

Results were evaluated using the Biacore Insight Evaluation software. The analyte binding late responses are reported in response units (RU) after reference subtraction, blank correction, molecular weight adjustment and controls adjustment.

Table 3. Compounds SPR binding responses to monomeric and aggregated alpha-synuclein

Compound	Aggregate binding late response (RU)	Monomer binding late response (RU)
Compound 1	674.7	0.7
Compound 2	123.3	0.2
Compound 4	194.4	0.7
Compound 6*	12.2	0.9
Compound 9*	44.2	0.2

*Compound concentration: 150nM

As can be seen from Table 3, the compounds of the invention show a strong binding preference for alpha-synuclein aggregates over monomers, as observed by the differences in analyte binding late responses. This data demonstrates the compounds' binding specificity towards aggregated forms of alpha-synuclein, and supports the good potential of the compounds for therapeutic use.

Assessment of compound binding to brain-derived alpha-synuclein by SPR

A similar SPR methodology was used to assess binding of compounds to alpha-synuclein aggregates extracted from brains of MSA and PD donors. Briefly, a monoclonal antibody targeting alpha-synuclein (clone 10D2) was immobilized on a sensor chip surface and brain-derived alpha-synuclein aggregates were flowed on the surface and captured via the antibody. Consequently, compounds were flowed on the chip's surface and a change of mass at the interface was measured, demonstrating binding of compounds to brain-derived aggregates.

Method

Alpha-synuclein aggregate-enriched fraction, or total brain homogenates, extracted from PD and MSA brain tissues as described previously (Spillantini et al., Proc Natl Acad Sci U S A, 1998 May 26; 95(11): 6469–6473). Alpha-synuclein antibody, clone 10D2 was immobilized onto a CM3 or CM5 chip by amine coupling: the flow cells were activated with EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) /NHS (N-hydroxysuccinimide) and antibody diluted in 10 mM sodium acetate pH 5.0 was flowed over the activated surface to achieve capture level of approximately 5'000 RU. All flow cells were saturated with an injection of 1 M ethanolamine-hydrochloric acid. Brain extracts from PD or MSA were flowed over the chip surface to achieve

immobilization levels of approximately 3'000 RU. Test compounds at 10 or 12.5 µM in PBS-P+ (Phosphate buffer saline + surfactant Polysorbate 20) 1.0 x and 3% DMSO were flowed over the captured alpha-synuclein brain-derived aggregates.

5 *Data processing*

Results were evaluated using the Biacore Insight Evaluation software. The analyte binding late responses are reported in response units (RU) after reference subtraction and blank correction.

Table 4. Compounds SPR binding responses to brain-derived aggregated alpha-synuclein

Compound	MSA brain-derived aggregate binding late response (RU)	PD brain-derived aggregate binding late response (RU)
Compound 1	**	***
Compound 3	***	**
Compound 5	**	**
Compound 9	**	**

10

*** RU ≥ 100, **5 ≤ RU < 50

As can be seen from Table 4, the compounds of the invention show binding to disease-relevant aggregated forms of alpha-synuclein derived from MSA or PD brains. This data demonstrates the compounds' target engagement towards disease-relevant proteoforms of alpha-synuclein, and further supports the good potential of the compounds for therapeutic use.

15

Primary neuron seeding assay for assessment of efficacy in prevention of alpha-synuclein aggregation

Potency of test compounds of the present invention in preventing alpha-synuclein aggregation was evaluated in a cellular model. In this model, rat primary neurons were exposed to the MSA-derived brain extracts, in the presence or absence of test compounds. The alpha-synuclein seeds present in the MSA-derived brain extracts trigger the formation of *de novo* aggregates by recruiting endogenous rat alpha-synuclein. Staining for alpha-synuclein phosphorylated at serine 129 (pS129) was used as a surrogate for *de novo* aggregate quantification. The reduction of alpha-synuclein pS129-positive

20

25

inclusions in the presence of compounds of the present invention was used to evaluate the compounds' potency.

Method

- 5 MSA insoluble fraction was extracted in 1% Triton-X-100-containing phosphate buffer supplemented with protease inhibitors, centrifuged at high speed, resuspended and recentrifuged. The resulting pellets containing the insoluble fraction were resuspended in PBS by sonication. 1 μ L of MSA total brain homogenate (prepared in phosphate buffer saline supplemented with protease inhibitors) or 1 μ L of MSA insoluble fraction was added to rat primary neurons plated in 96-well plates at day *in vitro* (DIV) 6, with or without test compounds. At DIV 15, the cells were fixed and stained for MAP2 and alpha-synuclein pS129 and imaged.

- This assay was used to evaluate the compound treatment effects when the compound was added to neurons either 2 hours before, or simultaneously with, or after the MSA insoluble fraction containing seeds at the following intervals: 2 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days or 8 days. When the compound was added to neurons after the seeds then the cell culture media was removed and substituted with conditioned cell culture media to remove any seeds that were not yet internalized by neurons.

20 Data processing

Alpha-synuclein pS129 and MAP2 channels were segmented and the total alpha-synuclein pS129 and MAP2 area per image was determined.

The aggregates-to-cell ratio was defined as the aggregate area per MAP2 area (sum over each well):

$$ratio = \frac{area\ aggregates}{area\ neurons}$$

25

The aggregates-to-cell ratio serves to determine the inhibition of *de novo* aggregate formation. The normalized inhibition was calculated for each compound at each dose using the following formula:

$$normalized\ inhibition = 1 - \frac{ratio_{compound}}{ratio_{DMSO}}$$

30

Table 5. Compounds potency in the primary neuron seeding assay

Compound	Inhibition of aggregation at 0.5 μM (%)	Inhibition of aggregation, IC ₅₀
Compound 1	69	**
Compound 2	61	**
Compound 3	65	***
Compound 4	88	***
Compound 5	43	**
Compound 6	59	**
Compound 9	73	***

*** IC₅₀ ≤ 0.1 μM, **0.1 < IC₅₀ ≤ 0.5 μM

5 As can be seen from Table 5, compounds of the invention show a strong potency in reducing the burden of intracellular alpha-synuclein aggregates with IC₅₀ in nanomolar range, demonstrating that the compounds are efficacious: 1) in a cellular environment, 2) in neuronal cells and 3) on brain-derived alpha-synuclein, further supporting the good potential of the compounds for therapeutic use in diseases, disorders or abnormalities associated with aggregated alpha-synuclein.

10

Table 6. Inhibition of aggregation in primary neurons treated with compounds 6 days after seeding

Compound	Inhibition of aggregation at 1 μM (%)
Compound 4	57.5
Compound 9	66

15 Results in Figure 1 and Table 6 show that compounds of the invention leads to significant inhibition of *de novo* aggregate formation and reduce alpha-synuclein aggregate burden even when treatment starts several days after the seed uptake and internalization, demonstrating that the compounds are inhibiting aggregation intracellularly.

Primary neuron seed amplification assay for assessment of efficacy in prevention of alpha-synuclein aggregation

Potency of test compounds of the present invention in preventing alpha-synuclein aggregation was evaluated in a seed amplification cellular model. In this model MSA brain-derived seeds were passaged twice in primary neuron cultures demonstrating that the alpha-synuclein seeds present in the MSA-derived brain extracts maintain their seeding activity after being propagated within neurons.

Method

10 μ L of MSA insoluble fraction (prepared as described above) was added to rat primary neurons plated in 6-well plates at DIV6 and collected at DIV25 in lysis buffer containing 1% Triton, as well as protease and phosphatase inhibitors. These 1st generation of seeds was used to treat new primary neuron cultures following the same process. At DIV25, the 2nd generation of seeds was collected. 1 μ L of 2nd generation neuron-amplified MSA seeds was added to rat primary neurons plated in 96-well plates at DIV 6, with or without test compounds. At DIV 15, the cells were fixed and stained for MAP2 and alpha-synuclein pS129, imaged and analysed as described above to calculate the aggregates-to-cell ratio, which serves to determine the inhibition of *de novo* aggregate formation.

Table 7. Compounds potency in the primary neuron seed amplification assay

Compound	Inhibition of aggregation, IC ₅₀
Compound 1	**
Compound 4	**
Compound 5	**
Compound 9	***

*** IC₅₀ $\leq$ 0.1 μ M, **0. 1 < IC₅₀ $\leq$ 0.5 μ M

Treatment of neurons with compounds of the present invention led to reduction of alpha-synuclein aggregates (measured by immunostaining for alpha-synuclein phosphorylated at serine 129 (pS129)) further demonstrating the compounds' potency on *de novo* aggregate formation. As can be seen from **Table 7**, compounds of the invention showed a strong potency in reducing burden of intracellular alpha-synuclein aggregates with IC₅₀ in nanomolar range, demonstrating that the compounds are efficacious on 2nd generation propagated aggregates in neuronal cells, further supporting the good

potential of the compounds for therapeutic use in diseases, disorders or abnormalities associated with aggregated alpha-synuclein.

Target engagement ratio

- 5 Target engagement ratio is defined as the compound unbound brain concentration following *per os* administration divided by the efficacious concentration (IC50) determined by the primary neuron seeding assay.

Method

- 10 Pharmacokinetic studies were performed in male CD-1 mice to assess the brain, CSF and plasma exposure profiles after single oral gavage administration of compounds at a dose of 20 mg/kg. Brains, plasma, and CSF samples were collected at the indicated timepoints and processed for LC-MS/MS analysis for the quantitative determination of the test compound concentrations in each biological compartment. The unbound brain concentration was determined for each time point by the following
15 formula:

$$C_{brain,u} = \frac{f_{u,b} \times C_{brain,t} \times 1000}{MW}$$

Where $C_{brain,u}$ is the unbound brain concentration; $f_{u,b}$ is the fraction unbound in brain; $C_{brain,t}$ is the total concentration in brain; MW is the molecular weight of the compound.

20 **Table 8. Target engagement ratio calculated by mouse PK studies single gavage administration of compounds at dose of 20mg/kg**

Compound	TER ($C_{max,brain,u} / IC_{50}$ neuron seeding assay)
Compound 4	>1
Compound 9	>1

$C_{max,brain,u}$: Maximum unbound brain concentration

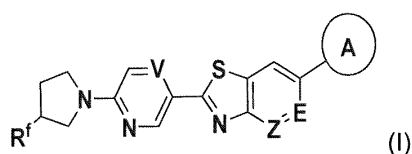
- 25 As shown in Figure 2A (Compound 4), Figure 2B (Compound 9) and Table 8, both compounds show favourable PK profiles. Good CNS exposure combined with the improved *in vitro* potency is resulting in compounds with desired TER at dose of 20mg/kg, demonstrating that it is feasible to expose

organisms at or above the efficacious concentrations, further supporting the good potential of the compounds for therapeutic use.

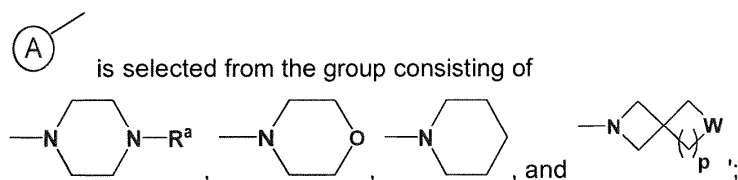
5

CLAIMS

1. A compound of formula (I):



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



15 wherein (A) is optionally substituted at any available position by one to three substituents R^A , wherein each R^A is independently selected from the group consisting of -OH, halogen, -C₁-C₃alkyl, -OC₁-C₃alkyl, -NR^bR^c, spiro-cycloalkyl including 3 to 6 ring atoms, and spiro-heterocycloalkyl including 3 to 6 ring atoms wherein 1 ring atom is O;

W is selected from NR^a and CR^dR^e;

p is 1 or 2;

for each occurrence **R^a** is selected from hydrogen and C₁-C₃ alkyl;

20 for each occurrence **R^b** is selected from hydrogen and C₁-C₃ alkyl;

for each occurrence **R^c** is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring atom is selected from O, S and NR^a;

for each occurrence **R^d** is selected from hydrogen, -OH, and C₁-C₃ alkyl;

for each occurrence **R^e** is selected from hydrogen and C₁-C₃ alkyl;

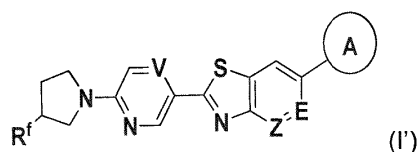
25 **V** is selected from CH or N;

Z is selected from CH or N;

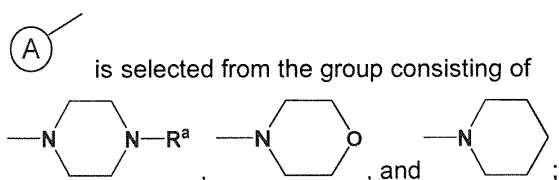
E is selected from CH or N; wherein at least one of **Z** and **E** is CH; and

R^f is selected from hydrogen, $-\text{OC}_1\text{-C}_3\text{alkyl}$ and halogen.

2. A compound of formula (I'):



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



10 wherein $\textcircled{\text{A}}$ is optionally substituted at any available position by one to three substituents R^A , wherein each R^A is independently selected from the group consisting of $-\text{OH}$, halogen, $-\text{NR}^b\text{R}^c$, spiro-cycloalkyl including 3 to 6 ring atoms, and spiro-heterocycloalkyl including 3 to 6 ring atoms wherein 1 ring atom is O;

for each occurrence R^a is selected from hydrogen and $\text{C}_1\text{-C}_3$ alkyl;

for each occurrence R^b is selected from hydrogen and $\text{C}_1\text{-C}_3$ alkyl;

15 for each occurrence R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring atom is selected from O, S and NR^a ;

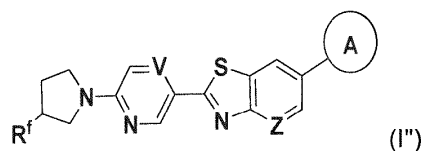
V is selected from CH or N;

Z is selected from CH or N;

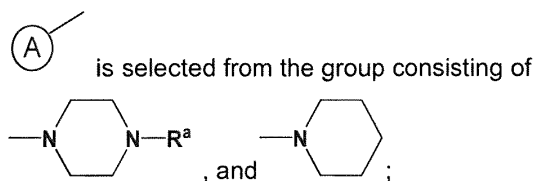
E is selected from CH or N; wherein at least one of Z and E is CH; and

20 R^f is selected from hydrogen and halogen.

3. A compound according to claim 1 or 2, having a formula (I''):



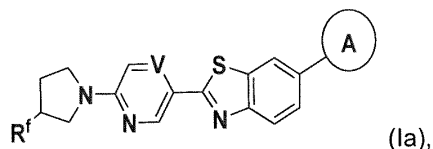
or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein



- 5 wherein (A) is optionally substituted at any available position by one to three substituents R^A , wherein each R^A is independently selected from the group consisting of -OH, halogen, $-NR^bR^c$, and spiro-heterocycloalkyl including 3 to 6 ring atoms wherein 1 ring atom is O; for each occurrence R^a is selected from hydrogen and C_1 - C_3 alkyl; for each occurrence R^b is selected from hydrogen and C_1 - C_3 alkyl; 10 for each occurrence R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring atom is selected from O, S and NR^a ; Z is selected from CH or N; V is selected from CH or N; and R^f is selected from hydrogen and halogen.

15

4. The compound according to any one of claims 1 to 3, having a formula (Ia):

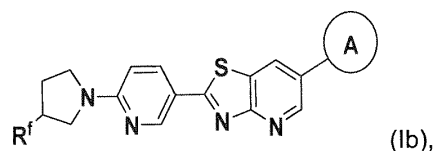


or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof,

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wherein (A), V and R^f are as defined in any one of claims 1 to 3.

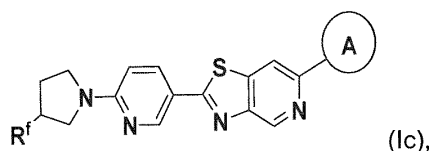
5. The compound according to any one of claims 1 to 3, having a formula (Ib):



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

- 5 wherein  and R^f are as defined in any one of claims 1 to 3.

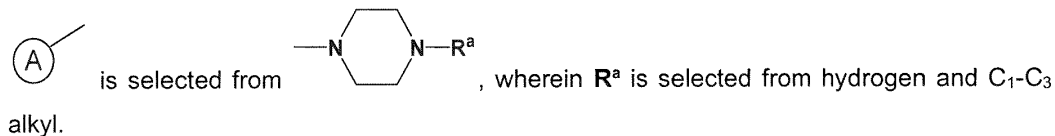
6. The compound according to claim 1 or 2, having a formula (Ic):



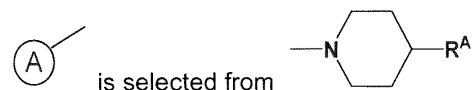
or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof,

- 10 wherein  and R^f are as defined in claim 1 or 2.

7. The compound according to any one of claims 1 to 6, wherein



8. The compound according to any one of claims 1 to 6, wherein

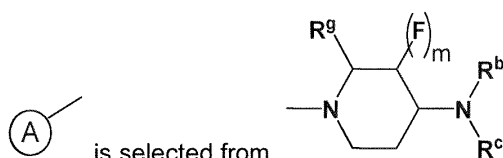


wherein R^a is independently selected from the group consisting of -OH and $-NR^bR^c$; wherein

- 20 R^b is selected from hydrogen and C_1 - C_3 alkyl; and

R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one ring atom is O.

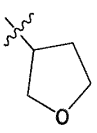
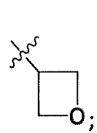
9. The compound according to any one of claims 1 to 6, wherein



wherein

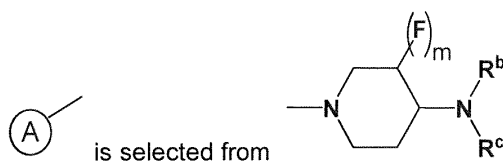
- 5 R^b is as defined in claim 1;

R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one

ring atom is O, preferably R^c is selected from hydrogen,  and ; R^g is selected from hydrogen and C_1 - C_3 alkyl; and m is 0, 1 or 2.

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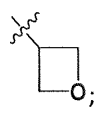
10. The compound according to claim 9 wherein



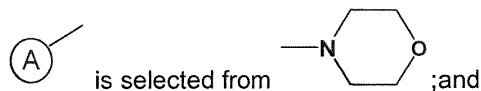
wherein

R^b is selected from hydrogen and C_1 - C_3 alkyl, and

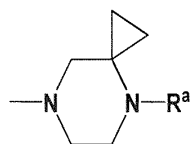
- 15 R^c is selected from hydrogen and heterocycloalkyl including from 3 to 8 ring atoms wherein one

ring atom is O, preferably R^c is selected from hydrogen or ; and m is 0, 1 or 2.

11. The compound according to any one of claims 1 to 6, wherein



20



, wherein R^a is selected from hydrogen and C_1 - C_3 alkyl.

12. The compound according to any one of claim 1 to 6, wherein



is selected from



wherein

- 5 W is selected from NR^a and CR^dR^e ;

p is 1 or 2;

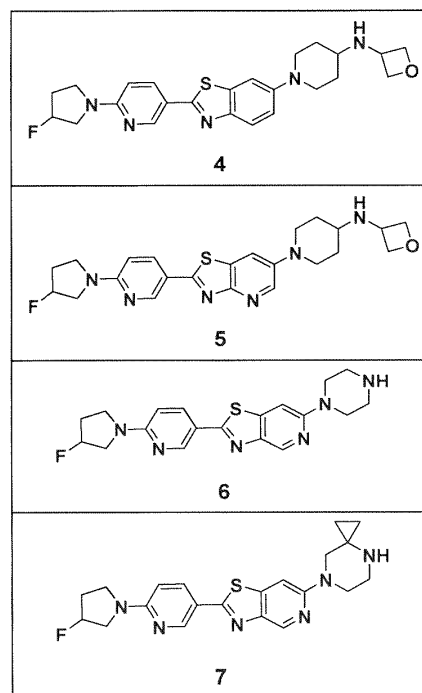
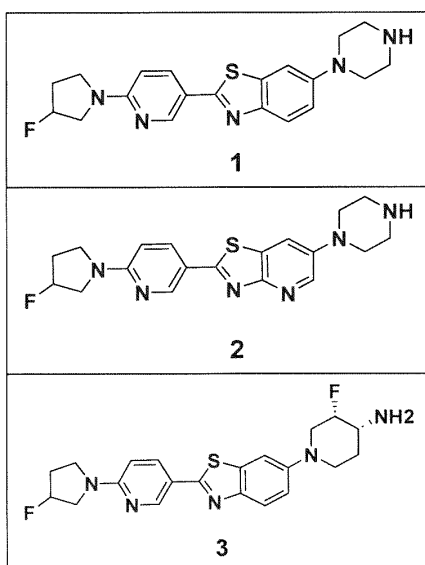
R^a is selected from hydrogen and C_1 - C_3 alkyl, preferably hydrogen;

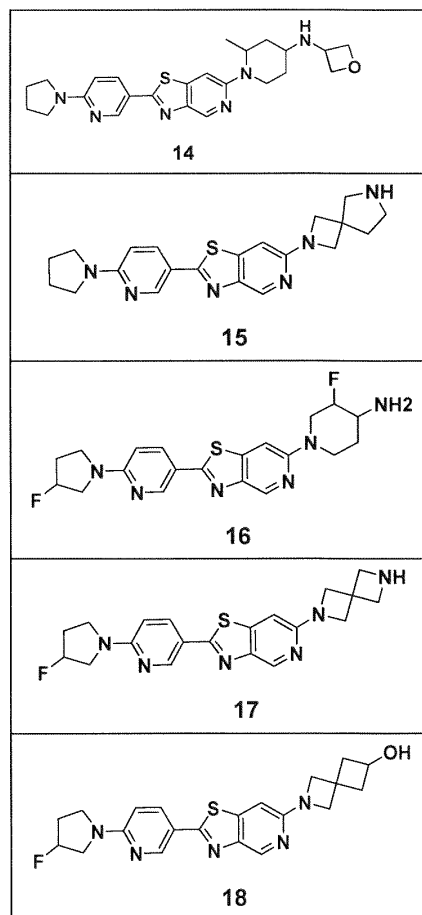
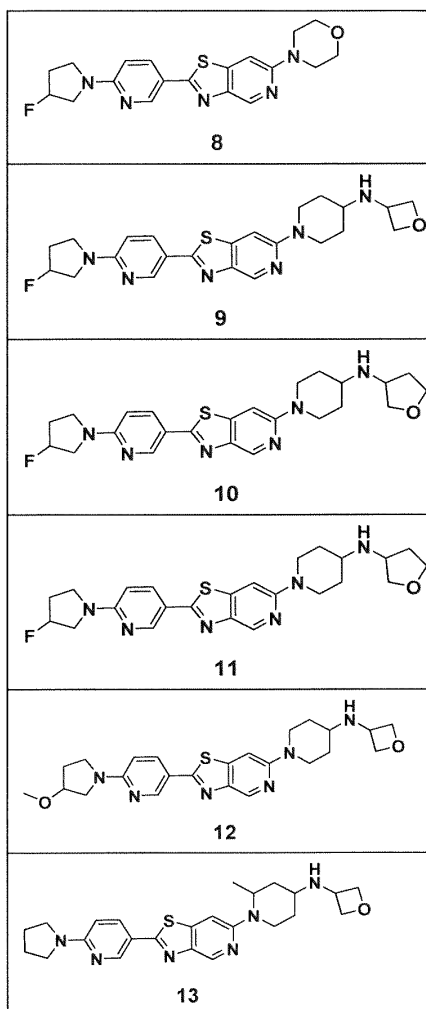
R^d is selected from hydrogen, $-OH$, and C_1 - C_3 alkyl, preferably $-OH$; and

R^e is selected from hydrogen and C_1 - C_3 alkyl, preferably $-hydrogen$.

10

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

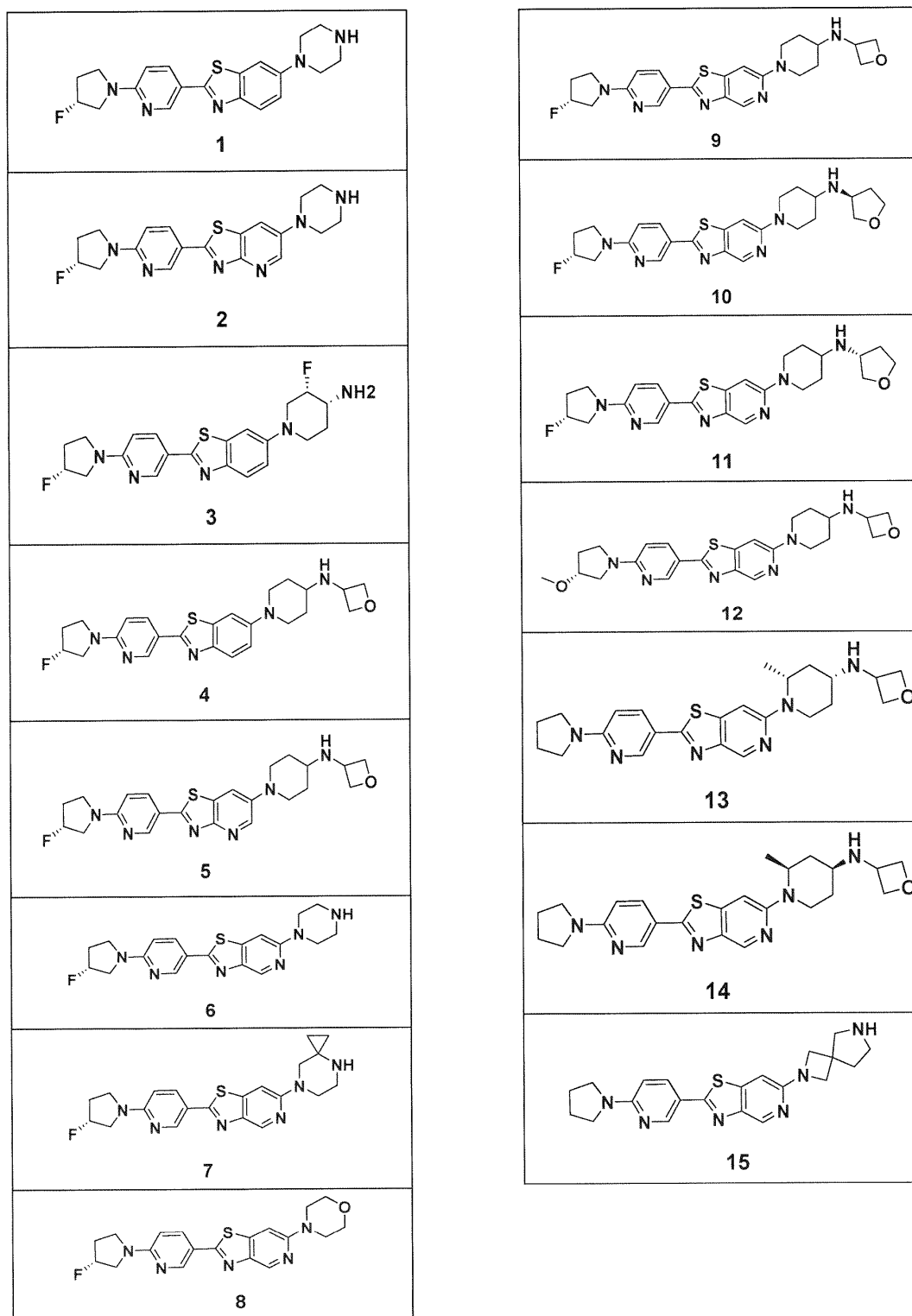


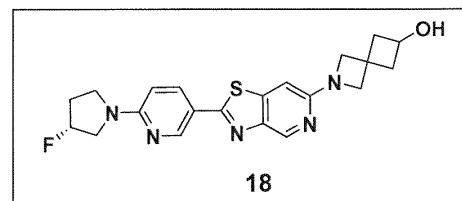
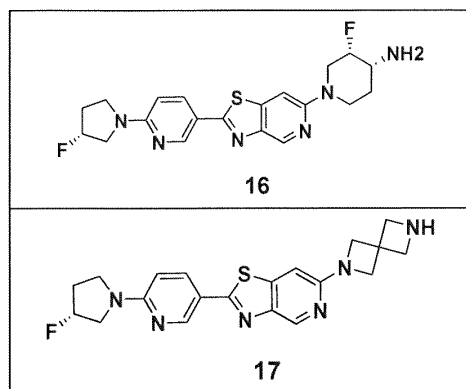


or a stereoisomer, racemic mixture, pharmaceutically acceptable salt, hydrate, or solvate thereof.

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





or a pharmaceutically acceptable salt, hydrate, or solvate thereof.

- 5 15. A pharmaceutical composition comprising a compound according to any one of claims 1 to 14, and optionally at least one pharmaceutically acceptable excipient, carrier, diluent and/or adjuvant.
- 10 16. The compound according to any one of claims 1 to 14, or the pharmaceutical composition according to claim 15, for use in the prevention, alleviation or treatment of a disease, disorder or abnormality associated with alpha-synuclein aggregates.
- 15 17. The compound for use or the pharmaceutical composition for use according to claim 16, wherein the disease, disorder or abnormality is selected from Parkinson's disease (including sporadic, familial with alpha-synuclein mutations, familial with mutations other than alpha-synuclein, pure autonomic failure or Lewy body dysphagia), SNCA duplication carrier, Lewy Body dementia (LBD), dementia with Lewy bodies (DLB) (including "pure" Lewy body dementia), Parkinson's disease dementia (PDD), diffuse Lewy body disease (DLBD), Alzheimer's disease, sporadic Alzheimer's disease, familial Alzheimer's disease with APP mutations, familial Alzheimer's disease with PS-1, PS-2 or other mutations, familial British dementia, Lewy body variant of Alzheimer's disease, Down syndrome, multiple system atrophy (MSA) (including Shy-Drager syndrome, striatonigral degeneration or olivopontocerebellar atrophy), traumatic brain injury, chronic traumatic encephalopathy, dementia puglistica, tauopathies (including Pick's disease, frontotemporal dementia, progressive supranuclear palsy, corticobasal degeneration, Niemann-Pick type C1 disease, frontotemporal dementia with Parkinsonism linked to chromosome 17), Creutzfeldt-Jakob disease, Huntington's disease, motor neuron disease, amyotrophic lateral sclerosis (including sporadic, familial or ALS-dementia complex of Guam), neuroaxonal dystrophy, neurodegeneration with brain iron
- 20
- 25

- 5 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 Kufoer-Rakeb syndrome and Sanfilippo syndrome) and rapid eye movement (REM) sleep behavior disorder.
18. The compound for use or the pharmaceutical composition for use according to claim 17, wherein the disease is Parkinson's disease.
- 10 19. The compound for use or the pharmaceutical composition for use according to claim 17, wherein the disease is multiple system atrophy.
20. The compound for use or the pharmaceutical composition for use according to claim 17, wherein the disease is dementia with Lewy bodies.
- 15 21. The compound for use or the pharmaceutical composition for use according to claim 17, wherein the disease is Parkinson's disease dementia.
22. The compound for use or the pharmaceutical composition for use according to claim 17, wherein the disease is SNCA duplication carrier.
- 20 23. The compound for use or the pharmaceutical composition for use according to claim 17, wherein the disease is Alzheimer's disease.
- 25 24. The compound for use or the pharmaceutical composition for use according to any one of claims 16 to 23, wherein the use is in a human.
25. The compound according to any one of claims 1 to 14, for use in inhibiting or preventing alpha-synuclein aggregation.
- 30 26. The compound according to any one of claims 1 to 14, for use in reducing the concentration of alpha-synuclein aggregates.
27. The compound according to any one of claims 1 to 14, for use in inhibiting *de novo* alpha-synuclein aggregate formation.
- 35

28. The compound according to any one of claims 1 to 14, for use as an *in vitro* analytical reference or an *in vitro* screening tool.
- 5 29. A method of treatment, alleviation or prevention of a disease, disorder or abnormality associated with alpha-synuclein aggregates, comprising administering a therapeutically effective amount of a compound according to any one of claims 1 to 14, or a pharmaceutical composition according to claim 15, to a subject in need thereof.
- 10 30. A mixture or a combination comprising a compound according to claims 1 to 14, and one or more therapeutic agent(s) different from the compound as defined in any one of claims 1 to 14, and optionally a pharmaceutically acceptable carrier, diluent, adjuvant and/or excipient.