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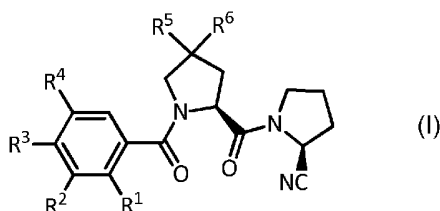
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(54) Title: 1-[1-(benzoyl)-pyrrolidine-2-carbonyl]-pyrrolidine-2-carbonitrile DERIVATIVES



(57) Abstract: The present invention relates to 1-[1-(benzoyl)-pyrrolidine-2-carbonyl]-pyrrolidine-2-carbonitrile derivatives having pharmacological activity formula (I) to processes of preparation of such compounds, to pharmaceutical compositions comprising them, and to their use in therapy and/or prophylaxis of a cognitive disorder.

## 1-[1-(BENZOYL)-PYRROLIDINE-2-CARBONYL]-PYRROLIDINE-2-CARBONITRILE DERIVATIVES

### FIELD OF THE INVENTION

The present invention relates to compounds having pharmacological activity, and more particularly to some 1-[1-(benzoyl)-pyrrolidine-2-carbonyl]-pyrrolidine-2-carbonitrile derivatives, to processes of preparation of such compounds, to pharmaceutical compositions comprising them, and to their use in therapy and/or prophylaxis of cognitive disorders.

### 10 BACKGROUND

Prolyl oligopeptidase (EC 3.4.21.26) (POP), also known as prolyl endopeptidase (PREP), is a serine protease that catalyses the hydrolysis of peptides at the C-terminal side of L-proline residues. It is widely distributed in mammals and can be purified from various organs, including the brain.

15 The enzyme plays an important role in the breakdown of proline-containing neuropeptides related to learning and memory functions (Wilk S et al., *Life Sci.* **1983**;33:2149-57; O'Leary RM, O'Connor B, *J. Neurochem.* **1995**;65:953-63).

The effects of prolyl oligopeptidase inhibition have been tested in the treatment of cognitive deficits related to neurodegenerative processes. Parkinson's disease was generated in monkeys by treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that produces depletion of substance P. Subsequent treatment with S-17092, a potent POP inhibitor, increased the performance of cognitive tasks (Schneider JS et al., *Neuropsychopharmacology* **2002**;26(2):176-82). It has also been found that POP inhibition prevents the oligomerization of  $\alpha$ -synuclein ex vivo [Myöhänen TT et al., *Br. J. Pharmacol.* **2012**;166(3):1097-113]. In the case of Alzheimer's disease (AD), several in vivo experiments in animal models showed that POP inhibition led to neuroprotective and cognition-enhancing effects (Kato A et al., *J. Pharmacol. Exp. Ther.* **1997**;283(1):328-35 and Toide K et al., *Rev. Neurosci.* **1998**;9(1):17-29). Neuroprotective effects were originally observed by Katsube's group, when cortical and cerebellar granule cells were prevented from age-induced apoptosis by treatment with the POP inhibitor ONO-1603 (Katsube N et al., *J. Pharmacol. Exp. Ther.* **1999**;288(1):6-13).

Clinical trials with POP inhibitors in the treatment of cognitive deficits have been performed only in a few cases. In a phase I clinical study Morain's group (Morain P et al., *Br. J. Clin. Pharmacol.* **2000**;50(4):350-9) found that S 17092, a new orally active prolyl endopeptidase inhibitor, showed cognition-enhancing properties in healthy  
5 elderly subjects and a clear dose-dependency; moreover, no adverse effects were detected. Later studies suggested additional slight mood-stabilizing properties for this compound (Morain P et al., *Neuropsychobiology* **2007**;55(3-4):176-83).

Prolyl oligopeptidase activity has been reported to be altered (post-mortem) in several neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease,  
10 Huntington's disease and multiple sclerosis (MS) (Mantle D et al., *Clin. Chim. Acta* **1996**;249(1-2):129-39).

There is also a substantial amount of evidence pointing to a role for neuroinflammation in the pathogenesis of neurodegenerative diseases such as AD, MS and Parkinson's disease (Hirsch EC et al., *Lancet Neurol.* **2009**;8(4):382-97, Philips T et al., *Lancet*  
15 *Neurol.* **2011**;10(3):253-63). POP has been considered to be the main enzyme implicated in the release of an anti-inflammatory tetrapeptide Ac-SDKP from T $\beta$ 4 in the brain (Yang F et al., *Hypertension* **2004**;43(2):229-36, Nolte WM et al., *Biochemistry* **2009**;48(50):11971-81). This suggests that the inhibition of POP may help reduce neuroinflammation and consequently POP inhibitors may be useful in the treatment of  
20 neurodegenerative diseases with an inflammatory component such as Alzheimer's and Parkinson's disease and in particular help improve the cognitive disorders associated with these diseases.

Senile plaques spreading over the cortical brain areas are typical neuropathological hallmarks of AD. The main protein component of these plaques is amyloid  $\beta$ -peptide  
25 (A $\beta$ ). Deposition of A $\beta$  triggers neuronal dysfunction and death in the brain. This peptide derives from the  $\beta$ -amyloid precursor protein (APP). Under normal conditions, APP is cleaved by  $\alpha$ -secretase to generate soluble APP $\alpha$  which precludes A $\beta$  generation.

Interestingly, POP inhibition increases intracellular IP3 levels, which may contribute to  
30 the stimulation of APP $\alpha$  production, which would in turn decrease A $\beta$  generation.

Additionally, Rossner (Rossner S et al., *Neurochem. Res.* **2005**;30(6-7):695-702) found less POP immunoreactive neurons in brain structures of AD patients affected by A $\beta$  plaques.

Additionally it seems that substance P can suppress neurotoxic action of  $\beta$ -amyloid protein (Kowall NW et al., *Proc. Natl. Acad. Sci. USA* **1991**;88(16):7247-51). Prolyl oligopeptidase inhibitors inhibit the metabolism of substance P helping to sustain levels of substance P that may suppress the neurotoxic action of  $\beta$ -amyloid protein.

- 5 In view of the above mentioned effects, it is thought that prolyl oligopeptidase inhibitors may be useful drugs for the treatment of Alzheimer's disease helping to improve the cognitive disorders associated with the disease.

Prolyl oligopeptidase has also been associated with several factors that might be relevant to multiple sclerosis (MS). For instance, POP is involved in the regulation of  
10 microglia toxicity (Klegeris A et al., *Glia* **2008**;56(6):675-85). Indeed, a recent report established a direct connection between POP and MS; the plasma POP activities of patients with RR-MS were significantly reduced (Tenorio-Laranga J et al., *J Neuroinflammation* **2010**;7:23). Interestingly, the reduction correlated with the severity of disease symptoms, but not with patient age. Instead, an inverse correlation between  
15 POP activity and age was observed in healthy controls, and in elderly controls the levels were comparable to those found in MS patients.

The neuropathological hallmark of Parkinson's disease is the progressive degeneration of melanised dopaminergic neurons in substantia nigra pars compacta together with intracellular inclusions known as Lewy bodies. A major component of the Lewy bodies  
20 is a 140 amino acid protein,  $\alpha$ -synuclein. Under certain conditions,  $\alpha$ -synuclein monomers interact to form prefibrillar aggregates or protofibrils, which can create cytotoxic insoluble fibrils. These fibrils cannot be degraded by the proteasome, and they impair the function of this intracellular proteolytic system. This leads to an accumulation of  $\alpha$ -synuclein protofibrils (and other proteins that are degraded by the  
25 proteasome) in the cytosol (Bennett MC, *Pharmacol. Ther.* **2005**;105(3):311-31) and as a consequence,  $\alpha$ -synuclein protofibrils are increased in brains of Parkinson's disease patients. These fibrils have been associated with neurotoxicity in  $\alpha$ -synuclein overexpressing cells and mouse models (Masliah E et al., *Science* **2000**;287(5456):1265-9; Gosavi N et al., *J. Biol. Chem.* **2002**;277(50):48984-92).  
30 Abnormal accumulation of misfolded  $\alpha$ -synuclein may lead to mitochondrial changes which can promote oxidative stress and evoke cell death (Hsu LJ et al., *Am. J. Pathol.* **2000**;157(2):401-10). Furthermore, three point mutations (A53T, A30P or E46K) in the  $\alpha$ -synuclein gene are known to be involved in the pathogenesis of familial form of

Parkinson's disease (Polymeropoulos MH et al., *Science* **1997**;276(5321):2045-7; Zarranz JJ et al., *Ann. Neurol.* **2004**;55(2):164-73).

It has been shown in vitro that the aggregation rate of  $\alpha$ -synuclein was enhanced when the protein was incubated with a clone of wild-type porcine POP, and this enhancement  
5 depended upon the POP concentration (Brandt I et al., *Peptides* **2008**;29(9):1472-8). Moreover, a mutated variant without POP activity (S544A) did not accelerate the aggregation rate.

Enhanced aggregation could also be prevented by the addition of POP inhibitors, suggesting that the effect was dependent on the POP enzymatic activity. Recent  
10 evidence has suggested that POP inhibitors can block the increased  $\alpha$ -synuclein aggregation induced by oxidative stress in human  $\alpha$ -synuclein overexpressing neuroblastoma SH-SY5Y cells Myöhänen TT et al., *Br. J. Pharmacol.* **2012**;166(3):1097-113. POP colocalizes with  $\alpha$ -synuclein in SH-SY5Y cells, and this colocalization disappears after incubation with POP inhibitors, pointing to an interaction  
15 between POP and  $\alpha$ -synuclein. A 5-day treatment with a POP inhibitor reduced the amount of soluble  $\alpha$ -synuclein in the brains of a A30P  $\alpha$ -synuclein transgenic mice.

Thus, inhibition of brain POP activity could prevent  $\alpha$ -synuclein aggregation and thus, prevent the formation of the cytotoxic protofibrils present in the Lewy bodies. Therefore, POP inhibitors could potentially have therapeutical value in the treatment of  
20 neurodegenerative disorders where accelerated  $\alpha$ -synuclein aggregation has been described.

Compounds capable of inhibiting POP are effective for preventing experimental amnesia induced by scopolamine in rats, inferring that POP inhibitors have functions in the alleviation of mnemonic dysfunctions (Yoshimoto T et al., *J. Pharmacobiodyn.*  
25 **1987**;10:730-5).

The effect of subchronic administration of rosmarinic acid, a non-competitive POP inhibitor (with a relatively high IC50 value of 63.7  $\mu$ M), was tested in the Morris water maze in rats, and an enhancement in spatial memory was reported (Park DH et al., *Fitoterapia* **2010**;81(6):644-8).

30 It has been found that patients with bipolar disorder have high levels of activity of the POP in serum. In recent years, POP has gained importance as a target for the treatment of this disease, especially due to his involvement in the metabolism of inositol-1,4,5-P3 (IP3). IP3 is a key molecule in the transduction of the signal in the

cascade of neuropeptides. Through the binding to specific receptors, neuropeptides induce an increase of IP3, which binds to its receptor on the membrane of the endoplasmic reticulum and induces the release of Ca<sup>2+</sup>, which is believed to play a crucial role in learning and memory. Recent findings have shown that the POP modulates the concentration of IP3 (Komatsu Y *J. Neurosci.* **1996**;16:6342-52). Thus it is known that a disruption of the gene of the POP in the eukaryotic *Dictyostelium discoideum* induces resistance to lithium via elevation of IP3 (Schulz I et al., *Eur. J. Biochem.* **2002**;269:5813-20), and also reduced the proteolytic activity of POP, which is responsible for the high concentration IP3 in glioma cells antisense human for POP.

10 This effect is also observed when these cells are treated with specific POP inhibitors (Williams RS et al., *EMBO J.* **1999**;18:2734-45).

The IP3 signaling pathway is involved in the action of several drugs therapeutic mood stabilizers (lithium, carbamazepine and valproic acid) and defects in the mechanisms that regulate the IP3 signaling may cause bipolar disorder. Moreover, the mood stabilizer drug that is commonly used to treat bipolar disorder, valproic acid, directly inhibits the activity of recombinant POP (Cheng L et al., *Mol. Cell. Neurosci.* **2005**;29:155-61). In summary, there is strong evidence that POP inhibitors are useful in the prevention and / or treatment of bipolar affective disorder in mammals. Thus, to provide novel inhibitors of POP is interesting in the therapy of this disorder or disease.

20 In summary, the effects of several POP inhibitors in various cognitive tasks have been characterized, and there is some kind of consensus that POP inhibitors have positive effects on learning and memory (Morain P et al., *CNS Drug. Rev.* **2002**;8(1):31-52; Shinoda M et al., *Eur. J. Pharmacol.* **1996**;305(1-3):31-8; Marighetto A et al., *Learn. Mem.* **2000**;7(3):159-69; Toide K et al., *Pharmacol. Biochem. Behav.* **1997**;56(3):427-34; Schneider JS et al., *Neuropsychopharmacology* **2002**;26(2):176-82).

Several patents and patent applications disclose POP inhibitors: WO 2008/077978 A1, WO 2005/027934 A1, JP 2011-037874 A2, WO 2005/002624 A1, WO 2004/060862 A2, WO 03/04468 A1; DE 196 03 510 A1, US 2006/0100253 A1 and US 6,159,938 A, but only a few compounds have undergone in vivo studies (JTP-4819, S 17092, Z-321, ONO-1603, Y-29794, ZTTA, Z-Pro-Prolinal, and KYP-2047), only the first three in the list have entered clinical trials and none of them has reached the market place.

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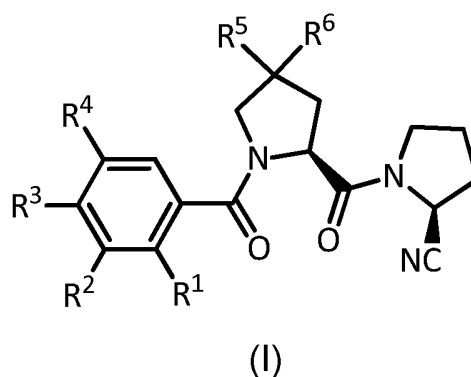
In spite of the existence of POP inhibitors, there is still a need in the art to provide alternative compounds with a high affinity to POP and a good capacity to cross the blood-brain barrier to reach the brain where the action of the inhibitor takes place when

used to treat cognitive disorders. This is an important feature for the compounds to be good candidates for use in the therapy of cognitive disorders.

### BRIEF DESCRIPTION OF THE INVENTION

The inventors have now successfully found that a series of 1-[1-(benzoyl)-pyrrolidine-2-carbonyl]-pyrrolidine-2-carbonitrile derivatives are not only capable of inhibiting POP with a high potency but are also capable of crossing a parallel artificial membrane which is a well accepted method for predicting the capacity to cross the blood-brain barrier. These two properties make the compounds of the present invention ideal candidates for use in the therapy of cognitive disorders.

Therefore, one aspect of the invention relates to compounds having the formula (I):



wherein

$R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  are independently selected from the group consisting of  $C_{1-4}$ alkoxy,  $C_{1-4}$ alkylcarbonyloxy, benzyloxy, phenylcarbonyloxy, naphthylcarbonyloxy, quinolinylcarbonyloxy, isoquinolinylcarbonyloxy, trifluoromethyl, halogen and hydrogen;

$R^5$  is selected from the group consisting of halogen, nitrile,  $C_{1-4}$ alkoxy,  $C_{1-4}$ alkylthio,  $C_{1-4}$ alkyl,  $C_{1-4}$ alkylcarbonyloxy, phenyl, phenoxy, phenylthio and trifluoromethyl;

$R^6$  is selected from the group consisting of hydrogen, fluor and methyl;

or a pharmaceutically acceptable salt, isomer, prodrug or solvate thereof.

Another aspect of this invention refers to processes for the preparation of a compound of formula (I) as defined above or a pharmaceutically acceptable salt, isomer, prodrug or solvate thereof.

Another aspect of this invention refers to a medicament or pharmaceutical composition comprising at least one compound of formula (I) as defined above, or a pharmaceutically acceptable salt, isomer, prodrug or solvate thereof and a pharmaceutically acceptable carrier, adjuvant or vehicle.

- 5 Another aspect of this invention refers to a compound of formula (I) as defined above, or a pharmaceutically acceptable salt, isomer, prodrug or solvate thereof, for use as a medicament, particularly for the prevention and/or treatment of cognitive disorders.

- Another aspect of this invention refers to a method for the treatment or prophylaxis of cognitive disorders in a mammal wherein a therapeutic amount of a compound of formula (I) as defined above, or a pharmaceutically acceptable salt, isomer, prodrug or solvate thereof, is administered to a patient in need of said treatment. In a particular embodiment the disorder is a cognitive disorder associated with a disease selected from the group consisting of schizophrenia, bipolar affective disorder, Alzheimer's disease and Parkinson's disease.
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15

- Another aspect of this invention refers to the use of a compound of formula (I) as defined above, or a pharmaceutically acceptable salt, isomer, prodrug or solvate thereof, for the preparation of a medicament, particularly for the prevention and/or treatment of cognitive disorders and more particularly a cognitive disorder associated with a disease selected from the group consisting of schizophrenia, bipolar affective disorder, Alzheimer's disease and Parkinson's disease. These aspects and preferred embodiments thereof are additionally also defined in the claims.
- 20

## DESCRIPTION OF THE FIGURES

- Figure 1 is graphic comparing the results obtained in the novel object recognition (NOR) test for PBS+vehicle, MK-801+ vehicle and MK-801 and the compound of example 4
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Figure 2 is graphic comparing the results obtained in the passive avoidance task test for PBS+vehicle, MK-801+ vehicle and MK-801 and the compound of example 4.

## 30 DETAILED DESCRIPTION OF THE INVENTION

In the context of the present invention, the following terms have the meaning detailed below.



As used herein C<sub>1-4</sub>alkyl, as a group or part of a group, defines straight or branched chain saturated hydrocarbon radicals having from 1 to 4 carbon atoms such as methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, sec-butyl and tert-butyl. Alkyl radicals may be optionally substituted by one or more substituents such as an aryl, halo, hydroxy,  
5 alkoxy, carboxy, cyano, carbonyl, acyl, alkoxy carbonyl, amino, nitro, mercapto, alkylthio, etc. If substituted by aryl we have an "Aralkyl" radical, such as benzyl and phenethyl.

The term C<sub>1-4</sub>alkoxy means C<sub>1-4</sub>alkyloxy or a C<sub>1-4</sub>alkyl ether radical, wherein the term C<sub>1-4</sub>alkyl is as defined above. Examples of suitable alkyl ether radicals include  
10 methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, i-butoxy, sec-butoxy and tert-butoxy.

The term C<sub>1-4</sub> alkylcarbonyloxy means a C<sub>1-4</sub>alkyl bound to a -C(=O)-O- group wherein the term C<sub>1-4</sub>alkyl is as defined above.

"Halogen", "halo" or "hal" refer to bromo, chloro, iodo or fluoro.

15 "Nitrile", "cyano" or "carbonitrile" refer to the group -C≡N.

The term C<sub>1-4</sub>alkylcarbonyl refers to a C<sub>1-4</sub>alkyl linked to a carbonyl -C(=O)- group.

The term phenoxy means phenyloxy or a phenyl ether radical.

The term phenylthio means a phenyl linked to the thioether group -S-.

It should be noted that the radical positions on any molecular moiety used in the  
20 definitions may be anywhere on such moiety as long as it is chemically stable.

Radicals used in the definitions of any variable herein include all possible isomers unless otherwise indicated.

The term "salt" must be understood as any form of an active compound used in accordance with this invention in which said compound is in ionic form or is charged  
25 and coupled to a counter-ion (a cation or anion) or is in solution. This definition also includes quaternary ammonium salts and complexes of the active molecule with other molecules and ions, particularly, complexes formed via ionic interactions. The definition includes in particular physiologically acceptable salts; this term must be understood as equivalent to "pharmacologically acceptable salts" or "pharmaceutically acceptable  
30 salts".

- The term “pharmaceutically acceptable salts” in the context of this invention means any salt that is tolerated physiologically (normally meaning that it is not toxic, particularly, as a result of the counter-ion) when used in an appropriate manner for a treatment, applied or used, particularly, in humans and/or mammals. These physiologically acceptable salts may be formed with cations or bases and, in the context of this invention, are understood to be salts formed by at least one compound used in accordance with the invention –normally an acid (deprotonated)– such as an anion, particularly when used on humans and/or mammals. These physiologically acceptable salts may also be formed with anions or acids and, in the context of this invention, are understood as being salts formed by at least one compound used in accordance with the invention – normally protonated, for example in nitrogen – such as a cation and at least one physiologically tolerated anion, particularly when used on humans and/or mammals. This definition specifically includes in the context of this invention a salt formed by a physiologically tolerated acid, i.e. salts of a specific active compound with physiologically tolerated organic or inorganic acids – particularly when used on humans and/or mammals. Examples of this type of salts are those formed with: hydrochloric acid, hydrobromic acid, sulphuric acid, methanesulfonic acid, formic acid, acetic acid, oxalic acid, succinic acid, malic acid, tartaric acid, mandelic acid, fumaric acid, lactic acid or citric acid.
- The term “solvate” in accordance with this invention should be understood as meaning any form of the active compound in accordance with the invention in which said compound is bonded by a non-covalent bond to another molecule (normally a polar solvent), including especially hydrates and alcoholates, like for example, methanolate. A preferred solvate is the hydrate.
- Any compound that is a prodrug of a compound of formula (I) is also within the scope of the invention. The term “prodrug” is used in its broadest sense and encompasses those derivatives that are converted in vivo to the compounds of the invention. Examples of prodrugs include, but are not limited to, derivatives and metabolites of the compounds of formula I that include biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Preferably, prodrugs of compounds with carboxyl functional groups are the lower alkyl esters of the carboxylic acid. The carboxylate esters are conveniently formed by esterifying any of the carboxylic acid moieties present on the molecule. Prodrugs can

typically be prepared using well-known methods, such as those described by Burger "Medicinal Chemistry and Drug Discovery 6th ed. (Donald J. Abraham ed., 2001, Wiley), "Design and Applications of Prodrugs" (H. Bundgaard ed., 1985, Harwood Academic Publishers) and Krogsgaard-Larsen et al. "Textbook of Drug design and  
5 Discovery" Taylor & Francis (April 2002).

The compounds of the present invention represented by the above described formula (I) may include enantiomers depending on the presence of chiral centres or isomers depending on the presence of multiple bonds (e.g. Z, E). The single isomers, enantiomers or diastereoisomers and mixtures thereof fall within the scope of the  
10 present invention.

Furthermore, any compound referred to herein may exist as tautomers. Specifically, the term tautomer refers to one of two or more structural isomers of a compound that exist in equilibrium and are readily converted from one isomeric form to another. Common tautomeric pairs are amine-imine, amide-imidic acid, keto-enol, lactam-lactim, etc.

Unless otherwise stated, the compounds of the invention are also meant to include isotopically-labelled forms i.e. compounds which differ only in the presence of one or more isotopically-enriched atoms. For example, compounds having the present structures except for the replacement of at least one hydrogen atom by a deuterium or tritium, or the replacement of at least one carbon by  $^{13}\text{C}$ - or  $^{14}\text{C}$ -enriched carbon, or the  
20 replacement of at least one nitrogen by  $^{15}\text{N}$ -enriched nitrogen are within the scope of this invention.

The compounds of formula (I), or their salts or solvates are preferably in pharmaceutically acceptable or substantially pure form. By pharmaceutically acceptable form is meant, inter alia, having a pharmaceutically acceptable level of  
25 purity excluding normal pharmaceutical additives such as diluents and carriers, and including no material considered toxic at normal dosage levels. Purity levels for the drug substance are preferably above 50%, more preferably above 70%, most preferably above 90%. In a preferred embodiment it is above 95% of the compound of formula (I), or of its salts, solvates or prodrugs.

As noted previously, the term "pharmaceutically acceptable salts, solvates, prodrugs" refers to any salt, solvate, or any other compound which, upon administration to the recipient, is capable of providing (directly or indirectly) a compound as described herein. However, it will be appreciated that non-pharmaceutically acceptable salts,  
30

solvates and prodrugs also fall within the scope of the invention since those may be useful in the preparation of pharmaceutically acceptable salts, solvates and prodrugs. The preparation of salts, solvates and prodrugs can be carried out by methods known in the art.

- 5 As used herein, the terms "treat", "treating" and "treatment" include the eradication, removal, reversion, alleviation, modification, or control of a disease or condition, such as a cognitive disorder.

As used herein, the terms "prevention", "preventing", "preventive", "prevent" and "prophylaxis" refer to the capacity of a compound of formula (I) to avoid, minimize or  
10 difficult the onset or development of a disease or condition, such as a cognitive disorder, before its onset.

Therefore, by "treating" or "treatment" and/or "preventing" or "prevention", as a whole, is meant at least a suppression or an amelioration of the symptoms associated with the condition afflicting the subject, where suppression and amelioration are used in a broad  
15 sense to refer to at least a reduction in the magnitude of a parameter, e.g., symptom associated with the condition being treated, such as a cognitive disorder. As such, the method of the present invention also includes situations where the condition is completely inhibited, e.g., prevented from happening, or stopped, e.g., terminated, such that the subject no longer experiences the condition. As such, the present method  
20 includes both preventing and managing a cognitive disorder.

The term "cognitive disorder," as used herein, means any condition characterized by a deficit in mental activities associated with thinking, learning, or memory. Examples of such disorders include agnosias, amnesias, aphasias, apraxias, deliriums, dementias, and learning disorders.

- 25 The cognitive disorder may be (and frequently is) associated with (that is, be caused by or occur in the presence of) other conditions characterized by damage to or loss of neurons or other structures involved in the transmission of signals between neurons. Hence, cognitive disorders may be associated with neurodegenerative diseases such as Alzheimer's disease, corticobasal degeneration, Creutzfeldt-Jacob disease,  
30 frontotemporal lobar degeneration, Huntington's disease, multiple sclerosis, normal pressure hydrocephalus, organic chronic brain syndrome, Parkinson's disease, Pick disease, progressive supranuclear palsy, or senile dementia (Alzheimer type).

Cognitive disorders may also be associated with other conditions which impair normal functioning of the central nervous system, including psychiatric disorders such as anxiety disorders, dissociative disorders, mood disorders such as bipolar affective disorder, schizophrenia, and somatoform and factitious disorders.

- 5 The compounds described here may be used to treat agnosias, amnesias, aphasias, apraxias, deliriums, dementias, learning disorders and other cognitive disorders.

Examples of dementias which may be treated with the methods of the invention include AIDS dementia complex, Binswanger's disease, dementia with Lewy Bodies, frontotemporal dementia, multi-infarct dementia, Pick's disease, semantic dementia,  
10 senile dementia, and vascular dementia.

Examples of learning disorders which may be treated with the methods of the invention include Asperger's syndrome, attention deficit disorder, attention deficit hyperactivity disorder, autism, childhood disintegrative disorder, and Rett syndrome.

Examples of aphasia which may be treated with the methods of the invention include  
15 progressive non-fluent aphasia.

The compounds described here may also be used to treat patient having deficits in mental activities that are mild or that otherwise do not significantly interfere with daily life. Mild cognitive impairment is an example of such a condition: a patient with mild cognitive impairment displays symptoms of dementia (e.g., difficulties with language or  
20 memory) but the severity of these symptoms is such that a diagnosis of dementia may not be appropriate. The compounds described here may be used to treat mild cognitive impairment and other, similarly less severe forms of cognitive disorders.

Thus, another aspect of the present invention is a method for the treatment or prophylaxis of cognitive disorders in a mammal wherein a therapeutic amount of a  
25 compound of the invention is administered to a patient in need of said treatment.

In a particular embodiment of the present invention the compounds described here may be used to treat patients having a cognitive disorder associated with schizophrenia, bipolar affective disorder, Alzheimer's disease or Parkinson's disease.

In a particular embodiment of the present invention in the compounds of formula (I) or a  
30 pharmaceutically acceptable salt, isomer, prodrug or solvate thereof, R<sup>1</sup> is hydrogen; R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are independently selected from the group consisting of C<sub>1-4</sub>alkoxy, C<sub>1-4</sub>alkylcarbonyloxy, benzyloxy, phenylcarbonyloxy, naphthyl-carbonyloxy,

quinolinylcarbonyloxy and isoquinolinylcarbonyloxy;  $R^5$  is selected from the group consisting of halogen, nitrile,  $C_{1-4}$ alkoxy,  $C_{1-4}$ alkylthio,  $C_{1-4}$ alkyl, phenyl, phenoxy, phenylthio and trifluoromethyl and  $R^6$  is selected from the group consisting of hydrogen, fluor and methyl.

- 5 In a particular embodiment,  $R^2$  and  $R^4$  are independently selected from the group consisting of hydrogen, halogen, trifluoromethyl and  $C_{1-4}$ alkoxy

In another particular embodiment,  $R^2$  is selected from the group consisting of hydrogen and methoxy and  $R^4$  is selected from the group consisting of fluor, trifluoromethyl and methoxy.

- 10 In another particular embodiment,  $R^5$  is fluor.

In a particular embodiment,  $R^2$  and  $R^4$  are independently selected from the group consisting of  $C_{1-4}$ alkoxy,  $C_{1-4}$ alkylcarbonyloxy and benzyloxy.

In a particular embodiment,  $R^2$  and  $R^4$  are methoxy.

In another embodiment,  $R^3$  is a benzyloxy.

- 15 In another embodiment  $R^1$  is hydrogen.

In another embodiment,  $R^5$  is selected from fluor, methoxy, methylthio and phenyl, preferably fluor.

In another embodiment,  $R^6$  is hydrogen or fluor.

- 20 In additional preferred embodiments, the preferences described above for the different substituents are combined. The present invention is also directed to such combinations of preferred substitutions in the formulae above.

Particular individual compounds of the invention falling under formula (I) include the compounds listed below:

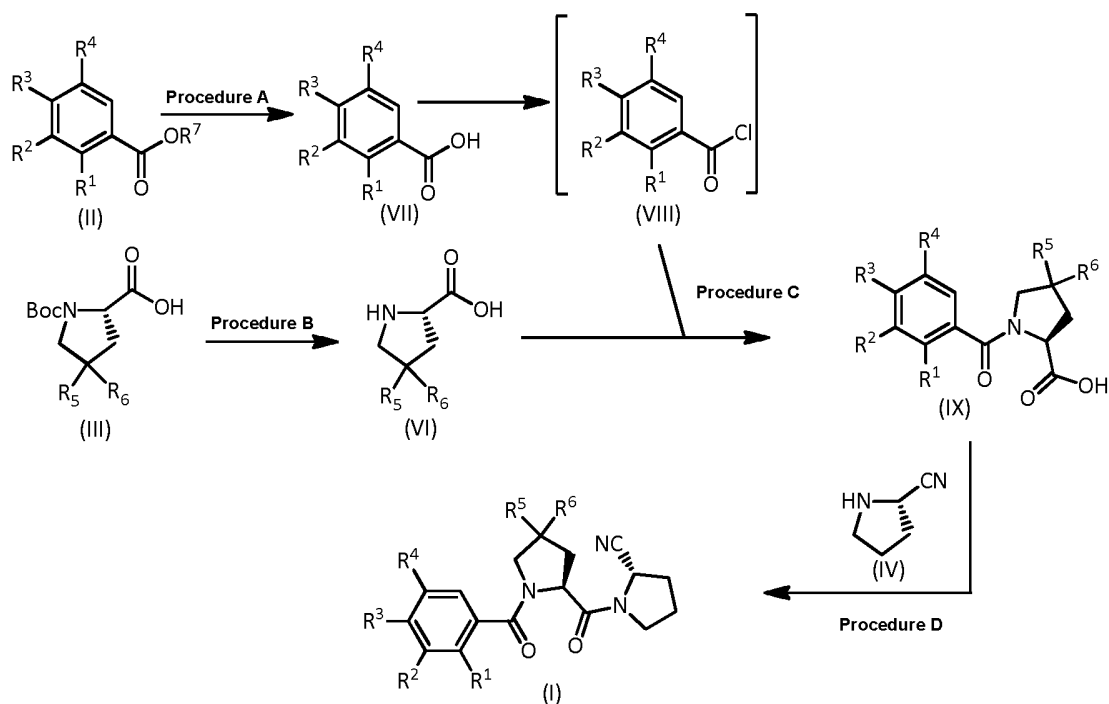
- 25
- (S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-methoxypyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
  - (S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-fluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
  - (S)-1-((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-phenylpyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile

- (S)-1-((2S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-(methylthio)pyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- 5      • (S)-1-((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-methylpyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-cyanopyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- 10      • (S)-1-((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-(trifluoromethyl)-pyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-(tert-butoxy)pyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-acetoxypyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- 15      • (S)-1-((2S)-1-(4-acetoxy-3,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S)-1-(4-benzoyloxy-3,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S)-1-(3,4-dibenzyloxy-5-methoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- 20      • (S)-1-((2S)-1-(3,4-dibenzoyloxy-5-methoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S)-1-(3-acetoxy-4,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- 25      • (S)-1-((2S)-1-(3-pivaloyloxy-4,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((S)-1-(4-(benzyloxy)benzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile

- (S)-1-((S)-1-(3-(benzyloxy)benzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((S)-1-(2-(benzyloxy)benzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- 5     • (S)-1-((S)-1-(4-(benzyloxy)-3-(trifluoromethyl)benzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((S)-1-(4-(benzyloxy)-3-fluorobenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile

or a pharmaceutically acceptable salt, isomer, prodrug or solvate thereof.

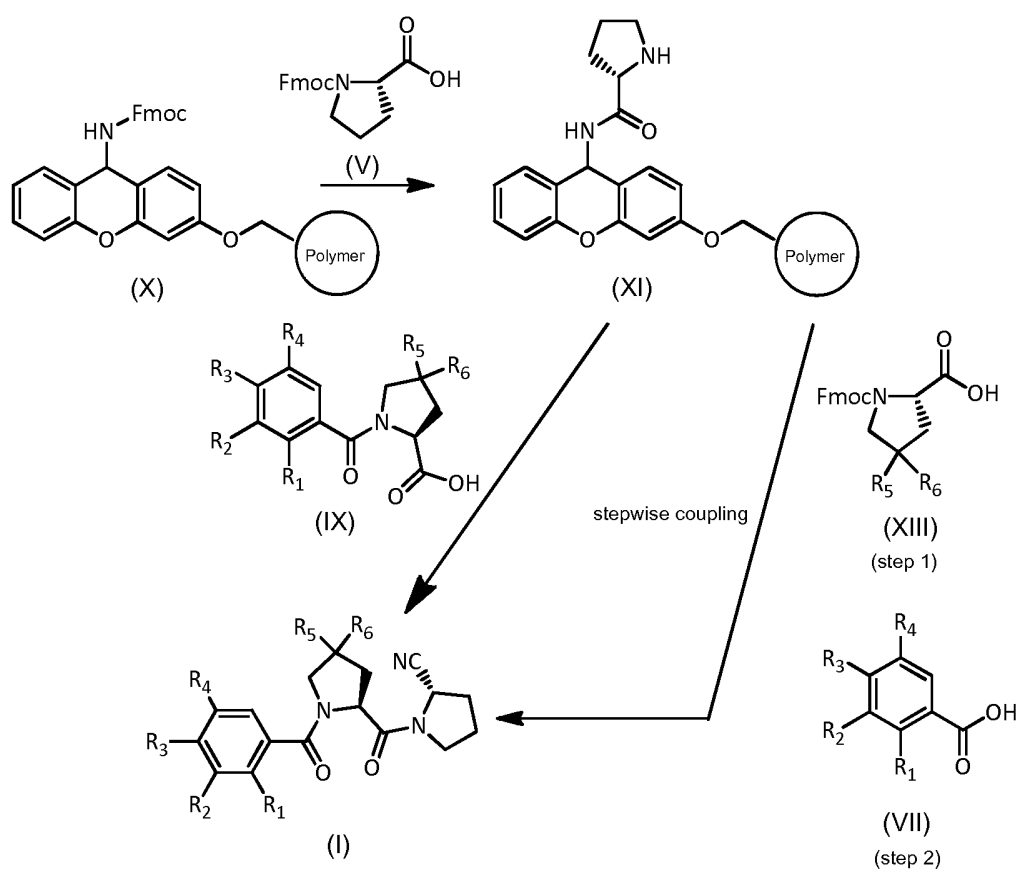
- 10 The compounds of formula (I) defined above can be obtained by available synthetic procedures as illustrated by the following general schemes:



#### DETAILED SCHEME FOR PROCEDURE E



16



In a first step the ester of formula (II) is dissolved or suspended in a polar organic solvent (preferably a protic polar organic solvent) such as ethanol (EtOH) or methanol or a mixture of polar organic solvents. An aqueous base solution is added and the hydrolytic reaction is performed by maintaining the mixture, typically under reflux, at a temperature comprised between room temperature and the reflux temperature of the solvent mixture until completion of the hydrolysis, typically for a period of 0.5 to 4 hours, preferably 1-2 hours. The base solution is preferably of inorganic nature, such as a dilute alkali, for example NaOH. Then the reaction mixture is left to reach room temperature and, preferably, concentrated to approximately a fifth of the reaction volume. The reaction mixture is then slowly added to an acid solution such as a 1M HCl solution to effect neutralization, while cooled in an ice bath. If acidification leads to precipitation, the solid is filtered and washed with water, providing the product of formula (VII). If no precipitate is obtained, the resulting solution is extracted several times with an appropriate organic solvent such as ethyl acetate, the organic phase is dried and evaporated. The crude product of formula (VII) is purified by flash chromatography.

Deprotection of the amine of formula (III) is achieved under mild acidic conditions, such as addition onto a hydrogen chloride solution in an organic solvent such as dioxane, or with a TFA/DCM mixture, at low temperature ranging from 0°C to room temperature. The reaction is stirred at room temperature for 1-3 hours. The solvent is then  
5 evaporated to dryness, to give the hydrochloride salt or the trifluoroacetate salt of the amine of formula (VI), depending on the acid used.

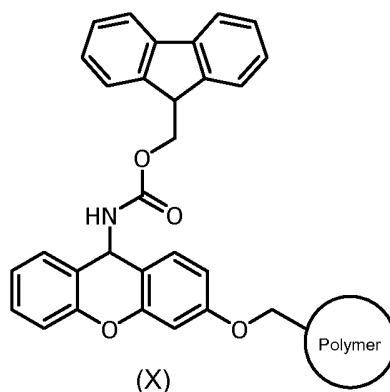
The compound of formula (IX) is prepared from the carboxylic acid of formula (VII) and amine of formula (VI) under Schotten-Baumann conditions. Thus, a chlorinating agent such as oxalyl chloride is added to a solution of the carboxylic acid of formula (VII) in  
10 an organic solvent such as toluene. The reaction is stirred at a temperature between 50°C and 80°C for 1 to 2 hours to allow for the formation of the carboxylic acid chloride of formula (VIII). After evaporation of the solvent, the resulting crude is solubilized in an organic solvent such as THF and added to an aqueous basic solution of the amine of formula (VI), typically an aqueous NaOH solution of the amine of formula (VI), at a low  
15 temperature such as 0°C. The reaction mixture is stirred at the low temperature for 1 to 2 hours and at room temperature during 2 to 4 hours. Then, the solvent is evaporated and the remaining aqueous fraction is adjusted to acid pH (3-4) by addition of a HCl solution and extracted with ethyl acetate. The organic phase is washed with brine, dried, filtered and evaporated. The crude product is purified by flash chromatography  
20 when necessary.

The product of formula (IX) is then coupled to (S)-pyrrolidine-2-carbonitrile of formula (IV) in the presence of a base, such as a N,N-diisopropylethylamine (DIEA), and aided by a coupling reagent, such as a carbodiimide. In particular, the compound of formula (IX) is dissolved in an aprotic organic solvent such as dichloromethane and added to a  
25 carbodiimide, for example a solid-supported carbodiimide such as N-cyclohexylcarbodiimide, N'-methyl polystyrene, together with DIEA. After 5 min, (S)-pyrrolidine-2-carbonitrile of formula (IV) and extra DIEA are added. The reaction is stirred at room temperature for 8 to 16 hours. Then, the reaction mixture is filtered and the remaining solid is washed with the aprotic organic solvent. The filtrate is  
30 evaporated to dryness. The crude product is then purified by preparative RP-HPLC.

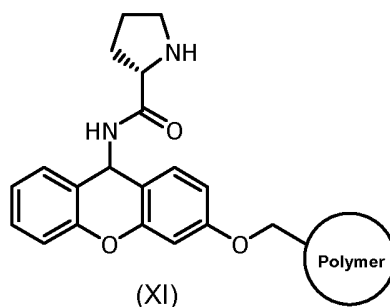
Alternatively, the compounds of formula (I) may be prepared as illustrated in Scheme E and described below:

An amine-functionalised resin such as Sieber amide resin of formula (X) is placed in a syringe fitted with a polyethylene porous disk. The resin is swelled by washes with

appropriate organic solvents such as dichloromethane (DCM) and dimethylformamide (DMF). When the amine group of the resin is protected (i.e. in the case of Sieber amide resin), removal of the protecting group (such as a fluorenylmethoxycarbonyl (Fmoc) protecting group) is achieved by treatment with an amine base solution such as a piperidine solution in DMF.

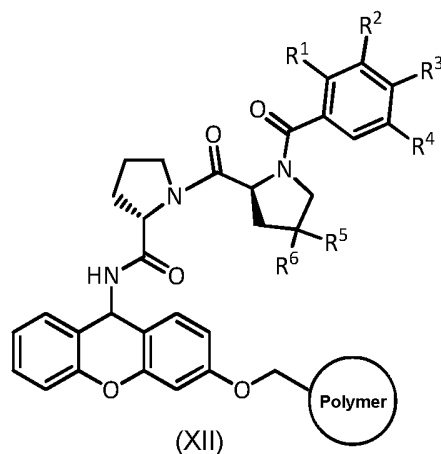


Following removal of the protecting group from the resin, Fmoc-protected L-Proline of formula (V) is attached to the resin using an activating agent such as a triazole (i.e. TBTU) and an amine base such as DIEA in an appropriate organic solvent such as DMF. The mixture is stirred during 1 to 2 hours. After filtration and washing, the extent of the coupling may be monitored using the Kaiser test, re-coupling is performed when required. Fmoc is removed to yield product of formula (XI) by a treatment with an amine base solution such as a piperidine solution in DMF and/or a mixture of piperidine/DBU/toluene/DMF. Fmoc removal may be assessed using the *p*-nitrophenyl ester NF31 test.



The product of formula (XI) is coupled to the product of formula (IX) to yield the product of formula (XII) using an activating agent such as PyBOP, in the presence or in the absence of an additive such as HOAt, and an amine base such as DIEA in an appropriate organic solvent such as DMF. The mixture is stirred manually during the total reaction time of 1 to 2 hours. A systematic re-coupling is done using the same

amounts and time. The extent of the coupling may be monitored using the *p*-nitrophenyl ester NF31 test.



Alternatively, the product of formula (XII) may also be obtained by stepwise coupling of  
5 product (XI) first to compound of formula (XIII), followed by the removal of the Fmoc protecting group and then coupling with compound of formula (VII).

The product of formula (XII), thoroughly washed with an appropriate organic solvent such as DCM and dried, is transferred to a flask, to which trifluoroacetic anhydride and pyridine are added in a small amount of an organic solvent. The mixture is kept at a  
10 temperature of 20 to 40° C for 8 to 16 hours. Then, the reaction mixture is filtered and the resin is washed with the same organic solvent. The filtrates are collected and the solvent is evaporated to dryness. The resulting crude is dissolved in an appropriate solvent such as ethyl acetate and washed with saturated NaHCO<sub>3</sub> solution and a 5% aq. KHSO<sub>4</sub> solution. The organic phase is dried, filtered, and evaporated. The crude is  
15 taken up in H<sub>2</sub>O:CH<sub>3</sub>CN and lyophilized to yield the peptide nitrile of formula (I).

Alternatively, the peptidyl-resin of formula (XII) may be treated with a mixture of TFA/H<sub>2</sub>O/TIS during 1-2 hours. Then, the resin is filtered and washed with TFA, the filtrates are collected and the solvent is evaporated to dryness. The crude is resuspended in a mixture of H<sub>2</sub>O:CH<sub>3</sub>CN and lyophilized. The resulting crude peptide  
20 amide is taken up in an appropriate organic solvent such as DCM and converted to the nitrile for example in the presence of phosphorus pentoxide, titanium tetrachloride, thionyl chloride, trifluoroacetic anhydride/pyridine or triphenylphosphine/carbontetrachloride. The mixture is kept at room temperature for 8 to 16 hours, the solvent is evaporated and the residue taken up in ethyl acetate. The organic solution is

subsequently washed with aq.  $\text{KHSO}_4$  solution and aq.  $\text{NaHCO}_3$  solution. Drying and evaporation of the organic phase yields the peptide nitrile of formula (I).

The crude product is purified by RP-HPLC.

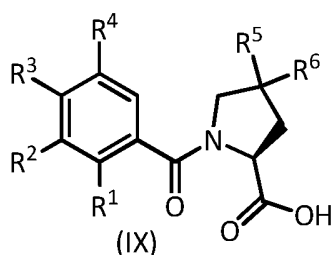
Where the above described processes for the preparation of compounds of the invention give rise to mixtures of stereoisomers, these isomers may be separated by conventional techniques such as preparative chromatography. If there are chiral centers, the compounds may be prepared in racemic form, or individual enantiomers may be prepared either by enantiospecific synthesis or by resolution.

The compounds of formulae (II), (III), (IV) and (V), as well as some of the compounds of formula (VII), used as starting products are either commercially available and may also be prepared using methods well known to the expert in the field.

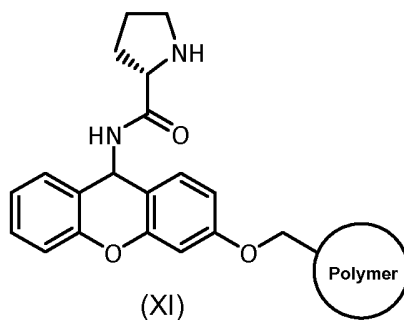
Thus, in one aspect the present invention provides for processes for the preparation of a compound of formula (I) or a pharmaceutically acceptable salt, isomer, prodrug or solvate thereof.

In one embodiment the process comprises the steps of:

a) reacting a compound of formula (IX):

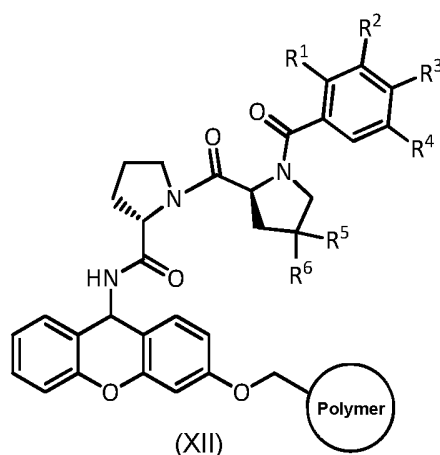


in which  $\text{R}^1$ ,  $\text{R}^2$ ,  $\text{R}^3$ ,  $\text{R}^4$ ,  $\text{R}^5$  and  $\text{R}^6$  are as defined above in formula (I), with a compound of formula (XI):

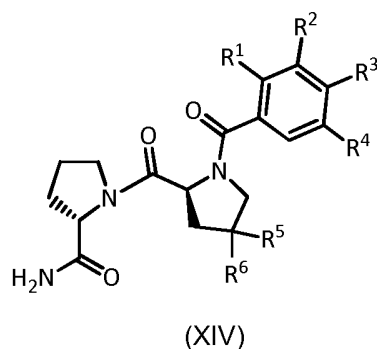


wherein polymer stands for a polymer which is inert under the reaction conditions of the synthetic method herein-disclosed and insoluble but swellable in the solvents herein-employed such as low cross-linked polystyrene and polyethyleneglycol-grafted polystyrene polymers.

5 to yield a compound of formula (XII);



b) hydrolising the compound of formula (XII) to yield the compound of formula (XIV)



10 and

c) subjecting the compound of formula (XIV) to conditions capable of transforming a carboxamide group into a nitrile group to yield the compound of formula (I);

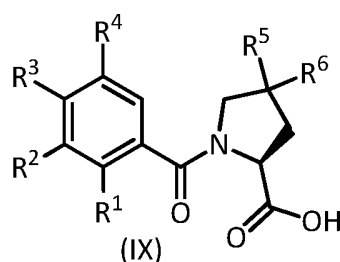
wherein steps b) and c) may be performed separately or in a one pot reaction.

In another embodiment of the present invention the process for the preparation of a compound of formula (I) or a pharmaceutically acceptable salt, isomer, prodrug or solvate thereof, comprises the steps of:

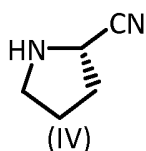
15

a) reacting a compound of formula (IX):

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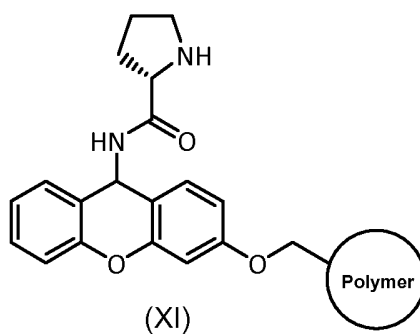
in which  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$  and  $R^6$  are as defined above in formula (I), with a compound of formula (IV)



5

In still another embodiment the process comprises the steps of:

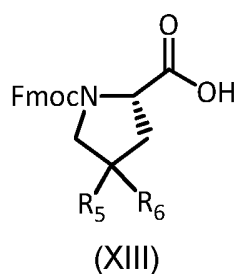
a) reacting a compound of formula (XI):



10 wherein polymer stands for a polymer which is inert under the reaction conditions of the synthetic method herein-disclosed and insoluble but swellable in the solvents herein-employed such as low cross-linked polystyrene and polyethyleneglycol-grafted polystyrene polymers.

with a compound of formula (XIII):

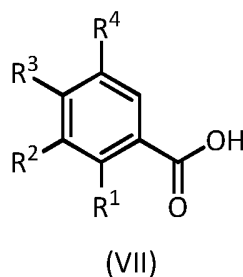
23



in which  $R^5$  and  $R^6$  are as defined above in formula (I)

b) removing the Fmoc protecting group

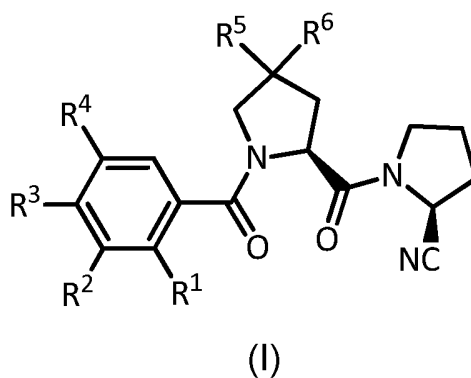
c) reacting with a compound of formula (VII);



5

in which  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  are as defined above in formula (I)

d) hydrolysing the resulting product from the supporting polymer to yield the compound of formula (I)



10 in which  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$  and  $R^6$  are as defined above.

It has been found that the compounds of general formula (I) are useful in the treatment of cognitive disorders, in particular cognitive disorders associated with other diseases or conditions of the central nervous system.



In a particular embodiment of the present invention, the cognitive disorder is a cognitive disorder associated with a disease selected from the group consisting of schizophrenia, bipolar affective disorder, Alzheimer's disease and Parkinson's disease.

The present invention further provides medicaments or pharmaceutical compositions comprising a compound of this invention, or a pharmaceutically salt, derivative, prodrug  
5 or stereoisomer thereof together with a pharmaceutically acceptable carrier, adjuvant, or vehicle, for administration to a patient.

The auxiliary materials or additives of a pharmaceutical composition according to the present invention can be selected among carriers, excipients, support materials,  
10 lubricants, fillers, solvents, diluents, colorants, flavour conditioners such as sugars, antioxidants, binders, adhesives, disintegrants, anti-adherents, glidants and/or agglutinants. In the case of suppositories, this may imply waxes or fatty acid esters or preservatives, emulsifiers and/or carriers for parenteral application. The selection of these auxiliary materials and/or additives and the amounts to be used will depend on  
15 the form of application of the pharmaceutical composition.

The medicament or pharmaceutical composition according to the present invention may be in any form suitable for the application to humans and/or animals, preferably humans including infants, children and adults and can be produced by standard procedures known to those skilled in the art. Therefore, the formulation in accordance  
20 with the invention may be adapted for topical or systemic application, particularly for dermal, transdermal, subcutaneous, intramuscular, intra-articular, intraperitoneal, intravenous, intra-arterial, intravesical, intraosseous, intracavernosal, intranasal, pulmonary, buccal, sublingual, ocular, intravitreal, percutaneous, rectal, vaginal, oral, epidural, intrathecal, intraventricular, intracerebral, intracerebroventricular,  
25 intracisternal, intraspinal, perispinal, intracranial, delivery via needles or catheters with or without pump devices, or other application routes.

In an embodiment the pharmaceutical compositions are in oral form, either solid or liquid. Suitable dose forms for oral administration may be tablets, pills, caplets, gel caps, chewing gums, capsules, granules, drops, syrups or solutions and may contain  
30 conventional excipients known in the art such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize starch, calcium phosphate, sorbitol or glycine; tableting lubricants, for example magnesium stearate; disintegrants, for example starch, polyvinylpyrrolidone,

sodium starch glycollate or microcrystalline cellulose; or pharmaceutically acceptable wetting agents such as sodium lauryl sulfate.

In another embodiment the pharmaceutical compositions are in the form of products for non-parenteral intranasal administration, preferably in the form of products for intranasal administration. Typically intranasal administration is carried out by using nasal sprays, squeeze bottles, and liquid droppers as delivery devices. To be used with these devices, the pharmaceutical compositions are advantageously liquid solutions or suspensions of the compounds of the invention.

The compositions may be prepared by conventional methods of blending, filling or tableting. Repeated blending operations may be used to distribute the active agent throughout those compositions employing large quantities of fillers. Such operations are conventional in the art. The tablets may for example be prepared by wet or dry granulation and optionally coated according to methods well known in normal pharmaceutical practice, in particular with an enteric coating.

The pharmaceutical compositions may also be adapted for parenteral administration, such as sterile solutions, suspensions or reconstitutable dry preparations, aerosols or sprays in the appropriate unit dosage form. Adequate excipients can be used, such as bulking agents, buffering agents or surfactants.

The composition of the invention may be formulated as deposits in dissolved form or in patches, for percutaneous application.

Skin applications include ointments, gels, creams, lotions, suspensions or emulsions.

Suitable form of rectal application is by means of suppositories.

The mentioned formulations will be prepared using standard methods such as those described or referred to in the Spanish and US Pharmacopoeias and similar reference texts.

In one embodiment of the invention it is preferred that compound of formula (I) is used in therapeutically effective amounts. The physician will determine the dosage of the present therapeutic agents which will be most suitable and it will vary with the form of administration and the particular compound chosen, and furthermore, it will vary with the patient under treatment, the age of the patient, the type of disease or condition being treated. When the composition is administered orally, larger quantities of the active agent will be required to produce the same effect as a smaller quantity given

parenterally. The compounds are useful in the same manner as comparable therapeutic agents and the dosage level is of the same order of magnitude as is generally employed with these other therapeutic agents. Active compounds will typically be administered once or more times a day for example 1, 2, 3 or 4 times daily,  
5 with typical total daily doses in the range of from 0.1 to 1000 mg/kg/day.

The compounds and compositions of this invention may be used with other drugs to provide a combination therapy. The other drugs may form part of the same composition, or be provided as a separate composition for administration at the same time or at different time.

10 Particularly, the combination of at least one compound of formula (I) and at least one another drug may be formulated for its simultaneous, separate or sequential administration, with at least a pharmaceutically acceptable carrier, additive, adjuvant or vehicle. This has the implication that the combination of the compound of formula (I) and the other drug may be administered:

15 a) As a combination that is being part of the same medicament formulation, both being then administered always simultaneously.

b) As a combination of two units, each with one of them giving rise to the possibility of simultaneous, sequential or separate administration. In a particular embodiment, the compound of formula (I) is independently administered from  
20 the other drug (i.e. in two units) but at the same time. In another particular embodiment, the compound of formula (I) is administered first, and then the other drug is separately or sequentially administered. In yet another particular embodiment, the other drug is administered first, and then the compound of formula (I) is administered, separately or sequentially, as defined.

25

In the context of the present invention, the following acronyms and abbreviations have been used, the meaning detailed below:

AcOEt	Ethyl acetate
AcSDKP	<i>N</i> -acetyl-seryl-aspartyl-lysyl-proline
30 AD	Alzheimer's disease
BBB	Blood-Brain Barrier
Boc	<i>tert</i> -Butoxycarbonyl
BSA	Bovine serum albumin

	DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
	DCM	Dichloromethane
	DIEA	<i>N,N'</i> -Diisopropylethylamine
	DMF	Dimethylformamide
5	DMSO	Dimethylsulfoxide
	DPPIV	Dipeptidyl peptidase IV
	EtOH	Ethanol
	Fmoc	9-Fluorenylmethoxycarbonyl
	FPLC	Fast protein liquid chromatography
10	HOAt	1-Hydroxy-7-azabenzotriazole
	IP3	Inositol triphosphate
	IPTG	Isopropyl $\beta$ -D-1-thiogalactopyranoside
	LB	Lysogeny broth
	MALDI-TOF	Matrix-assisted laser desorption/ionization - time-of-flight
15	MK-801	Dizocilpine (INN)
	MS	Multiple sclerosis
	OD	Optical density
	PAMPA	Parallel artificial membrane permeability assay
	PBS	Phosphate buffered saline
20	PC	Phosphatidylcholine
	PE	Phosphatidylethanolamine
	pETM10	Plasmid pETM10
	PI	Phosphatidylinositol
	POP	Prolyl oligopeptidase
25	hPOP	human Prolyl oligopeptidase
	PREP	Prolyl endopeptidase (please note that POP and PREP are synonyms)
	PS	Phosphatidylserine
	PyBOP	(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
	RP-HPLC	Reverse phase high performance liquid chromatography
30	SD	Standard deviation
	SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
	TBTU	O-(Benzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium tetrafluoroborate
	TFA	Trifluoroacetic acid
	THF	Tetrahydrofuran
35	TIS	Triisopropylsilane

Tris	Tris(hydroxymethyl)aminomethane
Tβ4	Thymosin beta-4 protein
Z-G-P-AMC	( <i>N</i> -Benzyloxycarbonyl-Gly-Pro-methylcoumarinyl-7-amide)

- 5 The following examples are merely illustrative of certain embodiments of the invention and cannot be considered as restricting it in any way.

### **EXAMPLES**

#### **SPECIFIC SYNTHETIC CONDITIONS USED FOR THE PREPARATIONS DESCRIBED IN THE EXAMPLES**

- 10 Procedure A: Hydrolysis of ester of formula (II) to the carboxylic acid of formula (VII)

The ester of formula (II) (1 mmol) is solubilized in 95% EtOH. NaOH (3.7 mmol) is added and the reaction is maintained at reflux for approximately 2 hours. Then it is left to reach room temperature. The reaction mixture is concentrated to approx. 15-20 mL and then this solution is slowly added onto a 1M HCl solution, while cooled in an ice bath. A white solid precipitates, which is collected by filtration, washed with water and dried well before the next synthetic step. In the case that no precipitate appears, the resulting solution is extracted with AcOEt (3x), the organic phase is dried and evaporated. The crude product is purified by flash chromatography, if needed.

- 20 Procedure B: Deprotection of a Boc protected amine of formula (III) to yield the amine of formula (VI)

The Boc protected amine of formula (III) (1 mmol) is slowly added onto 4M HCl in dioxane (20 ml) at 0°C. The reaction is stirred at room temperature for 2 hours. The solvent is then evaporated to dryness, to give the hydrochloride salt of the amine of formula (VI).

- 25 Procedure C: Coupling of an amine of formula (VI) to a carboxylic acid of formula (VII) through formation of the carboxylic acid chloride of formula (VIII).

- 30 Oxalyl chloride (1.5 mmol) is added to a solution of the carboxylic acid of formula (VII) (1 mmol) in toluene (5 ml). The reaction is stirred at 50°C for 1.5 hours to allow for the formation of the carboxylic acid chloride of formula (VIII). After evaporation of the solvent, the resulting crude is solubilized in THF and added to an aqueous NaOH solution of the amine of formula (VI) (1.1 mmol) at 0°C. The reaction mixture is stirred at 0°C for 1.5 hours and at room temperature during 3 hours. Then, THF is evaporated

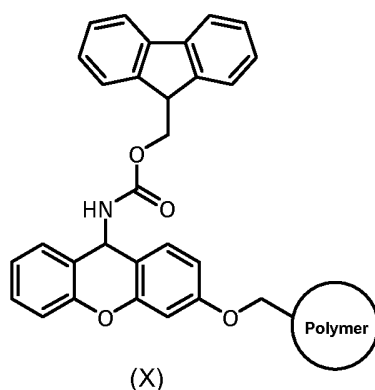
and the remaining aqueous fraction is adjusted to acid pH (3-4) by addition of 1M HCl solution and extracted with AcOEt. The organic phase is washed with brine, dried, filtered and evaporated. The crude product of formula (IX) is purified by flash chromatography when necessary.

- 5 Procedure D: Coupling of the product of formula (IX) to (S)-pyrrolidine-2-carbonitrile of formula (IV) in solution

The product of formula (IX) (1.2 mmol) is dissolved in DCM and added to N-Cyclohexylcarbodiimide, N'-methyl polystyrene (3 mmol), together with DIEA (1 mmol). After 5 min, (S)-pyrrolidine-2-carbonitrile of formula (IV) (1 mmol) and DIEA (1 mmol)  
10 are added. The reaction is stirred at room temperature overnight. Then, the reaction mixture is filtered and the remaining solid is washed with DCM. The filtrate is evaporated to dryness. The crude product is then purified by preparative RP-HPLC.

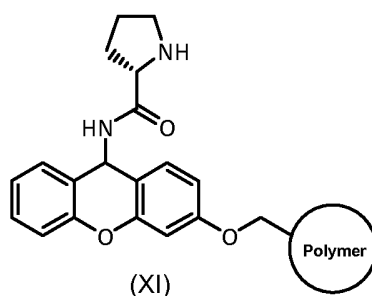
Procedure E: General procedure for synthesis on solid-phase:

Swelling/conditioning of the resin: Sieber amide resin of formula (X) (1 eq) is placed in  
15 a syringe fitted with a polyethylene porous disk. The resin is swelled by washes with DCM and DMF. Removal of the fluorenylmethoxycarbonyl (Fmoc) protecting group is achieved by treatments with a 20% piperidine solution in DMF.



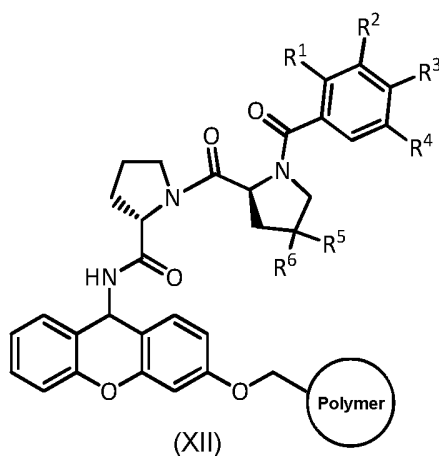
Then, Fmoc-protected L-Proline of formula (V) (4 eq) is attached to the resin using  
20 TBTU (4 eq) and DIEA (8 eq) in DMF. The mixture is intermittently stirred manually during 90 min. After filtration and washing, the extent of the coupling is monitored using the Kaiser test, re-coupling is performed when required. Fmoc is removed to yield product of formula (XI) by a treatment with a 20% piperidine solution in DMF and subsequently with a piperidine/DBU/toluene/DMF (20:5:5:70) solution. Fmoc removal is  
25 assessed using the *p*-nitrophenyl ester NF31 test (described in Maddar, A. et al., *Eur. J. Org. Chem.* **1999**; (11):2787-91).

30



The product of formula (IX) (2 eq) is coupled to the product of formula (XI) to yield the product of formula (XII) using PyBOP (2 eq), HOAt (6 eq) and DIEA (6 eq) in DMF. The mixture is intermittently stirred manually during the total reaction time, 90 min. A systematic re-coupling is done using the same amounts and time. The extent of the coupling is monitored using the *p*-nitrophenyl ester NF31 test.

Alternatively, the product of formula (XIII) (4 eq) is coupled to the product of formula (XI) using PyBOP (4 eq), HOAt (12 eq) and DIEA (12 eq) in DMF. The mixture is intermittently stirred manually during the total reaction time, 90 min. The extent of the coupling is monitored using the *p*-nitrophenyl ester NF31 test, and a re-coupling is done if necessary. The Fmoc group is removed by a treatment with a 20% piperidine solution in DMF and a treatment with a piperidine/DBU/toluene/DMF (20:5:5:70) solution. Subsequently, the product of formula (VII) (4 eq) is incorporated, using PyBOP (4 eq), HOAt (12 eq) and DIEA (12 eq) in DMF, to obtain the product of formula (XII). The mixture is intermittently stirred manually during the total reaction time, 90 min. The extent of the coupling is monitored using the *p*-nitrophenyl ester NF31 test, and a re-coupling is done if necessary.



The product of formula (XII), thoroughly washed with DCM and dried, is transferred to a round bottom flask, and trifluoroacetic anhydride (5 eq) and pyridine (10 eq) in DCM

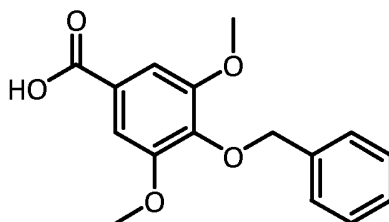
(approx. 2 mL/100 mg) are added. The mixture is kept at room temperature overnight. Then, the reaction mixture is filtered and the resin is washed with DCM. The filtrates are collected and the solvent is evaporated to dryness. The resulting crude is dissolved in AcOEt and washed with saturated NaHCO<sub>3</sub> solution and a 5% aq. KHSO<sub>4</sub> solution.

- 5 The organic phase is dried, filtered, and evaporated. The crude is taken up in H<sub>2</sub>O:CH<sub>3</sub>CN (1:1) and lyophilized to yield the peptide nitrile of formula (I).

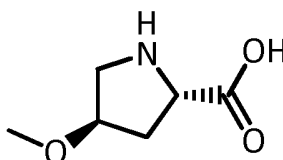
- Alternatively, the peptidyl-resin of formula (XII) may be treated with a mixture of TFA/H<sub>2</sub>O/TIS (95:2.5:2.5, approx. 2-5 mL/100 mg) during 1-2 hours. Then, the resin is filtered and washed with TFA, the filtrates are collected and the solvent is evaporated to dryness. The crude is resuspended in a mixture of H<sub>2</sub>O:CH<sub>3</sub>CN (1:1) and lyophilized. The resulting crude peptide amide is taken up in DCM and trifluoroacetic anhydride (5 eq) and pyridine (10 eq) are added. The mixture is kept at room temperature overnight, the solvent is evaporated and the residue taken up in AcOEt. The organic solution is subsequently washed with aq. 5% KHSO<sub>4</sub> solution and aq. 10% NaHCO<sub>3</sub> solution.
- 10
- 15 Drying and evaporation of the organic phase yield the peptide nitrile of formula (I).

The crude product is purified by RP-HPLC.

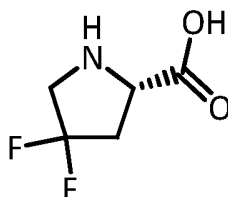


**SYNTHESIS OF INTERMEDIATE COMPOUNDS:****Intermediate 1: 4-benzyloxy-3,5-dimethoxybenzoic acid**

Methyl 3,5-dimethoxy-4-hydroxybenzoate (2.0 g, 9.4 mmol), potassium carbonate (3.2 g, 22.6 mmol) and potassium iodide (500 mg, 3.0 mmol) are introduced in a round-bottom flask. Acetone (200 mL) is added. The reaction is stirred at room temperature for 30 minutes. Then, benzyl chloride (4.3 mL, 37.7 mmol) is added to the reaction mixture and stirring is maintained at reflux during 8 hours. Afterwards, the reaction is left to cool to room temperature. Water is added and three extractions with diethyl ether are performed, the organic extract is washed with brine, dried and evaporated. The crude product is purified by flash chromatography, yielding 1.7 g (5.7 mmol). Subsequently, hydrolysis of the methyl ester is performed following Procedure A described above, to give 4-benzyloxy-3,5-dimethoxybenzoic acid (2.4 g, 7.9 mmol).

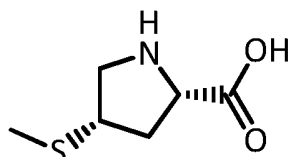
**Intermediate 2: (2S,4R)-4-methoxypyrrolidine-2-carboxylic acid**

Starting from commercially available (2S,4R)-1-(tert-butoxycarbonyl)-4-methoxypyrrolidine-2-carboxylic acid (221 mg, 1.5 mmol), the product is obtained in quantitative yield as the hydrochloride salt following Procedure B described above and used without further purification.

**Intermediate 3: (S)-4,4-difluoropyrrolidine-2-carboxylic acid**

Starting from commercially available (S)-1-(tert-butoxycarbonyl)-4,4-difluoropyrrolidine-2-carboxylic acid (150 mg, 1.0 mmol), the product is obtained in quantitative yield as the hydrochloride salt following Procedure B described above and used without further purification.

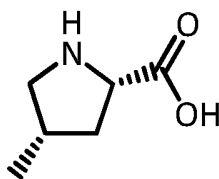
5 **Intermediate 4: (2S,4S)-4-(methylthio)pyrrolidine-2-carboxylic acid**



Starting from commercially available (2S,4S)-1-(tert-butoxycarbonyl)-4-methylthio-pyrrolidine-2-carboxylic acid (310 mg, 1.93 mmol), the product is obtained in quantitative yield as the hydrochloride salt following Procedure B described above and used without further purification.

10

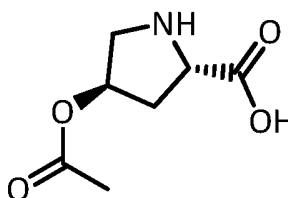
**Intermediate 5: (2S,4S)-4-methylpyrrolidine-2-carboxylic acid**



Starting from commercially available (2S,4S)-1-(tert-butoxycarbonyl)-4-methylpyrrolidine-2-carboxylic acid (500 mg, 2.18 mmol), the product is obtained in quantitative yield as the hydrochloride salt following Procedure B described above and used without further purification.

15

**Intermediate 6: (2S,4R)-4-acetoxypyrrolidine-2-carboxylic acid**

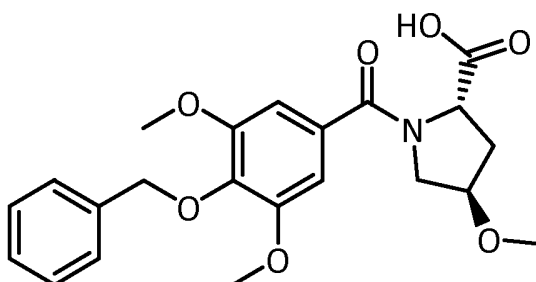


Commercially available *trans*-L-hydroxyproline (500 mg, 3.81 mmol) is dissolved in 6N hydrochloric acid (1 mL). Glacial acetic acid (1 mL) is added, and the solution is cooled to 0°C in an ice bath. Acetyl chloride (10 mL) is then added slowly. After a few minutes, the product is obtained through precipitation, which is helped by addition of ether. The

20

compound (626 mg, 2.98 mmol), in the form of hydrochloride salt, is isolated through filtration, washed with ether, dried and used directly in the next step.

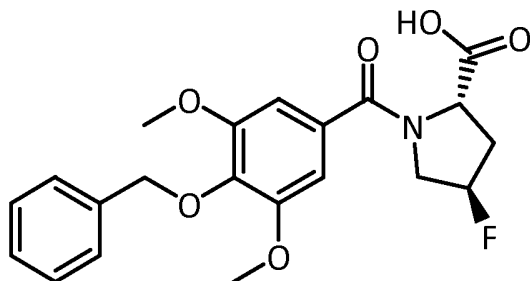
**Intermediate 7: (2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-methoxy-pyrrolidine-2-carboxylic acid**



5

Prepared following Procedure C described above from Intermediate 1 (4-benzyloxy-3,5-dimethoxybenzoic acid) (425 mg, 1.5 mmol) and Intermediate 2 ((2S,4R)-4-methoxypyrrolidine-2-carboxylic acid) (1.5 mmol). Purification by flash chromatography affords the desired product (428 mg, 1.0 mmol).

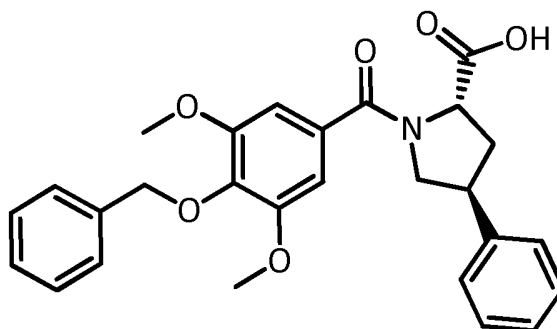
10 **Intermediate 8: (2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-fluoropyrrolidine-2-carboxylic acid**



15 Prepared following Procedure C described above from Intermediate 1 (4-benzyloxy-3,5-dimethoxybenzoic acid) (714 mg, 2.5 mmol) and commercially available (2S,4R)-4-fluoropyrrolidine-2-carboxylic acid (363 mg, 2.7 mmol). Purification by flash chromatography affords the desired product (670 mg, 1.7 mmol).

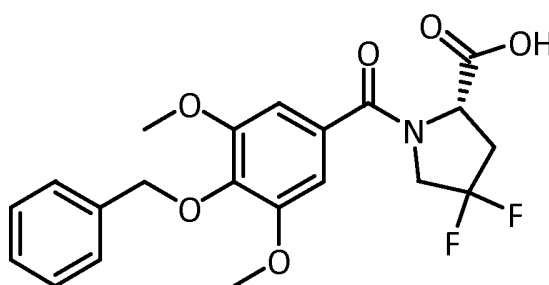
**Intermediate 9: (2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-phenylpyrrolidine-2-carboxylic acid**

35



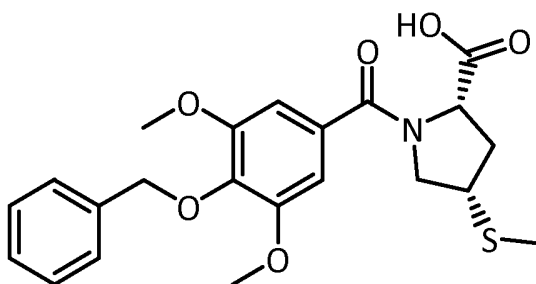
Prepared following Procedure C described above from Intermediate 1 (4-benzyloxy-3,5-dimethoxybenzoic acid) (700 mg, 2.4 mmol) and commercially available (2S,4S)-4-phenylpyrrolidine-2-carboxylic acid (608 mg, 2.7 mmol). Purification by flash chromatography affords the desired product (700 mg, 1.5 mmol).

**Intermediate 10: (S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carboxylic acid**



Prepared following Procedure C described above from Intermediate 1 (4-benzyloxy-3,5-dimethoxybenzoic acid) (260 mg, 0.9 mmol) and Intermediate 3 ((S)-4,4-difluoropyrrolidine-2-carboxylic acid) (1.0 mmol). Purification by flash chromatography affords the desired product (366 mg, 0.9 mmol).

**Intermediate 11: (2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-(methylthio)pyrrolidine-2-carboxylic acid**

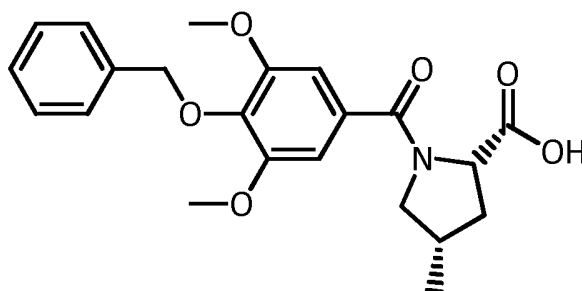


15

Prepared following Procedure C described above from Intermediate 1 (4-benzyloxy-3,5-dimethoxybenzoic acid) (505 mg, 1.75 mmol) and Intermediate 4 ((2S,4S)-4-

(methylthio)pyrrolidine-2-carboxylic acid) (1.93 mmol). Purification by flash chromatography affords the desired product (537 mg, 1.24 mmol).

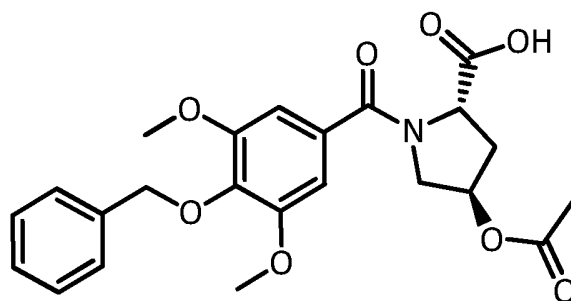
**Intermediate 12: (2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-methylpyrrolidine-2-carboxylic acid**



5

Prepared following Procedure C described above from Intermediate 1 (4-benzyloxy-3,5-dimethoxybenzoic acid) (384 mg, 1.33 mmol) and Intermediate 5 ((2S,4S)-4-methylpyrrolidine-2-carboxylic acid) (1.47 mmol). Purification by flash chromatography affords the desired product (342 mg, 0.85 mmol).

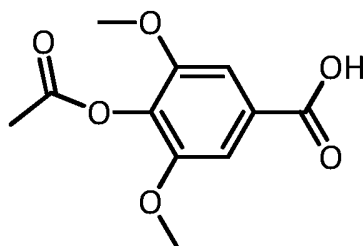
10 **Intermediate 13: (2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-acetoxypyrrolidine-2-carboxylic acid**



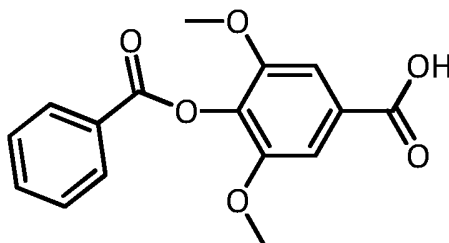
15 Prepared following Procedure C described above from Intermediate 1 (4-benzyloxy-3,5-dimethoxybenzoic acid) (400 mg, 1.39 mmol) and Intermediate 6 ((2S,4R)-4-acetoxypyrrolidine-2-carboxylic acid) (1.53 mmol). Purification by flash chromatography affords the desired product (342 mg, 0.85 mmol).

**Intermediate 14: 4-acetoxy-3,5-dimethoxybenzoic acid**

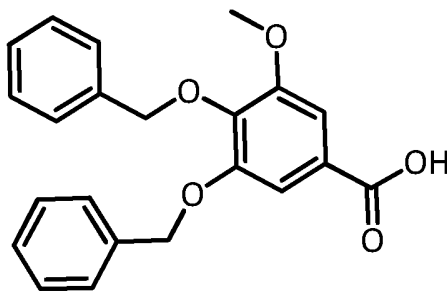
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4-Hydroxy-3,5-dimethoxybenzoic acid (300 mg, 1.51 mmol) is dissolved in pyridine (732  $\mu$ L, 9.08 mmol) at 0°C. Acetic anhydride (214  $\mu$ L, 2.27 mmol) is added dropwise while the mixture is stirred. The ice bath is kept for 2h, after which the mixture is poured  
5 into ice water. The mixture is extracted with DCM (3x), the organic phase is washed with 1N HCl solution (3x), with water and with brine, dried over sodium sulfate, filtered and evaporated, to give 4-acetoxy-3,5-dimethoxybenzoic acid (266 mg, 1.10 mmol).

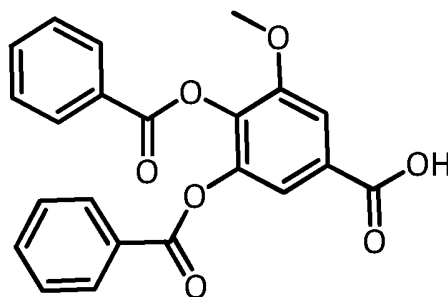
**Intermediate 15: 4-benzoyloxy-3,5-dimethoxybenzoic acid**

4-Hydroxy-3,5-dimethoxybenzoic acid (300 mg, 1.51 mmol) is dissolved in water (6 mL) and then isopropanol (2.5 mL) is added, followed by potassium carbonate (523 mg, 3.78 mmol). The mixture is kept under argon and cooled to 0°C. Then, benzoyl chloride (185  $\mu$ L, 1.59 mmol) is added dropwise to the vigorously stirred reaction mixture. A thick white precipitate is formed during the addition. The mixture is stirred for an  
15 additional 20 min before being quenched with 6M HCl, while keeping the reaction mixture cool. The solid is collected by filtration, washed with cold water and dried to give 4-benzoyloxy-3,5-dimethoxybenzoic acid as a white solid (401 mg, 1.33 mmol).

**Intermediate 16: 3,4-dibenzoyloxy-5-methoxybenzoic acid**

Methyl 3,4-dihydroxy-5-methoxybenzoate (300 mg, 1.51 mmol), potassium carbonate (1.0 g, 7.3 mmol) and potassium iodide (161 mg, 0.97 mmol) are introduced in a round-bottom flask. Acetone (60 mL) is added. The reaction is stirred at room temperature for 30 minutes. Then, benzyl chloride (1.39 mL, 12.1 mmol) is added to the reaction mixture and stirring is maintained at reflux during 8 hours. Afterwards, the reaction is left to cool to room temperature. Water is added and three extractions with diethyl ether are performed, the organic extract is washed with brine, dried and evaporated. The crude product is purified by flash chromatography, yielding 377 mg (1.0 mmol). Subsequently, hydrolysis of the methyl ester is performed following Procedure A described above, to give 3,4-dibenzoyloxy-5-methoxybenzoic acid (144 mg, 0.4 mmol).

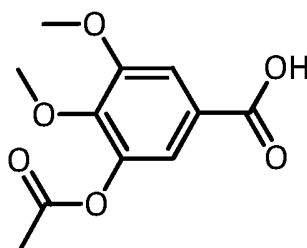
**Intermediate 17: 3,4-dibenzoyloxy-5-methoxybenzoic acid**



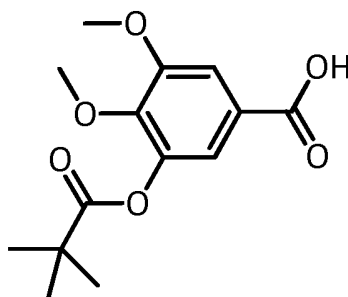
3,4-Dihydroxy-5-methoxybenzoic acid (300 mg, 1.63 mmol) is dissolved in water (6 mL) and then isopropanol (2.5 mL) is added, followed by potassium carbonate (1.13 g, 8.15 mmol). The mixture is kept under argon and cooled to 0°C. Then, benzoyl chloride (388 µL, 3.34 mmol) is added dropwise to the vigorously stirred reaction mixture. The mixture is stirred for an additional 20 min before being quenched with 6M HCl, while keeping the reaction mixture cool. Then, it is diluted with AcOEt and the phases are separated. The organic phase is washed successively with 1M HCl solution and with brine, dried over sodium sulfate, filtered and evaporated. Purification of the crude by flash chromatography gives 3,4-dibenzoyloxy-5-methoxybenzoic acid as a white solid (548 mg, 1.40 mmol).

**Intermediate 18: 3-acetoxy-4,5-dimethoxybenzoic acid**

39



3-Hydroxy-4,5-dimethoxybenzoic acid (300 mg, 1.51 mmol) is dissolved in pyridine (732  $\mu$ L, 9.08 mmol) at 0°C. Acetic anhydride (214  $\mu$ L, 2.27 mmol) is added dropwise while the mixture is stirred. The ice bath is kept for 2h, after which the mixture is poured  
5 into ice water. The mixture is extracted with DCM (3x), the organic phase is washed with 1N HCl solution (3x), with water (2x) and with brine (2x), dried over sodium sulfate, filtered and evaporated to give 3-acetoxy-4,5-dimethoxybenzoic acid (277 mg, 1.15 mmol).

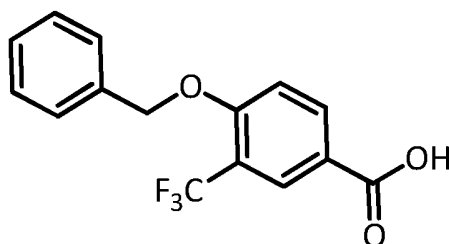
**Intermediate 19: 3-pivaloyloxy-4,5-dimethoxybenzoic acid**

10

A solution of 3-hydroxy-4,5-dimethoxybenzoic acid (300 mg, 1.51 mmol) and pyridine (244  $\mu$ L, 3.02 mmol) in chloroform (2 mL) is stirred for 30 min. To this reaction mixture is added dropwise a solution of pivaloyl chloride (196  $\mu$ L, 1.59 mmol) in chloroform (2 mL) at room temperature, and the reaction is stirred until its completion according to  
15 TLC (around 3h). Then, the reaction mixture is diluted with DCM, a 1M HCl solution is added and the phases are separated. The organic phase is successively washed with 1M HCl solution (2x), with water and with brine, dried over sodium sulfate, filtered and evaporated. Purification by flash chromatography affords 4-pivaloyloxy-3,5-dimethoxybenzoic acid (321 mg, 1.14 mmol).

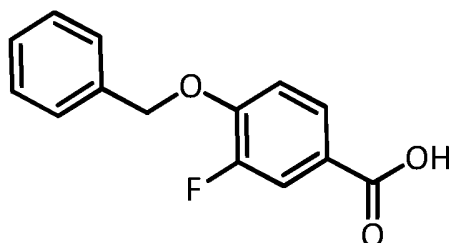
**20 Intermediate 20: 4-benzyloxy-3-trifluoromethylbenzoic acid**





4-hydroxy-3-trifluoromethylbenzoic acid (1.0 g, 4.9 mmol) and potassium carbonate (1.6 g, 11.6 mmol) are introduced in a round-bottom flask. DMF (10 mL) is added and the reaction is stirred at room temperature for 5 minutes. Then, benzyl chloride (2.2 mL, 19.4 mmol) is added to the reaction mixture is maintained at reflux during 4 hours. Afterwards, the reaction is left to cool to room temperature. Water is added and three extractions with ethyl acetate (3x50 mL) are performed, the organic extract is washed with brine, dried and evaporated. The crude product is purified by flash chromatography, yielding 1.3 g (3.4 mmol). Subsequently, hydrolysis of the benzyl ester is performed following Procedure A described above, to give 4-benzyloxy-3-trifluoromethylbenzoic acid (320 mg, 1.1 mmol).

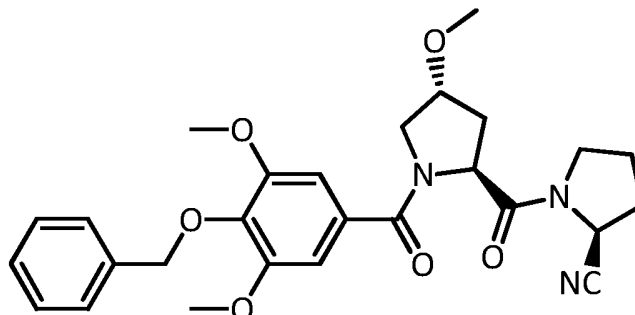
**Intermediate 21: 4-benzyloxy-3-fluorobenzoic acid**



4-hydroxy-3-fluorobenzoic acid (1.0 g, 6.4 mmol), potassium carbonate (2.7 g, 19.2 mmol) and potassium iodide (532 mg, 3.2 mmol) are introduced in a round-bottom flask. Acetone (140 mL) is added and the reaction is stirred at room temperature for 30 minutes. Then, benzyl bromide (3.8 mL, 32.0 mmol) is added to the reaction mixture is maintained at reflux during 12 hours. Afterwards, the reaction is left to cool to room temperature. Water is added and three extractions with ethyl acetate (3x50 mL) are performed, the organic extract is washed with brine, dried and evaporated. The crude product is purified by flash chromatography, yielding 1.2 g (3.5 mmol). Subsequently, hydrolysis of the benzyl ester is performed following Procedure A described above, to give 4-benzyloxy-3-fluoromethylbenzoic acid (612 mg, 2.5 mmol).

**Example 1:**

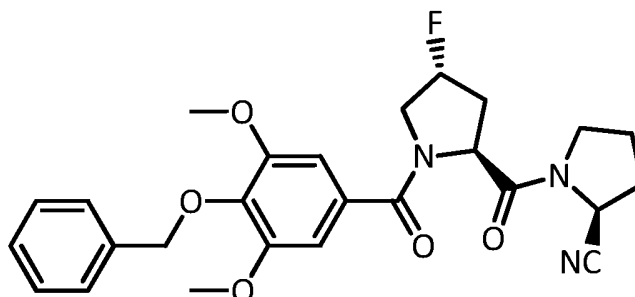
**(S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-methoxypyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



Commercially available (S)-pyrrolidine-2-carbonitrile (58 mg, 0.4 mmol) and  
 5 Intermediate 7 ((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-methoxypyrrolidine-2-carboxylic acid) (220 mg, 0.5 mmol) are coupled following Procedure D described above. Purification by RP-HPLC affords 10 mg (0.02 mmol) of final product.

**Example 2:**

**(S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-fluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



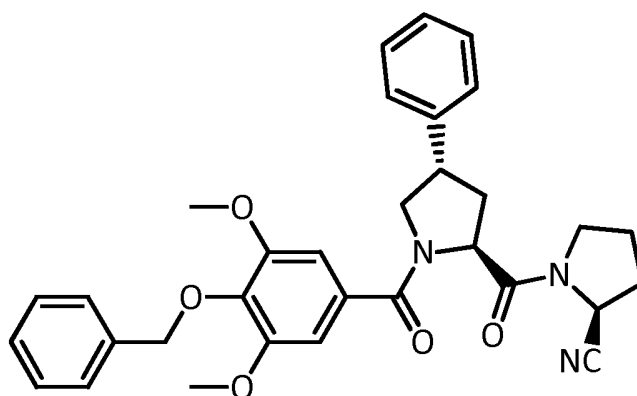
Starting from commercially available Sieber amide resin (500 mg, 0.30 mmol, 1 eq),  
 commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (400 mg, 1.2 mmol)  
 and Intermediate 8 ((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-  
 15 fluoropyrrolidine-2-carboxylic acid) (239 mg, 0.60 mmol), the product is prepared following Procedure E described above. Purification by RP-HPLC affords 80 mg (0.17 mmol) of final product.

**Example 3:**

**(S)-1-((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-phenylpyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**

20

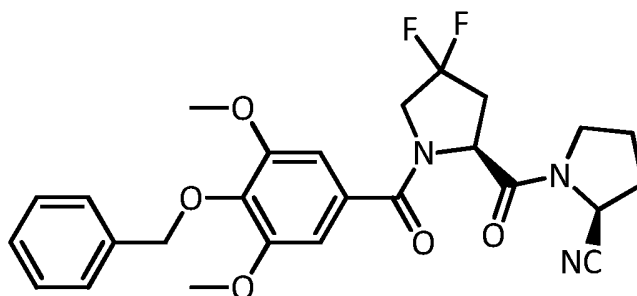
42



Starting from commercially available Sieber amide resin (500 mg, 0.38 mmol, 1 eq), commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (516 mg, 1.53 mmol) and Intermediate 9 ((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-phenylpyrrolidine-2-carboxylic acid) (351 mg, 0.76 mmol), the product is prepared following Procedure E described above. Purification by RP-HPLC affords 16 mg (0.03 mmol) of final product.

**Example 4:**

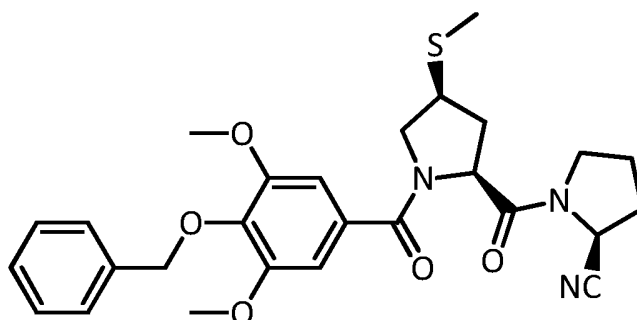
**(S)-1-((2S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



Starting from commercially available Sieber amide resin (250 mg, 0.19 mmol, 1 eq), commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (258 mg, 0.77 mmol) and Intermediate 10 ((S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carboxylic acid) (161 mg, 0.38 mmol), the product is prepared following Procedure E described above. Purification by RP-HPLC affords 18 mg (0.036 mmol) of final product.

**Example 5:**

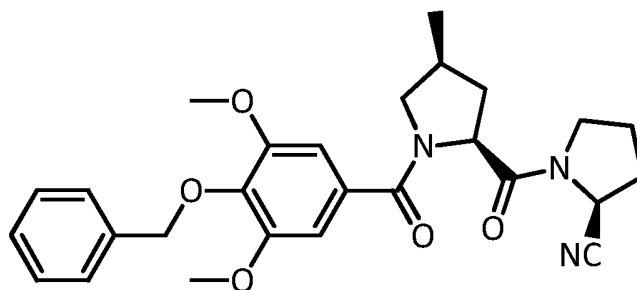
**(S)-1-((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-(methylthio)pyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



Starting from commercially available Sieber amide resin (500 mg, 0.38 mmol, 1 eq), commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (516 mg, 1.53 mmol) and Intermediate 11 ((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-(methylthio)pyrrolidine-2-carboxylic acid) (330 mg, 0.76 mmol), the product is prepared following Procedure E described above. Purification by RP-HPLC affords 22 mg (0.043 mmol) of final product.

#### Example 6:

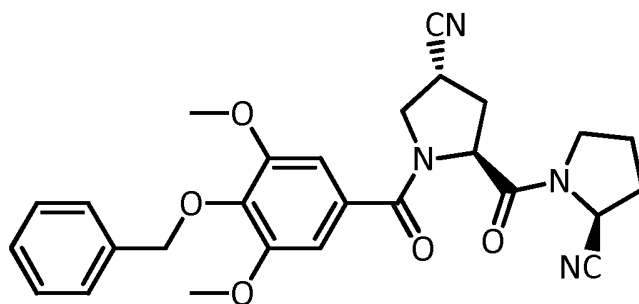
**(S)-1-((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-methylpyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



Starting from commercially available Sieber amide resin (300 mg, 0.18 mmol, 1 eq), commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (247 mg, 0.73 mmol) and Intermediate 12 ((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-methylpyrrolidine-2-carboxylic acid) (146 mg, 0.37 mmol), the product is prepared following Procedure E described above. Purification by RP-HPLC affords 10 mg (0.02 mmol) of final product.

#### Example 7:

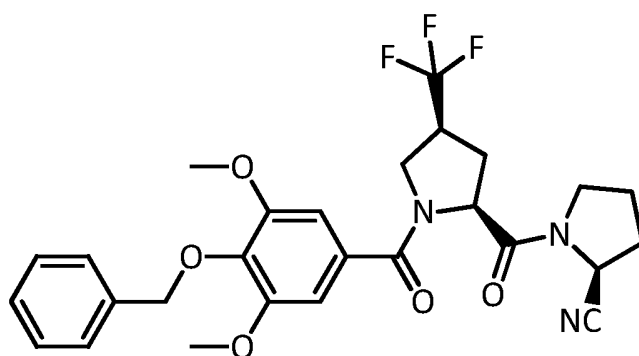
**(S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-cyanopyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



Commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (516 mg, 1.53 mmol) and Boc-trans-4-cyano-L-proline (368 mg, 1.53 mmol) are sequentially coupled onto commercially available Sieber amide resin (500 mg, 0.38 mmol, 1 eq), through stepwise coupling as described in Procedure E above. After cleavage of the dipeptide from the resin, Intermediate 1 (4-benzyloxy-3,5-dimethoxybenzoic acid) (145 mg, 0.5 mmol) is coupled to the resulting nitrile dipeptide following Procedure C, via formation of the carboxylic acid chloride. Purification of the crude by RP-HPLC affords 11 mg (0.03 mmol) of final product.

#### 10 Example 8:

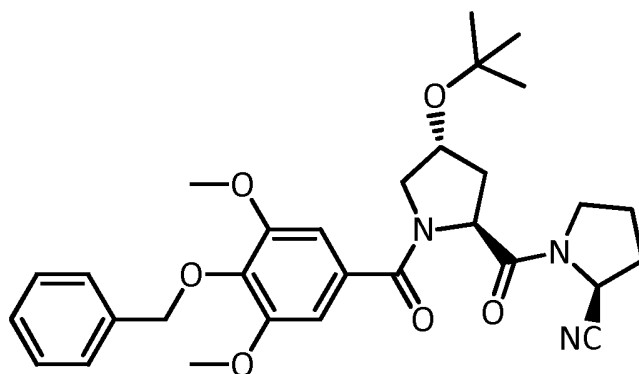
**(S)-1-((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-(trifluoromethyl)pyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



Commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (135 mg, 0.40 mmol), (2S,4S)-Fmoc-4-trifluoromethyl-pyrrolidine-2-carboxylic acid (162 mg, 0.40 mmol), and Intermediate 1 (4-benzyloxy-3,5-dimethoxybenzoic acid) (115 mg, 0.40 mmol) are sequentially coupled onto commercially available Sieber amide resin (165 mg, 0.10 mmol, 1 eq), through stepwise coupling as described in Procedure E above. Purification by RP-HPLC affords 13 mg (0.045 mmol) of final product.

**Example 9:**

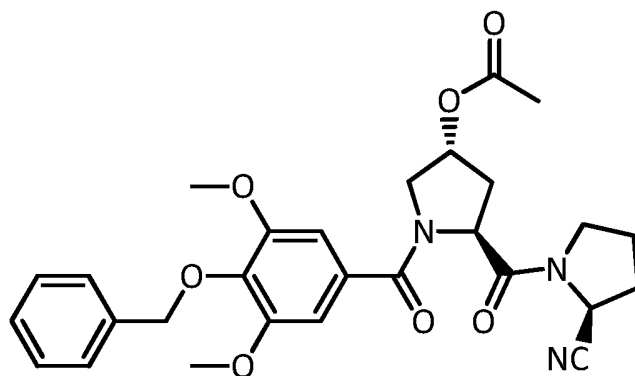
**(S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-(tert-butoxy)pyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



- 5 Commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (135 mg, 0.40 mmol), Fmoc-4-tert-butoxy-L-proline (164 mg, 0.40 mmol), and Intermediate 1 (4-benzyloxy-3,5-dimethoxybenzoic acid) (115 mg, 0.40 mmol) are sequentially coupled onto commercially available Sieber amide resin (165 mg, 0.10 mmol, 1 eq), through stepwise coupling as described in Procedure E above. Purification by RP-HPLC affords  
10 22 mg (0.076 mmol) of final product.

**Example 10:**

**(S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-acetoxypyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**

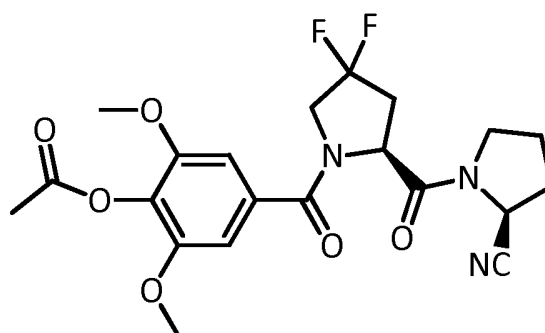


- 15 Starting from commercially available Sieber amide resin (165 mg, 0.10 mmol, 1 eq), commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (135 mg, 0.40 mmol) and Intermediate 13 ((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-

acetoxypyrrolidine-2-carboxylic acid) (90 mg, 0.20 mmol), the product is prepared following Procedure E described above. Purification by RP-HPLC affords 6.2 mg (0.012 mmol) of final product.

**Example 11:**

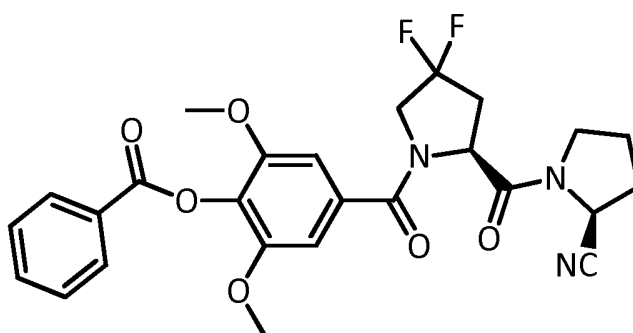
- 5 **(S)-1-((2S)-1-(4-acetoxy-3,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



- 10 Commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (135 mg, 0.40 mmol), Fmoc-4,4-difluoro-L-proline (149 mg, 0.40 mmol) and Intermediate 14 (4-acetoxy-3,5-dimethoxybenzoic acid) (96 mg, 0.40 mmol) are sequentially coupled onto commercially available Sieber amide resin (165 mg, 0.10 mmol, 1 eq), through stepwise coupling as described in Procedure E above. Purification by RP-HPLC affords 25 mg (0.054 mmol) of final product.

**Example 12:**

- 15 **(S)-1-((2S)-1-(4-benzoyloxy-3,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**

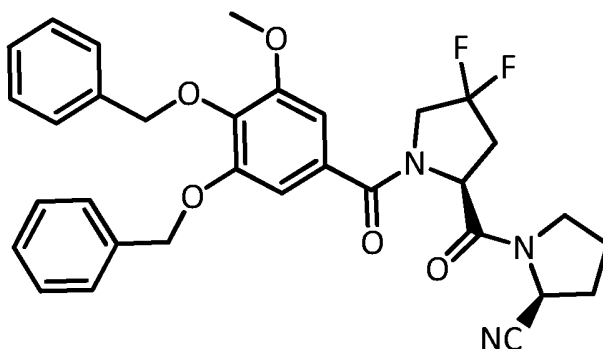


Commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (135 mg, 0.4 mmol), Fmoc-4,4-difluoro-L-proline (149 mg, 0.4 mmol) and Intermediate 15 (4-

benzyloxy-3,5-dimethoxybenzoic acid) (121 mg, 0.4 mmol) are sequentially coupled onto commercially available Sieber amide resin (165 mg, 0.1 mmol, 1 eq), through stepwise coupling as described in Procedure E above. Purification by RP-HPLC affords 26 mg (0.051 mmol) of final product.

5 **Example 13:**

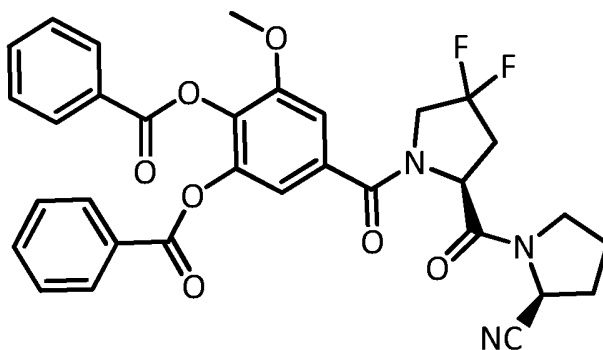
**(S)-1-((2S)-1-(3,4-dibenzyloxy-5-methoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



10 Commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (135 mg, 0.4 mmol), Fmoc-4,4-difluoro-L-proline (149 mg, 0.4 mmol) and Intermediate 16 (3,4-dibenzyloxy-5-methoxybenzoic acid) (146 mg, 0.4 mmol) are sequentially coupled onto commercially available Sieber amide resin (165 mg, 0.1 mmol, 1 eq), through stepwise coupling as described in Procedure E above. Purification by RP-HPLC affords 24 mg (0.041 mmol) of final product.

15 **Example 14:**

**(S)-1-((2S)-1-(3,4-dibenzoyloxy-5-methoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**

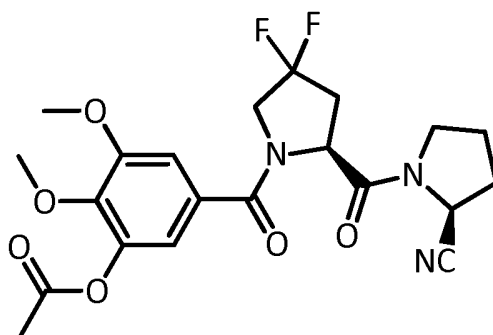




Commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (135 mg, 0.4 mmol), Fmoc-4,4-difluoro-L-proline (149 mg, 0.4 mmol) and Intermediate 17 (3,4-dibenzoyloxy-5-methoxybenzoic acid) (157 mg, 0.4 mmol) are sequentially coupled onto commercially available Sieber amide resin (165 mg, 0.1 mmol, 1 eq), through  
5 stepwise coupling as described in Procedure E above. Purification by RP-HPLC affords 31 mg (0.052 mmol) of final product.

**Example 15:**

**(S)-1-((2S)-1-(3-acetoxy-4,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



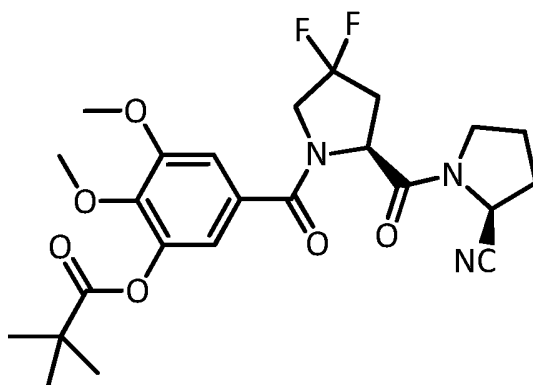
10

Commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (135 mg, 0.4 mmol), Fmoc-4,4-difluoro-L-proline (149 mg, 0.4 mmol) and Intermediate 18 (3-acetoxy-4,5-dimethoxybenzoic acid) (96 mg, 0.4 mmol) are sequentially coupled onto commercially available Sieber amide resin (165 mg, 0.1 mmol, 1 eq), through stepwise  
15 coupling as described in Procedure E above. Purification by RP-HPLC affords 22 mg (0.048 mmol) of final product.

**Example 16:**

**(S)-1-((2S)-1-(3-pivaloyloxy-4,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**

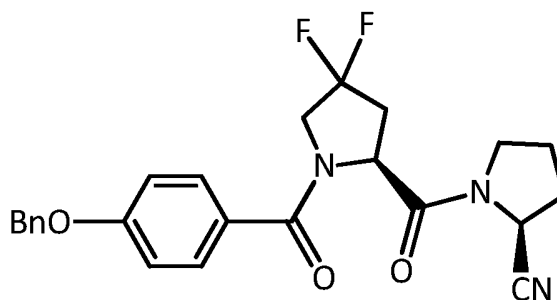
49



Commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (135 mg, 0.4 mmol), Fmoc-4,4-difluoro-L-proline (149 mg, 0.4 mmol) and Intermediate 19 (3-pivaloyloxy-4,5-dimethoxybenzoic acid) (113 mg, 0.4 mmol) are sequentially coupled  
 5 onto commercially available Sieber amide resin (165 mg, 0.1 mmol, 1 eq), through stepwise coupling as described in Procedure E above. Purification by RP-HPLC affords 19 mg (0.040 mmol) of final product.

#### Example 17:

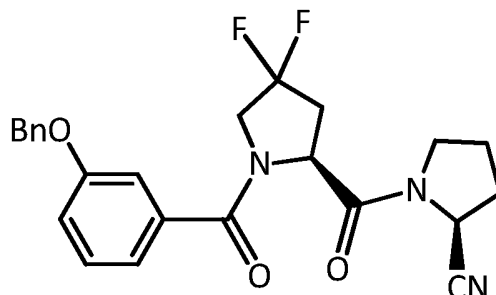
(S)-1-((S)-1-(4-(benzyloxy)benzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-  
 10 2-carbonitrile



Commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (150 mg, 0.45 mmol), Fmoc-4,4-difluoro-L-proline (166 mg, 0.45 mmol) and 4-benzyloxybenzoic acid (101 mg, 0.45 mmol) are sequentially coupled onto commercially available Sieber  
 15 amide resin (200 mg, 0.15 mmol, 1 eq), through stepwise coupling as described in Procedure E above. Purification by RP-HPLC affords 17 mg (0.038 mmol) of final product.

#### Example 18:

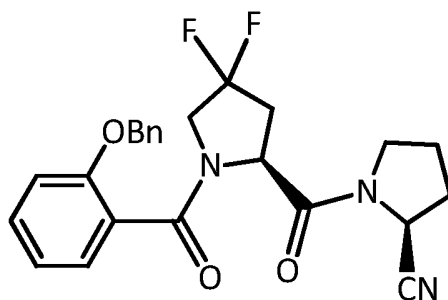
**(S)-1-((S)-1-(3-(benzyloxy)benzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



Commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (150 mg, 0.45 mmol), Fmoc-4,4-difluoro-L-proline (166 mg, 0.45 mmol) and 3-benzyloxybenzoic acid (101 mg, 0.45 mmol) are sequentially coupled onto commercially available Sieber amide resin (200 mg, 0.15 mmol, 1 eq), through stepwise coupling as described in Procedure E above. Purification by RP-HPLC affords 8 mg (0.018 mmol) of final product.

**10 Example 19:**

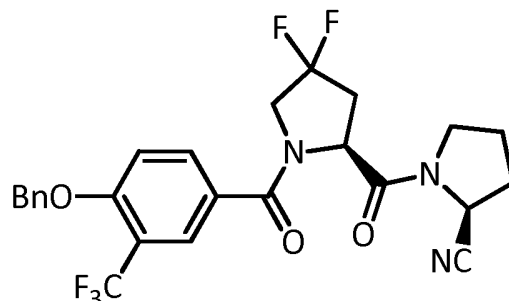
**(S)-1-((S)-1-(2-(benzyloxy)benzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



Commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (150 mg, 0.45 mmol), Fmoc-4,4-difluoro-L-proline (166 mg, 0.45 mmol) and 2-benzyloxybenzoic acid (101 mg, 0.45 mmol) are sequentially coupled onto commercially available Sieber amide resin (200 mg, 0.15 mmol, 1 eq), through stepwise coupling as described in Procedure E above. Purification by RP-HPLC affords 5 mg (0.011 mmol) of final product.

**20 Example 20:**

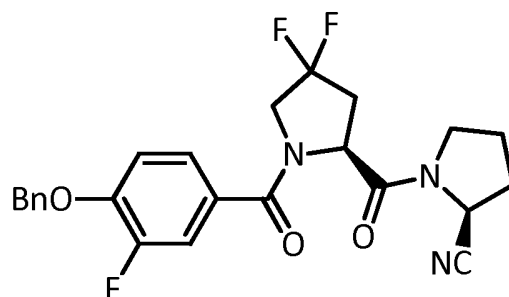
**(S)-1-((S)-1-(4-(benzyloxy)-3-(trifluoromethyl)benzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



Commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (150 mg, 0.45 mmol), Fmoc-4,4-difluoro-L-proline (166 mg, 0.45 mmol) and Intermediate 20 (4-benzyloxy-3-trifluoromethylbenzoic acid) (132 mg, 0.45 mmol) are sequentially coupled onto commercially available Sieber amide resin (200 mg, 0.15 mmol, 1 eq), through stepwise coupling as described in Procedure E above. Purification by RP-HPLC affords 32 mg (0.061 mmol) of final product.

**Example 21:**

**(S)-1-((S)-1-(4-(benzyloxy)-3-fluorobenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile**



Commercially available Fmoc-protected L-Proline (Fmoc-L-Pro-OH) (150 mg, 0.45 mmol), Fmoc-4,4-difluoro-L-proline (166 mg, 0.45 mmol) and Intermediate 21 (4-benzyloxy-3-fluorobenzoic acid) (109 mg, 0.45 mmol) are sequentially coupled onto commercially available Sieber amide resin (200 mg, 0.15 mmol, 1 eq), through stepwise coupling as described in Procedure E above. Purification by RP-HPLC affords 14 mg (0.030 mmol) of final product.

**20 PHARMACOLOGICAL DATA**

## DETERMINATION OF INHIBITORY EFFECT OF NOVEL COMPOUNDS ON (HUMAN) PROLYL OLIGOPEPTIDASE ACTIVITY

### Expression and purification of Prolyl oligopeptidase (POP)

POP was obtained by expression in *E. coli* and affinity purification using a His tail  
5 fusion according to a literature procedure (Tarragó T et al., *ChemBioChem* **2006**;7:827-33) summarized below:

hPOP expression: *E. coli* BL21 competent cells were transformed with pETM10 hPOP. To induce expression, a pre-culture of LB medium (50 mL) containing kanamycin (50 µg/mL) was inoculated with one colony and was grown overnight at 37 °C. Next day,  
10 two cultures of LB medium (500 mL) were inoculated with the overnight culture (10 mL). The inoculated cultures were grown at 37 °C and 220 rpm until the OD<sub>595</sub> was 1.2 (2.5–3 hours). IPTG was then added (final concentration of 1 mM) and induction was performed overnight at 25 °C. Cells were harvested (3500 g, 15 min, 4 °C) and the pellet was suspended in suspension buffer (50 mL) [Tris-HCl pH 8 (50 mM), NaCl (300  
15 mM), imidazole (1 mM)] and sonicated with use of four cycles (each consisting of 15 sec of sonication and 15 sec of rest) at an intensity of 50 % and 0.5 pulses, the sample being kept on ice. After sonication, the sample was centrifuged (40 000 g, 30 min, 4 °C) and the supernatant was used immediately for POP purification. An ÄKTA explorer FPLC system was used for purification. The supernatant was applied at a flow of 1  
20 mL/min to a HiTrapQuelating column (5 mL) previously equilibrated with 5 column volumes of suspension buffer. The column was washed with suspension buffer until the absorbance at 280 nm returned to basal level. The column was then rinsed with 5 volumes of washing buffer (50 mM Tris-HCl, pH 8, 300 mM NaCl, 30 mM imidazole). The elution was performed with 4 volumes of elution buffer (50 mM Tris-HCl, pH 8, 300  
25 mM NaCl, 500 mM imidazole). Fractions (4 mL) were collected during the entire elution. POP activity was checked in all fractions and positive ones were analyzed by SDS-PAGE and stained with Biosafe Comassie Stain G-250. Positive fractions were collected and desalted by use of a HiPrep 26/10 Desalting column with Tris-HCl (50 mM, pH 8) as buffer. Recombinant hPOP was quantified with the Bio-Rad Protein  
30 Assay with BSA as standard. Aliquots of the recombinant enzyme were prepared and immediately frozen with liquid nitrogen and stored at -80 °C.

### POP inhibition assays

POP activity was determined following the method described by Toide *et al* (Toide K et al., *J. Pharmacol. Exp. Ther.* **1995**;274:1370-8), using Z-G-P-AMC (*N*-

benzyloxycarbonyl-Gly-Pro-methylcoumarinyl-7-amide) as POP substrate. The reactions were performed in 96-well microtiter plates, which allowed simultaneous monitoring of multiple reactions. For each reaction, activity buffer (134  $\mu$ l, 100 mM Na/K phosphate buffer, pH 8.0) was pre-incubated for 15 min at 37 °C with hPOP (ranging from 20 to 60 nM, depending on the activity of the hPOP batch) and the corresponding new compound solution (3  $\mu$ l). A stock solution of new compound was prepared in DMSO (100 mM), and dilutions were prepared from this stock solution with DMSO. Alternatively, the reactions were performed using another activity buffer (141  $\mu$ L, 100 mM Tris-acetate, 10 mM BSA, 1mM DTT, pH 7.3), pre-incubating with hPOP (10 nM) and the corresponding new compound solution (3  $\mu$ l) (Conditions B).

After preincubation, Z-G-P-AMC (10  $\mu$ l, 3 mM in 40% 1,4-dioxane) was added (3  $\mu$ l, 1.5 mM in 40% of 1,4-dioxane, in Conditions B), and the reaction was incubated for 1 hour at 37 °C. The reaction was stopped by adding sodium acetate (150  $\mu$ l, 1 M, pH 4) and the formation of AMC was measured fluorimetrically. The excitation and emission wavelengths were 360/40 and 485/20 nm, respectively.

Several concentration points (ranging from 25 pM to 400  $\mu$ M) were measured for each compound. The inhibitory activity on prolyl oligopeptidase was calculated according to eq 1. For each new compound, the fluorescence in the presence (*a*) and in the absence of hPOP (*b*) was measured. The maximum fluorescence (0% inhibitory activity) was obtained from a sample of hPOP in the absence of inhibitory compounds. To estimate the inhibitory potency of the novel compound, activities were plotted against the log concentration of the compound, adjusting to a sigmoid curve using GraphPad Prism software, and the IC<sub>50</sub> value, defined as the concentration of compound required to inhibit 50% of POP activity, was determined from the resulting curve.

$$\text{Inhibitory activity (\%)} = \left[ 1 - \left( \frac{a-b}{c-d} \right) \right] \times 100 \quad (\text{Equation 1})$$

wherein:

*a* corresponds to fluorescence intensity in the presence of substrate + tested compound + hPOP

*b* corresponds to the fluorescence intensity in the presence of substrate + tested compound

*c* corresponds to the fluorescence intensity in the presence of substrate + hPOP

*d* corresponds to the fluorescence intensity of the presence of substrate.

The new compounds exhibit high inhibition potency against human prolyl oligopeptidase. The results are summarized in Table 1.

5 **Table 1:** Inhibition of human prolyl oligopeptidase.

Compound (Example n°)	IC <sub>50</sub> (nM)	SD
1	63.8	11.4
2	60.4	10.6
3	339.0	138.5
4	48.7	20.3
5	175.9	72.0
6	13.1 (*)	7.3
8	176.5	8.3
9	668.1	327.3
11	352	–
12	111	–
13	336	–
15	5600	–
16	2140	–
17	287.3 (*)	135.8
18	487.3 (*)	16.8
19	255.4 (*)	34.2
20	4.5 (*)	0.02
21	5.0 (*)	1.6

(\*) Measured in conditions B

### Inhibitory activity against related proline specific proteases

The inhibitory effect of the new compounds on the activity of dipeptidyl peptidase IV (DPPIV) was tested. The above described procedure for determining the inhibitory activity on prolyl oligopeptidase was followed, using G-P-AMC (H-Gly-Pro-methylcoumarinyl-7-amide) as substrate. After preincubation of DPPIV with the activity buffer and the corresponding compound solution, G-P-AMC (10 µl, 750 µM in 40% 1,4-dioxane) was added, and the reaction was incubated for 20 min at 37 °C. The reaction was stopped by adding sodium acetate (150 µl, 1 M, pH 4) and the formation of AMC was measured fluorimetrically. Several concentration points (ranging from 100 µM to 400 µM) were measured for each compound. The inhibitory activity on DPPIV was calculated according to eq 1. None of the novel compounds showed inhibitory activity against dipeptidyl peptidase IV (IC<sub>50</sub> values over 400 µM), and are therefore specific POP inhibitors.

Additionally, the inhibitory activity of the new compounds against fibroblast activation protein (FAP) was tested. A procedure similar to the one described above for the determination of the inhibitory activity on POP was followed. Z-G-P-AMC was used as a substrate, at a final concentration of 100  $\mu$ M. The buffer used in the assays was 50 mM Tris, 1M NaCl, 1mg/ml BSA pH: 7.5. Recombinant human FAP was used at a stock concentration of 2  $\mu$ g/mL in activity buffer, leading to a final concentration 0.1  $\mu$ g/mL in the assay. Stock solutions of the each new compound were prepared at 20 mM in DMSO and diluted conveniently. After preincubation of FAP with the activity buffer and the corresponding new compound solution at 37°C for 15 min, the substrate (50  $\mu$ L, 100  $\mu$ M in activity buffer) was added, and the reaction was incubated for 1h at 37 °C. The reaction was stopped by adding sodium acetate (150  $\mu$ L, 1 M, pH 4) and the formation of AMC was measured fluorimetrically. Several concentration points (ranging from 100  $\mu$ M to 400  $\mu$ M) were measured for each compound. The inhibitory activity on FAP was calculated according to eq 1. None of the novel compounds showed inhibitory activity against FAP ( $IC_{50}$  values over 400  $\mu$ M), and are therefore specific POP inhibitors.

## DETERMINATION OF PERMEABILITY PROPERTIES OF THE COMPOUNDS

### Parallel Artificial Membrane Permeability Assay (PAMPA)

Parallel artificial membrane permeability assay (PAMPA) described in Kansy M et al., *J. Med. Chem.* **1998**;41(7):1007-10 was used to determine the capacity of compounds to cross the Blood-Brain Barrier (BBB) by passive diffusion (Di L et al., *Eur. J. Med. Chem.* **2003**;38(3):223-32). The effective permeability ( $P_e$ ) of the compounds was measured at an initial concentration of 200  $\mu$ M. The buffer solution was prepared from a commercial concentrated one following the manufacturer's instructions. pH was adjusted to 7.4 using a 0.5 M NaOH solution. A stock solution of new compound was prepared in DMSO and diluted with buffer solution to a final 200  $\mu$ M concentration (0.5% DMSO content). The PAMPA sandwich was separated and each donor well was filled with 200  $\mu$ L of the compound solution. The acceptor plate was placed into the donor plate, ensuring that the underside of the membrane was in contact with buffer. 4  $\mu$ L of the mixture of phospholipids (20 mg/mL) in dodecane was added to the filter of each well, and 200  $\mu$ L of buffer solution was added to the each acceptor well. The plate was covered and incubated at room temperature in a saturated humidity atmosphere for 4 hours under orbital agitation at 100 rpm. After 4 hours, the contents of the



acceptor and donor compartments were analyzed by HPLC: 150  $\mu$ L of each well from the donor plate and 150  $\mu$ L of each well from the acceptor plate were transferred to HPLC vials, injecting each sample into a reverse-phase C<sub>18</sub> column (150 mm x 4.6 mm x 5  $\mu$ m, 100 Å) (100  $\mu$ L/injection from the acceptor wells, 10  $\mu$ L/injection from the donor wells and for  $t_0$  references). Transport was also confirmed by MALDI-TOF spectrometry.

The phospholipid mixture used was a porcine polar brain lipid extract, provided by Avanti polar lipids, with the following composition: 12.6% phosphatidylcholine (PC), 33.1% phosphatidylethanolamine (PE), 18.5% phosphatidylserine (PS), 4.1% phosphatidylinositol (PI), 0.8% phosphatidic acid and 30.9% of other compounds.

The effective permeability ( $P_e$ ) after 4 hours was calculated using equation 2 and the percentage of transport was calculated using equation 3:

$$P_e = \frac{-218.3}{t} \times \log \left[ 1 - \frac{2C_A(t)}{C_D(t_0)} \right] \times 10^{-6} \text{ cm/s} \quad (\text{Equation 2})$$

$$T\% = \frac{C_A(t)}{C_D(t_0)} \times 100 \quad (\text{Equation 3})$$

wherein:

$t$  is time (h)

$C_A(t)$  is the compound concentration in the acceptor well at time  $t$

and  $C_D(t_0)$  is the compound concentration in the donor well at  $t_0$ .

Based on the indicative  $P_e$  values shown in Table 2, the novel compounds show good permeability across the BBB (Table 3)

**Table 2:** Indicative  $P_e$  values

Indicative $P_e$ values (cm/s)	Transport inside CNS
$P_e \geq 4 \cdot 10^{-6}$	Good
$2 \cdot 10^{-6} \leq P_e < 4 \cdot 10^{-6}$	Questionable
$P_e < 2 \cdot 10^{-6}$	Bad

**Table 3:** Effective permeability ( $P_e$ ) and percentage of transport of the new compounds

Compound (Example N°)	$P_e$ ( $\times 10^{-6}$ cm/s)	SD	%T	SD
1	1.00	0.1	4.3	--
2	7.28	2.75	13.03	4.39
3	2.89	0.84	5.72	1.58
4	22.14	5.86	29.92	4.80
5	9.98	3.07	17.03	4.25
6	3.79	0.16	14.76	0.59
8	5.07	0.30	19.27	1.00
12	2.10	0.02	8.47	0.06
16	5.02	0.30	19.08	1.01
17	8.04	3.13	14.12	4.69
18	6.51	1.79	11.91	2.88
19	10.29	1.50	17.55	2.03
20	1.29	0.66	2.64	1.33
21	5.57	1.25	10.43	2.06

### EFFECT OF THE NEW COMPOUNDS ON LEARNING AND MEMORY IN A COGNITION IMPAIRMENT ANIMAL MODEL

The new compounds were evaluated for their efficacy as cognition enhancers in a pharmacological model for cognitive impairment. The effects of the new compounds were evaluated in untreated and MK-801-treated rodents (mice or rats). MK-801 is a non-competitive antagonist of the *N*-Methyl-D-aspartate (NMDA) receptor which impairs animal performance in various learning and memory paradigms (Castellano C et al., *Curr. Drug Targets* **2001**;2:273–83.; Riedel G et al., *Behav. Brain Res.* **2003**;140:1–47). MK-801 also produces various effects on rodent behavior, including deficits in sensory processing, hypermotility, stereotypy and ataxia. The behavioral phenotype induced by MK-801 treatment has been widely used as animal model of cognitive deficits (Bardgett ME et al., *Brain Res. Bull.* **2003**;60:131–42; Van der Staay FJ et al., *Behav. Brain Res.* **2011**;220:215–29; Mutlu O et al., *Pharmacol. Biochem. Behav.* **2011**;99:557–65).

In order to determine whether the tested compounds act as cognitive enhancer, their ability to restore normal cognitive behavior was tested through widely used tests such as the novel object recognition test (Dere E et al., *Neurosci. Biobehav. Rev.* **2007**;31:673–704; Boess FG et al., *J. Pharmacol. Exp. Ther.* **2007**;321:716–25); the passive or inhibitory avoidance task (Sarter M et al., *Psychopharmacology (Berl)*

1992;107:144–59); the Morris water maze (D’Hooge R et al., *Brain Res. Rev.* **2001**;36:60–90); and the T-maze alternation task (Boess FG et al., *Neuropharmacology* **2004**;47:1081–92; Spowart-Manning L et al., *Behav. Brain Res.* **2004**;151:37–46).

- 5 As a representative example for the evaluation of the new POP inhibitors, the protocol followed for each of the behavioral tests, as well as the results obtained in the object recognition test and the passive avoidance test are described.

### Novel object recognition task

- The **novel object recognition (NOR) task** is based on the natural preference of rodents to explore novel objects (Ennaceur A et al., *Behav. Brain Res.* **1988**;31:47–59). It is a relevant non-rewarded test for studying visual learning and memory deficits. Briefly, the NOR task procedure consisted of three trials: habituation, training and retention. Each animal was habituated to a 40-cm diameter circular arena for 10 min in the absence of objects (habituation session). The following day, the animal was placed for 10 min in the circular arena for the training trial, and two identical objects were placed in a symmetrical position. This step was done for two consecutive days. On the third day, one of the objects was replaced by a different object. The object not used in the training trial was used as the novel object in the retention trial. The animals were then allowed to explore freely for 10 min, and the time spent exploring each object was recorded. The animal is expected to spend more time exploring the novel object, which is a sign of intact recognition memory. An index of discrimination was calculated as follows: time spent exploring the new object *minus* time spent exploring the old object, divided by the total time exploring both objects, and multiplied by 100. A higher index of discrimination was considered to reflect greater memory retention.
- 25 The corresponding tested POP inhibitor, freshly dissolved in 5% Tween 80 in PBS, was given subcutaneously (s.c.) at a dose of 5 mg/Kg, in a volume of 0.1 mL per 10 g of animal body weight. Fifteen minutes later, MK-801 dissolved in PBS buffer was injected intraperitoneally (i.p.) at a dose of 0.2 mg/kg, in a volume of 0.1 mL per 10 g of animal body weight. A control group was administered i.p. with MK-801 and s.c. with the same volume of vehicle (PBS with a 5% of Tween 80). Another control group received PBS i.p. and the same volume of vehicle s.c. Drug doses were selected according to behavioral and neurochemical studies, showing that the drugs have the intended effect.
- 30

Animals were injected with the two drugs every day during the training period as well as prior to the test session.

The results obtained when administering the compound of Example 4, as a representative of the compounds of the invention, are shown in Figure 1.

- 5 As illustrated with the compound of Example 4, the compounds of the invention are able to reverse MK-801-induced memory impairment in the NOR test.

### Passive avoidance task

- For the evaluation of the **passive avoidance task**, a two-compartment box with a light compartment and a dark compartment of the same dimensions was used. The two compartments were separated by a guillotine door that could be raised. The apparatus used was according to standard procedures for this test. One shock session and an evaluation session were given, separated by intersession intervals of 24 h. In the shock session, the rodent was placed in the light compartment. After an accommodation period of 20 s, the guillotine door to the other compartment was opened and lowered once again, once the rodent had entered the dark compartment. Then, a short and weak foot shock was administered. The rodent was removed from the apparatus 60 s after shock termination and put back into its home cage. In the evaluation session, the time which the animal takes to enter the dark compartment (in seconds) was measured, as a sign of memory retention of the shock received in the dark compartment during the previous session. A second evaluation session was performed one week after the initial shock session. The corresponding evaluated compound was injected s.c. 35 min before the shock session, followed 15 minutes later by i.p. injection of MK-801, or PBS in the case of the control, in the same doses and volume as described for the object recognition test. The animals treated with MK-801 alone showed little retention of the memory of the shock session, while the animals which had additionally received a POP inhibitor showed a larger latency to enter the dark compartment, indicative of better memory retention.

The results obtained when administering the compound of Example 4, as a representative of the compounds of the invention, are shown in Figure 2.

- 30 As illustrated with the compound of Example 4, the compounds of the invention are able to reverse MK-801-induced memory impairment in the Passive avoidance test.

**Water maze (Morris escape test)**

Morris water escape performance was assessed in a water tank, according to standard procedures and dimensions for this test, filled with tap water stained with latex at a temperature of approximately 22°C. The escape platform consisted of a gray polyethylene cylinder, submerged 1.5 cm below the surface of the water. The corresponding evaluated POP inhibitor was administered s.c. 35 min before training and test sessions, followed after 15 min by i.p. injection of MK-801, or PBS in the case of the control, in the same dose and volume as described for the object recognition test. Animals were injected with the two compounds every day during the training sessions, as well as on evaluation sessions.

The rodents received two sets of training sessions during three consecutive days, with an interval of two-days between the two sets. Each training session consisted of two sets of three trials, which were run in close succession. A trial was started by placing the rodent into the pool, facing the wall of the tank. Four starting positions (north, east, south, and west) were used in randomized order. The escape platform was always in the same quadrant. A trial was terminated as soon as the animal had climbed onto the escape platform or when 60 s had elapsed, whichever event occurred first. Once rodents reached the platform, they were allowed to stay for 30 s in order to allow them to associate the escape platform with a specific position on the tank. Then it was taken from the platform and the next trial was started. If an animal did not find the platform within 60 s, it was put on the platform by the experimenter and was allowed to stay there for 30 s. During the first training session a visual clue was placed to mark the position of the platform. This clue was removed for the following sessions. During the training sessions the latency to reach the platform was recorded.

The day after the second set of training sessions was finished, an evaluation was performed: the platform was removed, and the time the rodent spent in the quadrant of the pool where the platform had been positioned during the training sessions (target quadrant) was measured during 60 s. In the probe trial, all animals were released from the same start position, opposite to the target quadrant. Animals treated with MK-801 were not able to effectively learn and remember where the platform stood, as shown by longer swum distances and escape latency, as well as the time these animals spent on the target quadrant, which was about average compared to the other quadrants. Animals which were treated with MK-801 and with the corresponding POP inhibitor showed a better performance on the test, learning the position of the platform (as

reflected on higher percentage of time spent in the target quadrant), thus showing that the effect of MK-801 was effectively reversed. The animals were left to rest for a week and trained afterwards for 4 additional days. On the fifth day the platform was removed and a second evaluation was performed.

## 5 T-maze alternation task

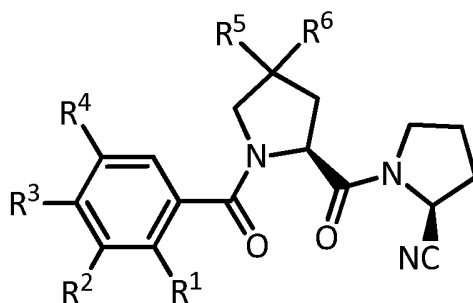
Working memory was tested using a T-maze alternation task. The experiments were performed in a T-maze constructed of wood and painted black, according to general dimensions and procedure. The side alleys were closed off from the main alley by movable doors. A week before habituation, all animals were partially food restricted and remained that way throughout the remaining part of the experiment, in order to keep the animals at 85% of their free-feeding body weight. A video camera was situated ~1 m above the T-maze to videotape the test session. The T-maze was cleaned between different animals but not between different trials. The full experiment consisted of three parts: habituation, training, and testing. During habituation, all animals were placed on the T-maze until they ate two pieces of food or 90 s had elapsed. This was repeated three times a day for 5 d. During training, all animals received six trials a day per day. Each trial consisted of two runs: a forced run and a free run. On the forced run, rodents were forced to obtain a piece of food from one goal arm of the T-maze, with the other goal arm blocked by its door. Animals were then placed back into the start arm for 10 s delay period. At the beginning of the free run, the animals were allowed to choose either goal arm. If the animals chose the arm opposite to the one they had been forced into during the forced run, they received the food reward. If the animals chose the same arm into which they had been forced, they received no food reward. There was a 5 min inter-trial interval. The training period ended after control animals made >70% correct choices on 2 consecutive days. Animals took 7–12 days to reach the criterion. Animals that did not reach the criterion by 14 days were rejected from the study. Rodents were then tested for their performance at 10 or 40 s delay periods. Animals were given three 10 s delay and three 40 s delay trials during the day of testing. For drug testing, rodents were given six 10 s delay trials 15 min after drug exposure. The sequence of delays and forced-run food locations (either left or right) were randomized each day, as long as the same delay or the same forced-arm location was not used for three trials in a row. Goal entries were defined as placing four paws in the arm.

The corresponding evaluated POP inhibitor was injected s.c. 35 min before the test session, followed 15 minutes later by i.p. injection of MK-801, or PBS in the case of the control, in the same doses and volume as described for the object recognition test.

Control animals showed learning curves with near-chance level performance (around  
5 50% of correct arm entries) between days 1 and 4 of training, and a gradual improvement between days 11 and 14 of the training until reaching a plateau of 70% correct arm entries. Their performance remained stable at 10 and 40 s delay trials. Animals treated with MK-801 were not able to effectively learn the alternation task and performed below chance level in the delay trials. Animals which were treated with MK-  
10 801 and with the corresponding POP inhibitor showed a better performance on the test, with similar learning curves as the control animals and retaining memory in the delay trials, thus showing that the effect of MK-801 was effectively reversed.

## CLAIMS

1. A compound of the formula (I)



(I)

wherein

- 5     R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are independently selected from the group consisting of C<sub>1-4</sub>alkoxy, C<sub>1-4</sub>alkylcarbonyloxy, benzyloxy, phenylcarbonyloxy, naphthylcarbonyloxy, quinolinylcarbonyloxy, isoquinolinylcarbonyloxy, trifluoromethyl, halogen and hydrogen;
- R<sup>5</sup> is selected from the group consisting of halogen, nitrile, C<sub>1-4</sub>alkoxy, C<sub>1-4</sub>alkylthio, C<sub>1-4</sub>alkyl, phenyl, phenoxy, phenylthio and trifluoromethyl;
- 10     R<sup>6</sup> is selected from the group consisting of hydrogen, fluor and methyl;
- or a pharmaceutically acceptable salt, isomer, prodrug or solvate thereof.
2. Compound according to claim 1 wherein R<sup>2</sup> and R<sup>4</sup> are independently selected from the group consisting of hydrogen, halogen, trifluoromethyl and C<sub>1-4</sub>alkoxy
- 15     3. Compound according to claim 2, wherein R<sup>2</sup> is selected from the group consisting of hydrogen and methoxy and R<sup>4</sup> is selected from the group consisting of fluor, trifluoromethyl and methoxy.
4. Compound according to any of claims 1 to 3, wherein R<sup>3</sup> is a benzyloxy.
5. Compound according to any of claims 1 to 4, wherein R<sup>5</sup> is fluor.
- 20     6. Compound according to any of claims 1 to 5, wherein R<sup>6</sup> is hydrogen or fluor.
7. Compound according to claim 1, wherein said compound is selected from:
- (S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-methoxypyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile



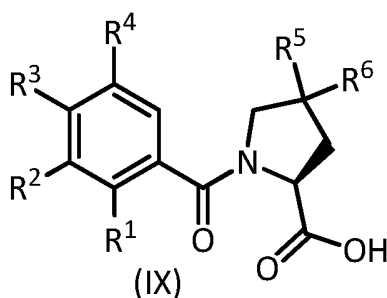
- (S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-fluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-phenylpyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- 5      • (S)-1-((2S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-(methylthio)pyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- 10      • (S)-1-((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-methylpyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-cyanopyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S,4S)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-(trifluoromethyl)-pyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- 15      • (S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-(tert-butoxy)pyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S,4R)-1-(4-(benzyloxy)-3,5-dimethoxybenzoyl)-4-acetoxypyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- 20      • (S)-1-((2S)-1-(4-acetoxy-3,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S)-1-(4-benzoyloxy-3,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S)-1-(3,4-dibenzyloxy-5-methoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- 25      • (S)-1-((2S)-1-(3,4-dibenzoyloxy-5-methoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((2S)-1-(3-acetoxy-4,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile

- (S)-1-((2S)-1-(3-pivaloyloxy-4,5-dimethoxybenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((S)-1-(4-(benzyloxy)benzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- 5      • (S)-1-((S)-1-(3-(benzyloxy)benzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((S)-1-(2-(benzyloxy)benzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((S)-1-(4-(benzyloxy)-3-(trifluoromethyl)benzoyl)-4,4-
- 10      difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile
- (S)-1-((S)-1-(4-(benzyloxy)-3-fluorobenzoyl)-4,4-difluoropyrrolidine-2-carbonyl)pyrrolidine-2-carbonitrile

or a pharmaceutically acceptable salt, isomer, prodrug or solvate thereof.

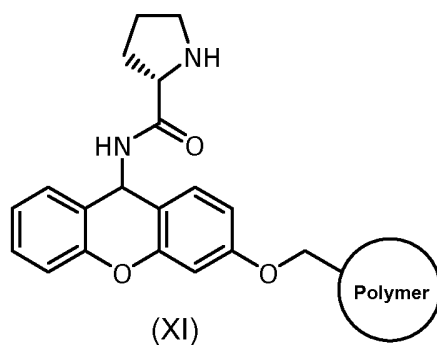
8. A process for the preparation of a compound of formula (I) as defined in any of  
 15      claims 1 to 7 or a pharmaceutically acceptable salt, isomer, prodrug or solvate thereof, which comprises

a) reacting a compound of formula (IX):

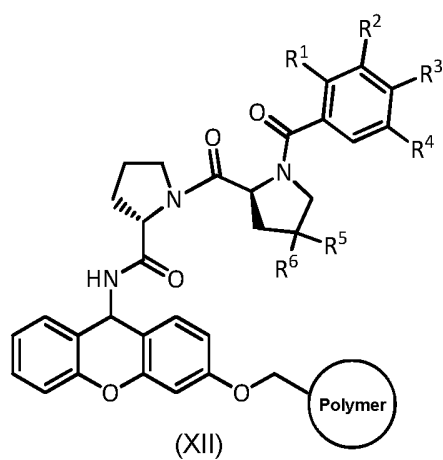


- 20      in which R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>6</sup> are as defined above in formula (I), with a compound of formula (XI):

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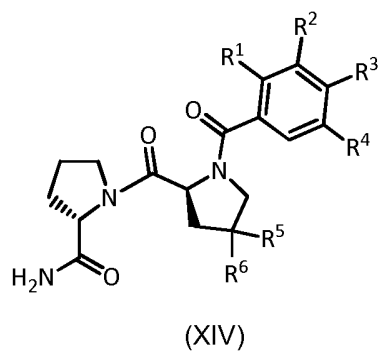


to yield a compound of formula (XII);



b) hydrolising the compound of formula (XII) to yield the compound of formula (XIV)

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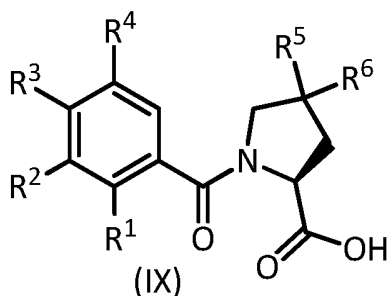
and

c) subjecting the compound of formula (XIV) to conditions capable of transforming a carboxamide group into a nitrile group to yield the compound of formula (I);

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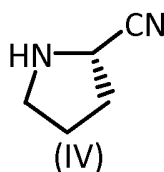
wherein steps b) and c) may be performed separately or in a one pot reaction.

9. A process for the preparation of a compound of formula (I) as defined in any of claims 1 to 7 or a pharmaceutically acceptable salt, isomer, prodrug or solvate thereof, which comprises reacting a compound of formula (IX):



5

in which  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$  and  $R^6$  are as defined above in formula (I), with a compound of formula (IV)



- 10 10. A pharmaceutical composition comprising at least one compound of formula (I) as defined in any of claims 1 to 7, or a pharmaceutically acceptable salt, isomer, prodrug or solvate thereof and a pharmaceutically acceptable carrier, adjuvant or vehicle.
11. A compound of formula (I) as defined in any of claims 1 to 7 for use as a medicament.
- 15 12. A compound of formula (I) as defined in any of claims 1 to 7 for use in the treatment and/or prophylaxis of a cognitive disorder.
13. A compound for use as defined in claim 12 wherein the cognitive disorder is a cognitive disorder associated with a disease selected from the group consisting of schizophrenia, bipolar affective disorder, Alzheimer's disease and Parkinson's disease.
- 20 14. A method for the treatment or prophylaxis of cognitive disorders in a mammal wherein a therapeutic amount of a compound as defined in any of claim 1 to 7 is administered to a patient in need of said treatment.

15. A method as defined in claim 14 wherein the cognitive disorder is a cognitive disorder associated with a disease selected from the group consisting of schizophrenia, bipolar affective disorder, Alzheimer's disease and Parkinson's disease.
- 5 16. Use of a compound of formula (I) as defined in any of claims 1 to 7 for the manufacture of a medicament.
17. Use of a compound of formula (I) as defined in any of claims 1 to 7 for the manufacture of a medicament for the treatment and/or prophylaxis of a cognitive disorder.
- 10 18. Use as defined in claim 17 wherein the cognitive disorder is a cognitive disorder associated with a disease selected from the group consisting of schizophrenia, bipolar affective disorder, Alzheimer's disease and Parkinson's disease.

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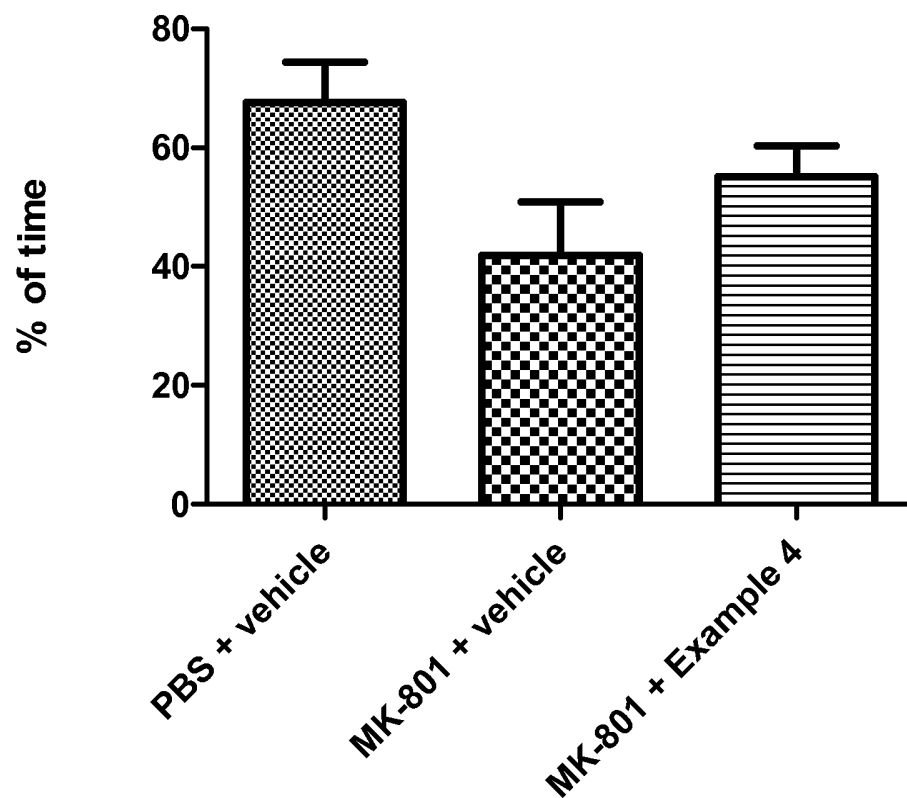


FIGURE 1

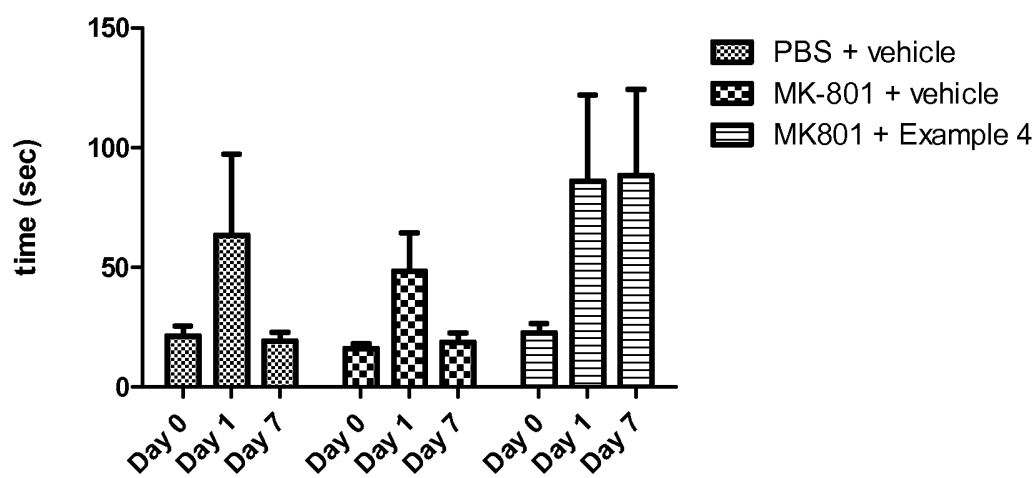


FIGURE 2

# INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/073460

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C07D403/06 A61K31/401 A61P25/00  
ADD.

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C07D A61K A61P

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

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

EPO-Internal, WPI Data, CHEM ABS Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 03/004468 A1 (ORION CORP [FI]; GYNTER JUKKA [FI]; MAENNISTOE PEKKA [FI]; WALLEN ERI) 16 January 2003 (2003-01-16) cited in the application the whole document	1-18
A	WO 2004/060862 A2 (ORION CORP [FI]; GYNTER JUKKA [FI]; WALLEN ERIK [FI]; JARHO ELINA [FI]) 22 July 2004 (2004-07-22) cited in the application the whole document	1-18



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents :

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

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

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

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

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Date of the actual completion of the international search

6 December 2013

Date of mailing of the international search report

16/12/2013

Name and mailing address of the ISA/

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Hacking, Michiel

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2013/073460

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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