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(54) **Title:** LABELLED CHROMONE DERIVATIVES

(57) **Abstract:** The present invention relates to compounds having selective binding for MAO-B as compared with MAO-A. The invention also provides radioactive versions of these compounds, and precursor compounds for the synthesis of these radioactive compounds. The radioactive compounds of the invention are particularly useful for in vivo imaging applications.



WO 2016/097345 A1

LABELLED CHROMONE DERIVATIVES

Technical Field of the Invention

The present invention concerns compounds that target monoamine oxidase B (MAO-B). Specifically, the compound of the invention is a chromone derivative. A labelled chromone derivative is also provided having use as an *in vivo* imaging agent for MAO-B. The present invention also provides a precursor compound useful in the synthesis of this *in vivo* imaging agent, as well as a method for synthesis of said precursor compound. Other aspects of the invention include a method for the synthesis of the *in vivo* imaging agent comprising use of the precursor compound, a kit for carrying out said method, and a cassette for carrying out an automated version of said method. In addition, the invention provides a radiopharmaceutical composition comprising the *in vivo* imaging agent, as well as methods for the use of said *in vivo* imaging agent.

Description of Related Art

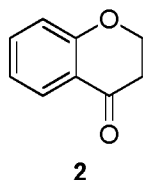
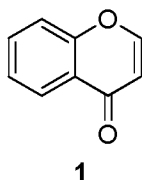
Monoamine oxidase B (MAO-B) is found in the brain primarily in nonneuronal cells such as astrocytes and radial glia (Westlund et al. (1988) Neuroscience 25: 20 439-456; Westlund et al. (1985) Science 230: 181-183; Levitt et al. (1982) Proc. Natl. Acad. Sci., USA, 79: 6385-6389). Its levels are known to increase with age and in association with neurodegenerative disease in both humans and mice (Saura et al. (1994) J Neural Transm Suppl 41: 89-94; Fowler et al. (1980) J Neural Transm 49: 1-20; Riederer et al. (1987) Adv Neurol 45: 111-118; Gerlach et al. (1996) Neurology 47: S137-145).

MAO-B activity levels have been found to be doubled in the substantia nigra in Parkinson's disease, and to correlate with the percentage of dopaminergic substantia nigra cell loss (Damier et al. (1996) Neurology 46: 1262-1269). Activated MAO-B has also been demonstrated in the brain of Alzheimer patients (Gulyás et al. Neurochem Int. 2011; 58(1): 60-8).

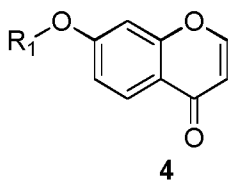
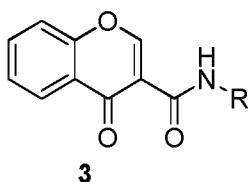
A number of radiolabeled compounds have been reported in literature such as [¹¹C] SL25.1188 and [¹⁸F]Fluororasagiline that are used for imaging MAO-B. They are selective for MAO-B over MAO-A. These known compounds have IC₅₀ values in the range of 11-27 nM and selectivity for MAO-B of ~100 (Nag *et al* Bioorg Med Chem 2012; 20: 3065-3071; Saba *et al* Synapse 2010; 64:61-69).

Chromone **1** below has been described as a scaffold for MAO inhibitors with some

analogues being particularly selective for MAO-B (Gaspar *et al* 2011 J Med Chem; 54: 5165; Legoabe *et al* 2012 Eur J Med Chem; 49: 343-5). This scaffold is similar to the flavones **2** below:

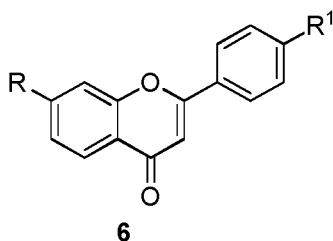
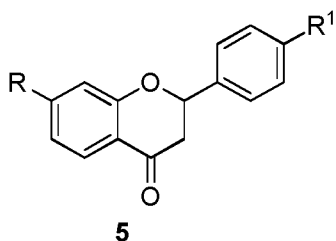


- 5 In particular, compounds having structures **3** and **4** below have been shown to have good selectivity for MAO-B over MAO-A:



Where R is typically substituted aryl and R¹ is a substituted aralkyl.

- 10 Chimenti *et al* (Bioorg Med Chem 2010; 18: 1273-1279) describes a series of flavone compounds of structure 5 below and chromone compounds of structure 6 below (in both cases where R and R¹ are selected from H, F, CH₃ or OCH₃):



- 15 In this paper, Chimenti *et al* note that, while the inhibitory activity of the chromone derivatives remains selective for MAO-B, there is a relevant decrease of inhibitory activity with the introduction of the double bond.

There is still scope for further agents having selective binding affinity for MAO-B and in particular for agents suitable as *in vivo* imaging agents.

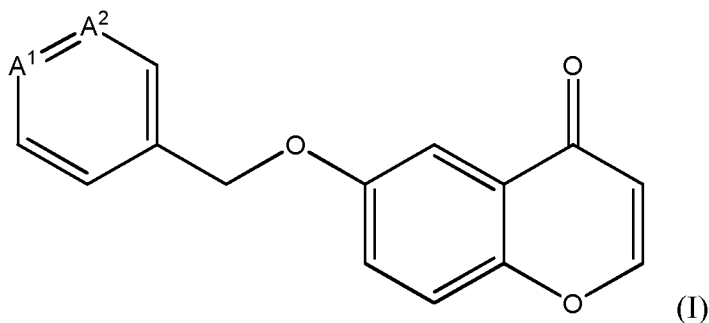
Summary of the Invention

- 20 The present invention relates to compounds having selective binding for MAO-B as compared with MAO-A. The invention also provides radioactive versions of these compounds, and precursor compounds for the synthesis of these radioactive

compounds. The radioactive compounds of the invention are particularly useful for *in vivo* imaging applications, such as in positron-emission tomography (PET). The compounds of the invention are novel over those of the prior art and display good properties for binding to MAO-B.

5 Detailed Description of the Preferred Embodiments

In a first aspect, the present invention relates to a compound of Formula I:



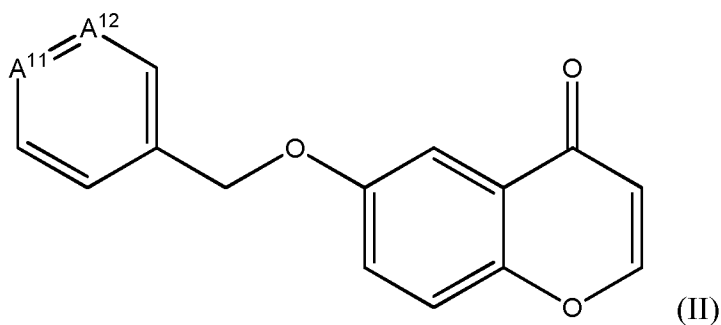
wherein one of A¹ and A² is N and the other is C-F.

In one embodiment of the compound of the invention A¹ is N and A² is C-F.

10 In one embodiment of the compound of the invention A² is N and A¹ is C-F.

In one embodiment of the compound of the invention C-F is C-¹⁸F.

In a second aspect, the present invention provides a precursor compound for the synthesis of the compound of the invention comprising ¹⁸F, wherein said precursor is a compound of Formula II:



wherein one of A¹¹ and A¹² is C-R¹ and the other is N, wherein R¹ is selected from nitro, quaternary ammonium salt, diazonium salt, iodonium salt or chloro.

A “precursor compound” comprises a non-radioactive derivative of a radiolabelled compound, designed so that chemical reaction with a convenient chemical form of the

detectable label occurs site-specifically; can be conducted in the minimum number of steps (ideally a single step); and without the need for significant purification (ideally no further purification), to give the desired *in vivo* imaging agent. Such precursor compounds are synthetic and can conveniently be obtained in good chemical purity.

- 5 The term “nitro” means the substituent $-\text{NO}_2$.

The term “quaternary ammonium salt” refers to a salt of a quaternary ammonium cation with an anion wherein a quaternary ammonium cation is a positively charged polyatomic ions of the structure NR_4^+ , R being an alkyl group or an aryl group.

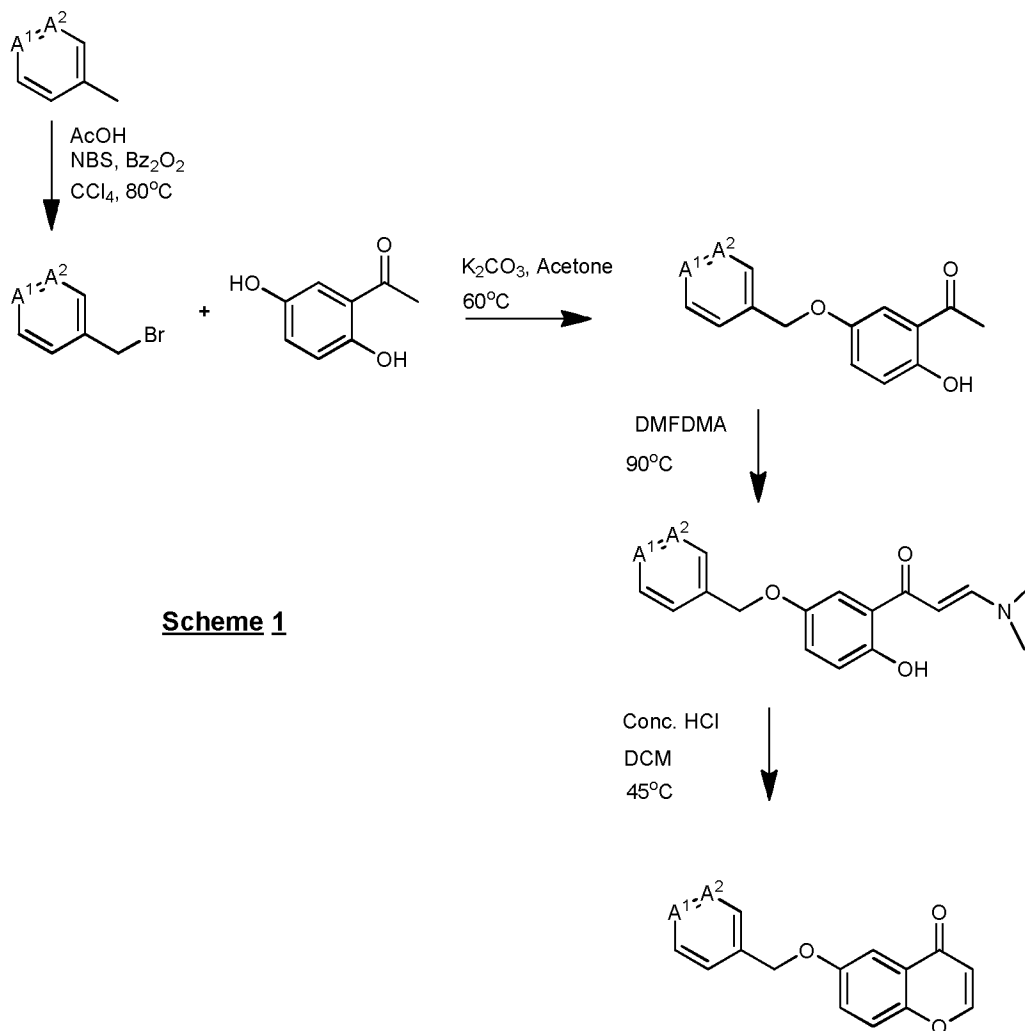
- 10 The term “diazonium salt” refers to the functional group $\text{R-N}_2^+ \text{X}^-$ where R can be any organic residue such alkyl or aryl and X is an inorganic or organic anion such as a halogen

The term “iodonium salt” refers to a salt of a halonium ion R-I^+ where R is a hydrocarbon.

- 15 The terms “comprising” or “comprises” have their conventional meaning throughout this application and imply that the agent or composition must have the essential features or components listed, but that others may be present in addition. The term ‘comprising’ includes as a preferred subset “consisting essentially of” which means that the composition has the components listed without other features or components being present.

- 20 In one embodiment of the precursor compound of the invention R^1 is chloro or nitro.

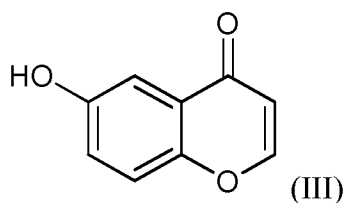
Precursor compounds and non-radioactive compounds of the present invention readily obtained by straightforward adaptation methods known in the art according to Scheme 1 below:



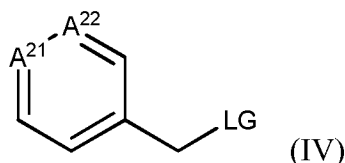
In a third aspect, the present invention provides a method for the synthesis of the compound of the invention comprising ¹⁸F wherein said method comprises reaction of
 5 the precursor compound of the invention with [¹⁸F]fluoride.

Precursor compounds and methods of introducing radioactive isotopes into organic molecules are well-known in the art. A good overview is provided in the “Handbook of Radiopharmaceuticals: Radiochemistry and Applications” (Wiley 2003; Welch and Redvanley, Eds.). Well-known synthetic routes to ¹⁸F-labelled derivatives are also
 10 described by Bolton (J Lab Comp Radiopharm 2002; 45: 485-528).

In a fourth aspect the present invention provides a method for the synthesis of the compound of the invention comprising ¹⁸F wherein said method comprises reaction of a compound of Formula III:



with a compound of Formula IV:



wherein LG is a leaving group.

- 5 A review by Dollé (2005 Curr Pharm Des; 11: 3221-3235) describes how a variety of [^{18}F]fluoropyridyl-containing compounds can be obtained by nucleophilic heteroaromatic substitution at the *ortho* position with [^{18}F]fluoride. These methods can be easily adapted by the person skilled in the art to arrive a method of the fourth aspect of the invention.
- 10 In a fifth aspect, the present invention provides a pharmaceutical formulation comprising the compound as defined hereinabove together with a biocompatible carrier in a form suitable for mammalian administration.

A “pharmaceutical formulation” is a composition comprising the compound of the invention, together with a biocompatible carrier in a form suitable for mammalian administration. In certain embodiments the “biocompatible carrier” is a fluid, especially a liquid, in which the compound is suspended or dissolved, such that the formulation is physiologically tolerable, i.e. can be administered to the mammalian body without toxicity or undue discomfort. The biocompatible carrier is suitably an injectable carrier liquid such as sterile, pyrogen-free water for injection; an aqueous solution such as saline (which may advantageously be balanced so that the final product for injection is either isotonic or not hypotonic); an aqueous solution of one or more tonicity-adjusting substances (e.g. salts of plasma cations with biocompatible counterions), sugars (e.g. glucose or sucrose), sugar alcohols (e.g. sorbitol or mannitol), glycols (e.g. glycerol), or other non-ionic polyol materials (e.g. polyethyleneglycols, propylene glycols and the like). The biocompatible carrier may also comprise biocompatible organic solvents such as ethanol. Such organic solvents are useful to

solubilise more lipophilic compounds or formulations. Preferably the biocompatible carrier is pyrogen-free water for injection, isotonic saline or an aqueous ethanol solution. The pH of the biocompatible carrier for intravenous injection is suitably in the range 4.0 to 10.5.

5 In a sixth aspect, the present invention provides an *in vivo* imaging method for determining the distribution and/ or extent of expression of monoamine oxidase B (MAO-B) in a subject comprising:

- (i) administration of the compound as defined herein comprising ^{18}F ;
- 10 (ii) detecting by an *in vivo* imaging procedure signals emitted by the radioactive isotope comprised in said compound; and,
- (iii) generating an image representative of said signals.

The term “*in vivo* imaging” as used herein refers to those techniques that noninvasively produce images of all or part of the internal aspect of a subject. One suitable *in vivo*
15 imaging procedures for use with this aspect of the invention is positron-emission tomography (PET).

The compound of the invention may be administered as the pharmaceutical formulation of the invention, e.g. parenterally, i.e. by injection. For parenteral administration, steps to ensure that the radiopharmaceutical composition is sterile and apyrogenic also need
20 to be taken.

The term “MAO-B condition” refers to any condition in which MAO-B activity is abnormal. In particular, MAO-B conditions include neurodegenerative diseases, non-limiting examples of which include Parkinson's disease and Alzheimer's disease.

For example, the *in vivo* imaging method of the invention can be carried out before,
25 during and after treatment with a drug to combat a MAO-B condition. In this way, the effect of said treatment can be monitored over time. PET is particularly suitable in this aspect as it has excellent sensitivity and resolution, so that even relatively small changes in a lesion can be observed over time, which is particularly advantageous for treatment monitoring.

30 In one embodiment, the *in vivo* imaging method of the invention is carried out

repeatedly during the course of a treatment regimen for said subject, said regimen comprising administration of a drug to combat a MAO-B condition.

In an alternative embodiment, said *in vivo* imaging method of the sixth aspect of the invention can be understood to be carried out from step (ii) wherein the subject is
5 already administered with said compound of the invention.

In a further alternative embodiment, the sixth aspect of the invention can be understood to be the compound of the invention for use in said *in vivo* imaging method.

In a yet further alternative embodiment, said *in vivo* imaging method can be understood to be the use of said compound of the invention in the manufacture of the
10 pharmaceutical composition of the invention for the *in vivo* imaging of a MAO-B condition.

In a seventh aspect the present invention provides a method of diagnosis of a condition in which MAO-B expression is abnormal wherein said method of diagnosis comprises the *in vivo* imaging method as defined hereinabove as well as the further step (iv) of
15 attributing the distribution and extent of MAO-B expression to a particular clinical picture.

In an alternative embodiment, the seventh aspect of the invention can be understood to be the compound of the invention for use in said method of diagnosis.

In yet further alternative embodiment of the seventh aspect of the invention, said
20 method of diagnosis can be understood to be the use of said compound of the invention in the manufacture of the pharmaceutical composition of the invention for the diagnosis of a MAO-B condition.

This written description uses examples to disclose the invention, including the best mode, and also to enable any person skilled in the art to practice the invention,
25 including making and using any devices or systems and performing any incorporated methods. The patentable scope of the invention is defined by the claims, and may include other examples that occur to those skilled in the art. Such other examples are intended to be within the scope of the claims if they have structural elements that do not differ from the literal language of the claims, or if they include equivalent structural
30 elements with insubstantial differences from the literal languages of the claims. All patents and patent applications mentioned in the text are hereby incorporated by

reference in their entireties, as if they were individually incorporated.

Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described
5 herein unless incompatible therewith.

Brief Description of the Figures

Figures 1 and 2 present NMR spectra for certain compounds synthesised as described in the examples.

Figures 3 and 4 show the results of an autoradiography study of certain compounds of
10 the invention.

Brief Description of the Examples

Example 1 describes the synthesis of 6-((6-fluoropyridin-3-yl)methoxy)-4H-chromen-4-one.

Example 2 describes the synthesis of 6-((2-fluoropyridin-4-yl)methoxy)-4H-chromen-4-
15 one.

Example 3 describes the synthesis of a fluorine-18 radiochemistry precursor for the compound of Example 2.

Example 4 describes the radiosynthesis of [^{18}F]6-((2-fluoropyridin-4-yl)methoxy)-4H-chromen-4-one.

20 Example 5 describes an assay to measure the *in vitro* inhibition of MAO-A and MAO-B.

List of Abbreviations used in the Examples

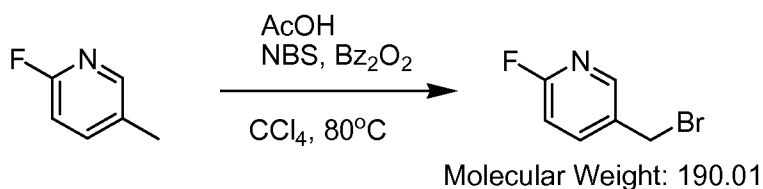
CRC:	concentration response curve
DCM:	dichloromethane
25 DMFDMA:	dimethylformamide dimethylacetal
DMSO:	dimethyl sulfoxide
MAO A:	monoamine oxidase A
MAO B:	monoamine oxidase B

Max: maximum
 MIN: minimum
 TLC: thin-layer chromatography

Examples

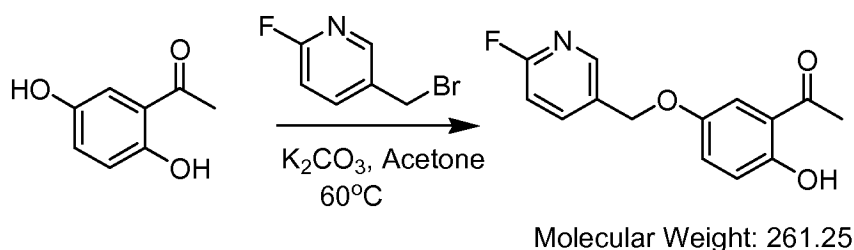
5 Example 1: 6-((6-fluoropyridin-3-yl)methoxy)-4H-chromen-4-one

Step 1:



To a stirred solution of 2-fluoro-5-methylpyridine (1.44 g, 12.95 mmol) in dry carbon tetrachloride (13 ml), N-bromo succinimide (2.32 g, 12.95 mmol) and Benzoyl peroxide (157 mg, 0.64 mmol) was added. The reaction mixture was stirred at 80° C for 2h. The progress of the reaction was monitored by TLC (1:9, Ethyl acetate : pet. ether). The reaction mixture was cooled to ambient temperature and filtered under suction. The solid was washed with Carbon tetrachloride. The solvent was removed under reduced pressure. The crude was purified by column chromatography (60-120 mesh silica gel) using 3% Ethyl acetate in pet ether as eluent to yield pure 5-(bromomethyl)-2-fluoropyridine (1.2 g, 48.7%) as a pale yellow oil.

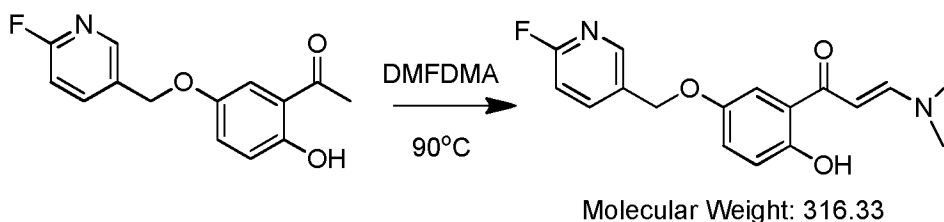
Step 2:



To a stirred solution of 2,5-dihydroxyacetophenone (0.4 g, 2.62 mmol) in dry acetone (10 ml) was added K₂CO₃ (0.36 g, 2.6 mmol). After stirring at ambient temperature for 30 minutes, 5-(bromomethyl)-2-fluoropyridine (0.494 g, 2.6 mmol) was added dropwise. The reaction mixture was heated to 60° C for 14h. The progress of the reaction was monitored by TLC (2:8, Ethyl acetate : pet. ether). After completion of the

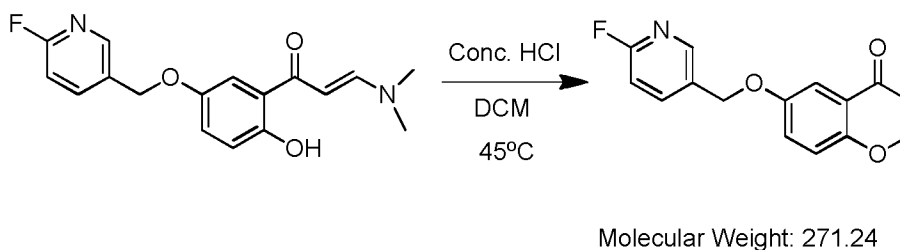
reaction the reaction mixture was cooled to room temperature and quenched with aqueous 10% NaHCO₃ solution (30 ml) and extracted with ethyl acetate (4 x 70 ml). The combined organic layer was dried over anhydrous sodium sulfate and removed under reduced pressure. The crude was purified by column chromatography (60-120 mesh silica gel) using 9% Ethyl acetate in pet ether as eluent to yield pure **1-(5-((6-fluoropyridin-3-yl)methoxy)-2-hydroxyphenyl)ethanone** (0.345 g, 50.4%) as a pale yellow solid.

Step 3:



A mixture of 1-(5-((6-fluoropyridin-3-yl)methoxy)-2-hydroxyphenyl)ethanone (0.345 g, 1.32 mmol, 1 eq) and DMFDMA (0.32 g, 2.64 mmol, 2 eq) was heated to 90° C for 2 h. Reaction progress was monitored by TLC (5:5, ethyl acetate : pet ether). After completion of reaction, reaction mixture was diluted with ethyl acetate (40 ml) and thoroughly washed with water (4 x 40 mL). The combined organic layer was washed with saturated NaCl (20 mL) solution, dried over anhydrous Na₂SO₄, filtered and concentrated. The crude was purified by column chromatography (60-120 mesh silica gel) using 28-37% Ethyl acetate in pet ether as eluent to yield **(E)-3-(dimethylamino)-1-(5-((6-fluoropyridin-3-yl)methoxy)-2-hydroxyphenyl)prop-2-en-1-one** (0.21 g, mixture of desired and the final) as a pale yellow solid.

Step 4:



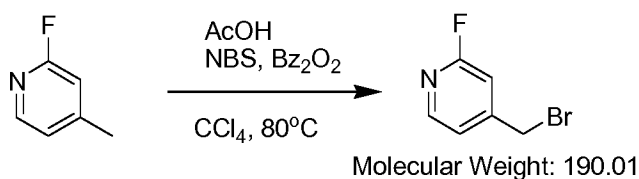
To a stirred solution of (E)-3-(dimethylamino)-1-(5-((6-fluoropyridin-3-yl)methoxy)-2-hydroxyphenyl)prop-2-en-1-one (0.21 g, 0.663 mmol, 1 eq), in dichloromethane (6 mL), was added conc. HCl (0.6 mL) drop wise at room temperature. The reaction

mixture was heated to reflux at 45° C for 2 h. Reaction progress was monitored by TLC (5:5, ethyl acetate: pet ether). After completion, the reaction mixture was cooled to room temperature and extracted with dichloromethane (2 x 30 mL). The combined organic layers were washed successively with saturated NaHCO₃ solution (20 mL) and saturated NaCl solution (20 mL). After drying over anhydrous Na₂SO₄, it was concentrated and the crude product was purified by column chromatography over 60-120 mesh silica gel (24% Ethyl acetate in pet ether) followed by crystallization from ethyl acetate afforded **6-((6-fluoropyridin-3-yl)methoxy)-4H-chromen-4-one** as off pale yellow solid (0.120 g, 66.72%).

Figure 1 shows the NMR characterization data for this compound.

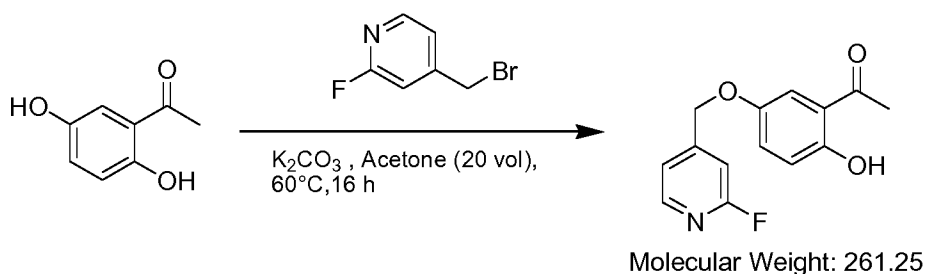
Example 2: Synthesis of 6-((2-fluoropyridin-4-yl)methoxy)-4H-chromen-4-one

Step-1:



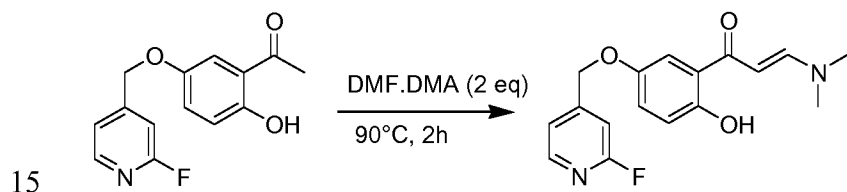
To a stirred solution of 2-fluoro-4-methylpyridine (5g, 44.99 mmol) in dry carbon tetrachloride (114 ml) was added glacial Acetic acid (3.3ml) at 25° C. Then reaction mixture was heated to 50° C and N-bromo succinimide (8 g, 44.9 mmol) followed by Benzoyl peroxide (1.1 g, 4.49 mmol) was added. After complete addition reaction mixture was stirred at 80° C for 20h. The progress of the reaction was monitored by TLC (1:9, Ethyl acetate : pet. ether). After completion of reaction, reaction mixture was cooled to room temperature quenched with cold water and extracted with DCM (4 x 200 mL). The combined organic layer was washed with saturated NaCl (100 mL) solution, dried over anhydrous Na₂SO₄, filtered and concentrated. The crude was purified by column chromatography (60-120 mesh silica gel) using 2% Ethyl acetate in pet ether as eluent to yield pure **4-(bromomethyl)-2-fluoropyridine** (2.8 g, 23.4%) as a pale yellow oil.

Step-2:

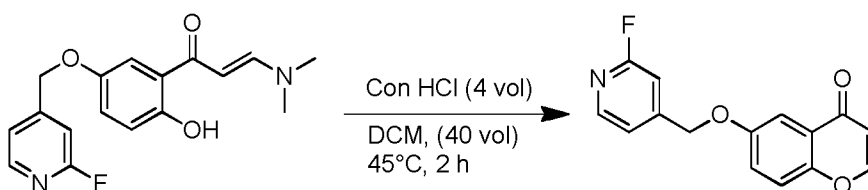


To a stirred solution of 2,5-dihydroxy acetophenone (0.6 g, 3.94 mmol, 1 eq), in acetone (15 mL), was added K_2CO_3 (0.54 g, 3.9 mmol, 0.99 eq). After stirring at room temperature for 30 minutes, 4-(bromomethyl)-2-fluoropyridine (0.74 g, 3.90 mmol, 0.99 eq) was added drop wise. The reaction mixture was heated to reflux at 60° C for 16 h. Reaction progress was monitored by TLC (2:8, ethyl acetate: pet ether). After completion of reaction, the reaction mixture was cooled to room temperature mixed with aq. $NaHCO_3$ solution (30 mL) and extracted with ethyl acetate (4 x 50 mL). The combined organic layer was washed with saturated $NaCl$ (100 mL) solution, dried over anhydrous Na_2SO_4 , filtered and concentrated. The crude was purified by column chromatography (60-120 mesh silica gel) using 13% Ethyl acetate in pet ether as eluent to yield **1-(5-((2-fluopyridin-4-yl)methoxy)-2-hydroxyphenyl)ethanone** (0.5 g, 48.2%) as a pale yellow solid.

Step-3:



A mixture of 1-(2-hydroxy-5-((4-methylbenzyl)oxy)phenyl)ethan-1-one (0.5 g, 1.91 mmol, 1 eq) and DMFDMA (0.456 g, 3.82 mmol, 2 eq) was heated to 90° C for 2 h. Reaction progress was monitored by TLC (5:5, ethyl acetate : pet ether). After completion of reaction, reaction mixture was diluted with ethyl acetate (40 ml) and thoroughly washed with water (4 x 40 mL). The combined organic layer was washed with saturated $NaCl$ (20 mL) solution, dried over anhydrous Na_2SO_4 , filtered and concentrated. The crude was purified by column chromatography (60-120 mesh silica gel) using 32-65% Ethyl acetate in pet ether as eluent to yield **(E)-3-(dimethylamino)-1-(5-((2-fluopyridin-4-yl)methoxy)-2-hydroxyphenyl)prop-2-en-1-one** (0.25 g, mixture of desired and the final) as a pale yellow solid.

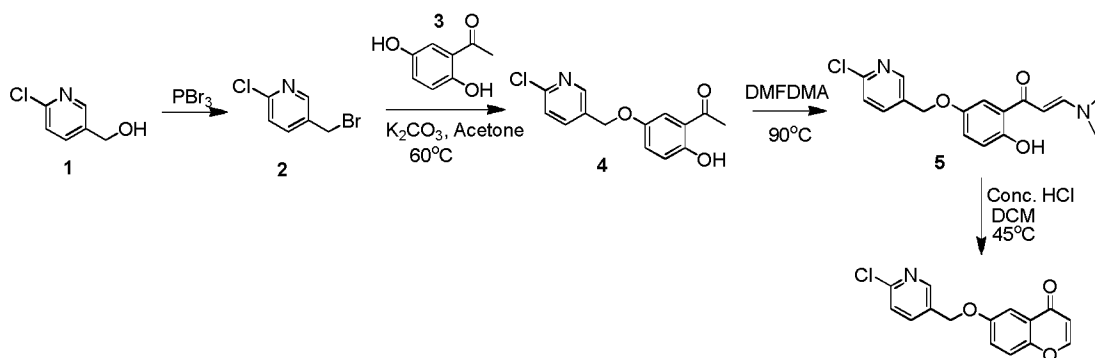
Step-4:

Molecular Weight: 271.24

To a stirred solution of *(E)*-3-(dimethylamino)-1-(5-((2-fluopyridin-4-yl)methoxy)-2-hydroxyphenyl)prop-2-en-1-one (0.25 g, 0.79 mmol, 1 eq), in dichloromethane (10 mL), was added conc. HCl (1 mL) drop wise at room temperature. The reaction mixture was heated to reflux at 45° C for 2 h. Reaction progress was monitored by TLC (5:5, ethyl acetate: pet ether). After completion, the reaction mixture was cooled to room temperature and extracted with dichloromethane (2 x 30 mL). Combined organic layer was washed successively with saturated NaHCO₃ solution (20 mL) and saturated NaCl solution (20 mL). After drying over anhydrous Na₂SO₄, it was concentrated and the crude was purified by column chromatography over 60-120 mesh silica gel using 32% Ethyl acetate in pet ether as eluent. The product was crystallized from ethyl acetate afforded 6-((2-fluoropyridin-4-yl)methoxy)-4H-chromen-4-one as pale yellow solid (0.092 g, 42.29%).

Figure 2 shows the NMR characterization data for this compound.

Example 3: Synthesis of a fluorine-18 radiochemistry precursor for the compound of Example 1.



20 **Step 1:**

To a stirred solution of (6-chloropyridin-3-yl)methanol (2 g, 13.93 mmol) in dry DCM (40 ml), PBr_3 (1.6 ml, 16.71 mmol) was added dropwise at 0° C. The reaction mixture was stirred at ambient temperature for 3h. The progress of the reaction was monitored by TLC (3:7, Ethyl acetate: pet. ether). After completion of the reaction the reaction mixture was cooled to room temperature and quenched with aqueous 10% NaHCO_3 solution (30 ml) and extracted with DCM (3 X 50 ml). The combined organic layer was dried over anhydrous sodium sulfate and removed under reduced pressure. The crude was purified by column chromatography (60-120 mesh silica gel) using 8% Ethyl acetate in pet ether as eluent to yield pure 5-(bromomethyl)-2-chloropyridine (2.2 g, 76.65%) as a yellow oil.

Step 2:

To a stirred solution of 2,5-dihydroxyacetophenone (1.48 g, 9.78 mmol) in dry Acetone (30 mL) was added K_2CO_3 (0.36 g, 1.33 mmol). After stirring at ambient temperature for 30 minutes, a solution of 5-(bromomethyl)-2-chloropyridine (2 g, 9.68 mmol) in acetone (15 mL) was added dropwise. The reaction mixture was heated to 60° C for 14h. The progress of the reaction was monitored by TLC (3:7, Ethyl acetate: pet. ether). After completion of the reaction the reaction mixture was cooled to room temperature and quenched with aqueous 10% NaHCO_3 solution (50 ml) and stirred for 15 minutes. The yellow solid appeared was filtered under suction. The solid obtained was suspended in EtOAc (70 ml) and inorganic was filtered under suction. The EtOAc layer was dried over anhydrous sodium sulfate and removed under reduced pressure. The crude was purified by column chromatography (60-120 mesh silica gel) using 14% Ethyl acetate in pet ether as eluent to yield pure **1-(5-((6-chloropyridin-3-yl)methoxy)-2-hydroxyphenyl)ethan-1-one** (1.15 g, 42.9%) as a pale yellow solid.

Step 3:

A mixture of 1-(5-((6-chloropyridin-3-yl)methoxy)-2-hydroxyphenyl)ethan-1-one (1.1 g, 3.96 mmol, 1 eq) and DMFDMA (2.63 g, 19.80 mmol, 5 eq) was heated to 90° C for 2 h. Reaction progress was monitored by TLC (5:5, ethyl acetate : pet ether). After completion of reaction, reaction mixture was diluted with water (30 ml), the reaction mass gets precipitating out then the reaction mixture was filtered and thoroughly washed with water (2 x 30 mL), followed by pet ether (30 mL). The solid was dried to yield **(E)-1-(5-((6-chloropyridin-3-yl)methoxy)-2-hydroxyphenyl)-3-**

(dimethylamino)prop-2-en-1-one (0.68 g, 42.9%) as a pale yellow solid.

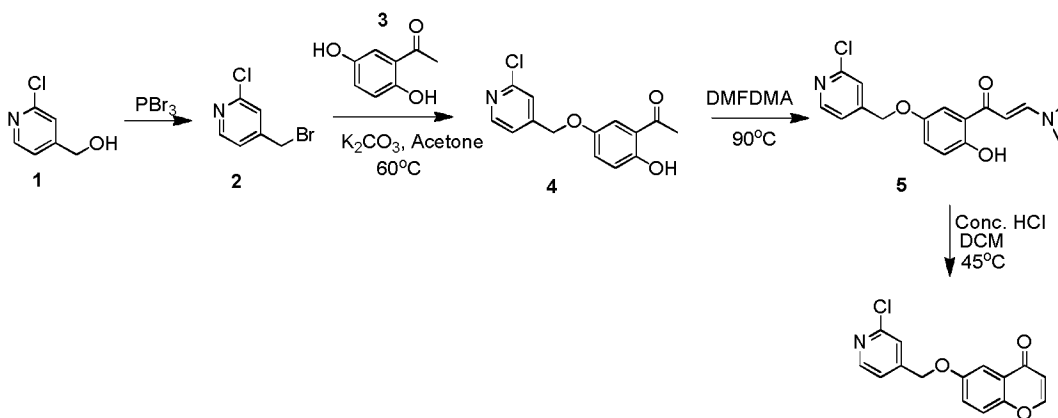
Step 4:

To a stirred solution of (E)-1-(5-((6-chloropyridin-3-yl)methoxy)-2-hydroxyphenyl)-3-(dimethylamino)prop-2-en-1-one (0.40 g, 1.20 mmol, 1 eq), in dichloromethane (20 mL), was added conc. HCl (1.8 mL) drop wise at room temperature. The reaction mixture was heated to reflux at 45° C for 2 h. Reaction progress was monitored by TLC (5:5, ethyl acetate: pet ether). After completion, the reaction mixture was cooled to room temperature and extracted with dichloromethane (2 x 40 mL). The combined organic layers were washed successively with saturated NaHCO₃ solution (60 mL) and saturated NaCl solution (70 mL). After drying over anhydrous Na₂SO₄, it was concentrated and the crude product was purified by column chromatography over 60-120 mesh silica gel (24% Ethyl acetate in pet ether) followed by crystallization from ethyl acetate afforded **6-((6-chloropyridin-3-yl)methoxy)-4H-chromen-4-one** (GE-MAO-B-03_4) as off pale yellow solid (0.245 g, 71.01%).

LCMS gave m.z = 288 [M+H]⁺

Example 4: Synthesis of a fluorine-18 radiochemistry precursor for the compound of

Example 2



Step-1:

To a stirred solution of (2-chloropyridin-4-yl)methanol (2g, 13.93 mmol) in dry in dry DCM (40 ml), PBr₃ (1.6 ml, 16.71 mmol) was added dropwise at 0° C. The reaction mixture was stirred at ambient temperature for 3h. The progress of the reaction was monitored by TLC (3:7, Ethyl acetate: pet. ether). After completion of the reaction the

reaction mixture was cooled to room temperature and quenched with aqueous 10% NaHCO₃ solution (30 ml) and extracted with DCM (3 X 50 ml). The combined organic layer was dried over anhydrous sodium sulfate and removed under reduced pressure. The crude was purified by column chromatography (60-120 mesh silica gel) using 8% Ethyl acetate in pet ether as eluent to yield pure **4-(bromomethyl)-2-chloropyridine** (1g, 34.77%) as a pale yellow oil.

Step-2:

To a stirred solution of 2,5-dihydroxyacetophenone (0.740 g, 4.89 mmol) in dry Acetone (20 mL) was added K₂CO₃ (0.668 g, 4.84 mmol). After stirring at ambient temperature for 30 minutes, a solution of 5-(bromomethyl)-2-chloropyridine (1 g, 4.84 mmol) in acetone (15 mL) was added dropwise. The reaction mixture was heated to 60° C for 14h. The progress of the reaction was monitored by TLC (3:7, Ethyl acetate: pet. ether). After completion of the reaction the reaction mixture was cooled to room temperature and quenched with aqueous 10% NaHCO₃ solution (50 ml) and stirred for 15 minutes. The yellow solid appeared was filtered under suction. The solid obtained was suspended in EtOAc (70 ml) and inorganic was filtered under suction. The EtOAc layer was dried over anhydrous sodium sulfate and removed under reduced pressure. The crude was purified by column chromatography (60-120 mesh silica gel) using 14% Ethyl acetate in pet ether as eluent to yield pure **1-(5-((2-chloropyridin-4-yl)methoxy)-2-hydroxyphenyl)ethan-1-one** (0.7 g, 52.23%) as a pale yellow solid.

Step-3:

A mixture of 1-(5-((2-chloropyridin-4-yl)methoxy)-2-hydroxyphenyl)ethan-1-one (0.7 g, 2.52 mmol, 1 eq) and DMFDMA (1.50 g, 12.60 mmol, 5 eq) was heated to 90° C for 2 h. Reaction progress was monitored by TLC (5:5, ethyl acetate : pet ether). After completion of reaction, reaction mixture was diluted with water (25 ml), the reaction mass gets precipitating out then the reaction mixture was filtered and thoroughly washed with water (2 x 25 mL), followed by pet ether (30 mL). The solid was dried to yield **(E)-1-(5-((2-chloropyridin-4-yl)methoxy)-2-hydroxyphenyl)-3-(dimethylamino)prop-2-en-1-one** (0.43 g, 51.2%) as a yellow solid.

Step-4:

To a stirred solution of (E)-1-(5-((2-chloropyridin-4-yl)methoxy)-2-hydroxyphenyl)-3-

(dimethylamino)prop-2-en-1-one (0.43 g, 1.29 mmol, 1 eq), in dichloromethane (25 mL), was added conc. HCl (1.9 mL) drop wise at room temperature. The reaction mixture was heated to reflux at 45° C for 2 h. Reaction progress was monitored by TLC (5:5, ethyl acetate: pet ether). After completion, the reaction mixture was cooled to
5 room temperature and extracted with dichloromethane (2 x 30 mL). Combined organic layer was washed successively with saturated NaHCO₃ solution (20 mL) and saturated NaCl solution (20 mL). After drying over anhydrous Na₂SO₄, it was concentrated and the crude was purified by column chromatography over 60-120 mesh silica gel using 32% Ethyl acetate in pet ether as eluent. The product was crystallized from ethyl acetate
10 afforded **6-((2-chloropyridin-4-yl)methoxy)-4H-chromen-4-one** (GE-MAO-B-03_5) as off white solid (0.22 g, 59.45%).

LCMS gave m.z = 288 [M+H]⁺

15 **Example 5: Radiosynthesis of [¹⁸F] 6-((6-fluoropyridin-3-yl)methoxy)-4H-chromen-4-one**

The radiosynthesis was carried out on an automated radiochemistry module. Briefly, no-carrier-added [¹⁸F]fluoride was produced via ¹⁸O(p,n)¹⁸F reaction by proton irradiation of an ¹⁸O-enriched water target of the cyclotron. The [¹⁸F]fluoride was
20 trapped on an ion exchange cartridge (QMA) then eluted using a mixture of potassium carbonate, Kryptofix [2.2.2], water and acetonitrile. The solution was dried with heating with nitrogen plus gas flow. A solution of precursor (5–10 mg) in was added and the mixture heated at 125 C for 10 minutes. The crude product was diluted with water and semi-preparative HPLC was performed (column was ACE 5 C18-HL(250x10mm);
25 eluent was acetonitrile:ammonium formate (0.1 M), 30:70); flow 5 mL/min; retention time: 25 minutes). The product peak was collected, diluted with water, trapped on a reversed-phase SPE cartridge and formulated.

Analytical HPLC (254 nm; ACE5 C18-HL 250x4.6mm column; flow = 2 mL/min; eluent is 0.1% TFA: acetonitrile 45%-90% gradient over 10 minutes: retention time = 4
30 minutes.

Example 6: In vitro autoradiography of [¹⁸F 6-((6-fluoropyridin-3-yl)methoxy)-4H-chromen-4-one

[¹⁸F 6-((6-fluoropyridin-3-yl)methoxy)-4H-chromen-4-one was applied to whole brain sections from healthy Rhesus monkey (20 µm thickness) or human (100 µm thickness).

- 5 Blocking was performed with either deprenyl (MAO-B) or pirlindole (MAO-A).
Blocking agents (deprenyl and Pirlindole) are available commercially from Sigma-Aldrich.

Conditions:

- Radioligand concentration 0.02 MBq/ml
- 10 • Blocker concentrations 10 µM
- Incubation 90 min, RT
- Wash 3 x 5 min, 4°C

Buffer:

- Tris HCl 50 mM, pH 7.4 incl
- 15 • 120 mM NaCl
- 5 mM KCl
- 2 mM CaCl₂
- 1 mM MgCl₂
- 0.1 % BSA

- 20 Figure 3 shows the results obtained for human tissue and Figure 4 shows the results obtained for Rhesus monkey.

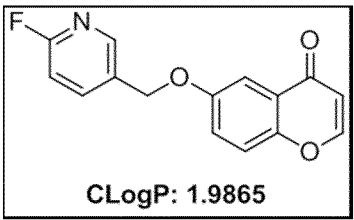
Example 7: In vitro inhibition of MAO-A and MAO-B

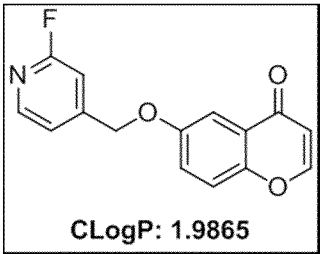
Assay development was carried out based on the standard protocol provided in product insert for Amplex Red Monoamine Oxidase Assay Kit from Life Technologies.

- 25 General parameters for screening of the compounds was as follows:
- ☐ Assay Type: Amplex Red Monoamine Oxidase Assay (Invitrogen Cat # A12214)
 - ☐ Assay Format: 96 well plate (Black, Corning)
 - ☐ Assay Volume: 200µL
 - ☐ Enzyme Source: MAO A and MAO B enzymes (Sigma-Aldrich Cat # M7316 and

M7441)

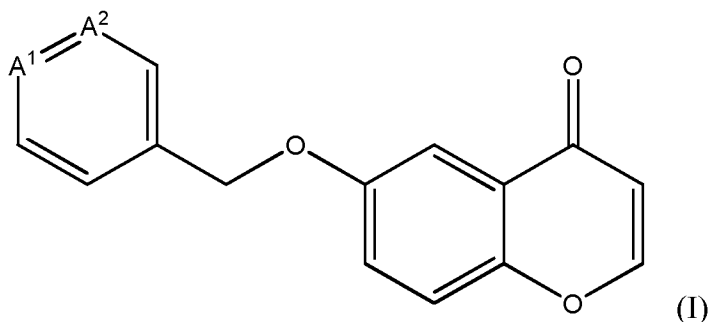
- ☐ Substrate : For MAO-A/B : Tyramine, For MAO-B : Benzylamine
 - ☐ Reference Compounds: For MAO-A: Clorgyline (Irreversible), Moclobemide (Reversible), for MAO-B: Pargyline (Irreversible), Deprenyl (Irreversible), Lazabemide hydrochloride (Reversible).
- 5
- ☐ Screening Format: Concentration Response Curve (CRC) for all compounds
 - ☐ Replicates: Duplicates (n=2)
 - ☐ Number of Runs: One (N=1)
 - ☐ Controls:
- 10
- ☐ Negative control/“MIN”: 1% DMSO Buffer without enzyme (substrate alone)
 - ☐ Positive control/ “Max”: 1% DMSO Buffer with enzyme and substrate
 - ☐ Reading: End point or Kinetic
 - ☐ Detection: Fluorescence Ex/Em - 560nm/590nm
 - ☐ Detection Instrument: Envision [Perkin Elmer]
- 15
- Stock solution will be prepared from solid samples and serial dilutions will be made on a semi-log scale while maintaining the final concentration of the DMSO in the assay as determined under tolerability study.

Compound	MAO-A IC ₅₀ (umol)	MAO-B IC ₅₀ (umol)	Selectivity index
 CLogP: 1.9865	4.120	0.077	53.22

 CLogP: 1.9865	>10	0.039	>258.55
Clorgyline	0.001		
Deprenyl		0.035	

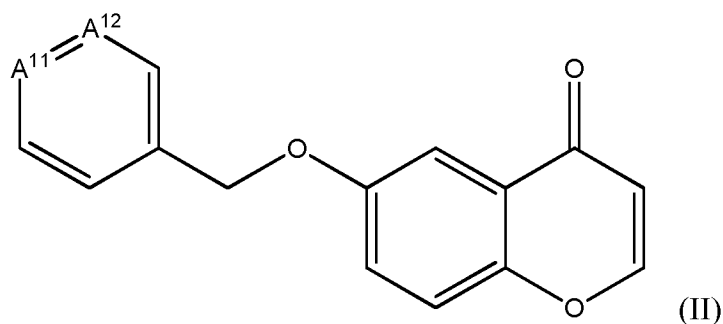
Claims

- (1) A compound of Formula I:



wherein one of A¹ and A² is N and the other is C-F.

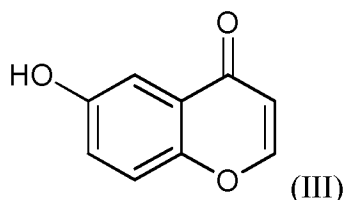
- 5 (2) The compound of Formula I as defined in Claim 1 wherein A¹ is N and A² is C-F.
- (3) The compound of Formula I as defined in Claim 1 wherein A² is N and A¹ is C-F.
- (4) The compound as defined in any one of Claims 1-3 wherein C-F is C-¹⁸F.
- 10 (5) A precursor compound for the synthesis of the compound as defined in Claim 4 wherein said precursor compound is a compound of Formula II:



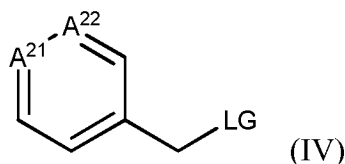
wherein one of A¹¹ and A¹² is C-R¹ and the other is N, wherein R¹ is selected from nitro, quaternary ammonium salt, diazonium salt, iodonium salt or chloro.

- 15 (6) The precursor compound as defined in Claim 5 wherein R¹ is chloro or nitro.
- (7) A method for the synthesis of the compound as defined in Claim 4 comprising reaction of the precursor compound of Formula II as defined in Claim 5 or Claim 6 with [¹⁸F]fluoride.
- (8) A method for the synthesis of the compound as defined in Claim 4 comprising

reaction of a compound of Formula III:

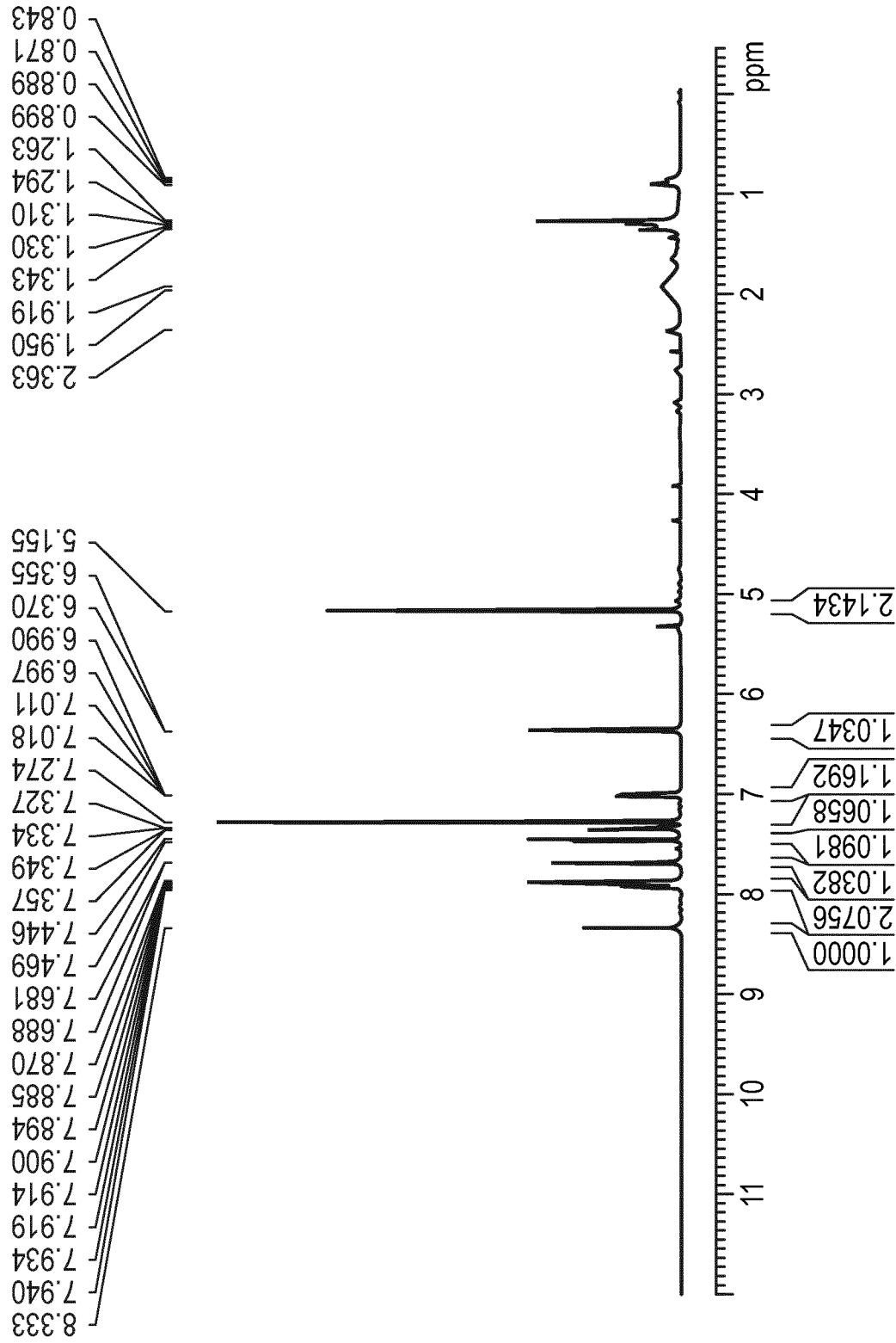


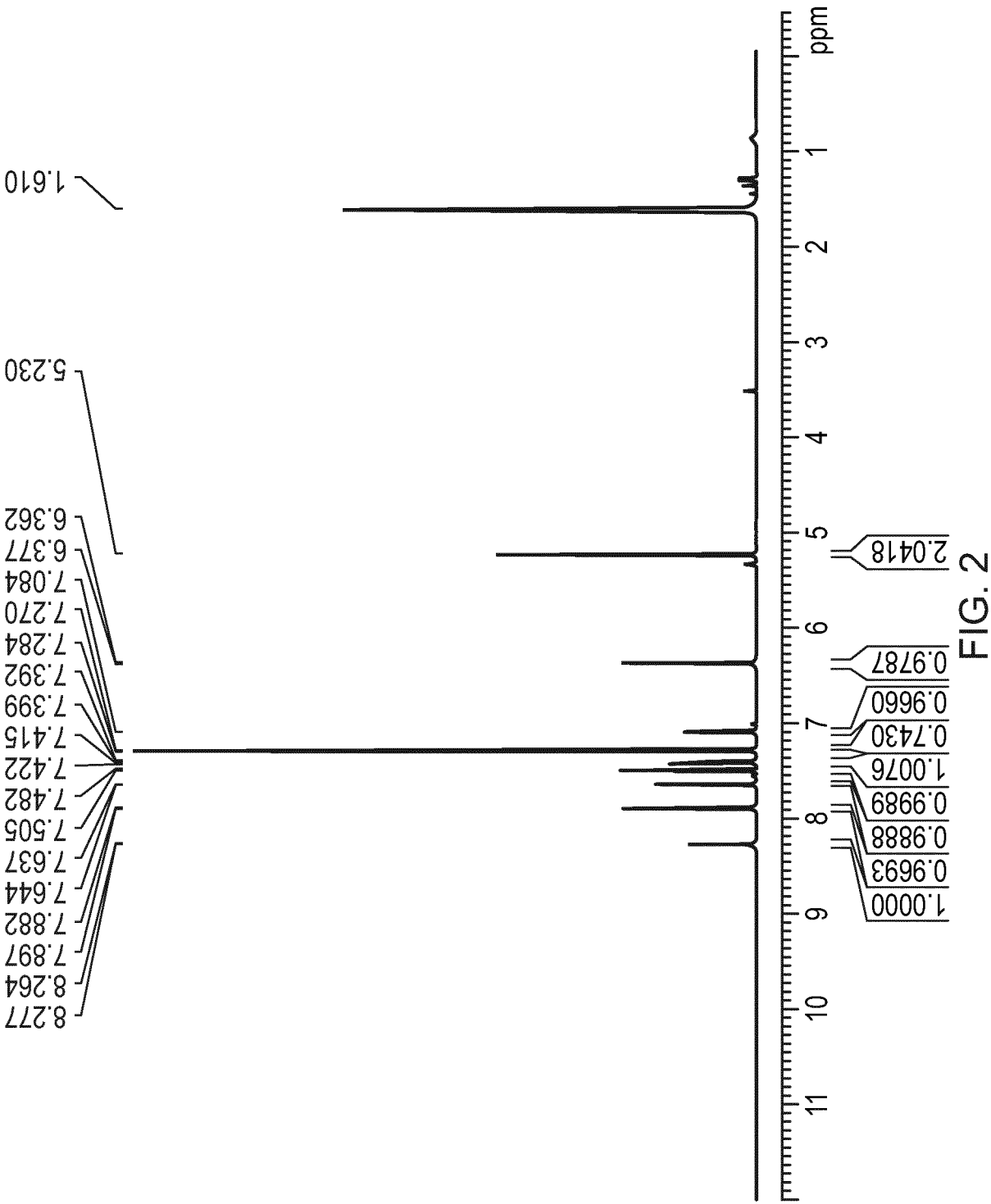
with a compound of Formula IV:



5 wherein LG is a leaving group.

- (9) A pharmaceutical formulation comprising the compound as defined in any one of Claims 1-4 together with a biocompatible carrier in a form suitable for mammalian administration.
- (10) An *in vivo* imaging method for determining the distribution and/ or extent of expression of monoamine oxidase B (MAO-B) in a subject comprising:
- 10 (i) administration of the compound as defined in Claim 4;
- (ii) detecting by an *in vivo* imaging procedure signals emitted by the radioactive isotope comprised in said compound; and,
- (iii) generating an image representative of said signals.
- 15 (11) The *in vivo* imaging method as defined in Claim 10 wherein said method is carried out repeatedly during the course of a treatment regimen for said subject, said regimen comprising administration of a drug to combat a MAO-B condition.
- (12) A method of diagnosis of a condition in which MAO-B expression is abnormal
- 20 wherein said method of diagnosis comprises the *in vivo* imaging method as defined in Claim 10 as well as the further step (iv) of attributing the distribution and extent of MAO-B expression to a particular clinical picture.





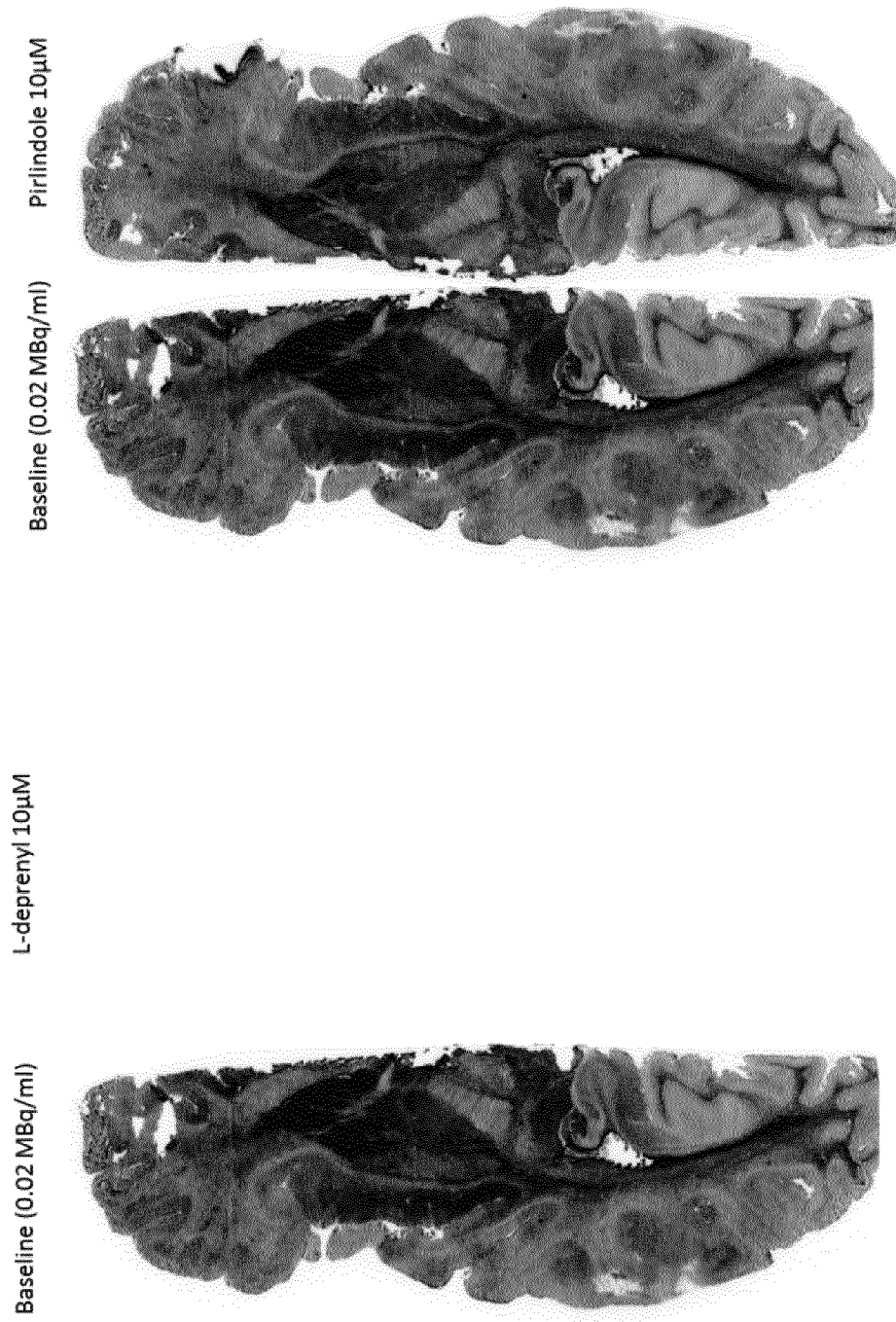


FIG. 3

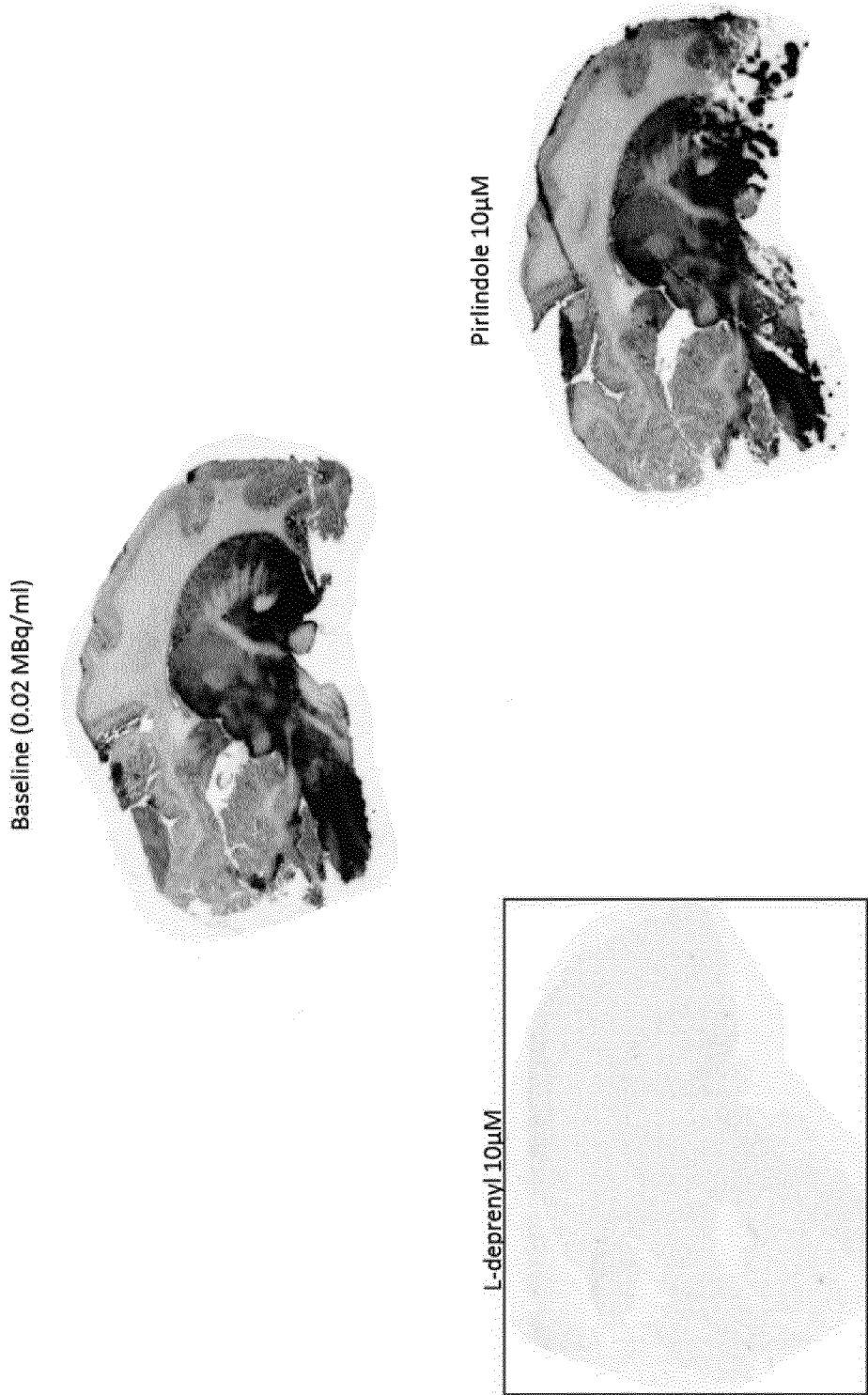


FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/080600

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D405/12 A61K31/35 A61P25/16
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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LESETJA J LEGOABE ET AL: "Inhibition of monoamine oxidase by selected C6-substituted chromone derivatives", EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, EDITIONS SCIENTIFIQUE ELSEVIER, PARIS, FR, vol. 49, 17 January 2012 (2012-01-17), pages 343-353, XP028458568, ISSN: 0223-5234, DOI: 10.1016/J.EJMECH.2012.01.037 [retrieved on 2012-01-24] example 3h; table 1 -----	1-12
A	WO 2013/016580 A2 (UNIV SOUTHERN CALIFORNIA [US]; SHIH JEAN C [US]; CHUNG LELAND [US]; ZH) 31 January 2013 (2013-01-31) the whole document -----	1-12



Further documents are listed in the continuation of Box C.



See patent family annex.

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

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

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"&" document member of the same patent family

Date of the actual completion of the international search

15 February 2016

Date of mailing of the international search report

25/02/2016

Name and mailing address of the ISA/

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Authorized officer

Baston, Eckhard

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2015/080600

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013016580 A2	31-01-2013	CA 2843359 A1	31-01-2013
		CN 103826625 A	28-05-2014
		EP 2736506 A2	04-06-2014
		JP 2014525914 A	02-10-2014
		US 2013039854 A1	14-02-2013
		WO 2013016580 A2	31-01-2013
