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Closing in on the AMPA receptor: Synthesis and evaluation of 2-acetyl-1-(4′-chlorophenyl)-6-methoxy-7-[^{11}C]methoxy-1,2,3,4-tetrahydroisoquinoline as a potential PET tracer

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Abstract—2-Acetyl-1-(4′-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, one of the most potent non-competitive AMPA antagonists described to date, has been labelled with carbon-11 and tritium and evaluated as a potential ligand for in vivo imaging of AMPA receptors using PET. The carbon-11 labelled compound showed good initial brain uptake in rats, but with rapid clearance and relatively homogenous distribution. In saturation binding studies, the tritiated racemic ligand was found to be highly potent with a K_d of 14.8 ± 1.8 nM. We conclude that the low receptor density labelled with this compound, its rapid clearance from the CNS and low specific binding makes it unsuitable as an in vivo PET imaging agent for AMPA receptors.

1. Introduction

Glutamate is the principal excitatory neurotransmitter in the central nervous system (CNS) and mediates its actions via activation of three families of ionotropic receptors (iGluRs): *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and kainate, as well as by G-protein-coupled metabotropic receptors (mGluRs).[1,2] Of these, AMPA receptors principally mediate the majority of fast excitatory amino acid transmission in the CNS and play a key role in memory, cognition and learning, as well as synaptic plasticity and neurodevelopment.[3] In the diseased brain, the AMPA receptors are implicated in a wide range of pathological processes, including epilepsy, Parkinson's disease, multiple sclerosis and schizophrenia.[4–9] Consequently, AMPA receptors are attractive for therapeutic intervention and particular attention has been devoted to selective AMPA receptor antagonists as potential neuroprotective agents and to AMPA receptor modulators (ampakines) as cognitive enhancers.[4]

AMPA receptors are composed of a four-subunit family (GluR1–4) that are products of separate genes and are believed to assemble as functional tetramers.[10] Additional complexity is introduced by RNA editing and alternative splicing, with all AMPA subunits being present as two alternatively spliced forms known as flip and flop. This heterogeneity of AMPA is important therapeutically because it conveys cation selectivity and rate and extent of desensitization.[2]

Non-invasive in vivo imaging using selective AMPA radioligands with positron emission tomography (PET) offers a unique opportunity to study these receptors in the living human brain and may therefore facilitate drug development and provide new diagnostic tools.

While considerable effort has been made to develop PET tracers for the related NMDA receptor family,[11] AMPA imaging has remained largely unexplored. Compared with NMDA, AMPA receptors are less well understood

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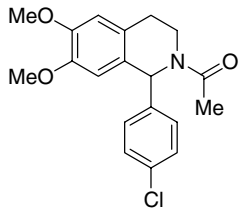

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Figure 1.

and few high affinity ligands are available. Non-competitive antagonists are in general preferred as ligands for PET imaging as they do not cause receptor internalization and their binding is unaffected by endogenous neurotransmitters. The recent discovery of the non-competitive AMPA antagonist 2-acetyl-1-(4′-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**1**, Fig. 1) as one of the most potent non-competitive AMPA antagonists described to date[12,13] indicated that this compound might have the required brain uptake, affinity and selectivity to allow in vivo imaging of AMPA receptors. Herein we report the novel synthesis and evaluation of *N*-acetyl-1-(4′-chlorophenyl)-6-methoxy-7-[^{11}C]methoxy-1,2,3,4-tetrahydroisoquinoline ([^{11}C]**1**) as a potential PET ligand. In addition, the tritiated derivative was prepared and used to determine affinity for the AMPA receptor, specific binding and receptor population in the rat brain. While this paper was under review, the synthesis of the title compound and a related fluorine-18 derivative was published elsewhere, albeit without biological evaluation.[14]

2. Results and discussion

2.1. Chemistry

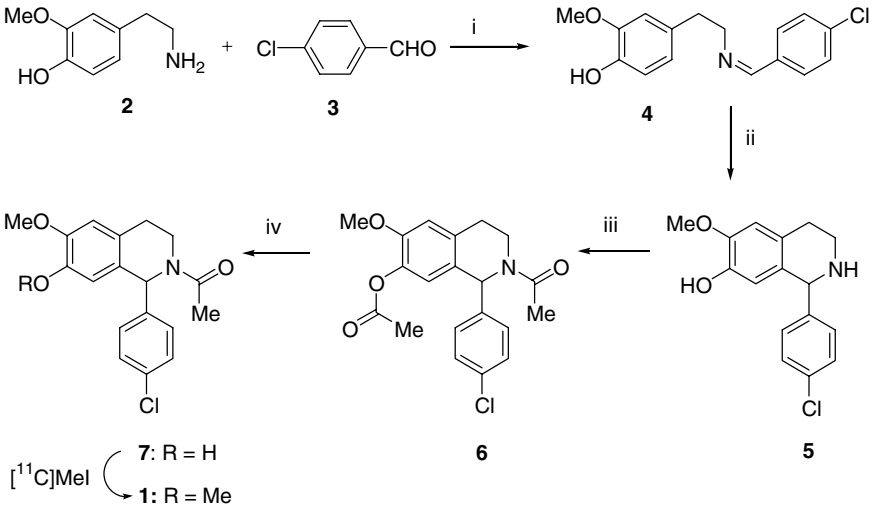
Racemic 2-acetyl-1-(4′-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**1**) was prepared as previously described.[12] The 7-desmethyl precursor was synthesized in 4 steps from commercially available 3-methoxytyramine (**2**) as outlined in Scheme 1. In short, 3-methoxytyramine (**2**) was reacted with 4-chlorobenzaldehyde (**3**) to give the corresponding imine **4**, which upon treatment with trifluoroacetic acid (TFA) underwent intramolecular cyclization to afford the racemic tetrahydroisoquinoline **5**. Compound **5** was further subjected to reaction with acetic anhydride to afford 2-acetyl-1-(4′-chlorophenyl)-7-acetoxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**6**). Derivative **6** was converted into 2-acetyl-1-(4′-chlorophenyl)-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**7**) under basic hydrolysis.

2.2. Radiochemistry

Treatment of the phenolic precursor **7** with [^{11}C]CH_3I in DMSO in the presence of sodium hydroxide followed by HPLC purification provided the ^{11}C labelled isoquinoline in 17 ± 5% (n = 8) decay corrected yield within 35 min after end-of-bombardment. The radiochemical purity was 99% with a specific activity of 56 GBq/μmol. The tritiated derivative 2-acetyl-1-(4′-chlorophenyl)-6-methoxy-7[^{3}H]methoxy-1,2,3,4-tetrahydroisoquinoline ([^{3}H]**1**) was obtained by replacing [^{11}C]CH_3I with [^{3}H]CH_3I (custom synthesis by Tritium Custom Preparation Group, Amersham Biosciences).

2.3. Biology

High selectivity, good uptake and retention in target tissue, and low unspecific binding are key requirements for PET tracers. Our initial concern was therefore to confirm the selectivity of compound **1** for the AMPA receptor system. A receptor binding assay covering 18 receptors and subtypes was therefore carried out (NovaScreen) and at a concentration of 10 μM no cross activity was observed. Next, [^{11}C]**1** was evaluated in adult male Sprague–Dawley rats. The peripheral distribution of the compound is shown in Figure 2. Of the tissues sampled, skeletal muscle and skin had low initial contents of less than 1 uptake unit[15] ('uptake units' = (cpm g^{-1} wet weight tissue)/(injected cpm g^{-1}



Scheme 1. Reagents and conditions: (i) dry toluene, Δ, 3 h; (ii) TFA, Δ, 1.5 h; (iii) Ac_2O, Δ, 1.5 h; (iv) K_2CO_3, MeOH, rt, 1 h.

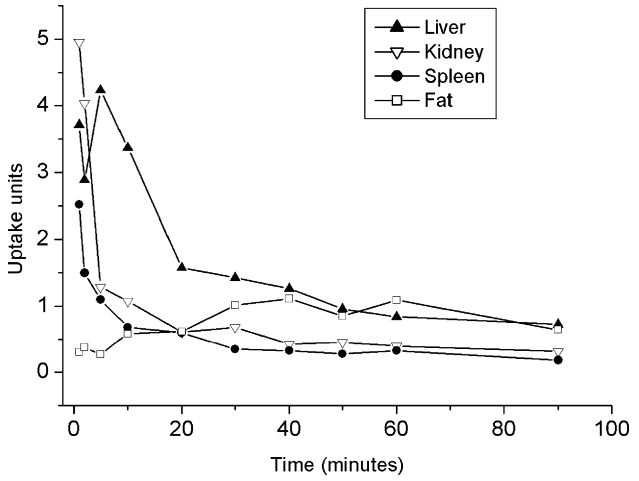


Figure 2. The time course of radioactivity in representative peripheral tissues after injection of [^{11}C]**1**. Uptake is given in 'uptake units', see text and reference.[14]

body weight)$^{-1}$) and this concentration decreased to ~0.1 over 90 min. Similar profiles were observed in stomach, thymus, intestine and liver although each had higher initial contents of between 1 and 4 uptake units. Kidney, spleen, heart and lung had an initial content of between 2 and 5 uptake units followed by a rapid clearance. Fat was the only tissue that accumulated radioactivity, from an initial low uptake of 0.30 uptake units to 1.11 at 40 min after injection. During the course of the experiment, there was only a small amount of clearance via urine but high and rapid clearance via the small intestine content. Initial clearance from body fluids was rapid – dropping from 10 to 0.76 uptake units from 0.2 to 2 min, thereafter clearance was slow reaching 0.22 uptake units at the final sample time of 90 min.

The time course of radioactivity in several brain regions after injection of [^{11}C]**1** was also examined (Fig. 3). The highest initial uptake (1 min p.i.) was found in inferior colliculi with 3.12 uptake units followed by superior colliculi (2.52) and cingulate cortex (2.48), with uptake in olfactory tubercles, thalamus and prefrontal cortex closely following with about 2.40 uptake units. Hippocampus had the lowest uptake (1.84), comparable with plasma concentration (1.40) at this time point. After the initial uptake, no brain tissue showed any further accumulation of radioactivity. Although there was slightly slower loss in 'pons with medulla', the remaining tissues showed a rapid clearance and relatively homogeneous distribution throughout the experiment. This is in contrast to the known distribution of brain AMPA receptors, with hippocampus, outer layers of cortex, olfactory regions, lateral septum, basal ganglia and amygdala enriched in GluR1, GluR2 and GluR3, while reticular thalamic nuclei and cerebellum are enriched in GluR4.[3]

To assess the impact of metabolism on brain uptake and distribution, the composition of radioactive species in brain tissue and plasma was analysed. In plasma, 94% was found to be intact tracer at 2 min, dropping to 81% at 5 min and 69% after 10 min. In brain, 91% of the tracer was intact at 10 min, dropping to 86% after 30 min. A total of four metabolites were detected, all more polar than the parent. This suggests that, at least for the earlier time points, metabolism had a limited effect on the observed uptake and distribution of the compound according to our recent studies.[16] However, the metabolic stability of compound **1** is likely be insufficient for PET studies in humans.

To gain further understanding of the properties of [^{11}C]**1**, the tritiated racemic derivative [^{3}H]**1** was subjected to a saturation binding study (MDS Pharma Services) using rat cortex membranes (Sprague–Dawley derived rats, 6–8 weeks) (Fig. 4). In these experiments, the K_d value for [^{3}H]**1** was found to be 14.8 ± 1.8 nM, specific to unspecific binding was 1:2 and B_{max} was 148 ± 33 fmol/mg protein.

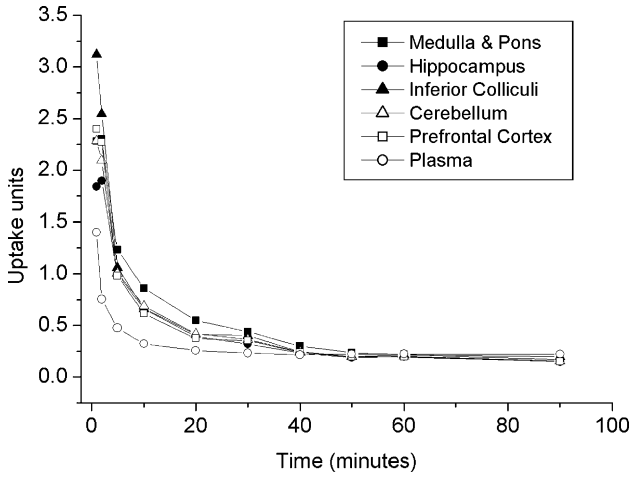


Figure 3. The time course of radioactivity in representative brain regions after injection of [^{11}C]**1**. Uptake is given in 'uptake units', see text and reference.[14]

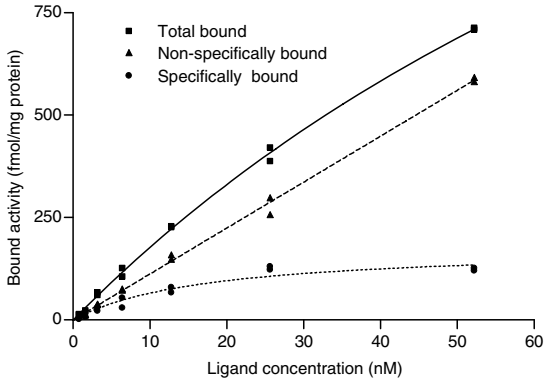


Figure 4. Characterization of [^{3}H]**1** binding to rat cortex membranes. These are representative experiments repeated 4 times. [^{3}H]**1** (0.61–52 nM) was incubated for 90 min at 4 °C before filtration. Total binding (■), non-specific binding (▲) measured with [^{3}H]**1** (5 nM) in the presence of 100 μM **1** and specific binding (●) are expressed in fmol/mg protein. K_i was 14.8 ± 1.8 nM, specific binding was 50% and B_{max} was 148 ± 33 fmol/mg protein. Data are means ± SD.

Although the receptor density measured with [^{3}H]**1** is comparable to that found for the high affinity site of the ligand AMPA itself (B_{max} 200 fmol/mg),[17] it is significantly lower than for other selective high affinity AMPA antagonists such as Ro 48-8587 (B_{max} 1000 fmol/mg)[18] and CP-526,427 (B_{max} 7000 fmol/mg).[19] Neuroimaging using PET requires a certain proportion of the radioligand to be receptor bound in order to give an accumulation or increased retention of the ligand in target tissue. As the concentration of the radioligand itself is minute, the binding potential (BP) is dictated by the affinity of the ligand for the receptor and the receptor density according to the formula $BP = B_{max}/K_d$.[20] In order to obtain a sufficient signal in vivo, the binding potential should exceed 0.5.[21] Although results obtained in vitro only serve as an indication of what might be found in vivo, the substantial difference between the measured B_{max} and K_d for [^{3}H]**1** clearly suggests insufficient binding potential and may explain the rapid clearance of the radioligand [^{11}C]**1** from the CNS, observed in our experiments. Hence, the binding pattern of [^{11}C]**1** in the CNS is likely to represent a combination of regional blood flow and unspecific binding.

This study raises several issues for future development of PET imaging of AMPA receptors. First, it is clear that the measured receptor density varies substantially between AMPA specific antagonists, despite the current notion that only AMPA modulators are subtype selective.[4] The assessment of future PET candidates should therefore be based on a combination of their affinity and the density of the receptor population they bind to. Second, as different AMPA antagonists label different populations of AMPA receptors, a detailed pharmacological profile will be required for any future imaging agent for AMPA receptors. Finally, the low density of AMPA receptors that in general are found with currently available high affinity AMPA ligands suggests that a ligand with sub-nanomolar affinity might be required for successful in vivo imaging of AMPA receptors.

3. Conclusion

2-Acetyl-1-(4′-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**1**), one of the most potent non-competitive AMPA antagonists described to date, has been labelled with carbon-11 and tritium and evaluated as a potential ligand for in vivo imaging of AMPA receptors using PET. Labelling with carbon-11 was achieved in 17% decay corrected yield (35 min after end-of-bombardment) by [^{11}C]methylation of the desmethyl precursor. In biodistribution studies in rats, the labelled compound showed good brain uptake and rapid clearance from the periphery; however, the distribution in the CNS was uniform and no retention was observed in regions known to be rich in AMPA receptors. In saturation binding studies, the tritiated racemic ligand was found to be highly potent with a K_d of 14.8 ± 1.8 nM, but the AMPA receptor population labelled with this compound was only 148 ± 33 fmol/mg protein. This suggests a low binding potential, which may explain the rapid clearance of the ligand from the CNS. In conclusion, the low receptor density labelled with this compound, its rapid clearance from the CNS and low specific binding make it unsuitable as an in vivo imaging agent for AMPA receptors using PET.

4. Experimental

4.1. General

4.1.1. Chemistry. 4-Hydroxy-3-methoxyphenylethylamine was obtained from Fluorochem limited, UK. All other reagents and solvents were obtained from Aldrich Chemical Co. or Fisher Scientific and used without further purification. Melting points were determined on a Stuart SMP10 apparatus and are uncorrected. Elemental analyses (C, H, N) were carried out on a Carlo Erba Model 1106 Elemental Analyzer and the results are within ±0.4% of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC; column chromatography was performed on Merck silica gel 60 (70–230 mesh). ^{1}H NMR spectra were measured in $CDCl_3$ with a Varian Gemini 300 spectrometer; chemical shifts are expressed in δ (ppm) relative to TMS as internal standard and coupling constants (*J*) in Hz. All exchangeable protons were confirmed by addition of D_2O.

HPLC was carried out using a Beckman 110B HPLC pump connected to a SA 6506 UV detector (Severn Analytical) set at 254 nm, a GM tube and a Servogor 124 twin pen chart recorder (Spectronic). Analytical HPLC for quality control was carried out on an Agilent 1100 series HPLC system equipped with a Bioscan GM tube detector.

4.1.2. Radiochemistry. [^{11}C]Methyl iodide was prepared using the standard 'wet' method as described by Turton et al.[22] The radio-synthesis was carried out using a remotely controlled apparatus housed within a lead shielded enclosure. [^{3}H]**1** was synthesized by Amersham Biosciences (The Maynard Centre, UK) by reaction of precursor **7** with [^{3}H]CH_3I. The radiochemical purity was 99% and the specific activity was 3.1 GBq/μmol.

4.1.3. Biology

4.1.3.1. Biodistribution. Male Sprague–Dawley rats (n = 10; body weight range, 256–290 g ± SE = 275 ± 4 g) were given tail vein and artery catheters under isoflurane and N_2O/O_2 anaesthesia. The animals were allowed to recover whilst lightly restrained in Bollman cages. Each rat received ~12 MBq of [^{11}C]**1** in a volume of 0.20 ml associated with a mass of co-injected compound 1 of 0.77 ± 0.03 nmol kg^{-1}. Radiochemical purity was ~99% and, at the time of injection, specific activity was ~56 MBq nmol^{-1}. At graded times 5–6 blood samples per animal with an arterial catheter (n = 4) were withdrawn at designated times, composite blood and plasma curves for radioactivity as a function of time were obtained by pooling data from animals. At times ranging between 1 and 90 min after radioligand injection, rats were killed and the following brain tissues were sampled; olfactory bulbs, olfactory tubercles, hypothalamus,

thalamus, prefrontal cortex, septal nuclei, cingulate cortex, striata, somatosensory cortex, amygdala with piriform cortex, inferior colliculi, superior colliculi, hippocampus, visual with temporal cortex, pons with medulla and cerebellum. In addition, the following peripheral tissues were sampled; skeletal muscle, skin, urine, fat, testis, small intestine, small intestine content, large intestine, large intestine content, spleen, liver, kidney, stomach, thymus, lung and heart ventricle. Radioactivity content was measured using a Wallac gamma-counter, with automatic correction for radioactive decay, and results normalized for the amount injected relative to body weight, giving[15]

$$\text{'uptake units'} = (\text{cpm/g wet weight tissue}) / (\text{injected cpm/g body weight}).$$

All biological work was carried out by licensed investigators in accordance with the UK Home office's "Guidance on the operation of Animals (Scientific procedures) Act 1986" (HMSO, Feb 1990).

4.1.3.2. Radiolabelled metabolite analysis. Blood samples were taken at 2, 5, 10 and 30 min after i.v. injection of [^{11}C]**1**). An aliquot of blood was taken for measurement of radioactivity, the remaining sample was dispensed into a heparinized tube and centrifuged (2000*g* for 2 min). Plasma samples (450 μL) were deproteinated with ice-cold acetonitrile (10 mL) and centrifuged (2000*g* for 2 min). The resulting pellets and duplicate aliquots (100 μL) of acetonitrile supernatant were taken for measurement of radioactivity. The remaining acetonitrile supernatant containing the extracted [^{11}C]**1** was rotary evaporated, the residue dissolved in the HPLC mobile phase (2.5 mL, acetonitrile/0.1 M ammonium formate 6:4 volume/volume) and filtered. Duplicate aliquots were taken for counting. Brain samples (cerebellum) were homogenized with ice-cold acetonitrile (14 mL) and the resulting mixture was centrifuged (2000*g* for 2 min). Duplicate aliquots (100 μL) were taken for counting. The acetonitrile supernatant was concentrated in vacuo, the residue dissolved in HPLC eluent (2.5 mL) and filtered.

The processed samples were injected into an HPLC column ('μ' Bondapak C_{18}). The column was eluted at a flow rate of 3 mL/min. The HPLC eluent was monitored sequentially for radioactivity and UV absorbance at 254 nm. Both detectors were linked to a computer-based integrator that recorded the chromatogram and allowed the correction of the data for physical decay, background radioactivity. The methodology has been reported elsewhere.[16,23]

4.1.3.3. In vitro studies. The radioligand binding studies were carried out by NovaScreen, USA. Determination of AMPA affinity, B_{max} and specific binding for compound [^{3}H]**1** was carried out by MDS Pharma Services, Taiwan, using literature procedures[17,24] with the following modifications: rat cortex membranes from Sprague–Dawley derived rats (6–8 weeks) were used in the experiments. Non-specific binding was measured with 100 μM compound **1** in place of 1 mM glutamic acid. The incubations were carried out for 90 min at 4 °C. The results provided are the average of four experiments, each carried out in doublets with 6 concentrations of compound **1**. The data were analysed using GraphPad Prism (GraphPad Prism version 4.03, San Diego, CA, USA). All animal procedures were carried out in full compliance with institutional guidelines relating to the conduct of animal experiments.

4.1.3.4. Receptor screen. Adenosine, A2; Adrenergic; Alpha 1, non-selective; Adrenergic, Beta, non-selective; Dopamine, D1; Dopamine, D2s; GABA A; GABA B; Muscarinic, Non-selective, Central; Nicotinic; Opioid, Non-selective; Serotonine, 5HT1, Non-selective; Glutamate, AMPA site; Glutamate, Chloride Dependent site; Glutamate, Kainate Site; Glutamate, MK-801 site; Glutamate, NMDA Agonist site; Glutamate, PCP site; Glutamate, NMDA, Glycine site.

4.1.4. Synthesis of 1-(4′-chlorophenyl)-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (5). A mixture of 2-(3′-methoxy-4′-hydroxyphenyl)ethylamine (**2**) (1.8 g, 10 mmol) and 4-chlorobenzaldehyde (**3**) (1.68 g, 12 mmol) in anhydrous toluene (50 mL) was refluxed for 3 h, then cooled and evaporated in vacuo. The oil residue was treated with diethyl ether to give a solid crude product, which was crystallized from EtOH to afford the desired imine **4**. Trifluoroacetic acid (10 mL) was added to a solution of 4-chlorobenziliden[2-(3′-methoxy-4′-hydroxyphenyl)ethyl]amine (**4**) (0.924 g, 3.2 mmol), and the mixture was refluxed for 90 min. By adding water the reaction was quenched, and the mixture was basified (pH 8–9) with NaOH to give the isoquinoline derivative **5** as a solid. The crude product was collected by filtration and purified by crystallization with MeOH. Mp 90–94 °C. Yield 80%. ^{1}H NMR: δ: 2.74–3.24 (m, 5H, CH_2–CH_2 + NH), 3.87 (s, 3H, MeO-6), 4.97 (s, 1H, H-1), 6.26 (s, 1H, H-5), 6.60 (s, 1H, H-8), 7.19 (d, 2H, J = 8.2, H2′–H6′), 7.29 (d, 2H, J = 8.2, H3′–H5′).

4.1.5. Synthesis of 7-acetoxy-2-acetyl-1-(4′-chlorophenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline (6). A solution of 1-(4′-chlorophenyl)-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**5**) (0.375 g, 1.3 mmol) in Ac_2O (10 mL) was refluxed for 90 min and then cooled, the reaction was quenched by adding water and the organic layer was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, and the solvent was removed until dryness under reduced pressure. The oil residue was washed with Et_2O, and the crude was crystallized with EtOH to afford compound **6**. Mp 82–84 °C. Yield 64%. ^{1}H NMR: δ: 2.16 (s, 3H, *Me*CON), 2.27 (s, 3H, *Me*COO), 2.71–3.71 (m, 4H, CH_2–CH_2), 3.84 (s, 3H, MeO-6), 6.72 (s, 1H, H-5), 6.77 (s, 1H, H-8), 6.81 (s, 1H, H-1), 7.18 (d, 2H, J = 8.5, H2′–H6′), 7.24 (d, 2H, J = 8.5, H3′–H5′).

4.1.6. Synthesis of 2-acetyl-1-(4′-chlorophenyl)-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline (7). A mixture of 7-acetoxy-2-acetyl-1-(4′-chlorophenyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline (**6**) (0.1 g, 0.268 mmol) and K_2CO_3 (0.081 g, 0.587 mmol), in MeOH (10 mL), was stirred at room temperature for

1 h. The reaction mixture was filtered and then evaporated in vacuo. The residue was made acidic (pH 2–3) with HCl to give the desired compound **7** as a white solid, which was collected by filtration. Mp 64–66 °C. Yield 60%. ^{1}H NMR: δ: 2.16 (s, 3H, *Me*CON), 2.70–3.74 (m, 4H, CH_2–CH_2), 3.90 (s, 3H, MeO-6), 5.56 (s, 1H, OH), 6.59 (s, 1H, H-5), 6.65 (s, 1H, H-8), 6.76 (s, 1H, H-1), 7.17 (d, 2H, J = 8.5, H2′–H6′), 7.23 (d, 2H, J = 8.5, H3′–H5′).

4.1.7. 2-Acetyl-1-(4′-chlorophenyl)-6-methoxy-7-[^{11}C]methoxy-1,2,3,4-tetrahydroisoquinoline ([^{11}C]1). To the phenolic precursor **7** (2 mg) were added NaOH (4 mg) and DMSO (200 μl). [^{11}C]MeI was bubbled into the reaction mixture for 2–3 min, the reaction vial was sealed and heated to 70 °C for 3 min. The resulting mixture was diluted with water, concentrated by means of a Sep-Pak column (C_{18} classic), the column was washed with water and eluted with MeOH. The methanolic fraction was purified by HPLC (Jupiter Semi prep C_{18}, MeCN/100 mM $(NH_4)_2HPO_4$ 45/55, 4 ml/min) to give the title compound in 17 ± 5% (n = 8) decay corrected yield. In this system, the retention time of the product was 12.0 min and that of the precursor **7** was 8.3 min. The radioactive product co-eluted with an authentic sample of compound **1**.

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