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Synthesis of [$^{13}C_2$, ^{15}N]-1,3-2*H*-1-benzyl-(*Z*)-3-(benzylidene)indolin-2-one

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the accumulation of α-synuclein into Lewy bodies. 3-Benzylidine-indolin-2-one represent a class of compounds, which are known to inhibit the accumulation of α-synuclein. In this paper we report the synthesis of [^{13}C] and [^{15}N] labelled 1-benzyl-(*Z*)-3-(benzylidene)indolin-2-one from commercially available [$^{13}C_2$]-chloroacetic acid and [^{15}N]-aniline in five steps. The product will be used to study its metabolites in human liver microsomes by liquid chromatography-tandem mass spectrometry.

KEYWORDS: α-synuclein, 3-(benzylidene)indolin-2-one, isotope label, synthesis

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1 | INTRODUCTION

Neurodegenerative diseases are characterized by progressive dysfunction, neuronal death and showing specific protein inclusion.[1,2] For example, Alzheimer's disease (AD), the most common form of dementia is diagnosed post-mortem by the appearance of extracellular plaques composed of amyloid beta (Aβ) peptide and intracellular tangles composed of tau protein aggregated into paired helical filaments (PHFs).[3,4] The diagnosis of neurodegenerative diseases is challenging in the earlier stages. Molecular imaging by nuclear medicine techniques, and in particular positron emission tomography (PET) molecular imaging, has considerably evolved providing useful biomarkers for neurodegenerative disease.[5] Amyloid plaques and neurofibrillary tangles in AD and their increased deposition can be detected by various PET-tracers, for example Pittsburgh Compound B (PiB), a derivative of Thioflavin-T (ThioT), has been highly useful as a positron emission tomography (PET) probe for selectively quantifying amyloid plaques in vivo.[6-9] Recently a number of ^{18}F and ^{11}C-labeled agents for imaging Aβ are commercially available permitting large scale clinical use and several tau radiotracers are undergoing evaluation in human imaging studies.[10-13]

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease.[14] Its main clinical symptoms including tremors, muscle rigidity, drooping posture and walking difficulty.[15,16] PD affects 1% of people over the age of 65 and approximately 4% of the population aged over 80 years.[17,18] Since PD is becoming a serious concern for many countries,[15] early therapy and disease prevention is essential, which can only be accomplished with early diagnosis. Neuropathological hallmarks of PD are loss of dopaminergic neurons in the substantia nigra and the formation of intraneuronal protein inclusions termed Lewy bodies, composed primarily of α-synuclein.[19,20] Small molecules that have suitable pharmaceutical properties as fibrillar α-synuclein ligands and that can be labelled with position emission

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tomography (PET) radionuclides will have great opportunity to serve as PET probes for quantifying α-synuclein aggregation in the brain.[21] But no suitable PET tracer has been reported that can be used to assess aggregated α-synuclein accumulation in the brain in vivo.

2-Oxindoles are an important class of heterocyclic compounds and possess a larger range of pharmaceutical activities.[22-24] Recently Professor Robert H. Mach synthesized a series of indolinone-diene analogues and their binding assays for α-syn, Aβ and tau fibrils were conducted. (*Z*)-1-(4-(2-Fluoroethoxy)benzyl)-3-((*E*)-3-(4-nitrophenyl)-allylidene)indolin-2-one showed high affinity (2 nM) and excellent selectivity for α-syn versus Aβ and tau fibrils and could be used as lead compound to study PET-based radiotracers for imaging α-syn aggregates which occur in Parkinson's disease.[25] Indolinone derivatives also exhibit high affinity towards receptor tyrosine kinases. SU5416 (Semaxinib) and SU11248 (Sutent) (Figure **1**) have entered preclinical and multi-center clinical studies for anti-angiogenic and antitumor activity. Torsten Kniess et al synthesized 3-[4′-[^{18}F]fluorobenzylidene]indolin-2-one (Figure **2**) and studied its biodistribution, clearance and found two more polar metabolites in arterial blood plasma of Wistar rats.[26] However, indolinone metabolites have not been studies systematically. To study metabolites of indolinone analogues in human liver microsomes by liquid chromatography-tandem mass spectrometry, we prepared [$^{13}C_2$, ^{15}N]-1,3-2*H*-1-benzyl-(Z)-3-(benzylidene)indolin-2-one.

FIGURE 1 Structures of tyrosine kinase inhibitors U5416 and SU11248

FIGURE 2 Structure of 3-[4′-[^{18}F]fluorobenzylidene]indolin-2-one

2 | RESULTS AND DISCUSSION

The synthesis of [$^{13}C_2$, ^{15}N]-1,3-2*H*-1-benzyl-(Z)-3-(benzylidene)indolin-2-one is outlined in Scheme **1**. Treatment of commercially available [$^{13}C_2$]-chloroacetic acid with oxalyl chloride afforded [$^{13}C_2$]-chloroacetyl chloride (**1**). Then compound **1** reacted with [^{15}N]-aniline to give [$^{13}C_2$, ^{15}N]-2-chloro-

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N-phenyl-acetamide (**2**). After cyclization in presence of $AlCl_3$ via Friedel-Crafts reaction, the key intermediate [$^{13}C_2$, ^{15}N]-1,3-2*H*-indol-2-one (**3**) was obtained. Condensation of [$^{13}C_2$, ^{15}N]-1,3-2*H*-indol-2-one (**3**) with benzaldehyde gave (*Z*)-isomer and (*E*)-isomer, with separation by column chromatography, [$^{13}C_2$, ^{15}N]-1,3-2*H*-(*Z*)-3-(benzylidene)indolin-2-one (**4**) and [$^{13}C_2$, ^{15}N]-1,3-2*H*-(*E*)-3-(benzylidene)indolin-2-one (**5**) were obtained. The configurations of **4** and **5** were assigned by comparison of their ^1H NMR spectrum with unlabeled compounds.[27-29] It is reported that *Z*-isomer has a higher affinity for α-syn. Finally compound **4** reacted with benzyl bromide to give [$^{13}C_2$, ^{15}N]-1,3-2*H*-1-benzyl-(*Z*)-3-(benzylidene)indolin-2-one (**6**).

3 | CONCLUSION

The [^{13}C] and [^{15}N] labelled 1,3-2*H*-1-benzyl-(*Z*)-3-(benzylidene)indolin-2-one was prepared in overall yield 2.9% via a synthetic route involving five steps with [$^{13}C_2$]-chloroacetic acid as starting material. The product will be useful in experiment to study its metabolites in human liver microsomes by liquid chromatography-tandem mass spectrometry.

SCHEME 1 Synthesis of [$^{13}C_2$, ^{15}N]-1,3-2*H*-1-benzyl-(*Z*)-3-(benzylidene)indolin-2-one

4 | EXPERIMENTAL

[$^{13}C_2$]-Chloroacetic acid (99%) and [^{15}N]-aniline **(**98%+) were purchased from Cambridge Isotope Laboratories **(**Andover, United State**)**. Chemicals were of analytical grade and used without further purification. Reactions were monitored by thin layer chromatography on pre-coated silica 60 F254 aluminium plates (Merck, Darmstadt, Germany). Spots were visualized by UV light 254 nm. Melting points were determined on XT4A microscopic digital melting-point apparatus and are uncorrected. ^1H NMR (400 MHz) spectra were recorded on a Bruker Avance 400 NMR spectrometer in $CDCl_3$ (TMS as internal standard). HRMS Spectra were obtained with Agilent Q-TOF 6520 spectrometer.

4.1 | [$^{13}C_2$]-chloroacetyl chloride (1)

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A solution of [$^{13}C_2$]-chloroacetic acid (250 mg, 2.6 mmol) in dichloromethane (5 mL) was stirred in a cooling bath under nitrogen gas atmosphere. Oxalyl chloride (0.16 mL, 1.9 mmol) in dry dichloromethane (2 mL) was added dropwise and the mixture was stirred at 0°C for 1 hour followed by stirring at ambient temperature for 3 hours. After completion of the reaction, colorless solution of [$^{13}C_2$]-chloroacetyl chloride (**1**) was obtained and used to next step with no further purification.

4.2 | [$^{13}C_2$, ^{15}N]-2-chloro-N-phenyl-acetamide (2)

[^{15}N]-Aniline (160 mg, 1.7 mmol) and triethylamine (0.5 mL) were dissolved in dry dichloromethane (2 mL). The resulting solution was cooled to 0°C, then **1** in DCM was added dropwise in 20 minutes. The reaction mixture was stirred at room temperature for 1 hour. The reaction was quenched by water (30 mL). The mixture was extracted with DCM (25 mL×4) and washed with saturated ammonium chloride (20 mL×3). The combined organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by column chromatography (petroleum ether/EtOAc, 10:1) to give **2** as a white solid (337 mg, 92.6%). m.p. 131.3-133.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, J_{N-H} = 90.4 Hz, 1H), 7.49-7.47 (d, J = 8.0 Hz, 2H), 7.32-7.28 (t, J = 7.4 Hz, 2H), 7.13-7.09 (t, J = 7.2 Hz, 1H), 4.13 (dd, J_{C-H} = 153.4, J_{H-H} = 3.8 Hz, 2H). HRMS (ESI+) *m/z* calcd. for $^{12}C_6{}^{13}C_2H_8Cl^{15}NO$ (M+H) 173.0410, found 173.0407.

4.3 | [$^{13}C_2$, ^{15}N]-1,3-2*H*-indol-2-one (3)

[$^{13}C_2$, ^{15}N]-2-Chloro-N-phenyl-acetamide (**2**) (337 mg, 2.0 mmol) was combined with aluminum chloride (1.2 g, 9.0 mmol) as a mixture of solid. The mixture was then heated in an oil bath at 230°C for 15 minutes. After cooling to room temperature, diluted hydrochloric acid (50 mL) was added dropwise and the mixture was extracted with ethyl acetate (30 mL×5). The organic layer was washed with brine, water and dried over Na_2SO_4. The solvent was removed under vacuum and the residue was purified by flash column

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chromatography (petroleum ether/EtOAc, 10:1) to give [$^{13}C_2$, ^{15}N]-1,3-2*H*-indol-2-one (**3**) (172.5 mg, 66.6%) as a white solid. m.p. 125.5-126.3°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, J_{N-H} = 94.4 Hz, J_{C-H} = 4.8 Hz, 1H), 7.19-7.13 (m, 2H), 6.97-6.93 (t, *J* = 7.4 Hz, 1H), 6.82-6.80 (d, *J* = 8.0 Hz, 1H), 3.47 (dd, J_{C-H} = 133.4, J_{H-H} = 5.4 Hz, 2H). HRMS (ESI+) *m*/*z* calcd. for $^{12}C_6{}^{13}C_2H_7{}^{15}NO$ (M+H) 137.0643, found 137.0627.

4.4 | [$^{13}C_2$, ^{15}N]-1,3-2*H*-(*Z*)-3-(benzylidene)indolin-2-one (4)

Benzaldehyde (0.14 mL, 1.4 mmol) and piperidine (0.1 mL, 1.1 mmol) were added to a solution of [$^{13}C_2$, ^{15}N]-1,3-2*H*-indol-2-one (**3**) (172.5 mg, 1.3 mmol) in ethanol. The reaction mixture was refluxed for 1 hour and cooled to room temperature. The obtained suspension was filtered, washed with cold ethanol and [$^{13}C_2$, ^{15}N]-1,3-2*H*-(*E*)-3-(benzylidene)indolin-2-one (**5**) was obtained. Evaporation of the solvent under vacuum afforded crude product. Purification of the crude product by flash column chromatography (petroleum ether/EtOAc, 8:1) gave two isomers.

[$^{13}C_2$, ^{15}N]-1,3-2*H*-(*Z*)-3-(benzylidene)indolin-2-one (**4**): 32.2 mg, 11.4%. m.p. 160.5-164.8°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21-8.19 (d, *J* = 6.8 Hz, 2H), 8.00 (dd, J_{N-H} = 95.2, J_{C-H} = 6.0 Hz, 1H), 7.50-7.37 (m, 4H), 7.18-7.14 (m, 2H), 6.99-6.95 (t, *J* = 7.4 Hz, 1H), 6.79-6.77 (m, 1H). HRMS (ESI+) *m*/*z* calcd. for $^{12}C_{13}{}^{13}C_2H_{11}{}^{15}NO$ (M+H) 225.0956, found 225.0933.

[$^{13}C_2$, ^{15}N]-1,3-2*H*-(*E*)-3-(benzylidene)indolin-2-one (**5**): 229.4 mg, 80.5%. m.p. 178.5-179.5°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (dd, J_{N-H} = 87.2, J_{C-H} = 5.6 Hz, 1H), 7.78-7.75 (m, 1H), 7.60-7.56 (t, *J* = 9.0 Hz, 3H), 7.42-7.35 (m, 3H), 7.17-7.13 (m, 1H), 6.82-6.79 (t, *J* = 7.8 Hz, 2H). HRMS (ESI+) *m*/*z* calcd. for $^{12}C_{13}{}^{13}C_2H_{11}{}^{15}NO$ (M+H) 225.0956, found 225.0944.

4.5 [$^{13}C_2$, ^{15}N]-1,3-2*H*-1-benzyl-(*Z*)-3-(benzylidene)indolin-2-one (6)

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A 60% dispersion of sodium hydride (38 mg, 1.6 mmol) was added to a solution of **4** (134 mg, 0.6 mmol) in DMF (3 mL) at room temperature and stirred for 15 minutes. Then benzyl bromide (290 mg, 1.6 mmol) was added. After completion of the reaction (1 hour), the solution was quenched by water (20 mL). The mixture was extracted with methyl tert-butyl ether (20 mL×5) and washed with saturated sodium chloride (20 mL×3). The organic phase was dried with Na_2SO_4 and concentrated in vacuo. The yellow oil residual was purified by flash column chromatography (petroleum ether/EtOAc, 80:1). The yellow solid [$^{13}C_2$, ^{15}N]-1,3-2*H*-1-benzyl-(*Z*)-3-(benzylidene) indolin-2-one (**6**) (84.6 mg, 44.8%) was obtained. m.p. 131.8-132.5°C. ^1H NMR (400 MHz, $CDCl_3$) δ 8.26-8.24 (m, 2H), 7.54-7.46 (m, 2H), 7.41-7.33 (m, 3H), 7.28-7.15 (m, 5H), 7.13-7.09 (t, *J* = 7.6 Hz, 1H), 6.98-6.94 (t, *J* = 7.4 Hz, 1H), 6.66-6.64 (d, *J* = 7.6 Hz, 1H), 4.92-4.91 (d, *J* = 2.4 Hz, 2H). HRMS (ESI+) *m/z* calcd. for $^{12}C_{20}{}^{13}C_2H_{17}{}^{15}NO$ (M+H) 315.1426, found 315.1425.

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CONFLICT OF INTEREST

The authors did not report any conflict of interest.

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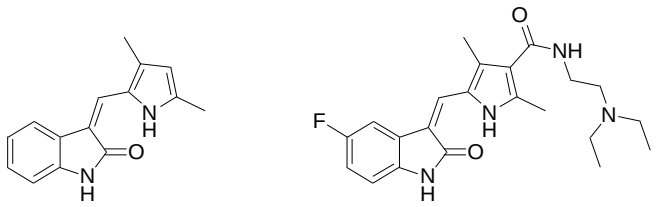

SU5416 (Semaxinib) SU11248 (Sutent)

FIGURE 1 Structures of tyrosine kinase inhibitors U5416 and SU11248

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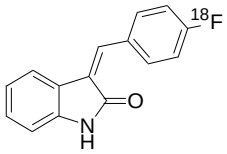

FIGURE 2 Structure of 3-[4′-[^{18}F]fluorobenzylidene]indolin-2-one

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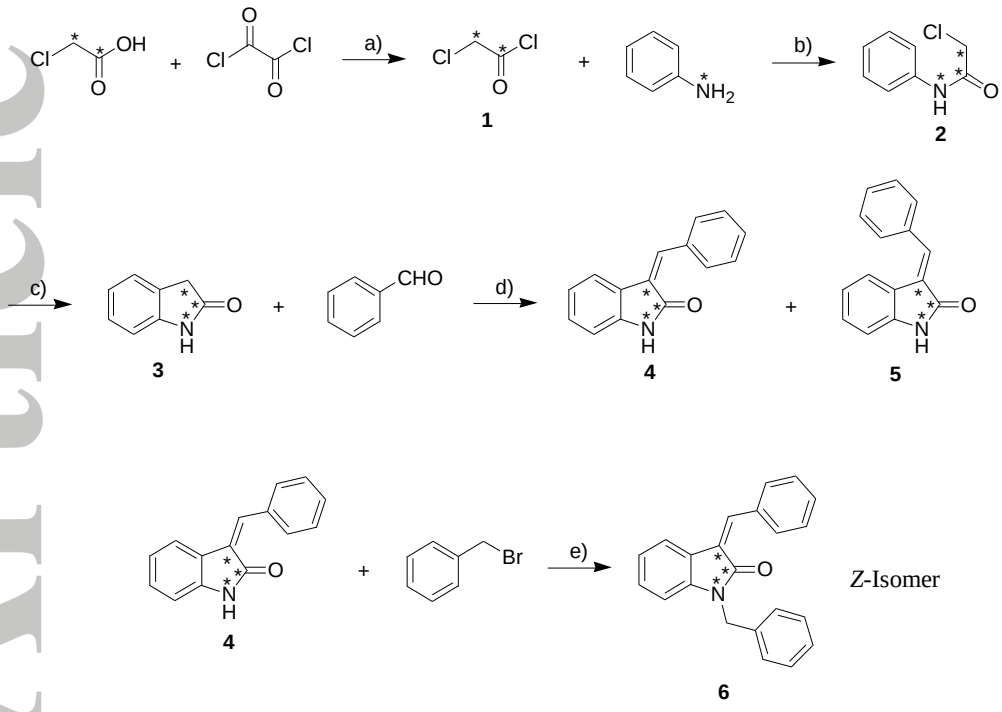

SCHEME 1 Synthesis of [$^{13}C_2$, ^{15}N]-1,3-2*H*-1-benzyl-(*Z*)-3-(benzylidene)indolin-2-one

a) DCM, DMF, 0°C-rt, 4 hours; b) DCM, triethylamine, 0°C-rt, 1 hour; c) anhydrous alchlor, 230°C, 15 min; d) piperidine, ethanol, reflux, 1 hour; e) 60% NaH, DMF, rt, 1 hour

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