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Syntheses of dual-radioisotope-labeled CP-I, a $GABA_A$ receptor partial agonist

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CP-I is a potent subtype-selective $GABA_A$ receptor partial agonist. Owing to its significant metabolic cleavage at C_8 observed in preliminary biotransformation studies with non-radiolabeled CP-I, the syntheses of CP-I labeled at the right or left hand side with ^{14}C or labeled with 3H at the right hand side were required. The two compounds labeled with ^{14}C at the left or right hand side were synthesized in 2 and 5 radio-synthetic steps using [^{14}C]2-chloroacetyl chloride and [^{14}C]NaCN as starting radiolabeled materials, respectively. CP-I was labeled with tritium at the right hand side by a tritium de-halogenation method. Batches of radiolabeled CP-I were mixed to give dual-radioisotope-labeled CP-I. An efficient approach to [^{14}C]fluoropyridinyl imidazole was developed, and a short synthesis of iodo-substituted fluoropyridinyl imidazole was also achieved. The details of these syntheses are discussed.

Keywords: C-14; tritium; dual-radioisotope labeling; halogen dance reaction; $GABA_A$ receptor partial agonist

Introduction

CP-I, 2-{[2-(3-fluoropyrid-2-yl)-1*H*-imidazol-1-yl] methyl}-1-propyl-5-cyano-1*H*-benzimidazole (compound **1**), is a potent subtype-selective partial agonist at the $GABA_A$ receptor complex. This agonist significantly reduces in rate both spontaneous locomotor activity and the latency to assume a sleep posture without the well-known side effects of commonly available hypnotics. Significant metabolic scission of CP-I to benzimidazole alcohol **2** and fluoropyridine imidazole **3** in an NADPH-dependent manner was observed from initial *in vitro* biotransformation studies using non-labeled CP-I (Figure 1).[1]

The further metabolic stability studies of compounds **2** and **3** in the presence of NADPH showed that **2** was inert while **3** was extensively metabolized. Therefore, both C-14- and H-3-labeled CP-I were synthesized for use in *in vivo* biotransformation studies. For the ^{14}C-labeled compounds, the label was placed on either side of C_8, the site of metabolic fragmentation, whereas the ^{3}H labeled was placed on the right-hand side. These labeled compounds were used to determine if all microsomal metabolites formed after C_8 N-dealkylation (or its core-intact metabolites) could be detected and quantified adequately (Figure 2).

By labeling CP-I with different isotopes, it was possible to monitor simultaneously the two radio-isoforms without the confusion of changes in specific activity of mono-radiolabeled metabolites.[1,2]

The retro-synthetic analysis for the incorporation of a single ^{14}C label at both sides of CP-I at position 2 on the substituted imidazole rings is shown in Figure 3. The disconnection of the C_8-N_1 bond gives the retro-targets **A** and **B.** Further disconnection of **A** provides labeled building block **D** and unlabeled intermediate **C**, which can be synthesized from commercially available compound **E**. The further disconnection of the labeled **B** gives the target cyanopyridine synthon **F**, which can be prepared from building block **G.** The carbon-14 radiolabel can be incorporated into **F** by reacting synthon **G** with [^{14}C]KCN or [^{14}C]NaCN. On the other hand, tritium-labeled CP-I could be derived from iodo- or bromo-substituted analog **H** by tritium de-halogenation. The compound **H** should be disconnected to give unlabeled **A** and **I,** while the intermediate **I** might be synthesized from **J (**unlabeled **B**).

More specifically, [^{14}C]chloroacetylchloride, a commercially available radioactive starting material, was utilized to synthesize the C-14-labeled CP-I at the left hand side via compound A by following a known synthetic route (Scheme 1). [^{14}C]NaCN was used to introduce a cyano group into the position 2 of pyridine. A new synthetic approach to the imidazole ring was developed to give compound **B/14** (Scheme 2). The unknown 4-iodo-substituted fluoropyridine imidazole **I/20** was synthesized by a halogen dancing approach and led to the preparation of iodo-substituted CP-I. The latter was converted to H-3-labeled CP-I by palladium catalyzed de-iodination with tritium gas (Scheme 3). The dual-radiolabeled CP-I was prepared by combining two single isotope-labeled compounds ([^{14}C]CP-I, [^{3}H]CP-I) in the desired ratio of C-14 and H-3 (Scheme 4).

Experimental

General methods

All reactions were carried out under an atmosphere of nitrogen unless otherwise stated. LC-MS data were obtained on a Water

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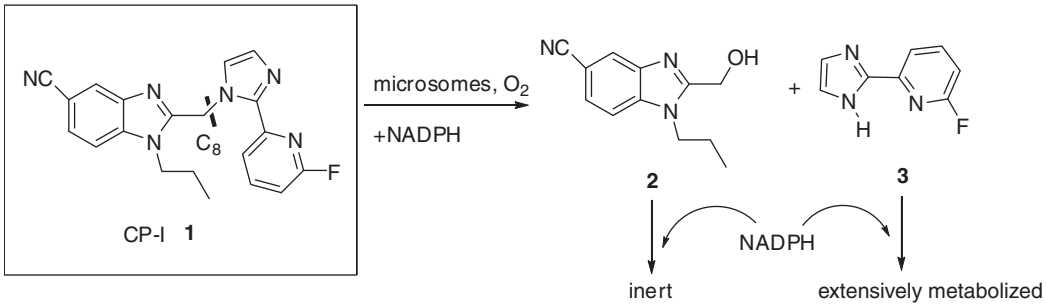


Figure 1. The NADPH-dependent microsomal-mediated metabolic scission of **1** into **2** and **3**.

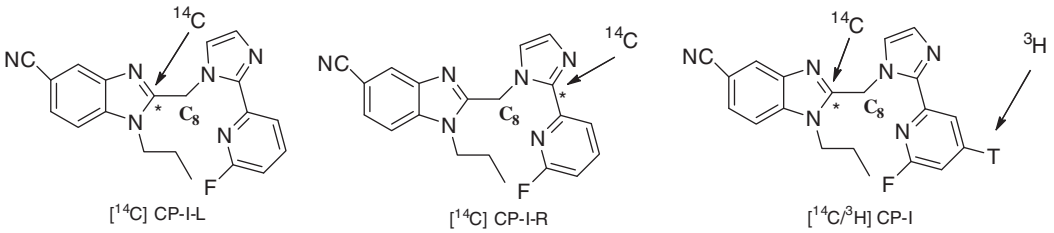


Figure 2. Positions of C-14 and H-3 labels.

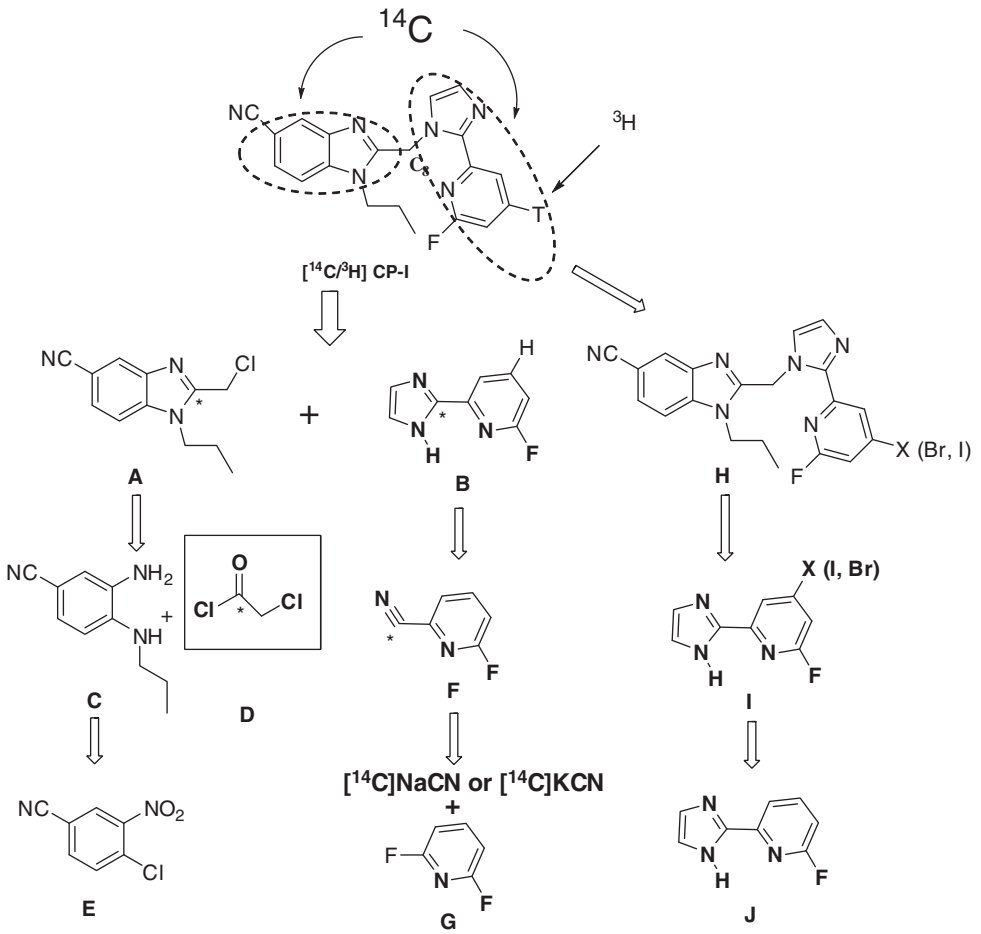
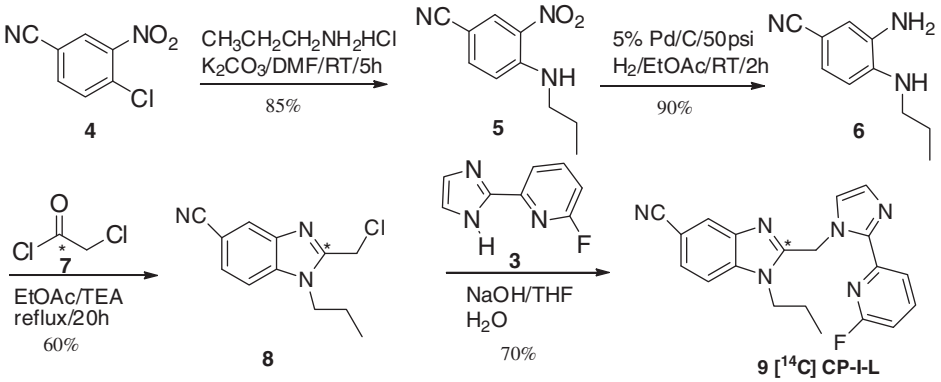
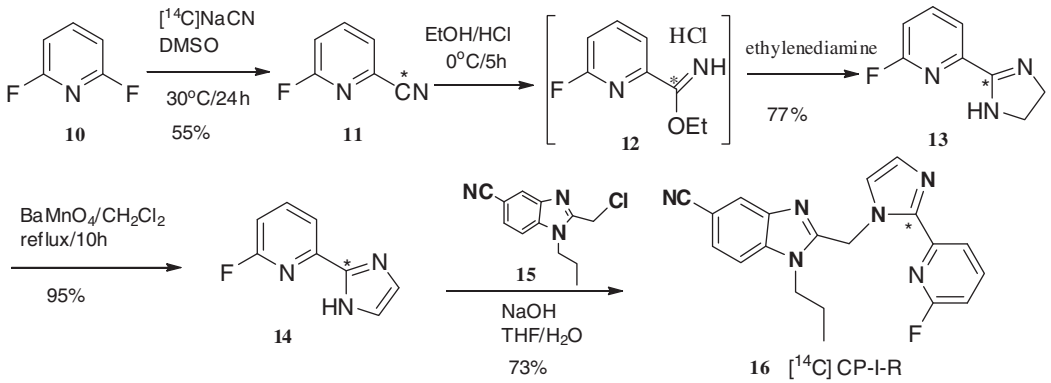


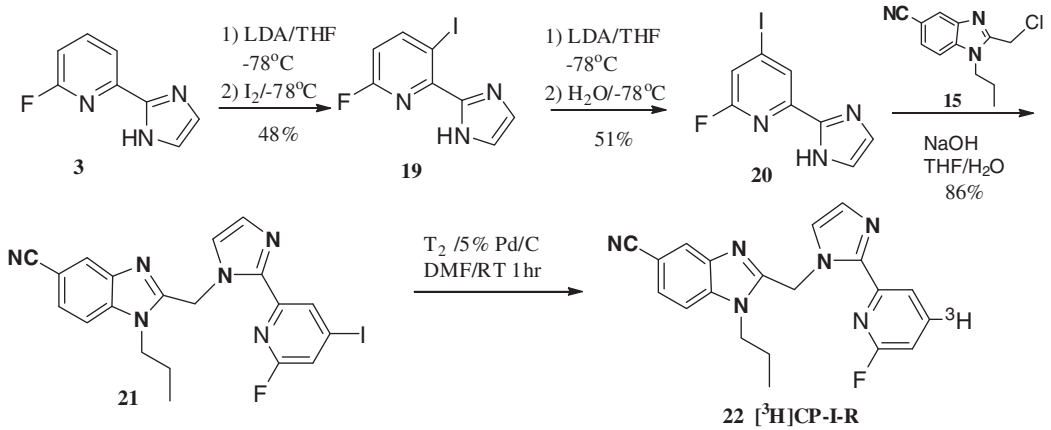
Figure 3. Retro-synthetic pathway of [^{14}C]CP-I and [3H]CP-I.



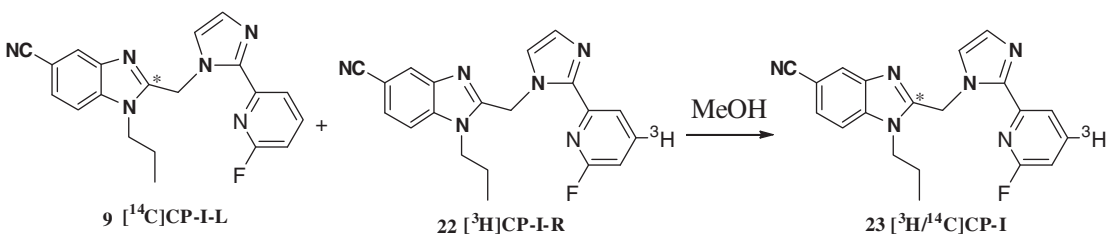
Scheme 1. Synthesis of [^{14}C]CP-I-L.



Scheme 2. Synthesis of [^{14}C]CP-I-R.



Scheme 3. Synthesis of [^{3}H]CP-I-R.



Scheme 4. Preparation of [^{3}H/^{14}C]CP-I.

Micromass LCZ mass spectrometer with flow injection analysis and electrospray ionization (ESI). $^{1}H/^{3}H$ and ^{13}C NMR spectra were recorded on a Varian Gemini 400 MHz instrument. Chemical purity of all labeled compounds was determined by HPLC and LC-MS. Purifications were done by flash column chromatography on Biotage Flash 40 system. Quantitation of radioactivity of C-14- and H-3-labeled compounds was performed using a Packard 2200CA liquid scintillation analyzer, with Scintiverse BD cocktail used throughout. Commercial reagents and solvents were purchased from Aldrich and used as-received unless otherwise noted. [^{14}C]Chloroacetyl chloride (50 mCi, 50 mCi/mmol) and [^{14}C]NaCN (60 mCi, 54 mCi/mmol) were purchased from American Radiolabeled Chemicals, Inc. Intermediates **3** and **15** and compound **1** were provided by Chemical R&D, Groton Lab, Pfizer Inc. 3-Amino-4-n-propylaminobenzonitrile **6** was synthesized according to the procedures described by Li.[3] All known compounds were identified by comparison of NMR spectra to those reported in the literature.

2-Chloromethyl-1-propyl-1H-benzol[d][2-^{14}C]imidazole-5-carbonitrile 8

To a solution of 3-amino-4-*n*-propylaminobenzonitrile **6** (0.515 g, 2.94 mmol) and triethylamine (0.45 ml) in ethyl acetate (6 ml) was slowly added [^{14}C]chloroacetyl chloride (50 mCi, 50 mCi/mmol, 1.0 mmol) at room temperature. The reaction mixture was stirred at room temperature for 30 min and then at 60°C for 20 h. After cooling to room temperature, the mixture was diluted with water (5 ml). The organic solution was washed with 1.0 M NaOH (2 × 5 ml), then with 0.25 M KH_2PO_4 (5 ml) followed by brine (5 ml). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give the crude product, which was recrystallized from EtOAc/hexane (1/2) to afford the titled compound as brown crystals (151.7 mg, 32.5 mCi, 50 mCi/mmol, 60%). ^{1}H NMR of [^{14}C]**8** was consistent with the unlabeled compound reported in the literature.[2]

1-Propyl-2-2-(2-fluoropyrid-6-yl)-1H-imidazol-1-yl-methyl-5-cyano-1H-[2-^{14}C]benzimidazole 9 ([^{14}C]CP-I-L)

To a solution of compound **8** (142.6 mg, 30.5 mCi, 0.61 mmol) and compound **3** (106.1 mg, 0.65 mmol) in THF (30 ml) was added a solution of NaOH (81 mg) in water (31 ml) slowly at room temperature. The resulting mixture was stirred at room temperature overnight and at 45°C for 1 h. After cooling the reaction mixture at 0°C for 30 min, the precipitate was collected by filtration and dried under reduced pressure to give the crude product. The pure product was obtained by flash chromatography (EtOAc/hexane = 1/4) as a off-white solid (160 mg, 22 mCi, 50 mCi/mmol, 70%). ^{1}H NMR (CD_3OD): δ 8.17 (q), 8.03 (q), 7.93 (q), 7.83 (s), 7.62 (q), 7.21(q), 6.36 (s), 4.43(t), 1.97(q), 1.04(t); ^{13}C NMR (CD_3OD): δ 162.4 (d), 152.5 (s), 148.3, 138.3, 130.1, 126.6, 125.3, 124.1, 120.4, 120.0, 111.2, 108.5, 105.6, 46.1, 44.6, 23.4, 11.3; HPLC condition for purity: YMC ODS-AQ, 5 μm, 250 × 4.6 mm, column temperature: 30°C, Mobile Phase A: 0.01 M TEA pH 2.5 w/$HClO_4$; Mobile Phase B: CH_3CN, 15% B linear gradient to 60% over 20 min, hold A:B 40:60 to 45 min. Flow rate = 1.0 ml/min, UV detection: 220 nm.

6-Fluoro[2-^{14}C]picolinonitrile 11

To a solution of [^{14}C]NaCN (1.1 mmol, 60 mCi, 54 mCi/mmol) in dry DMSO (1 ml) at 35°C was added 2,6-difluoropyridine (1.266 g, 11 mmol). The resulting mixture was stirred at 30°C for 24 h. After cooling to room temperature, the solid was filtered off and washed with CH_2Cl_2 (3 × 3 ml). The filtrate was washed with brine (3 × 2 ml) and dried over $MgSO_4$ and concentrated *in vacuo* to give the crude product. The pure product was obtained by flash chromatography (THF/hexane = 1/4) (73.8 mg, 33 mCi, 54 mCi/mmol, 55%). ^{1}H NMR ($CDCl_3$): δ 7.97 (dd, $^{3}J_{H\text{-}H}$ = 7.6 Hz, $^{4}J_{H\text{-}F}$ = 5.8 Hz), 7.62 (dd, $^{3}J_{H\text{-}H}$ = 7.3 Hz, $^{4}J_{H\text{-}F}$ = 1.7 Hz), 7.21 (dd, $^{3}J_{H\text{-}H}$ = 8.5 Hz, $^{3}J_{H\text{-}F}$ = 2.6 Hz); The by-product was isolated and identified as 2,6-[$^{14}C_2$]dicyanopyridine (8.2 mg). ^{1}H NMR ($CDCl_3$): δ 8.06 (t, 1H), 7.91 (d, 2H). ^{1}H NMRs of both C-14-labeled product and by-product were consistent with the unlabeled authentic compounds.

6-Fluoro-2-(4,5-dihydro-1H-[2-^{14}C]imidazol-2-yl)pyridine 13

To a solution of 6-fluoro[2-^{14}C]picolinonitrile **11** (73 mg, 32.4 mCi, 0.6 mmol) in EtOH (1.5 ml) and CH_2Cl_2 (1.5 ml) at 0°C was added dry HCl gas during 15 min. The reaction solution was stirred at room temperature for 4 h and then bubbled with dry nitrogen gas to remove the excess HCl gas and some solvent. A solution of EtOH/THF (1/1.5 ml) was added and the resulting mixture was cooled to 0°C, then ethylenediamine (200 mg, 3.33 mmol) was added. The resulting suspension was stirred at 5°C for 1 h and at room temperature for 15 h. The solvent was evaporated *in vacuo*. The oily residue was diluted with CH_2Cl_2 (15 ml) and washed with brine (2 × 4 ml). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give the titled product as an off-white solid (76.6 mg, 24.9 mCi, 54 mCi/mmol, 77%). The pure product was obtained by flash chromatography (5% MeOH in CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 7.98 (dd, $^{3}J_{H\text{-}H}$ = 7.98 Hz, $^{4}J_{H\text{-}F}$ = 2.2 Hz), 7.80 (dd, $^{3}J_{H\text{-}H}$ = 7.90 Hz, $^{4}J_{H\text{-}F}$ = 15.6 Hz), 6.94 (dd, $^{3}J_{H\text{-}H}$ = 8.0 Hz, $^{3}J_{H\text{-}F}$ = 2.6 Hz), 3.77 (b, 4H); ^{13}C NMR (CD_3OD): δ 164.5 (d, $^{4}J_{C\text{-}F}$ = 4.1 Hz), 161.5 (d, $^{1}J_{C\text{-}F}$ = 239.5 Hz), 144.3 (d, $^{3}J_{C\text{-}F}$ = 12.2 Hz), 142.1 (d, $^{3}J_{C\text{-}F}$ = 7.5 Hz), 118.6 (s), 109.5 (d, $^{2}J_{C\text{-}F}$ = 36.2 Hz), 50.2 (b); HRMS (ESI) *m/z* Found 165.0708, calcd for $C_8H_8FN_3$: 165.0702.

6-Fluoro-2-(1H-[2-^{14}C]imidazol-2-yl)pyridine 14

To a solution of compound **13** (76 mg, 0.46 mmol, 24.7 mCi) in CH_2Cl_2 (12 ml) was added $BaMnO_4$ (868.8 mg, 3.39 mmol). The suspension was stirred at 55°C for 10 h. After cooling to room temperature, the precipitate was filtered off and washed with CH_2Cl_2 (3 × 10 ml). The filtrate was concentrated in vacuo to give the crude product. The pure product was obtained by flash chromatography (5% MeOH in CH_2Cl_2) (71.2 mg, 23.5 mCi, 54 mCi/mmol, 95%). ^{1}H NMR (CD_3OD): δ 7.98 (q, $^{3}J_{H\text{-}H}$ = 8.0 Hz, 1H), 7.89 (ddd, $^{5}J_{H\text{-}F}$ = 2.4, $^{3}J_{H\text{-}H}$ = 8.0 Hz, $^{4}J_{H\text{-}H}$ = 0.66 Hz, 1H), 7.19 (b, 2H), 7.0 (ddd, $^{3}J_{H\text{-}F}$ = 2.4, $^{3}J_{H\text{-}H}$ = 8.13 Hz, $^{4}J_{H\text{-}H}$ = 0.66 Hz 1H); ^{13}C NMR (CD_3OD): δ 163.5 (d, $^{1}J_{C\text{-}F}$ = 240.5 Hz), 147.0 (S), 144.9 (s), 142.6 (d, $^{3}J_{C\text{-}F}$ = 7.6 Hz), 116.6 (d, 2C), 108.5 (d, $^{2}J_{C\text{-}F}$ = 38 Hz). HRMS (ESI) *m/z* Found 163.0542, calcd for $C_8H_6FN_3$: 163.0546.

1-Propyl-2-2-(2-fluoropyrid-6-yl)-1H-[2-^{14}C]imidazol-1-yl-methyl-5-cyano-1H-benzimidazole, 16 ([^{14}C]CP-I-R)

To a solution of compound **14** (71.2 mg, 23.5 mCi, 0.435 mmol) and compound **15** (102.8 mg, 0.44 mmol) in THF (20 ml) was added a solution of NaOH (60 mg) in water (22 ml) slowly at room temperature. The resulting mixture was stirred at room temperature overnight and at 45°C for 1 h. After cooling the reaction mixture at 0°C for 30 min, the precipitate was collected

by filtration, dried under reduced pressure to give the crude product. The pure product **16** was obtained by flash chromatography (EtOAc/hexane = 1/4) as a off-white solid (115 mg, 17.2 mCi, 53.8 mCi/mmol, 73%). ^{1}H NMR (CD_3OD): δ 8.17 (q), 8.03 (q), 7.93 (q), 7.83 (S), 7.62 (q), 7.21(q), 6.36 (S), 4.43(t), 1.97(q), 1.04(t); ^{13}C NMR (CD_3OD): δ 162.4 (d), 152.5 (s), 148.3, 138.3, 130.1, 126.6, 125.3, 124.1, 120.4, 120.0, 111.2, 108.5, 105.6, 46.1, 44.6, 23.4, 11.3; HPLC condition for purity: YMC ODS-AQ, 5 μm, 250 × 4.6 mm, column temperature: 30°C, Mobile Phase A: 0.01 M TEA pH 2.5 w/$HClO_4$; Mobile Phase B: CH_3CN, 15% B linear gradient to 60% over 20 min, hold A:B 40:60 to 45 min. Flow rate = 1.0 ml/min, UV detection: 220 nm.

6-fluoro-2-(1H-imidazol-2-yl)-3-iodopyridine 19

To a solution of diisopropylamine (2.9 ml) in dry THF (10 ml) was added a solution of *n*-BuLi (2.5 M, 12 ml) at 0°C. The solution was stirred at 0°C for 30 min and further cooled to −78°C. To this cold solution was added a solution of compound **3** (2.0 g, 12.3 mmol) in THF (5 ml) at −78°C to give a yellow suspension. The suspension was stirred at −78°C for 4 h and then to this cold suspension was added a solution of I_2 (3.68 g, 14.6 mmol) in THF (5 ml). The resulting mixture was stirred at −78°C for 10 h and slowly warmed to room temperature over 10 h. To the reaction mixture was added water (5 ml) and aqueous saturated $NaHCO_3$ (5 ml). The mixture was extracted with ether (4 × 25 ml). The combined ether layers were dried with $MgSO_4$ and concentrated in vacuo to give the crude product. The pure product was obtained by flash chromatography (EtOAc/hexane = 1/4) as a white solid (1.71 g, 48%). ^{1}H NMR (CD_3OD): δ 8.38 (t, 1H), 7.66 (q, 1H), 7.20 (b, 2H); ^{13}C NMR (CD_3OD): δ 163.5 (d, $^1J_{C\text{-}F}$ = 240.5 Hz), 153.0 (d, $^3J_{C\text{-}F}$ = 7.6 Hz), 148.4 (d, $^3J_{C\text{-}F}$ = 15.9 Hz), 144.9 (s, 2C), 120.2 (d, $^4J_{C\text{-}F}$ = 3.8 Hz), 76.3 (d, $^2J_{C\text{-}F}$ = 45.4 Hz); HRMS (ESI) *m/z* Found 288.9514, calcd for $C_8H_{10}FIN_3$: 288.9512.

6-fluoro-2-(1H-imidazol-2-yl)-4-iodopyridine 20

To a solution of diisopropylamine (0.725 ml) in dry THF (5 ml) was added a solution of *n*-BuLi (2.5 M, 3 ml) at 0°C. The solution was stirred at 0°C for 30 min and then cooled to −78°C. To this cold solution was added a solution of compound **19** (1.0 g, 3.46 mmol) in THF (2 ml) at −78°C to give a yellow suspension. The suspension was stirred at −78°C for 4 h and then to this cold suspension was added water (10 ml). The resulting mixture was stirred at room temperature for 2 h and then extracted with ether (4 × 20 ml). The combined ether layers were dried with $MgSO_4$ and concentrated *in vacuo* to give the crude product. The pure product was obtained by flash chromatography (EtOAc/hexane = 1/4) as a white solid (0.55 g, 51%). ^{1}HNMR (CD_3OD): δ 8.29 (t, $^5J_{H\text{-}F}$ = 1.0 Hz, $^4J_{H\text{-}H}$ = 1.1 Hz. 1H), 7.46 (q, $^3J_{H\text{-}F}$ = 2.9 Hz, $^4J_{H\text{-}H}$ = 1.1 Hz, 1H), 7.21(b, 2H); ^{13}CNMR (CD_3OD): δ 163.4 (d, $^1J_{C\text{-}F}$ = 253.1 Hz), 147.1 (d, $^3J_{C\text{-}F}$ = 15.9 Hz), 143.5 (s), 126.0 (s), 125.9 (s), 117.9 (s), 117.5(s), 109.0 (d, $^3J_{C\text{-}F}$ = 8.0 Hz); HRMS (ESI) *m/z* Found 288.9514, calcd for $C_8H_{10}FIN_3$: 288.9512.

1-Propyl-2-2-(2-fluoro-4-iodo-pyrid-6-yl)-1H-imidazol-1-yl-methyl-5-cyano-1H-benzimidazole 21

To a solution of compound **15** (102.8 mg, 0.44 mmol) and compound **20** (132.9 mg, 0.46 mmol) in THF (20 ml) was added a solution of NaOH (60 mg) in water (22 ml) slowly at room temperature. The resulting mixture was stirred at room temperature overnight and at 45°C for 1 h. After cooling the reaction mixture at 0°C for 30 min, the precipitate was collected by filtration, dried under reduced pressure to give the crude product. The pure product was obtained by flash chromatography (EtOAc/hexane = 1/4) as a off-white solid (183.8 mg, 86%). ^{1}H NMR (DMSO-d_6): δ 8.32, 7.91. 7.75 (d), 7.57 (d), 7.49, 7.45, 7.16 (s,1H), 6.04 (s), 4.30 (t), 2.48 (q), 0.85 (t), ^{13}C NMR (DMSO-d_6): δ 162.4 (d), 152.5 (s), 148.3, 138.3, 130.1, 126.6, 125.3, 124.1, 120.4, 120.0, 111.2, 108.5, 105.6, 46.1, 44.6, 23.4, 11.3; HRMS (ESI) *m/z* Found 486.0462, calcd for $C_{20}H_{16}FIN_6$: 486.0465.

2-[2-(6-Fluoro[4-^{3}H]pyrid-2-yl)1H-imidazol-1-yl]methyl-1-propyl-5-cyano-1H-benzoimidazole, 22 ([^{3}H]CP-I-R)

A trisorber flask was charged with compound **21** (1.0 mg) and 5% Pd/C (1 mg) in MeOH (0.5 ml). The reaction mixture was reduced with 800 mCi carrier free tritium gas on a commercial tritiation manifold (TRI-SORBER R Tritiation Manifold, IU/US System, Inc.) for 2 h. After completion, the reaction mixture was diluted with MeOH (1 ml) and the Pd/C catalyst was removed by filtration. Labile products were removed by co-evaporation with ethanol and the crude product was subjected to reverse phase HPLC purification. The fractions containing the desired pure product were pooled, concentrated, and reconstituted in ethanol to give 74.7 mCi of the final product **22** with a specific activity of 23.7 Ci/mmol and radiochemical purity of 99.52%. HPLC condition for purity: YMC ODS-AQ, 5 um, 250 × 4.6 mm, column temperature: 30°C, Mobile Phase A: 0.01 M TEA pH 2.5 w/$HClO_4$; Mobile Phase B: CH_3CN, 15% B linear gradient to 60% over 20 min, hold A:B 40:60 to 45 min. Flow rate = 1.0 ml/min, UV detection: 220 nm; HPLC condition for purification: YMC ODS-AQ, 5 μm, 250 × 6 mm, column temperature:25°C, Mobile Phase A: 0.1% TFA in water, Mobile Phase B: 0.1% TFA in CH_3CN, 15% B linear gradient to 30% over 5 min, hold A:B 70:30 to 22 min, hold A:B 30:70 to 30 min Flow rate = 2.0 ml/min, UV detection: 220 nm. ^{3}H NMR (CD_3OD): δ 8.10; ^{1}H NMR (CD_3OD): δ 8.08 (q), 8.02 (d), 7.85 (s), 7.76 (d), 7.70 (b), 7.61 (d), 7.258 (b), 7.07 (d), 6.28 (s), 4.43(t), 1.93(q), 1.01(t).

2-[2-(6-Fluoro[4-^{3}H]pyrid-2-yl)1H-imidazol-1-yl]methyl-1-propyl-5-cyano-1H-[2-^{14}C]benzoimidazole, 23 ([^{3}H/^{14}C]CP-I)

A solution of [^{14}C]CP-I-L (**9**, 250 μCi, 14 μCi/mg) in MeOH (2.5 ml) was combined with a solution of [^{3}H]CP-I-R (**22,** 2500 μCi) in MeOH (7.5 ml) to form a solution of [^{3}H/^{14}C]CP-I (250 μCi of C-14 and 2500 μCi of H-3 in 10 ml of MeOH) with ^{3}H-radiochemical purity of 99.4% and ^{14}C radiochemical purity of 99.6%. The final specific activity was determined to be 14.0 μCi/mg for ^{14}C and 138 μCi/mg for ^{3}H. HPLC condition for purity: YMC ODS-AQ, 5 μm, 250 × 4.6 mm, column temperature: 30°C, Mobile Phase A: 0.01 M TEA pH 2.5 w/$HClO_4$; Mobile Phase B: CH_3CN, 15% B linear gradient to 60% over 20 min, hold A:B 40:60 to 45 min. Flow rate = 1.0 ml/min, UV detection: 220 nm.

Results and discussion

Synthesis of [^{14}C]CP-I labeled at the left hand side

Our first goal was to introduce a C-14 label into the benzimidazole ring system at the left hand side of CP-I. Scheme 1 illustrates a route to [^{14}C]CP-I-L (**9**) via coupling of [^{14}C]chloromethyl compound **8** and aryl imidazole **3**.[3] Thus, aryl chloride **4** was reacted with *n*-propylamine in the presence of K_2CO_3 to give amino adduct **5** in 85% yield. The selective

reduction of the nitro group in amine **5** with 5% Pd/C in EtOAc yielded diamine **6** in 90% yield. The diamine **6** can be reacted with 2-chloro-acetimidic acid methyl ester, or a similar electrophile such as 2-chloro-1,1,1-trimethoxy-ethane, chloroacetic acid anhydride or chloroacetyl chloride to form the chloromethyl compound **8**.[3] However, [^{14}C]chloroacetyl chloride was selected because it was commercially available and easily prepared. The cyclization of the diamine **6** with [^{14}C]chloroacetyl chloride formed the desired [^{14}C]**8** in 60% isolated yield.

In the original synthesis of unlabelled CP-I (**1**), the final N-alkylation used NaH as base and DMF as solvent. In our radiosynthesis, the N-alkylation was carried out using an aqueous NaOH solution in THF as a more environmentally friendly alternative, and this afforded 70% of the final [^{14}C]CP-I-L. This route only involved two radiosynthetic steps and gave a 42% overall radiochemical yield with a radiochemical purity of 99.7% and a specific activity of 49.9 mCi/mmol.

Synthesis of [^{14}C]CP-I labeled at the right hand side

During drug development of CP-I, [^{14}C]CP-I labeled at right hand side was also required to support animal and human studies. Scheme 2 shows a 4-step radiosynthesis of [^{14}C]CP-I-R via N-alkylation of a key C-14-labeled imidazole derivative **14** with the unlabeled chloromethyl compound **15**.

According to literature,[4] the unlabeled imidazole derivative **3** can be prepared by the reaction of 6-fluoro-pyridine-2-carbaldehyde with glyoxal and ammonium hydroxide (route I in Figure 4). However, none of the C-14-labeled compounds could be easily synthesized because both [^{14}C]aldehydes are unstable. In our lab, during the preparation of isotope-labeled midazolam, we developed an efficient approach to imidazole derivatives via diamine coupling with acetimidic acid ethyl ester hydrochloride (Figure 4, route II).[5] The later can be easily prepared *in situ* from a nitrile. Therefore, we were focused on the new route II and explored a way to introduce a cyano group into position 2 of the pyridine.

The literature showed that 2-fluoro-5,6-dichloro pyridine could be selectively converted to 2-cyano-5,6-dichloropyridine using excess NaCN as a cyanation reagent and DMSO as solvent.[6] These reaction conditions were adopted to make the desired 2-cyano-6-fluropyridine. Unfortunately, the undesired di-cyano substituted pyridine **18** was isolated as a major product. However, after changing the molar ratio of 2,6-difluoropyridine **10** and NaCN from 1/3 to 10/1 and the temperature from 60 to 30°C, we were able to synthesize the 2-cyano-6-fluoropyridine **17** in 65% yield and its C-14-labeled analog **11** in 55% yield (see entries 5 & 6 in Table 1). Using CuCN as a cyanating reagent (entry 3 in Table 1) gave a very poor yield of **17** after 24 h of heating at 100°C.

The desired [^{14}C]2-cyano-6-fluoropyridine **11** was then easily converted to the [^{14}C]acetimidic acid ethyl ester hydrochloride **12** by treating **11** with dry HCl gas in EtOH at 0°C for 5 h. Compound **12** was not isolated but reacted directly with ethylenediamine to give [^{14}C]dihydroimidazole **13** in 77% yield after flash chromatography. The compound **13** was then oxidized by $BaMnO_4$ to desired imidazole **14** in 95% yield. The final N-alkylation of compound **14** with compound **15** in an aqueous NaOH/THF solution furnished the target, [^{14}C]CP-I-R, in 73% yield. Overall radiochemical yield for 5 steps was 33% with a radiochemical purity of 99.2% and a specific activity of 54.0 mCi/mmol.

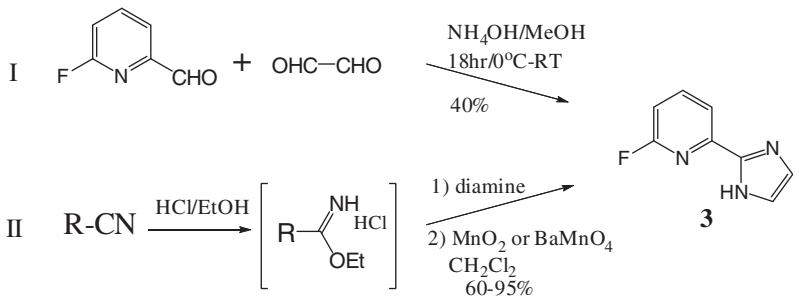
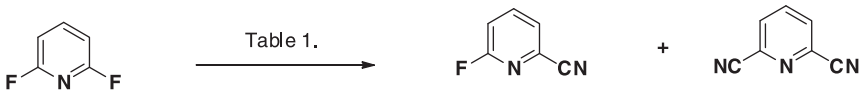


Figure 4. Possible approaches to 2-fluoro-6-(1*H*-imidazol-2-yl)pyridine **3**.

Table 1. Study on cyanation of 2,6-difluoropyridine with metal cyanides



10 17 18

Entry	Mole ♯ MCN	Temperature (°C)	Time (h)	Ratio **17**/**18**	Yields (major)
1	1 eq NaCN	60	4	1/8	45% **(18)**
2	1 eq NaCN	40	15	1/5	38% **(18)**
3	1 eq CuCN	100	24	1/1	10% **(17)**
4	0.1 eq NaCN	40	15	6/1	32% **(17)**
5	**0.1 eq NaCN**	**30**	**24**	**9/1**	**65% (17)**
6	**0.1 eq [^{14}C]NaCN**	**30**	**24**	**9/1**	**55% (17)**

Synthesis of [^{3}H]P-I labeled at the right hand side

The preliminary metabolic mechanism study suggested that only position 4 in the pyridine ring system was metabolically stable. Therefore, the tritium label has to be introduced into position 4 to avoid the loss of tritium *in vivo*. It was decided that a dehalogenation approach would be the easiest way to incorporate a tritium into the desired position. To synthesize the 4-iodo or bromo substituted CP-I (**21**), the 4-iodo- or bromo-substituted derivative of compound **3** was desired.

Normal halogenation reactions, such as treatments with Br_2 and NBS, failed to introduce a halogen (I or Br) into the desired position 4 in the compound **3**. However, a 'halogen-dance' phenomenon reported by several chemists in the literature[7] led us to the desired 6-fluoro-2-(1*H*-imidazol-2-yl)-4-iodopyridine **20**. Treatment of compound **3** with 2.2 eq of LDA in THF at −78°C followed by quenching with I_2 at −78°C gave 6-fluoro-2-(1*H*-imidazol-2-yl)-3-iodopyridine **19** in a 48% isolated yield. The isolated 3-iodo substituted pyridine **19** was then treated with 2.2 eq of LDA in THF again at −78°C followed by quenching with water to afford the desired 4-iodo substituted pyridine **20** in a 51% isolated yield. Nuclear Overhauser Effect analysis of regioisomers **19** and **20** further proved the structures.

Having the desired 4-iodo-substituted pyridine **20** in hand, the synthesis continued with N-alkylation of **20** using chloromethyl compound **15** under the same condition as the preparation of [^{14}C]CP-I to furnish 4-iodo-substituted CP-I (**21**). The de-iodination of **21** was carried out with 800 mCi of tritium gas and 5% Pd/C as a catalyst. A total of 74 mCi of pure [3 H]CP-I was isolated at a specific activity of 23.7 Ci/mmol and a radiochemical purity of 99.5% after preparative HPLC. ^{3}H NMR showed tritium was located at the expected position.

Preparation of [^{3}H/^{14}C]CP-I

Generally speaking, there are two ways to prepare dual radiolabeled compounds, i.e. (1) synthesize two radiolabeled intermediates and then couple them to make a dual-labeled target; (2) prepare single-labeled final compounds separately and then combine them in the desired ratio. Of these, method 2 is used more often and is more convenient for obtaining the desired ratio of two isotopomers. In addition, the single-isotope-labeled compounds were required for *in vitro* and *in vivo* studies.

In our case, C-14- and H-3-labeled CP-I were synthesized as described, and combined together as a 1/10 ratio of the total activity of C-14 to H-3 to support the dual-radiolabeled studies. The final dual-radioisotope-labeled CP-I was prepared as a methanol solution with ^{3}H-radiochemical purity of 99.4% and ^{14}C radiochemical purity of 99.6%. The final specific activity was determined to be 14.0 μCi/mg for ^{14}C and 138 μCi/mg for ^{3}H.

Conclusion

Having established valid routes for the key labeled intermediates, two C-14-labeled CP-I isotopomers and one H-3-labeled CP-I isotopomer were synthesized in good overall yields and >99% radiochemical purity. An efficient route to the C-14-labeled fluoropyridine imidazole was developed via the acetimidic acid ester approach. This new approach to the imidazole ring system can be easily expanded to prepare other isotopically labeled drugs. A short synthesis of iodo substituted fluoropyridine imidazole was also achieved via a 'halogen-dancing' approach.

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