Efficient syntheses of dual radioisotope-labelled PF-00217830, a D_2-partial agonist for the treatment of schizophrenia disorder

Yinsheng Zhang[a], Keith T. Garnes[b] and Donna Brown[c]

[a]*Radiochemistry Group, Chemical RandD, Groton Labs, Pfizer Inc. Groton, CT, USA*

[b]*Synthesis Services, Radiosynthesis, ABC Laboratories Inc. Columbia, MO, USA*

[c]*Donna Brown Consulting, Groton, CT, USA*

PF-00217830 is a D_2-partial agonist developed for the treatment of schizophrenia. The dual $^{14}C/^{14}C$ and $^{14}C/^{3}H$-labelled PF-00217830 were required for animal and human absorption, distribution, metabolism and elimination (ADME) studies. This report describes the development of the syntheses of dual $^{14}C/^{14}C$- and dual $^{14}C/^{3}H$-labelled PF-00217830. [^{14}C]PF-00217830-L labelled at the naphthalene ring was prepared in three steps starting with [^{14}C]naphthalene, while [^{3}H]PF-00217830-R was synthesised by Pd-catalysed tritium de-bromination of brominated PF-00217830 in a single radiosynthetic step. The dual [$^{14}C/^{3}H$]PF-00217830 was made by mixing 1:10 radioactivity ratio of [^{14}C]PF-00217830-L and [^{3}H]PF-00217830-R. The chemistry for [^{14}C]PF-00217830-R labelled at the pyridine ring was also described.

Keywords: radiochemistry, $^{14}C/^{14}C$ and $^{14}C/^{3}H$ labelling, pyridine formation, N-alkylation, tritium de-bromination,schizophrenia

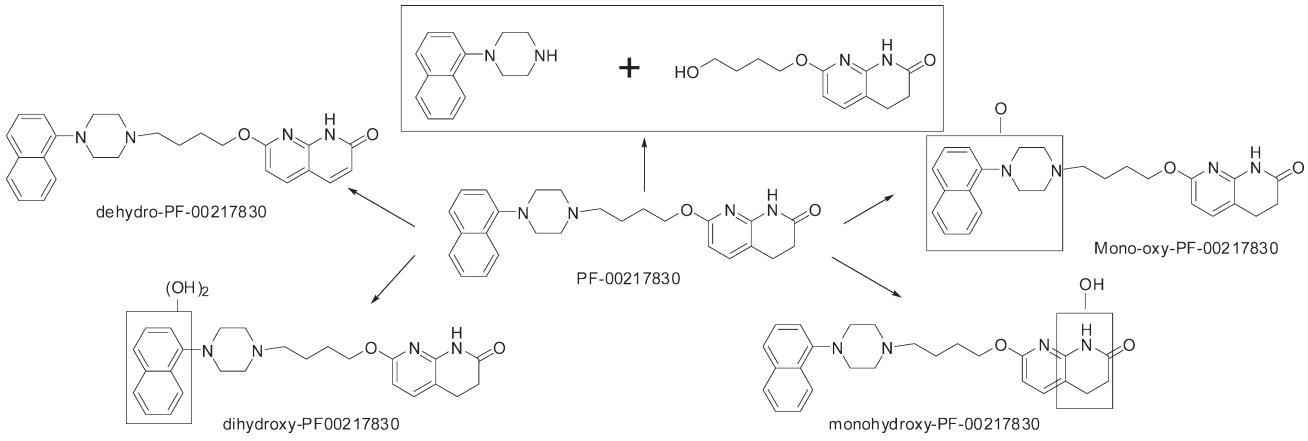
Schizophrenia is a mental disorder that is characterised by positive symptoms such as delusions, hallucinations and disorganised speech/behaviour and negative symptoms including apathy, withdrawal, lack of pleasure and impaired attention. PF-00217830, a D_2-partial agonist, was developed at phase II for the treatment of schizophrenia and bipolar disorder.[1]

The initial *in vitro* biotransformation studies using non-radiolabelled drug suggested that PF-00217830 underwent significant scission as well as oxidation at various sites of the intact pentacyclic compound (Scheme 1). The complex nature of this metabolic situation limited the utilisation of mono-radiolabelled PF-00217830 for animal and human absorption, distribution, mechanism and elimination (ADME) studies. In order to investigate both scission products, $^{14}C/^{14}C$-labelled PF-00217830 at the naphthalene ring (left hand side=LHS or L) and the pyridine ring (right hand side = RHS or R) and dual $^{14}C/^{3}H$-labelled PF-00217830 were required. A mixture of two radioisoforms (^{14}C and ^{3}H) can be monitored simultaneously without the confusion of change in specific activity of mono-radiolabelled metabolites that occurs using dual $^{14}C/^{14}C$ labelled PF-00217830.

This report describes the syntheses of mono-radiolabelled (^{14}C, ^{3}H) and dual-radiolabelled ($^{14}C/^{14}C$, $^{14}C/^{3}H$) PF-00217830 for use in pre-clinical studies. The synthesis of unlabelled PF-00217830 has been previously reported (Scheme 2).[1] [^{14}C]PF-0021783-R (**18**) labelled at the pyridine ring (where R indicates RHS)was synthesised in eight steps starting with [^{14}C]KCN by modifying the literature procedures. Our development of a palladium-catalysed N-arylation reaction of unprotected piperazine with aryl halide allowed us to prepare a key radio-labelled intermediate **27** efficiently. Therefore, [^{14}C]PF-00217830-L labelled at the naphthalene ring was prepared in three radiosynthetic steps starting with [^{14}C]naphthalene. Aselective bromination at the pyridine ring was achieved and resulted in a quick access to the bromo-substituted analogue of PF-00217830. [^{3}H]PF-00217830-L (where L indicates LHS) labelled at the 5-position of pyridine ring was then synthesised in one radiosynthetic step using a Pd-catalysed tritium de-bromination process. The dual $^{14}C/^{14}C$ and $^{14}C/^{3}H$-labelled PF-00217830 were prepared by combining mono-radiolabelled versions **18** and **23**, **23** and **26** based on 1:1 and 1:10 ratio of radioactivity, respectively.

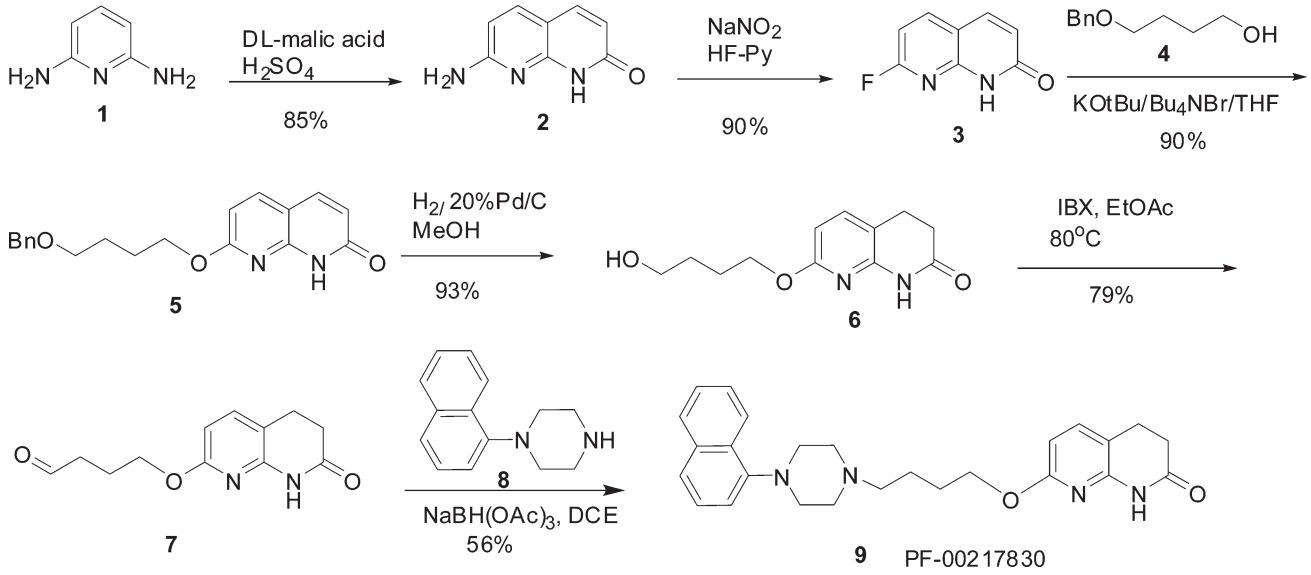
Results and discussion

A dual $^{14}C/^{14}C$-labelled PF-00217830 was the first target, with the labelling located at the naphthalene and pyridine ring systems. The synthesis of unlabelled PF-00217830 involves a total of eight steps from commercially available starting materials (Scheme 2).[1] Since the radio-labelled starting materials **1** and **8** were not commercially available, the synthesis of each compound was devised as described below.



Scheme 1 Metabolism of PF-00217830.

* Correspondent. E-mail: yinsheng.zhang@yahoo.com



Scheme 2 Synthesis of unlabelled PF-00217830.

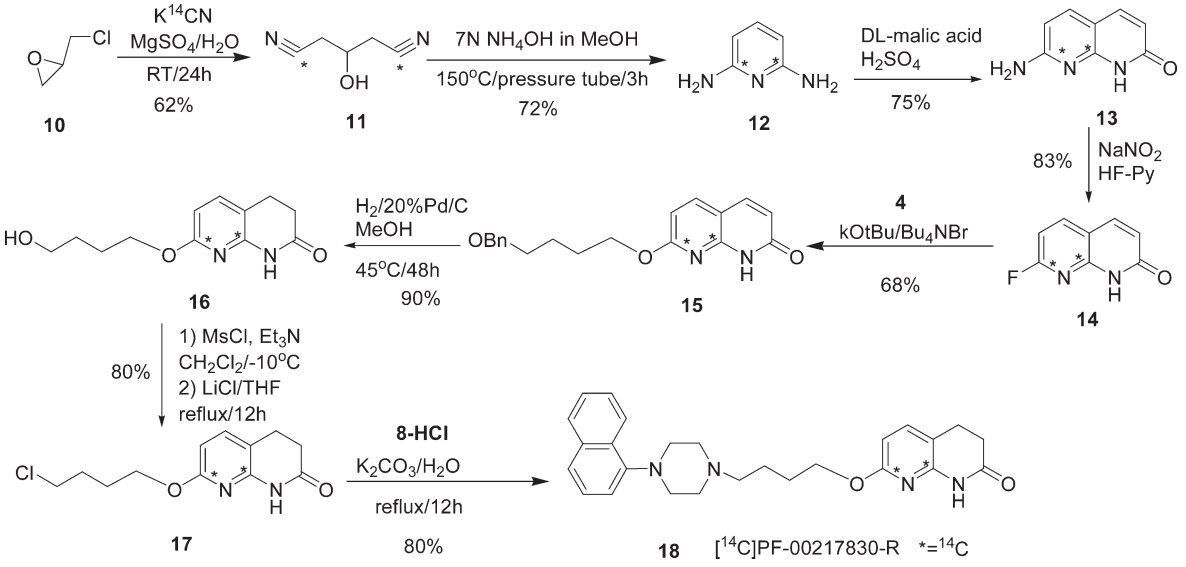
Synthesis of [^{14}C]PF-00217830-R labelled at the pyridine ring

The radiosynthetic route (Scheme 3) was developed using [^{14}C]KCN as radio-labelled source. The cyanide substitution of epichlorohydrin in an aqueous solution buffered with $MgSO_4$ gave the dinitrile alcohol **11** in a good yield (62%). Treatment of the dinitrile alcohol **11** with ammonia in MeOH (7 N) instead of ammonia gas[1] in a pressure tube formed the desired 2,6-diamino[2,6-$^{14}C_2$]pyridine **12** in 72% yield. Cyclisation of **12** with D,L-malic acid in concentrated H_2SO_4 provided aminonapthyridinone **13** in 75% yield. Diazotisation of **13** with $NaNO_2$ in the presence of HF in pyridine gave fluoronapthyridinone **14** in 83% yield. Coupling reaction of **14** with 4-(benzyloxy)butan-1-ol using potassium *t*-butoxide as a base afforded **15**. The yield was improved by addition of a catalytic amount of Bu_4NBr (68% *versus* 42%). Palladium-catalysed hydrogenation of **15** resulted in reduction to the dihydronapthyridinone with concomitant removal of the benzyl group to form alcohol **16** in 90% yield following purification.

According to the original synthesis (Scheme 2), the unlabelled alcohol **16** was oxidised with IBX to aldehyde **7** followed by reductive amination with aryl piperazine **8** to furnish the target, PF-00217830. However, the radiolabelled aldehyde formed from **16** was found to be unstable and the yield of the subsequent reductive amination was low. In our radiosynthesis, treatment of alcohol **16** with methanesulfonyl chloride first and then with lithium chloride in one pot produced chloride **17** in 80% yield. The final N-alkylation of the chloride **17** with 1-napthylenyl-piperazine hyrochloride in basic aqueous solution provided the desired **18**, [^{14}C]PF-00217830-R, in 80% yield with a radiochemical purity of 98.8% and a specific activity of 53 mCi mmol^{-1} after purification.

Synthesis of [^{14}C]PF-00217830-L labelled at the naphthalene ring

[^{14}C]1-Napthylenyl-piperazine is a key intermediate for the synthesis of [^{14}C]PF-00217830-L labelled at the naphthalene ring. The synthesis of unlabelled 1-napthylenyl-piperazine **8** was reported previously.[2,3] One possible way was to use 1-amino-naphthalene, an OEB 5 compound, to combine with bis(chloroethyl)amine-HCl in dichlorobenzene at 140 °C to give 1-napthylenyl-piperazine. Alternatively, 1-bromonaphthalene could be aminated with mono-N protected piperazine using Buchwald-Hartwig conditions following by conc. HCl hydrolysis to furnish 1-napthylenyl-piperazine. Both known



Scheme 3 Synthesis of [^{14}C]PF-00217830 labelled at the pyridine ring.

approaches could be adapted to prepare ^{14}C labelled **8**, however, both labelled starting materials, 1-aminonaphthalene and 1-bromonaphthalene were not commercially available, and the radiosynthesis would involve in multiple radiolabelling steps would give poor overall yields.

Our interest has been in seeking a mild, selective method for mono-N-arylation of piperazine with aryl halides to cut the reaction steps and make the synthesis more environmentally friendly, which can be applied to the synthesis of [^{14}C]PF-00217830 and other potential drug candidates. After extensive study on the Pd-catalysed amination of 1-halo-naphthalene with unprotected piperazine,[4] we found that PXPd, dichlorobis(chlorodi-*t*-butylphosphine) palladium (II), was the most efficient catalyst for C–N bond coupling reaction of halonaphthalene and piperazine compared with other catalysts reported in the literature.[5] Therefore, [1-^{14}C]naphthalene, a commercially available compound, was brominated with $CuBr_2/Al_2O_3$ in CCl_4 to produce the desired [^{14}C]1-bromonaphthalene **20** in 65% yield. Since four positions (1,4,5,8) of the naphthalene ring are chemically equivalent, after the bromination carbon-14 is equally distributed in the 4 positions. Then, **20** was aminated with piperazine in the presence of PXPd to furnish **21** in 84% yield as a free base after purification (Scheme 4).

In the synthesis of [^{14}C]PF-00217830-R (Scheme 3), the HCl salt of 1-napthylenyl-piperazine was used, and there was no solubility issue of N-alkylation reaction in an aqueous solution. However, by addition of 20% CH_3CN to the aqueous reaction mixture, we were able to get the free base **21** into the reaction solution and react with chloride **22** to achieve a similar yield (86%) to the original reaction (80%). The [^{14}C]PF-00217830-L labelled at the naphthalene ring was prepared in only three radiosynthetic steps in a 47% overall yield with a radiochemical purity of 99.5% and a specific activity of 54.5 mCi mmol^{-1}.

Synthesis of [^{3}H]PF-00217830-R labelled at the pyridine ring

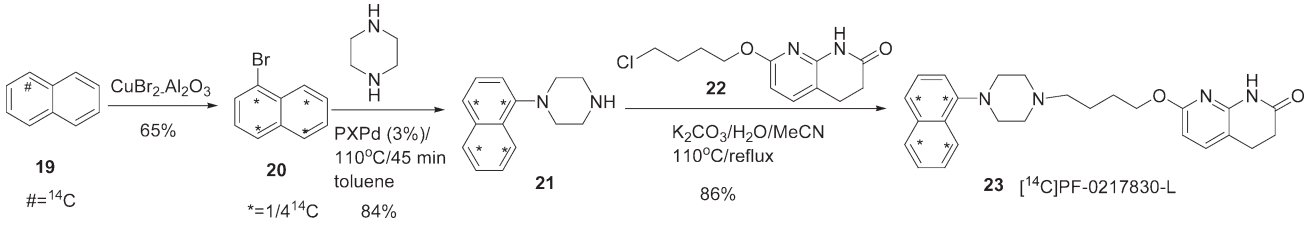
Tritium-labelled PF-00217830 was required for receptor binding and animal ADME studies. Based on the original synthetic route for PF-00217830, the shortest way to prepare the required [^{3}H]PF-00217830-R is to reduce the halide precursor of PF-00217830 with tritium gas (Scheme 5). We could either brominate the final molecule PF-00217830 or the intermediate **22**. However, the bromination of the final API, PF-00217830, did not offer only mono substituted bromide **25** since the naphthalene ring is more electron-rich than pyridine ring. Therefore, the bromination of **22** with NBS in $CHCl_3$ was carried out and indeed afforded the desired bromide **24** in 88% yield. The N-alkylation of 1-napthylenyl-piperazine **8** with the bromide **24** under the same conditions as the synthesis of [^{14}C]PF-00217830 gave the desired brominated precursor **25** in 62% yield after purification. In order to ensure that tritiation occurred only at the desired position, $Pd(PPh_3)_4$ was utilised as a reduction catalyst instead of 10% Pd/C. The latter might cause the undesired T/H exchange at other positions according to previous studies. So, the bromide **25** was hydrogenated with T_2 gas in the presence of $Pd(PPh_3)_4$ and KOAc at 100 °C for 2 h to give the desired tritiated PF-00217830 (**26**) in high yields. [^{3}H]PF-00217830-R (**26**) was obtained with a specific activity of 25.3 Ci mmol^{-1} and a >99% radiochemical purity.

Preparation of [$^{14}C/^3H$]PF-00217830 and [$^{14}C/^{14}C$]PF-00217830

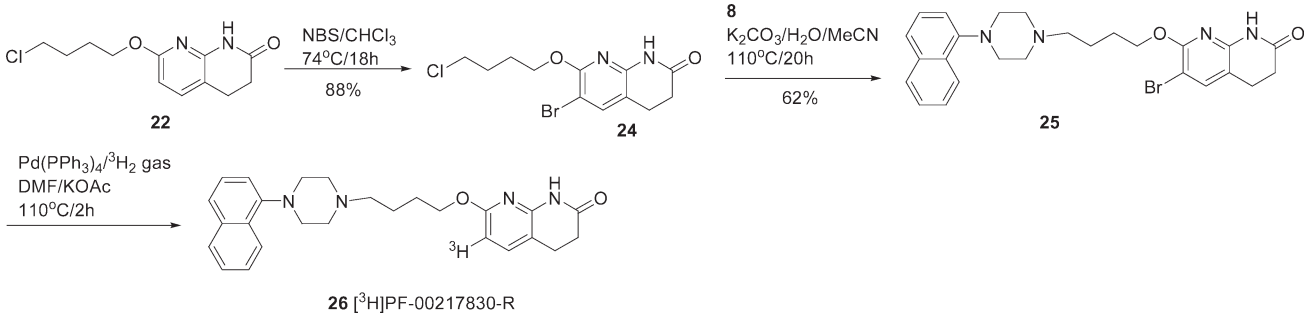
Two different methods have been utilised to prepare dual $^{14}C/^{14}C$ and $^{14}C/^3H$-labelled compounds. Method 1 is to synthesise two radiolabelled intermediates first and then couple them together to make a dual labelled target. Method 2 is to prepare single-labelled final compounds separately and then combine them in the desired ratio. Among these, method 2 is used more often and is more convenient for obtaining the desired ratio of two isotopomers. In addition, the single-isotope labelled compounds were needed for *in vitro* and *vivo* studies.

In our case, ^{14}C and ^3H-labelled PF-00217830 were synthesised separately, and combined together as a 1:10 ratio of the total activity of ^{14}C to ^3H and a 1:1 ratio of the activity of ^{14}C to ^{14}C, respectively to support the dual-radiolabelled studies (Scheme 6). The final [$^{14}C/^3H$]PF-00217830 (**27**) was prepared as a methanol solution with ^3H-radiochemical purity of 99.3% and ^{14}C radiochemical purity of 99.5%. The final specific activity was determined to be 13.9 μC mg^{-1} for ^{14}C and 140 μCi mg^{-1} for ^3H after radiochemical dilution. Similarly, the final [$^{14}C/^{14}C$]PF-00217830 (**28**) was obtained as a solid with a radiochemical purity of 98.8% and a specific activity of 53.6 mCi mmol^{-1}.

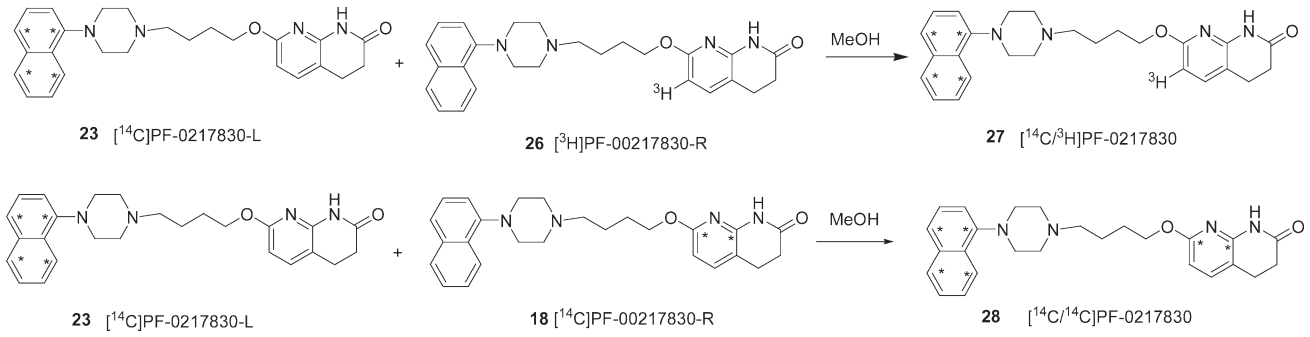
In conclusion, we have reported the efficient three-step radiosynthesis of [^{14}C] PF-00217830 labelled at the naphthalene ring and one-step radiosynthesis of [^{3}H]PF-00217830



Scheme 4 Synthesis of [^{14}C]PF-00217830 labelled at the naphthalene ring.



Scheme 5 Synthesis of [^{3}H]PF-00217830.



23 [^{14}C]PF-0217830-L **26** [^{3}H]PF-00217830-R **27** [^{14}C/^{3}H]PF-0217830

23 [^{14}C]PF-0217830-L **18** [^{14}C]PF-00217830-R **28** [^{14}C/^{14}C]PF-0217830

Scheme 6 Preparation of [^{14}C/^{3}H]PF-0217830 and [^{14}C/^{14}C]PF-00217830.

labelled at the pyridine ring. The key radiolabelled intermediate **27** was prepared in two steps in 55% chemical and radiochemical yields using the N-aryl amination reaction with unprotected piperazine in the presence of a new palladium catalyst, PXPd. The bromide precursor of PF-00217830 was also synthesised in two steps in 75% overall yield. We also described the synthesis of [$^{14}C_2$] PF-00217830 labelled at the pyridine ring. The modified 2-step approach to [2, 6-$^{14}C_2$] 2,6-diaminopyridine **12** was developed and will be useful for other labelled drug candidates having a pyridine core. In addition, a new chlorination and N-alkylation sequence was developed to avoid the stability issue of the labelled aldehyde intermediate **7**.

Experimental

All reactions were carried out under an atmosphere of nitrogen unless otherwise stated. Microwave reactions were carried out using CEM-discover microwave reactor. LC-MS data were obtained on a Waters Micromass LCT mass spectrometer with flow injection analysis and electrospray ionisation (ESI). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 400 MHz instrument. Chemical purity of all compounds was determined by HPLC and LC-MS. Purifications were done by flash column chromatography on Biotage Flash 40 system. Quantitation of radioactivity of ^{14}C and ^{3}H labelled compounds was performed using a Packard 2200CA liquid scintillation analyser, with Scintiverse BD cocktail used throughout. Commercial reagents and solvents were purchased from Aldrich and used as-received unless otherwise noted. [^{14}C]naphthalene (200 mCi, 54 mCi mmol^{-1}) and [^{14}C]KCN (200 mCi, 54 mCi mmol^{-1}) were purchased from American Radiolabelled Chemicals, Inc. Intermediates **4, 8** and **22** and final API **1** were provided by Chemical RandD, Groton Lab, Pfizer Inc. All known compounds were identified by comparison of their NMR spectra with those reported in the literature.

3-Hydroxy-[1,5-$^{14}C_2$]glutaronitrile (**11**): This compound was prepared according to a literature procedure.[3] From [^{14}C]KCN (200 mCi, 54 mCi mmol^{-1}), the title compound was obtained as an oil (162 mCi, 105 mCi mmol^{-1}, 81%). ^{1}H NMR was consistent with that of the authentic sample.

2,6-Diamino-[2,6-$^{14}C_2$]pyridine (**12**): A mixture of 3-hydroxy-[1,5-$^{14}C_2$]glutaronitrile (162 mCi, 1.54 mmol), 3-hydroxy-glutaronitrile (170 mg, 1.54 mmol), and copper(I) chloride (40 mg, 0.4 mmol) in ammonia solution in MeOH (7 M, 10 mL) was heated at 150 °C in an autoclave for 4 h. After the reaction mixture had been cooled down and filtered, the solvent was evaporated under vacuum to give a crude material, which was purified by flash column chromatography (20% MeOH/CH_2Cl_2). The title compound was obtained as a brown solid (117.5 mCi, 72%, 53 mCi mmol^{-1}, 241.7 mg). ^{1}H NMR was consistent with that of the authentic sample.

[$^{14}C_2$]7-Amino-1H-[1,8]naphthyridin-2-one (**13**): To a cold mixture of 2,6-diamino-[2,6-$^{14}C_2$]pyridine (240 mg, 2.2 mmol) and D/L-malic acid (324.5 mg, 2.42 mmol) was added dropwise conc. H_2SO_4 (3.0 mL) at 0 °C. The resulting mixture was heated at 110 °C for 4 h and then cooled to 0 °C. To the cold reaction mixture was added ice water (5 mL) slowly. The pH of the mixture was adjusted to >10 by gradual addition of 6N NaOH with ice-bath cooling. The suspension was stirred at room temperature for 30 min. The solid in the suspection was collected by filtration, washed with water (5 mL × 3) and dried under vacuum at 45 °C to give the title compound (265 mg, 75%, 53 mCi mmol^{-1}). ^{1}H NMR and ^{13}C NMR were consistent with published spectra.[1]

[$^{14}C_2$]7-Fluoro-1H-[1,8]naphthyridin-2-one (**14**): To a mixture of [$^{14}C_2$]7-amino-1H-[1,8]naphthyridin-2-one (260 mg, 1.61 mmol, 53 mCi mmol^{-1}) and HF-pyridine (70%, 15 mL) was added sodium nitrite (260 mg, 3.7 mmol) at 0 °C. The resulting solution was stirred vigorously with ice bath cooling for 3 h. To this cold solution was added ice water (3 mL) to form a suspension. The precipitate was collected by filtration, and then resuspended in ethyl acetate (5 mL). The resulting suspension was filtered to give the title product as a yellow solid (221.8 mg, 84%, 53 mCi mmol^{-1}). ^{1}H NMR and ^{13}C NMR were consistent with published spectra.[1]

[$^{14}C_2$]7-(4-Benzyloxy-butoxy)-1H-[1,8]naphthyridin-2-ol (**15**): A suspension of [$^{14}C_2$]7-fluoro-1H-[1,8]naphthyridin-2-one (220 mg, 1.34 mmol, 53 mCi mmol^{-1}), tetrabutylammonium bromide (218 mg, 0.7 mmol), 4-benzyloxybutanol (0.26 mL, 1.48 mmol) and THF (4 mL) was stirred at 25 °C for 30 min and cooled to 0 °C. To this cold mixture was added a solution of potassium *t*-butoxide in THF (1M, 3 mL, 3 mmol) at 0 °C. After completion of addition, the thick slurry became a solution and was stirred at room temperature for 4 h. HCl solution (1N, 3.2 mL) was slowly added to the reaction mixture at 25 °C and the resulting mixture was stirred at room temperature for 30 min. THF in the reaction mixture was evaporated under vacuum and the residue was treated with ethyl acetate (10 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and evaporated under vacuum to give the product as a yellow solid (295.2 mg, 68%, 53 mCi mmol^{-1}). ^{1}H NMR and ^{13}C NMR were consistent with published spectra.[1]

[$^{14}C_2$]7-(4-Hydroxy-butoxy)-3,4-dihydro-1H-[1,8]naphthyridin-2-one (**16**): [$^{14}C_2$]7-(4-benzyloxy-butoxy)-[1,8]naphthyridin-2-ol (290 mg, 0.89 mol, 53 mCi mmol^{-1}) and MeOH (5 mL) were charged to a pressure reactor with 20% palladium on carbon (522 mg) and hydrogenated for 48 h at 45 °C and 50 psi. Upon completion, the palladium catalyst was filtered and the filtrate was concentrated to give the product as an off-white solid (189 mg, 90%, 53 mCi mmol^{-1}). ^{1}H NMR and ^{13}C NMR were consistent with published spectra.[1]

[$^{14}C_2$]7-(4-Chloro-butoxy)-3,4-dihydro-1H-[1,8]naphthyridin-2-one (**17**): To a solution of [$^{14}C_2$]7-(4-hydroxy-butoxy)-3,4-dihydro-1H-[1,8]naphthyridin-2-one (180 mg, 0.76 mmol, 53 mCi mmol^{-1}) in THF (2 mL) was added methanesulfonyl chloride (0.1 mL, 1.29 mmol) at –10 °C with an acetone-ice bath. Triethylamine (0.2 mL) was added at a rate to keep the internal temperature below 0 °C. Following completion of addition, the reaction was warmed to room temperature. LiCl (65 mg, 1.55 mmol) was added to the reaction suspension. The resulting mixture was refluxed for 12 h. THF was removed by vacuum distillation and then ethyl acetate (10 mL) was added to the residue. The organic layer was washed with water (2 mL), sat. $NaHCO_3$ (2 mL) and brine (2 mL), and dried over $MgSO_4$. Concentration under vacuum gave the crude product, which was purified by flash column chromatography (10% EtOAc/hexane). The title compound was obtained as a white solid (153.8 mg, 80%, 53 mCi mmol^{-1}) with a radiochemical purity (RCP) of 98.2% and a specific activity of 53 mCi mmol^{-1}. ^{1}H NMR and ^{13}C NMR were consistent with published spectra.[5]

*[$^{14}C_2$]7-[4-(4-Naphthalen-1-yl-piperazin-1-yl)-butoxy]-3,4-dihydro-1H-[1,8]naphthyridin-2-one (***18***, [^{14}C]F-00217830-R):* A mixture of [$^{14}C_2$]7-(4-chloro-butoxy)-3,4-dihydro-1H-[1,8]naphthyridin-2-one (150 mg, 0.59 mmol, 53 mCi mmol^{-1}), 1-naphthalen-1-yl-piperazine hydrochloride (161. 2 mg, 0.65 mmol) and potassium carbonate (284 mg, 2.0 mmol) in water (1.5 mL) was heated at 110 °C for 20 h.

The reaction mixture was cooled to room temperature, then diluted with ethyl acetate (20 mL) and stirred for 20 min. The organic layer was separated, and the aqueous layer was extracted with additional ethyl acetate (10 mL). The combined organic layers were washed with brine (15 mL) water (15 mL), and dried over sodium sulfate. Concentration under vacuum gave the crude product, which was purified by flask column chromatography (ethyl acetate:hexane, 1:1). The title compound was obtained as a white solid (152 mg, 60%, 53 mCi mmol^{-1}, 18.7 mCi, radiochemical purity (RCP) and chemical purity (CP): 98.8% and 98.5% by HPLC assay). ^{1}H NMR and ^{13}C NMR were consistent with published spectra.[1] HPLC condition for purity: Water X-Bridge C18, 4.6 × 50, 5 µm, Mobile Phase A: 0.1% Formic acid; Mobile Phase B: MeCN, 0% B linear gradient to 80% over 8 min, hold A:B 20:80 to 10 min. Flow rate = 1.5 mL min^{-1}, UV detection: 210 nm.

[^{14}C]1-Bromonaphthalene (**20**): To a solution of copper (II) bromide (17.22 g, 0.077 mol) in HPLC grade water (60 mL) was added neutral alumina (40.0 g, Brockmann I neutral, Aldrich product # 199974) at room temperature. The water was removed by rotary evaporation at 80 °C under reduced pressure. The resulting dark brown reagent was further dried under vacuum at 100 °C for 15 h. The mass obtained was 56.867 g (theoretical yield) after drying.

To a 125 mL round bottom flask was added [1-^{14}C]naphthalene (484 mg, 200 mCi, 3.7 mmol, 54 mCi mmol^{-1}), carbon tetrachloride (33 mL) and copper (II) bromide-aluminum oxide reagent (6.2 g) described above. The reaction mixture was heated at 80 °C for 1.5 h. The reaction was cooled to room temperature and the solids were removed by filtration. The filtrate was concentrated to give the crude product as a yellow oil. Purification of the crude material by flash chromatography (hexane) offered the title compound **20** (498.3 mg, 130.0 mCi, 65%, 54.5 mCi mmol^{-1}, 98.3% RCP by HPLC). ^{1}H NMR was consistent with that of the authentic sample.

[^{14}C]N-naphthenyl-piperazine (**21**): In a 125 mL flask was charged with [^{14}C]1-bromonaphthalene (492.0 mg, 2.37 mmol, 128 mCi, 54 mCi mmol^{-1}) in THF (27 mL), piperazine (304.0 mg, 3.53 mmol), PXPd catalyst [Dichlorobis(chlorodi-*tert*-butylphosphine) palladium (II), 38 mg, 0.071 mmol] and sodium *t*-butoxide (346 mg, 3.6 mmol). The reaction mixture was heated at reflux for 60 min. After completion of the reaction (checked by HPLC), the reaction mixture was cooled to room temperature, diluted with ethyl acetate (40 mL) and stirred at room temperature for 20 min. The mixture was filtered and the filtrate was concentrated under vacuum to give the crude product as a brown oil. Purification of the crude material by flash chromatography (ethyl acetate and ethyl acetate:methanol, 4:3) gave the title compound **21** (326.5 mg, 83.2 mCi, 65%, 54.5 mCi mmol^{-1}). ^{1}H NMR was consistent with that of the authentic sample.

*[^{14}C] 7-[4-(4-Naphthalen-1-yl-piperazin-1-yl)-butoxy]-3,4-dihydro-1H-[1,8]naphthyridin-2-one (***23***, [^{14}C]PF-00217830-L):* To a mixture of [^{14}C]*N*-naphthenyl-piperazine (**21**, 184 mg, 0.859 mmol, 46.4 mCi, 54 mCi mmol^{-1}), 7-(4-chloro-butoxy)-3,4-dihydro-1*H*-[1,8]naphthyridin-2-one (**22**, 227 mg, 0.893 mmol) and acetonitrile (2.5 mL) was added a solution of potassium carbonate (356 mg, 2.58 mmol) in water (9.5 mL) at room temperature. The resulting mixture was heated at 115 °C for 21 h, and then cooled to room temperature and diluted with ethyl acetate (35 mL). After the mixture was stirred for 20 min, the organic layer was separated, and the aqueous layer was extracted with additional ethyl acetate (15 mL). The combined organic layers were washed with brine (15 mL) and water (15 mL) and dried over sodium sulfate. Concentration gave the crude product, which was purified by a 40 g Redi-Sep silica column (ethyl acetate:hexane, 1:1) to give the title compound **23** (316.3 mg, 39.9 mCi, 86%, 54.5 mCi mmol^{-1}) at 99.5% RCP by HPLC (HPLC conditions for purity: Water X-Bridge C18, 4.6 × 50, 5 µm, Mobile Phase A: 0.1% Formic acid; Mobile Phase B: MeCN, 0% B linear gradient to 80% over 8 min, hold A:B 20:80 to 10 min. Flow rate = 1.5 mL min^{-1}, UV detection: 210 nm). ^{1}H NMR and ^{13}C NMR were consistent with published spectra.[1]

7-(4-Chloro-butoxy)-3,4-dihydro-1H-6-bromo-[1,8]naphthyridin-2-one (**24**): A solution of 7-(4-chloro-butoxy)-3,4-dihydro-1*H*-[1,8]naphthyridin-2-one **22** (124 mg, 0.486 mmol) and NBS (96 mg, 0.54 mmol) in $CHCl_3$ (3 mL) was heated at 74 °C for 18 h. Upon completion, the reaction mixture was diluted with $CHCl_3$ (5 mL) and washed with water (5 mL × 3). Concentration under vacuum gave the crude product, which was purified by a flash column (50% ethyl acetate in hexane). The tilted compound **24** was obtained as a brown solid (142 mg, 89%). ^{1}H NMR ($CDCl_3$): δ 7.56 (s, 1H), 4.30 (t, 2H), 3.63 (t, 2H), 2.84 (t, 2H), 2.63 (t, 2H), 1.95 (m, 4H); ^{13}C NMR ($CDCl_3$): δ 170.2 (1C), 160.3(1C), 150.3(1C), 140.6 (1C), 115.3 (1C), 98.93(1C), 67.4(1C), 44.6(1C), 30.1 (1C), 29.1(1C), 26.8(1C), 23.5(1C). HRMS (ESI) *m/z* Found 331.9924; calcd for $C_{12}H_{14}BrClN_2O_2$: 331.9927.

7-[4-(4-Naphthalen-1-yl-piperazin-1-yl)-butoxy]-3,4-dihydro-1H-6-bromo-[1,8]naphthyridin-2-one (**25**): A mixture of 7-(4-chloro-butoxy)-3,4-dihydro-1H-6-bromo-[1,8]naphthyridin-2-one **24** (140 mg, 0.55 mmol), 1-naphthalen-1-yl-piperazine hydrochloride **8** (160 mg, 0.65 mmol) and potassium carbonate (284 mg, 2.0 mmol) in water (1.5 mL) was heated at 110 °C for 20 h. The reaction mixture was cooled to room temperature, then diluted with ethyl acetate (20 mL) and stirred for 20 min. The organics were separated, and the aqueous layer was extracted with additional ethyl acetate (10 mL). The combined organics were washed with brine (10 mL) and = water (15 mL), and dried over sodium sulfate. Concentration under vacuum gave the crude product, which was purified by flash chromatography (ethyl acetate:hexane, 1:1). The tilted compound was obtained as a white solid (173.5 mg, 62%). ^{1}H NMR ($CDCl_3$): δ 8.17 (d, 1H), 7.80 (d, 1H), 7.56 (s, 1H), 7.54 (d, 1H), 7.45 (m, 1H), 7.42 (m, 1H), 7.38 (t, 1H), 7.08 (d, 1H), 4.3 (t, 2H), 3.2 (b, 2H), 2.82 (t, 2H), 2.6 (t, 2H), 1.85 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 170.2, 160.1, 151.2, 150.1, 140.3, 134.9, 129.3, 128.1, 126.4, 125.8, 125.6, 125.0, 123.5, 116.7, 115.2, 98.9, 67.3, 57.5, 53.4 (2C), 50.9 (2C), 30.1, 27.8, 26.0, 23.7. HRMS (ESI) *m/z* Found 508.1474, calcd for $C_{26}H_{29}BrN_4O_2$: 508.1473.

*7-[4-(4-Naphthalen-1-yl-piperazin-1-yl)-butoxy]-3,4-dihydro-1H-[6-^{3}H]-[1,8]naphthyridin-2-one (***26***, [^{3}H]PF-00217830-R):* A flask was charged with 7-[4-(4-naphthalen-1-yl-piperazin-1-yl)-butoxy]-3,4-dihydro-1H-6-bromo-[1,8]naphthyridin-2-one (**25**, 5 mg, 0.0098 mmol), KOAc (3 mg) and $Pd(PPh_3)_4$ (5 mg) in DMF (0.5 mL). The reaction mixture was purged with nitrogen gas for three times and connected to tritium gas (2 Ci). The resulting mixture was heated at 100 °C for 2 h under tritium gas atmosphere. The solvent was removed under vacuum. The crude material was purified by prep HPLC to give [^{3}H]PF-00217830 (50 mCi, RCP and CP>98%, specific activity: 25.3 Ci mmol^{-1}). ^{1}H NMR (CD_3OD) δ 7.93 (d, 1H), 7.75 (d, 1H), 7.52 (d, 1H), 7.41 (m, 3H), 7.31 (t, 1H), 7.04 (d, 1H), 4.08 (t, 2H), 3.48 (m, 2H), 3.30 (m, 2H), 3.13 (m, 4H), 2.96 (m, 2H), 2.66 (t, 2H), 2.41 (t, 2H), 1.72 (m, 4H); ^{3}H NMR (CD_3OD): δ 6.41. HPLC conditions for purity: Water X-Bridge C18, 4.6 × 50, 5 µm, Mobile Phase A: 0.1% formic acid; Mobile Phase B: MeCN, 0% B linear gradient to 80% over 8 min, hold A:B 20:80 to 10 min. Flow rate = 1.5 mL min^{-1}, UV detection: 210 nm.

[^{14}C/^{3}H] PF-00217830 (**27**): A solution of [^{14}C]PF-00217830 (**23**, 250 µCi) in MeOH (2.5 mL) was combined with a solution of [^{3}H]PF-00217830 (**26,** 2500 µCi) in MeOH (7.5 mL) to form a solution of [^{3}H/^{14}C]PF-00217830 (250 µCi of ^{14}C and 2500 µCi of ^{3}H in 10 mL of MeOH) with ^{3}H radiochemical purity of 99.3% and ^{14}C radiochemical purity of 99.5%. The final specific activity was determined to be 13.9 µCi mg^{-1} for ^{14}C and 140 µCi mg^{-1} for ^{3}H. HPLC condition for purity: Water X-Bridge C18, 4.6 × 50, 5 µm, Mobile Phase A: 0.1% Formic acid; Mobile Phase B: CH_3CN, 0% B linear gradient to 80% over 8 min, hold A:B 20:80 to 10 min. Flow rate = 1.5 mL min^{-1}, UV detection: 210 nm.

[^{14}C/^{14}C] PF-00217830 (**28**): A solution of compound **18** (50.1 mg, 6.17 mCi) and compound **23** (49.8 mg, 6.31 mCi) in MeOH (10 mL) was stirred at 30 °C for 30 min and then filtered through a 0.2 µm syringe filter. The filtrate was evaporated to dryness and dried to constant weight under high vacuum to give the dual ^{14}C/^{14}C radiolabelled compound (99.2 mg, 12.36 mCi, 99.3%) with a RCP of 98.8% and a specific activity of 53.6 mCi mmol^{-1}.

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