Preparation of tritium-labeled PF-622, a novel fatty acid amide hydrolase inhibitor

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Abstract

In order to make a detailed characterization of the mechanism of inhibition and selectivity of a novel fatty acid amide hydrolase inhibitor **PF-622,** three tritium isotopomers were prepared. [^{3}H]**PF-622a** labeled at the piperazine ring B and [^{3}H]**PF-622b** labeled at both the ring B and phenyl ring A were synthesized via catalytic H(hydrogen)-T(tritium) exchange, utilizing 1 equiv. and excess of Crabtree's catalyst, respectively. The preparation of [^{3}H]**PF-622c** labeled only at the phenyl ring A was achieved via tritiodebromination of the bromide precursor, using $Pd(PPh_3)_4$ as a catalyst. The observations from these tritiation reactions might open new perspective in the labeling for the targets having a similar moiety.

Keywords

tritium-labeling, Crabtree's catalyst, H-T exchange, palladium, tritiodebromination

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Introduction

Fatty acid amide hydrolase (FAAH) is an integral membrane enzyme that degrades the fatty acid ethanolamide family of signaling lipids, including the endocannabinoid anandamide. Pharmacological inactivation of FAAH leads to analgesic, anti-inflammatory, anxiolytic, and antidepressant effects. These bioactivities indicate that FAAH may represent an attractive therapeutic target for treatment of pain, inflammation, and other central nervous system disorders.[1]

N-phenyl-4-(quinolin-2-ylmethyl)piperazine-1-carboxamide (**PF-622**) was discovered as a novel mechanistic class of FAAH inhibitors.[1,2] In order to make a detailed characterization of the mechanism of inhibition and selectivity of **PF-622** using enzyme kinetic and functional proteomic methods, three different tritium-labeled compounds ([^{3}H]**PF-622a**, [^{3}H]**PF-622b** and [^{3}H]**PF-622c**) were prepared. The tritium labeling of both *N*-substituents of the urea moiety helped us to determine whether the inhibitor **PF-622** is reversible or irreversible and which moiety of the molecule is bound to the enzyme if it is irreversible.

Althouth tritium-labeled compounds can be prepared by multi-step chemical synthesis, as are carbon-14-labeled compounds, a more ideal approach is to use the method of metal catalysed hydrogen isotope exchange to introduce tritium into the final product. Several effective metal catalysts were developed for "fast" tritium labeling of drugs, such as H-T exchange[3,4,5,6,7,8,9] and tritiodehalogenation.[10,11,12,13] This paper details the specific radiosynthesis of three tritium isotopomers (Fig 1.) in the presence of commercially available catalysts. Some intersting results from the fast tritum labeling of PF-622 are also discussed.

Results and Discussion

The desired tritium labeled **PF-622** can be prepared either by multi-step radiosynthesis or directly by H-T exchange of **PF-622** with tritium and by tritiodehalogenation of its halide derivatives. However, the direct H-T exchange and tritiodehalogenation strategies were my first choices because they are more efficient and produce less radio waste. Since **PF-622** is an aromatic amide derivative, it is amenable to direct tritium-hydrogen exchange in the presence of Crabtree's catalyst Ir[(COD)(PCy_3)(Py)]PF_6. [3,4,5] Therefore, the final product **PF-622** was first prepared to test the direct H-T exchange strategy.

PF-622 can be synthesized by following the literature method[2] which includes urea derivative formation, de-protection and reductive amination. However, crude **PF-622** from the final step was hard to purify and the overall yield (24.5%) was low. An alternate approach was then developed (Scheme 1). Thus, commercially available Boc protected piperazine **2** was directly alkylated by alcohol derivative **1** in the presence of an iridium catalyst-pentamethylcyclopentadienyliridium (III) chloride dimer [(Cp*$IrCl_2)_2$] to give C-N coupling product **3** in a high yield according to the literature methods.[14,15] Without further purification of **3**, it was de-protected easily by using trifluoroacetic acid to give the desired compound **4**. The treatment of piperizine derivative **4** with phenyl isocyanate furnished the final product **PF-622**. The overall chemical yield (59.8%) was better than the literature reported (24.5%).[2]

With the unlabled product **PF-622** in hand, the H-T exchange reaction between PF-622 and tritium gas in the presence of Crabtree's catalyst was first carried out. Based on previous research data,[3,4,5] the ortho protons to the amide functionality in the phenyl ring (A) would be predicted to exchange with tritium. However, the treatment of an equivalent of unlabeled **PF-622** and Crabtree's catalyst in CH_2Cl_2 with either a trace amount of tritium gas (45 mCi) or large amount of tritium gas (1.1 Ci) at room temperature provided a tritium-labeled compound ([^{3}H]**PF-622a**) with around 2.67 ppm of tritium NMR chemical shift (Fig. 2)

which responds to hydrogens on 3,5-positions (sp^3 carbon, see ^{1}H NMR of PF-622 in experimental section) of piperazine moiety (ring B, Scheme 2).

It was previously reported that heteroaryl nitrogen ligand such as pyridine and pyrimidine can effectively mediate hydrogen isotope exchange at sp^3 carbons[6,16,17,18] and also its mediating functions are more effective than amide carbonyls.[6,17] After further analyzing the structure of **PF-622**, it is possible that quinoline's nitrogen can direct the H-T exchange of 3,5-positions (sp^3 carbon) of the piperazine moiety (ring B) in the *in situ* assembling agostic complexes (**5a**) and then iridium six-membered metallacycle (**5a'**). The carbonyl group of the urea moiety is in similar distance to aromatic (*ortho* carbons ring A) and aliphatic (C3 & C5 ring B) carbons , but the carbonyl group participation of the direction of H-T exchange at sp^3 carbon may be unlikely because previous research data[17] shows that the quinoline's nitrogen co-ordinates the iridium center more effectively than the aminocarbonyl's oxygen and also the agostic complexe **5b** is less stable than **5a** based on the energy calculation (MOE). Our result of the alkyl-versus-aryl selectivity might further indicate that the iridium chelation between nitrogen at quinoline ring C and sp^3 carbon at the piperazine ring B was more effective than that between the carbonyl group of the urea moiety and sp^2 carbon at the aromatic ring A (**5c'**). As a result, the tritiation of **PF-622** with the limitted Crabtree's catalyst (1 equiv.) only gave a labeled compound [^{3}H]**PF-622a** (7.32 mCi, 7.7Ci/mmol, 99.1% RCP).

Furthermore, when the molar ratio of the catalyst and reaction time were increased tritium labels on both *N*-substituents of the urea moiety were observed based on tritium NMR data (Fig. 3). One equivalent of **PF-622** was treated with 2.24 equivalents of Crabtree catalyst in CH_2Cl_2 at room temperature for 18 hours ⏐⟶ to provide a new-labeled compound [^{3}H]**PF-622c** with 2.67 and 7.45 ppm of tritium NMR chemical shifts. The interesting result indicates that once excess catalyst (>2 equiv.) and longer reaction time were applied the H-T exchange unequally occurred both on the sp^3 carbon of the piperazine ring B (60.6%T) and

the sp^2 carbon of the phenyl ring A (39.4%T) as well. Under much higher catalyst loading there is nothing to prevent unproductive coordination of the iridium center to less effective functional groups (**5c**).

In order to specifically label the phenyl ring A, we have to utilize tritiodehalogenation of a halide precursor of **PF-622**. We could either brominate the final molecule **PF-622** or use 4-bromophenyl isocyanate as a starting material to synthesize a halide precursor **7** of **PF-622**. There are several halogenation methods available in literatures.[18,19,20,21,22,23] Some of the methods[18,19,20,21,22] were tried and did not introduce halogen only into the phenyl ring A of the final molecule **PF-622**. Krishna and her co-workers[23] reported that an aniline and anisole can be brominated by the electrophilic substitution of bromine generated in situ from ammonium bromide as a bromine source and hydrogen peroxide as an oxidant. This method indeed allowed us to introduce a bromo group only into position 4 of phenyl ring A. The desired bromide **7** was prepared in 75% yield.

De-bromination of **7** was first tried with tritium gas in the presence of 10% Pd/C according to the literature method.[12] In addition to the expected tritium in the 4 position in the A ring, tritium NMR (Fig. 4, [^3H_n]**PF-622d**) analysis also indicated additional tritium was present in the benzylic position which is not unexpected from the literature precedence. The result further indicated that under 10% Pd/C catalytic condition the catalytic H-T exchange as a side reaction took place at certain active positions mentioned above, a similar observation of which was reported by Devillers.[13]

In order to test the tetrakis $Pd(PPh_3)_4$ catalyst,[24] a trace tritiation run was carried out with **PF-622** and $Pd(PPh_3)_4$ in DMF at 110^{o}C for 1.5 hours. No H-T exchange was found under such condition. Therefore, the tritium de-bromination reaction was performed again with bromide precursor **7** and $Pd(PPh_3)_4$ in DMF at 110^{o}C for 1.5 h. The crude [^{3}H]**PF-622c** was purified by preparative HPLC to afford 16.2 mCi of [^{3}H]**PF-622c** with a specific activity of 24.7 Ci/mmol and radiochemical purity of 99.0%.

Conclusions

In summary, an alternate approach to **PF-622** was developed based on a direct Ir-catalyzed *N*-alkylation of an amine with an alcohol. The direct H-T exchange between **PF-622** and tritium gas was performed in the presence of Crabtree's catalyst. With using 1 equiv. of the catalyst, we were able to specifically synthesize the desired tritium isotopomer [^{3}H]**PF-622a** only labeling on 3,5-positions of piperazine moiety. While with a large amount of the catalyst (>2 equiv.) and longer reaction time, we prepared another tritium isotopomer [^{3}H]**PF-622b** labeling on both *N*-substituents of the urea moiety in 2:3 ratio (phenyl ring A to piperazine ring B).

Our observations further indicates that the Ir chelation between nitrogen at quinoline ring C and sp^3 carbon at the piperazine ring B was more effective than that between the carbonyl group of the urea moiety and sp^2 carbon at the aromatic ring A. The tritium de-bromination of the bromide derivative **7** with $Pd(PPh_3)_4$ offered the only reduction product [^{3}H]**PF-622c** without labeling on any other positions caused by H-T exchange. However, with 10% Pd/C the tritiodebromination provided a new product [^{3}H]**PF-622d** labeled at one desired position and other unexpected positions**.** These new observations will be applied in the tritium (or deuterium) labeling for the targets having a similar moiety.

Experimental

General methods: All reactions were carried out under an atmosphere of nitrogen unless otherwise stated. LC-MS data were obtained on a Water Micromass LCZ mass spectrometer with flow injection analysis. ^{1}H and ^{3}H NMR spectra were recorded on a Bruker 300 MHz instrument. Chemical purity of all compounds was determined by HPLC and LC-MS. Purifications were done by flash column chromatography on Biotage system. Quantitation of radioactivity of tritium labeled compounds was performed using a Packard 2200CA liquid scintillation analyzer, with Sciniverse BD cocktail. Commercial reagents, solvents and known intermediates **1** & **2** were purchased from Sigma-Aldrich and used as-received unless

otherwise noted. Tritium gas was purchased from American Radiochemical, Inc. Tritiation was done using TRI-SORBER ® Tritiation Manifold, IN/US systems.

2-(Piperazin-l-ylmethyl)quinoline 4[1,2]

To a solution of 2-hydroxymethylquinoline **1** (3.22 g, 20 mmol) in toluene (200 mL) were added, tert-butyl piperazine-1-carboxylate **2** (3.82g, 20.5 mmol), potassium carbonate powder (138 mg, 1 mmol) and pentamethylcyclopentadienyliridium (III) chloride dimer (80 mg, 0.1 mmol). The reaction mixture was purged with nitrogen gas and then sealed and heated to an internal temperature of 110^{o}C and held for 24 hr. Reaction was cooled to room temperature and filtered through Celite. Filtrate was concentrated under vacuum, and the crude **3** in trifluoroacetic acid (5 mL) and CH_2Cl_2 (80 mL) was stirred at room temperature for 6 hr. The reaction was concentrated under vacuum and the crude residue was purified on SiO_2 using Biotage SPl to give a pure 2-(piperazin-l-ylmethyl)quinoline **4** (3.27 g, 72%, pale yellow solid). ^{1}H NMR ($CDCl_3$): δ 8.13 (d, J = 9 Hz, 1H), 8.08 (d, J = 9 Hz, 1H), 7.81 (d, J = 8 Hz, 1H), 7.69 (t, J = 7 Hz, 1H), 7.66 (d, J = 9 Hz, 1H), 7.51 (t, J = 7 Hz, 1H), 3.84 (s, 2H), 2.93 (t, J = 5 Hz, 4H), 2.54 (bs, 4H); HRMS (EI) *m/z* found 227.1424, calcd for $C_{14}H_{17}N_3$: 227.1422.

4-Quinolin-2-ylmethyl-piperazine-1-carboxylic acid phenylamide (PF-622)[1,2]

A solution of 2-(piperazin-l-ylmethyl)quinoline **4** (5.7 g, 25.1 mmol) in CH_2Cl_2 (50 mL) was cooled in an ice bath and treated with phenyl isocyanate (3.3 mL). The reaction mixture was stirred at 0^{o}C for 1 hr and then at room temperature for 15 hr. The solid in the resulting mixture was filtered out and washed with CH_2Cl_2 (2 x 100 mL) to give the title compound as a white amorphous solid (7.2 g, 83%).^{1}H NMR (MeOD): δ 8.37 (d, J = 8.5 Hz, 1H), 8.05 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.1 Hz, 1H), 7.81-7.76 (m, 2H), 7.61 (t, J = 7.5 Hz, 1H), 7.36 (d, J = 7.5 Hz , 2H), 7.27 (t, J = 7.5 Hz, 2H), 7.03(t, J = 7.0 Hz, 1H), 3.90 (s, 2H), 3.65-3.55 (m, 4H, positions 2 and 6 of piperazine), 2.68-2.55 (m, 4H, positions 3 and 5 of piperazine). HRMS (EI) *m/z* found 346.1792, cald for $C_{21}H_{22}N_4O$: 346.1794

4-Quinolin-2-ylmethyl-[3,5-^{3}H]piperazine-1-carboxylic acid phenylamide (PF-622a)

The unlabeled **PF-622** (1 mg, 2.9 μmol) was treated with 1.1 Ci carrier free tritium gas and Crabtree's catalyst (2.3 mg, 2.9 μmol) in CH_2Cl_2 (0.5 ml) on a commercial tritiation manifold (TriSorber) for 2.5 hr at room temperature. The labile tritium was removed by repeated evaporation of methanol solution and the crude product was subjected to reverse phase HPLC purification. The fractions containing pure product was pooled and reconstituted in methanol to give 8.0 mCi of the final product with the following specifications: specific activity (SA) =7.7 Ci/mmol (by MS); radiochemical purity= 98.4%. Reverse-phase HPLC conditions: column phenomenex luna C18(2), 3μm, 4.6x150 mm; mobile Phase: A= water, B=CH_3CN, 20% B linear gradient to 50% B over 30 minutes; flow rate: 1.0 mL / min; UV detection: 230 nm; retention Time: 9.6 minutes. MS (ESI, M+H^+) *m/z*: 347 (100%), 348(24%), 349(13.5%). ^{3}H NMR (MeOD) :δ 2.67; ^{1}H NMR (MeOD): δ 8.36 (d, J = 8.5 Hz, 1H), 8.04 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.1 Hz, 1H), 7.81-7.76 (m, 2H), 7.61 (t, J = 7.5 Hz, 1H), 7.36 (d, J = 7.5 Hz , 2H), 7.27 (t, J = 7.5 Hz, 2H), 7.03 (t, J = 7.0 Hz, 1H), 3.90 (s, 2H), 3.65-3.55 (m, 4H), 2.68-2.55 (m, <4H) (consistent with the spectrum of PF-622).

4-Quinolin-2-ylmethyl-[3,5-^{3}H]piperazine-1-carboxylic acid [2,5-^{3}H] phenylamide (PF-622b)

The unlabeled **PF-622** (1 mg, 2.9 μmol) was treated with 1.1 Ci carrier free tritium gas and Crabtree's catalyst (5.1 mg, 6.5 μmol) in CH_2Cl_2 (0.6 mL) on a commercial tritiation manifold (TriSorber) for 18 hr at room temperature. The labile tritium was removed by repeated rotary evaporation of methanol solution and the crude product was subjected to reverse phase HPLC purification. The fractions containing pure product was pooled and reconstituted in methanol to give 10.0 mCi of the final product with the following specifications: specific activity (SA) =15.8 Ci/mmol (by MS); radiochemical purity:=98.2%. Reverse-phase HPLC conditions: column: phenomenex luna C18(2), 3μm, 4.6x150 mm; mobile Phase: A= water, B=CH_3CN, 20% B linear gradient to 50% B over 30 minutes; flow Rate: 1.0 mL / min; UV detection: 230 nm; retention time: 9.6 minutes. MS (ESI, M+H) *m/z*: 347 (100%), 348(24%), 349(23.5%), 350(12.1%). ^{3}H NMR (MeOD): δ 2.67, 7.45; ^{1}H NMR

(MeOD): δ 8.37 (d, J = 8.5 Hz, 1H), 8.04 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.1 Hz, 1H), 7.82-7.77 (m, 2H), 7.61 (t, J = 7.5 Hz, 1H), 7.40 (d, J = 7.5 Hz , <2H), 7.27 (t, J = 7.5 Hz, 2H), 7.03 (t, J = 7.0 Hz, 1H), 3.90 (s, 2H), 3.65-3.55 (m, 4H), 2.68-2.55 (m, <4H).

4-Quinolin-2-ylmethyl-piperazine-1-carboxylic acid 4-bromophenylamide 7

30% H_2O_2 (2.2 mmol) was added dropwise to the reaction mixture of **PF-622** (0.692 g, 2 mmol) and ammonium bromide (0.215g, 2.2 mmol) in acetic acid (4 mL). The resulting mixture was stirred at room temperature for 4 hr. After the completion of the reaction, the reaction mixture was treated with saturated sodium bicarbonate solution and extracted with CH_2Cl_2. The organic extract was dried over anhydrous sodium sulfate and solvent evaporated under reduced pressure. The crude product was purified by column chromatography (5% MeOH in CH_2Cl_2) to give the titled compound **7** as an off-white solid (0.638 g, 75%), ^{1}H NMR (MeOD): δ 8.37 (d, J = 8.4Hz, 1H), 8.05 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.1 Hz, 1H), 7.81-7.76 (m, 2H), 7.62 (t, J = 7.6 Hz, 1H), 7.40 (d, J = 8.8 Hz, 2H), 7.32(d, J = 9.0 Hz, 2H), 3.90 (s, 2H), 3.61-3.59 (m, 4H), 2.63-2.60 (m, 4H). HRMS (EI) *m/z* found 424.0901, calcd for $C_{21}H_{21}BrN_4O$: 424.0899

4-Quinolin-2-ylmethyl-piperazine-1-carboxylic acid [4-^{3}H]phenylamide (PF-622c)

The bromide precursor **7** of **PF-622** (0.75 mg, 1.7 µmol) was reduced with 1.1 Ci carrier free tritium gas and Pd $(PPh_3)_4$ (0.6 mg, 0.5 µmol) in DMF (0.15 mL) on a commercial tritiation manifold (TriSorber) at 110^{o}C for 1.5 hr. The labile tritium was removed by repeated rotary evaporation of methanol solution and the crude product was subjected to reverse phase HPLC purification. The fractions containing pure product were pooled and reconstituted in methanol to give 16.2 mCi of the final product with the following specifications: SA=24.7 Ci/mmol (by MS); radiochemical purity=97.0%. Reverse-phase HPLC conditions: column phenomenex luna C18(2), 3µm, 4.6x150 mm; mobile phase: A= water, B=CH_3CN, 20% B linear gradient to 50% B over 30 minutes; flow rate: 1.0 mL / min; UV detection: 230 nm; retention time: 9.6 minutes. MS (ESI, $M+H^+$) *m/z*: 347 (16%), 349 (100%). ^{3}H NMR (MeOD): δ 7.12; ^{1}H NMR ^{1}H NMR (MeOD): δ 8.37 (d, J = 8.5 Hz, 1H), 8.04 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.1 Hz, 1H), 7.82-7.77 (m, 2H), 7.61 (t, J = 7.5 Hz, 1H), 7.40 (d, J = 7.5 Hz , 2H), 7.27 (t, J = 7.5 Hz,

2H), 3.90 (s, 2H), 3.65-3.55 (m, 4H), 2.68-2.55 (m, 4H).

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Conflict of Interest

The author did not report any conflict of interest.

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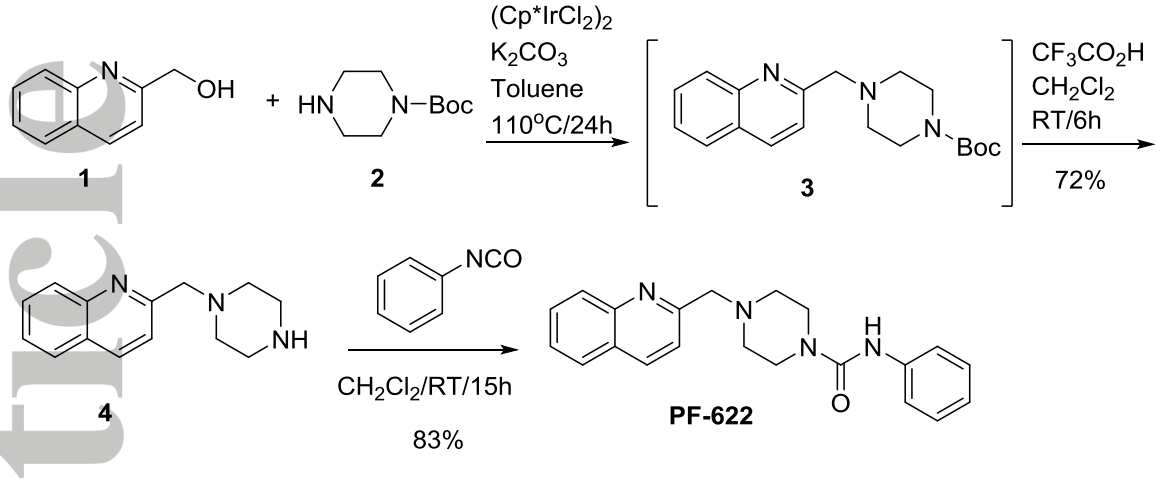
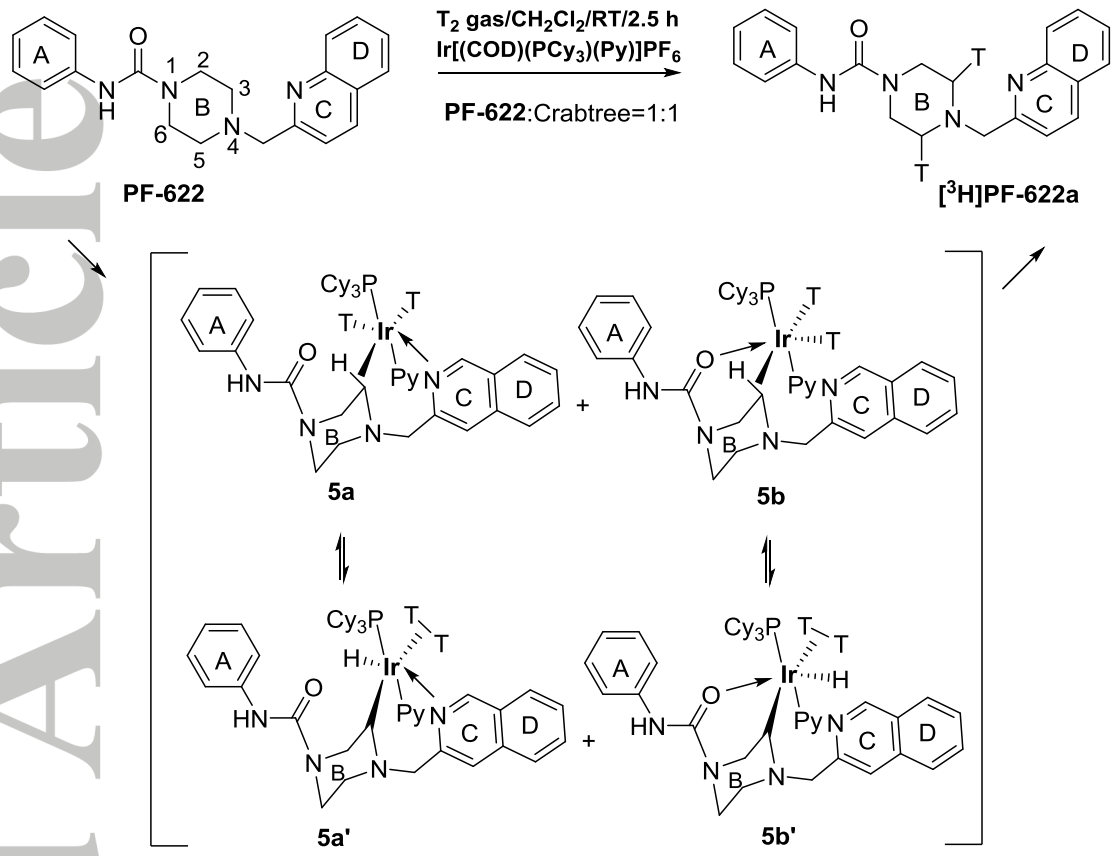
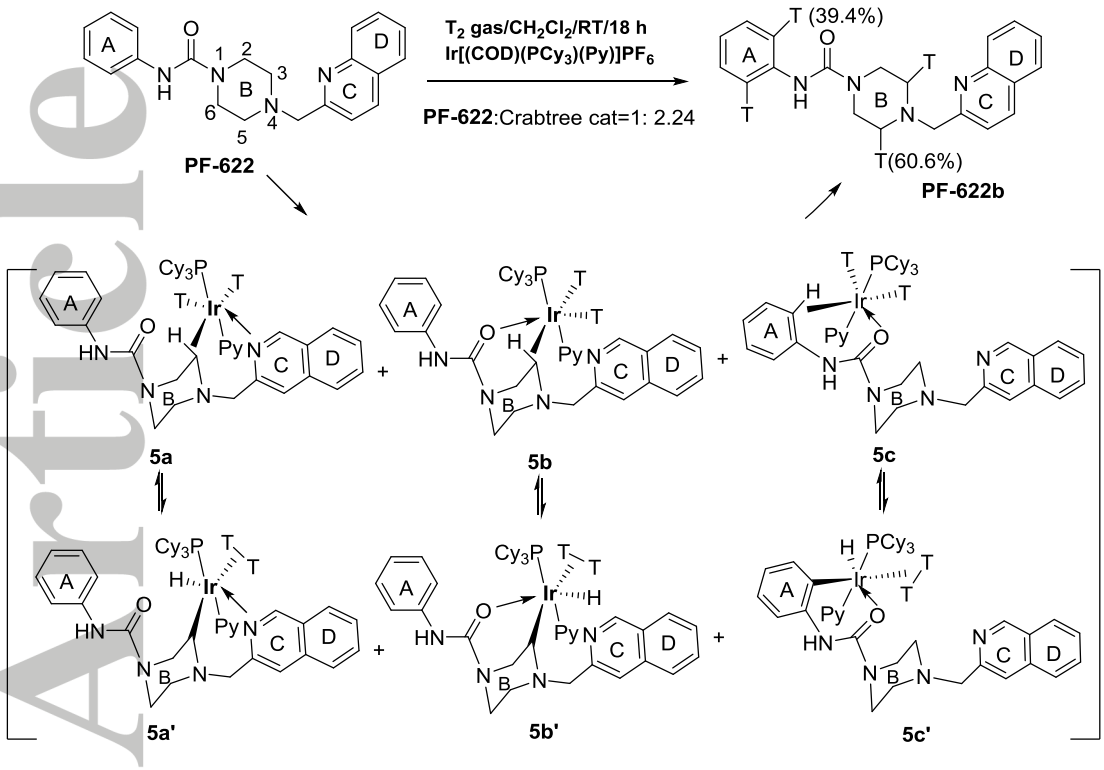


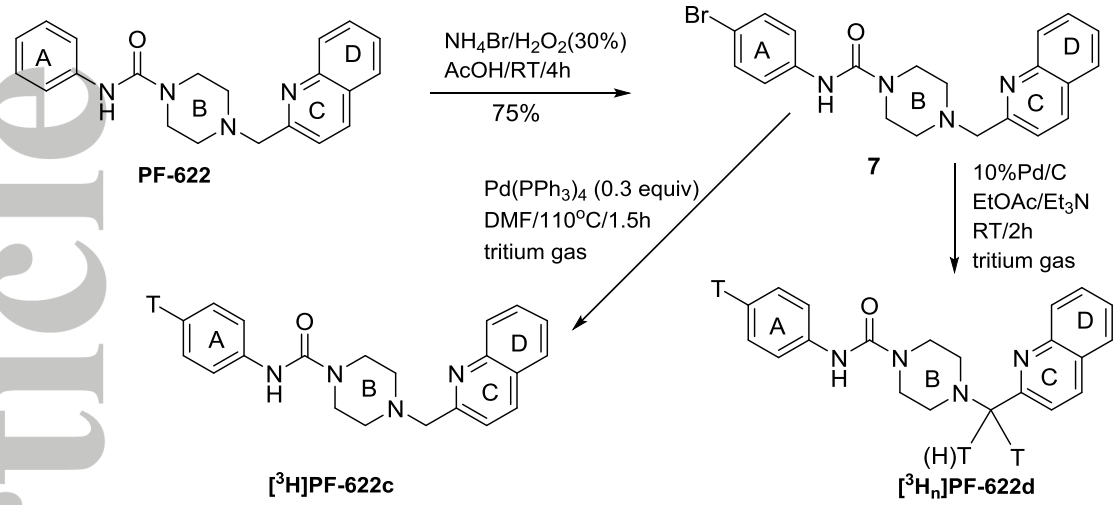
Scheme1. An alternate synthesis of **PF-622**



Scheme 2. A specific synthesis of [^{3}H]**PF-622a** with 1 equiv. of Crabtree's catalyst



Scheme 3. Synthesis of [^{3}H]**PF-622b** with excess Crabtree's catalyst



Scheme 4. A specific radiosynthesis of [^{3}H]**PF-622c** with $Pd(PPh_3)_4$

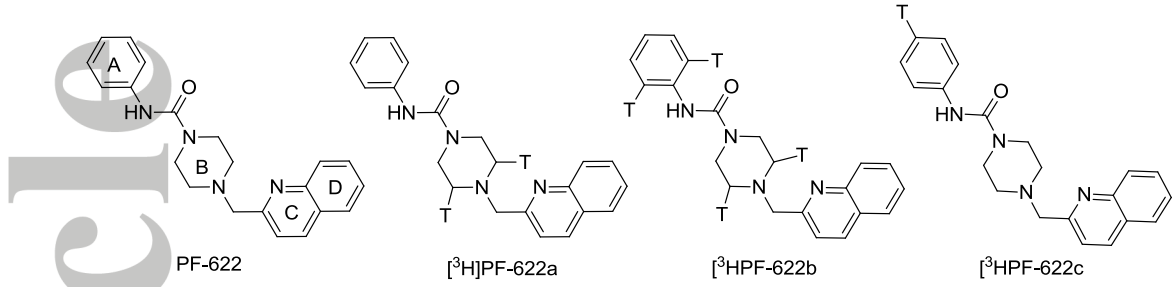


Fig. 1 Structures of **PF-622**, [^{3}H]**PF-622a**, [^{3}H]**PF-622b** and [^{3}H]**PF-622c**

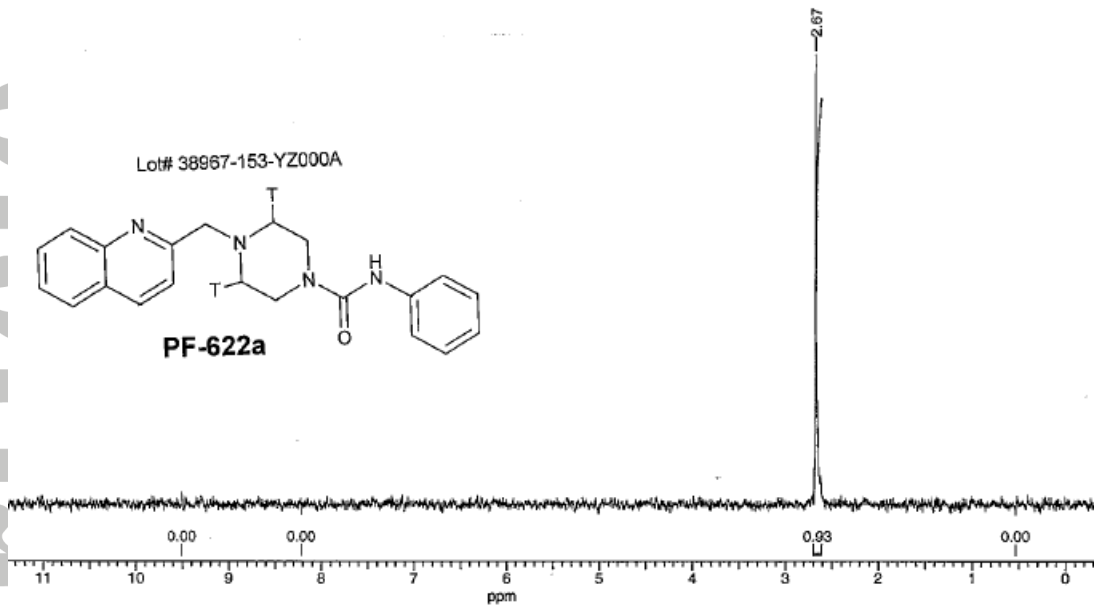


Fig 2. Proton-decoupled tritium NMR spectrum of PF-622a

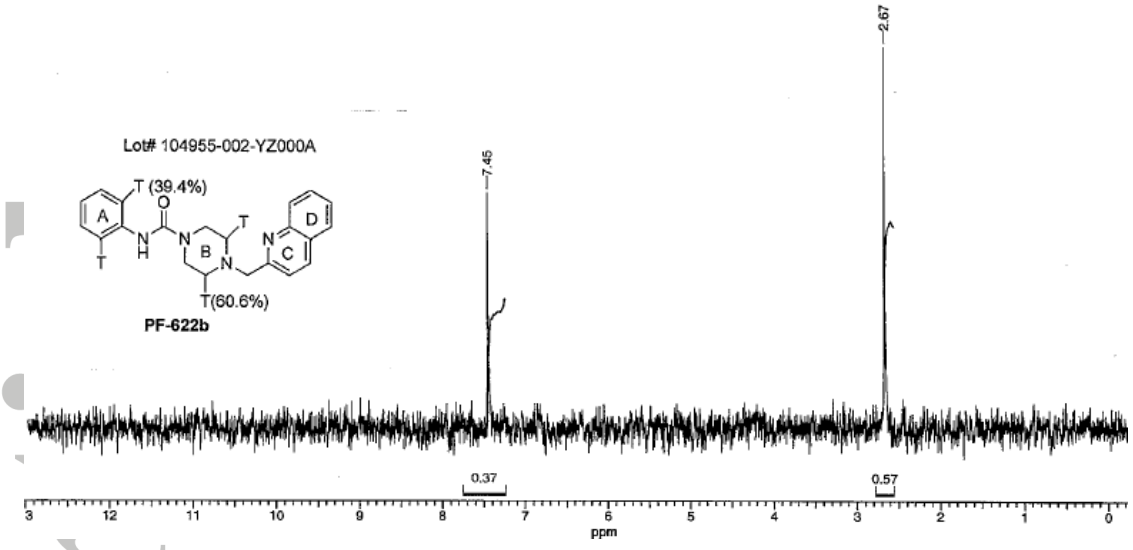


Fig.3 Proton-decoupled tritium NMR spectrum of **PF-622b**

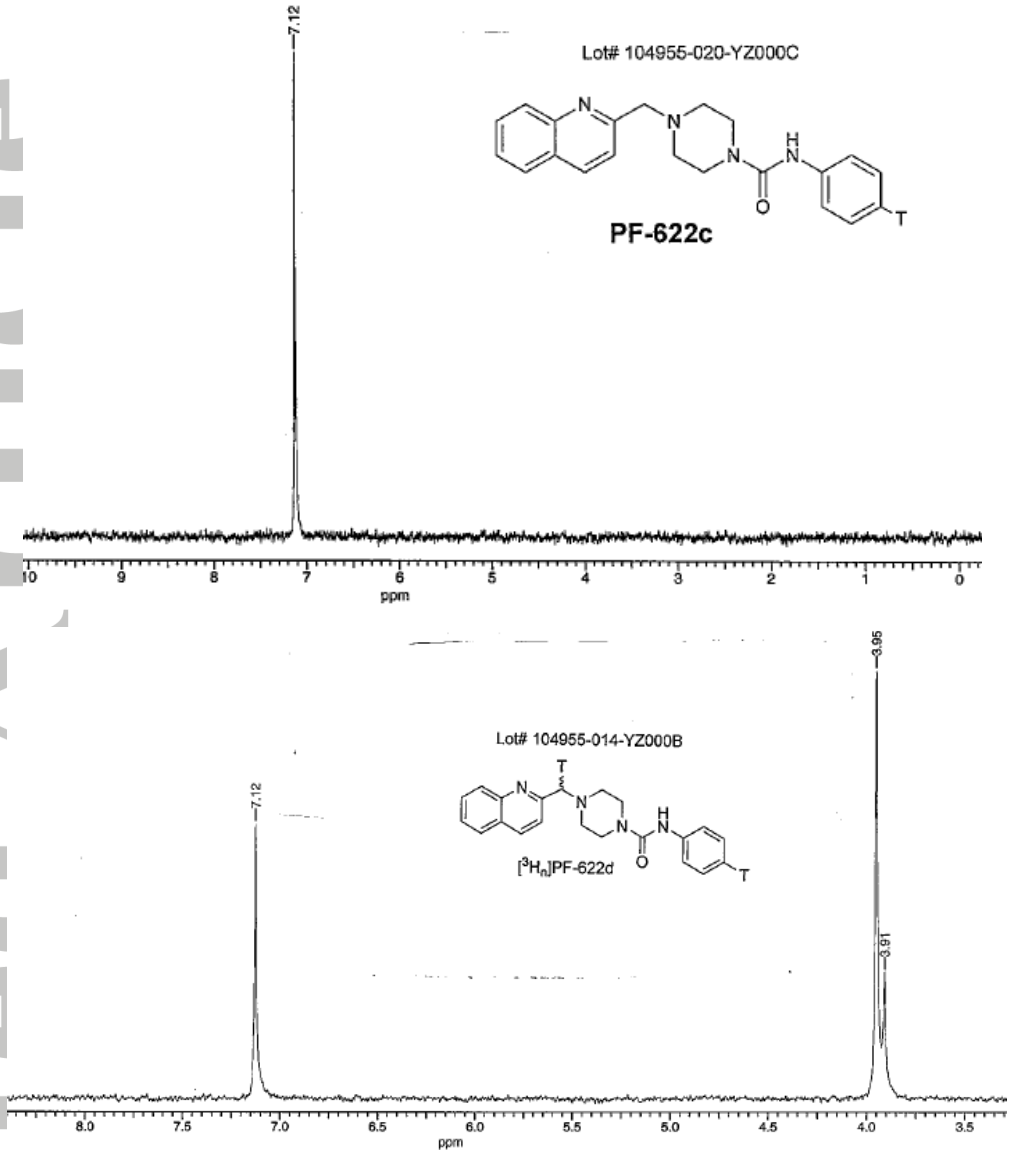


Fig.4 Proton-decoupled tritium NMR spectrum of PF-622c and PF-622d