Isotope Labelling by Reduction of Nitriles: Application to the Synthesis of Isotopologues of Tolmetin and Celecoxib

Short title: Isotope Labelling by Reduction of Nitriles

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Abstract

The aryl methyl group is found in many drug-like compounds, but there are limited ways of preparing compounds with an isotope label in this methyl position. The process of cyanation of an aryl halide followed by complete reduction of the nitrile to a methyl group was investigated as a route for preparing stable and radiolabelled isotopologues of drug-like compounds. Using this methodology, carbon-13, deuterium, carbon-14 and tritium labelled isotopologues of the non-steroidal anti-inflammatory drug (NSAID) tolmetin were produced, as well as carbon-13, deuterium and carbon-14 labelled isotopologues of another NSAID, celecoxib. The radiolabelled compounds were produced at high specific activity and the stable isotope labelled compounds with high incorporation making them suitable for use as internal standards in mass spectrometry assays. This approach provides a common synthetic route to multiple isotopologues of compounds using inexpensive and readily available labelled starting materials.

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Introduction

The complete reduction of aromatic nitrile groups under palladium-catalysed hydrogenation conditions to give a methyl group is a known but little-used process.[1] The aromatic nitrile compounds required for this process are readily formed by palladium-catalysed cross coupling reaction of the corresponding aromatic halides with cyanide.[2] Combining these two reactions leads to a synthetic route in which the carbon of the methyl group can be isotopically labelled by the use of labelled cyanides and the hydrogen positions by carrying out the reduction with hydrogen isotopes (Scheme 1). A common route can therefore be used to make carbon-14, stable isotope and tritium labelled isotopologues of organic compounds.

The tolyl group is a common motif found in many drug-like compounds (Figure 1), including the non-steroidal anti-inflammatory drugs (NSAIDs) tolmetin **1a** and celecoxib **2a**, the tyrosine kinase inhibitor imatinib (Gleevec) **3**, and the local anaesthetic lidocaine **4**.

Labelling in the methyl position of a tolyl group should provide labelled compounds suitable for a variety of applications. Synthesis of a stable isotope labelled compound with both carbon-13 and three deuterium atoms would give a mass increase of +4 over the unlabelled compound. In most cases this would make it suitable for use as an internal standard in mass spectrometry bioanalysis assays, as long as sufficient levels of deuterium incorporation were achieved. The methyl position is known to be susceptible to metabolism involving oxidation to the alcohol and the carboxylic acid by the cytochrome P450 enzymes CYP3A4 and CYP2C9.[3,4] This means that in metabolism studies hydrogen isotopes could be lost from the methyl position, and for this reason compounds with tritium labels in the methyl position would often need to find applications in studies that do not involve metabolism. It is rare for an aryl methyl to be removed by metabolism, so a carbon-14 labelled methyl would in general be suitable for ADME studies.

Other potential methods for labelling these methyl positions include the use of labelled methyl iodide following metal-halogen exchange on the aryl ring,[5] Stille coupling of the organotin compound with labelled methyl iodide,[6] or Suzuki-type coupling with labelled methyl iodide[7] or a labelled methyl borinate.[8]

Due to the relative ease of preparing the halogenated starting materials, the drugs tolmetin **1a** and celecoxib **2a** were chosen as examples to test this synthetic route. Tolmetin **1a** has previously been labelled with carbon-14 in the ketone carbon[9] and with tritium in multiple positions on the toluene and pyrazole rings.[10,11] Celecoxib has been labelled with carbon-14 in the toluene[12] and pyrazole rings[13] and with deuterium in the toluene[14] and phenylsulfonamide rings.[15]

Results and Discussion

Tolmetin

The iodinated tolmetin analogue **7** was prepared by the condensation of 4-iodobenzoyl chloride **5** and the substituted pyrrole **6** in refluxing xylene (Scheme 2).[16] The reaction proceeded in moderate yield (43%) but attempts to improve on this by the addition of DBN[17] or LiI[18] led to lower recoveries. For the cyanation step, initially potassium cyanide was investigated as the cyanide source as this is the cheapest source of isotopically labelled cyanide. Although this worked in acceptable yield (typical conditions: KCN, $Pd(PPh_3)_4$, CuI, 70°C for 18 h) it was found that the subsequent reduction step did not work with material

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prepared in this way despite purification of the nitrile by both normal and reverse phase chromatography. For this reason, alternative conditions using zinc cyanide as the cyanide source along with palladium catalyst and Xantphos ligand under microwave heating were used to give the nitrile derivatives **8a-c** in good yield (72-76%).[19] In the case of the carbon-13 labelled nitrile **8b**, zinc [^{13}C,^{15}N]cyanide was used despite the fact that the nitrogen-15 label would be lost in the reduction step.

The conditions for the chemoselective reduction of the nitrile were optimised with different solvents and reduction times. In protic solvents the reduction of the nitrile **8a** proceeded smoothly with palladium on carbon under an atmosphere of hydrogen; however, extended reaction times led to reduction of the ketone. When the reduction of the carbon-13 labelled nitrile **8b** was carried out with deuterium gas, isotope incorporation of only 0.5 d/mol was achieved. Changing the solvent to d_4-methanol gave **9b** with incorporation of 5 deuterium atoms, the extra two being alpha to the ester. These exchanged out rapidly during purification and in the subsequent hydrolysis step to give the [^{13}C, $^{2}H_3$]tolmetin **1b** with no unlabelled tolmetin detected by mass spectrometry. The reduction of the unlabelled nitrile compound **8a** with tritium gas was carried out at sub-atmospheric pressures and after hydrolysis gave [^{3}H]tolmetin **1d** with a specific activity of 20.5 Ci/mmol, with tritium incorporated exclusively in the methyl position. Synthesis of [^{14}C]tolmetin **1c** by cyanation with zinc [^{14}C]cyanide to give the nitrile **8c**, followed by reduction with hydrogen gas and hydrolysis gave material at high specific activity (55.6 mCi/mmol) and radiochemical purity.

Celecoxib

The iodinated celecoxib analogue **14** was prepared in a two-step process (Scheme 3).[20,21] Condensation of the iodoacetophenone **10** with ethyl trifluoroacetate **11** gave the 1,3-dicarbonyl **12** which cyclised regioselectively with the hydrazine **13** to give the iodinated intermediate **14**. The rest of the reaction sequence was initially trialled with unlabelled reagents. The same cyanation protocol as used for tolmetin worked well to give the nitrile **15a**, although longer reaction times were required for complete reaction. The subsequent hydrogenation gave mainly partial reduction to the amine **16** after 4.5 h. Resubmitting the mixture to the reaction conditions for an additional 22 h resulted in complete reduction to the methyl **2a**.

Using this methodology, [^{13}C,$^{2}H_3$]celecoxib **2b** was produced with high deuterium incorporation (2.9 d/mol) when d_4-methanol was used as the solvent in the reduction step. [^{14}C]Celecoxib **2c** was prepared in high specific activity (55.8 mCi/mmol) and radiochemical purity (>99%). [^{3}H]Celecoxib was not prepared, but this methodology should provide a route to this compound if required.

Conclusion

Cyanation followed by complete reduction has proven to be a useful approach to the synthesis of drug-like compounds containing aryl methyl groups. It allows for carbon-14, carbon-13, deuterium and tritium labelled isotopologues suitable for use in ADME and mass spectrometry assays to be prepared using a common synthetic route and using readily available and inexpensive labelled starting materials.

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Experimental

Microwave reactions were carried out using a CEM Explore (CEM Corporation, NC, USA). Column chromatography was carried out using pre-packed silica gel cartridges (SiliCycle, Quebec, Canada) on an Isco Companion (Teledyne Isco, NE, USA). ^{1}H NMR spectra were recorded on a Bruker (600 MHz) using the stated solvent. Chemical shifts (δ) in ppm are quoted relative to $CDCl_3$ (δ 7.26 ppm) and DMSO-d_6 (δ 2.50 ppm). Liquid chromatography-mass spectrometry (LC-MS) data was collected using a Waters Alliance LC (Waters Corporation, MA, USA) with Waters ZQ mass detector. Analytical HPLC data was recorded using Agilent 1200 HPLC system with a β-Ram Flow Scintillation Analyser, using the following conditions: Waters Sunfire C_{18}, 3.5 µm, 4.6 x 100 mm column at 40°C, eluting with 5% acetonitrile/water + 0.1% TFA to 95% acetonitrile/water + 0.1% TFA over a 32 minute gradient. Specific activities were determined gravimetrically with a Packard TriCarb 2100CA Liquid Scintillation Analyser (Packard Instrument Company Inc., IL, USA) using Ultima Gold^{TM} cocktail. Reactions with tritium gas were carried out on a steel manifold obtained from RC Tritec AG (Teufen, Switzerland). LCMS and specific activity measurements were obtained by electrospray ionization using a single quadrupole mass spectrometer with Masslynx v3.5 acquisition and data manipulation software. Specific activity was calculated by comparison of the ratio of tritium/hydrogen or carbon-14/carbon-12 for the tracer against the unlabelled reference.
Zinc [^{14}C]cyanide (gravimetric specific activity: 796 µCi/mg equivalent to 95.9 mCi/mmol) was obtained from Quotient Bioresearch (Radiochemicals) (Cardiff, UK) and zinc [$^{13}C_2$,$^{15}N_2$]cyanide was obtained from Sigma-Aldrich. Tritium gas was supplied absorbed onto a depleted uranium bed by RC Tritec AG (Teufen, Switzerland). All other reagents and solvents were obtained from Sigma-Aldrich and Fisher and were used without further purification.

Synthesis of Tolmetin Isotopologues

Methyl 2-(5-(4-iodobenzoyl)-1-methyl-1*H*-pyrrol-2-yl)acetate (7)

4-Iodobenzoyl chloride **5** (5.33 g, 20.0 mmol) was added to a solution of methyl 2-(1-methyl-1*H*-pyrrol-2-yl)acetate **6** (1.53 g, 10.0 mmol) in xylenes (15 ml). The reaction mixture was heated to reflux and stirred for 24 h then cooled to room temperature, concentrated and diluted with dichloromethane (15 ml). *N*,*N*-Dimethyl-1,3-propanediamine (3 ml) was added and the solution stirred at room temperature for 30 min. The solution was washed with water (15 ml), the organic layer was diluted with DCM (25 ml) and washed sequentially with 1M HCl (15 ml), 10% potassium carbonate solution (15 ml), and saturated brine (15 ml), dried over $MgSO_4$, filtered and concentrated. The precipitate was collected by filtration and product remaining in the filtrate was purified by flash silica chromatography, elution gradient 0 to 40% EtOAc in heptane. Fractions containing product were pooled and evaporated and combined with the precipitate collected earlier. The combined batches were crystallised from EtOAc and heptane to yield **7** (1.66 g, 4.32 mmol, 43%) as a tan solid. LCMS (ESI) *m/z* 384 $[M+H]^+$. ^{1}H NMR (DMSO-d_6): δ 3.67 (s, 3H), 3.82 (s, 3H), 3.92 (s, 2H), 6.14 (d, *J* = 4.1 Hz, 1H), 6.60 (d, *J* = 4.1 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 2H), 7.88 (d, *J* = 8.5 Hz, 2H).

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Methyl 2-(5-(4-cyanobenzoyl)-1-methyl-1*H*-pyrrol-2-yl)acetate (8a)
N,*N*,*N*′,*N*′-Tetramethylethylenediamine (TMEDA) (16.0 µL, 0.10 mmol), $Zn(CN)_2$ (36.8 mg, 0.31 mmol), $Pd_2(dba)_3$ (1.20 mg, 1.30 µmol) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (Xantphos) (3.02 mg, 5.22 µmol) were added to **7** (200 mg, 0.52 mmol) in DMF (1.2 ml) and sealed into a microwave tube. The reaction mixture was heated to 160°C for 200 s in the microwave reactor. The reaction mixture was cooled to room temperature, filtered and washed through with DCM. The solution was concentrated and the residue was azeotroped with toluene. The crude product was purified by flash silica chromatography, elution gradient 0 to 40% EtOAc in heptane to yield **8a** (112 mg, 0.40 mmol, 76% based on **7**, 65% based on $Zn(CN)_2$) as a white solid. LCMS (ESI) *m/z* 283 $[M+H]^+$. ^{1}H NMR (DMSO-d_6): δ 3.67 (s, 3H), 3.86 (s, 3H), 3.94 (s, 2H), 6.17 (d, *J* = 4.1 Hz, 1H), 6.59 (d, *J* = 4.1 Hz, 1H), 7.83 (d, *J* = 8.1 Hz, 2H), 7.97 (d, *J* = 8.1 Hz, 2H).

Methyl 2-(5-(4-cyanobenzoyl)-1-methyl-1H-pyrrol-2-yl)acetate (9a)
8a (30.0 mg, 0.11 mmol) and 10 % palladium on carbon (10% Pd/C) (18.1 mg, 0.02 mmol) were suspended in methanol (5 ml). The reaction mixture was stirred under hydrogen gas for 3 h. The reaction mixture was filtered to give a crude reaction mixture containing **9a** (69% conversion by analytical HPLC) which was used directly in the next stage. LCMS (ESI) *m/z* 272 $[M+H]^+$.

2-(1-Methyl-5-(4-methylbenzoyl)-1*H*-pyrrol-2-yl)acetic acid (tolmetin 1a)
9a (37.3 mg, 0.13 mmol) was dissolved in water (350 µL), methanol (500 µL) and THF (500 µl) and 1M NaOH (157 µL, 0.16 mmol) were added. The reaction was stirred at room temperature for 16 h. The reaction was concentrated and the aqueous mixture was acidified with 1M HCl to pH 6 then extracted with DCM (2 x 10 ml), the organic layer was dried over $MgSO_4$, filtered and evaporated to afford **1a** (23.6 mg, 0.092 mmol, 70%) as a white solid. LCMS (ESI) *m/z* 258 $[M+H]^+$. ^{1}H NMR (DMSO-d_6): δ 2.42 (s, 3H), 3.78 (s, 2H), 3.95 – 3.97 (m, 3H), 6.14 (d, *J* = 4.0 Hz, 1H), 6.69 (d, *J* = 4.0 Hz, 1H), 7.25 (d, *J* = 7.9 Hz, 2H), 7.71 (d, *J* = 7.9 Hz, 2H).

Methyl 2-(5-(4-[^{13}C,^{15}N]cyanobenzoyl)-1-methyl-1*H*-pyrrol-2-yl)acetate (8b)
TMEDA (16.0 µL, 0.11 mmol), Zn([^{13}C,^{15}N]CN)$_2$ (38.0 mg, 0.31 mmol), $Pd_2(dba)_3$ (1.20 mg, 1.30 µmol) and Xantphos (3.02 mg, 5.22 µmol) were added to **7** (200 mg, 0.52 mmol) in DMF (1.2 ml) and sealed into a microwave tube. The reaction mixture was heated to 160°C for 200 s in the microwave reactor. The reaction mixture was cooled to room temperature, filtered and washed through with DCM. The solution was evaporated and the crude product was purified by flash silica chromatography, elution gradient 0 to 40% EtOAc in heptane to yield **8b** (108 mg, 0.38 mmol, 73% based on **7**, 61% based on Zn([^{13}C,^{15}N]CN)$_2$) as a cream solid. LCMS (ESI) *m/z* 285 $[M+H]^+$.

Methyl 2-[1-methyl-5-[4-[$^2H_3$,^{13}C]methylbenzoyl]pyrrol-2-yl][2-^{2}H]acetate (9b)
8b (108 mg, 0.38 mmol) and 10% Pd/C (64.7 mg, 0.06 mmol) were suspended in d_4-methanol (16 ml) and stirred under deuterium gas for 5 h. The reaction mixture was filtered and evaporated then purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.1% NH_3) and acetonitrile as eluents to yield **9b** (42.8 mg, 0.15 mmol, 41%) as a white solid. LCMS (ESI) *m/z* 276 $[M+H]^+$.

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2-[1-Methyl-5-[4-[$^{2}H_3$,^{13}C]methylbenzoyl]pyrrol-2-yl]acetic acid ([$^{2}H_3$,^{13}C]tolmetin, 1b)
9b (42.8 mg, 0.15 mmol) was dissolved in water (575 µL) and THF (825 µL) and 1M NaOH (185 µL, 0.19 mmol) was added. The reaction mixture was stirred at room temperature for 2 h then concentrated and acidified with 1M HCl to pH 6. The mixture was extracted with DCM (2 x 10 ml), the organic layer was dried with $MgSO_4$, filtered and evaporated to afford **1b** (39.3 mg, 0.15 mmol, 97%). HPLC purity >99% (UV detection at 254 nm). LCMS (ESI) *m/z* 262 $[M+H]^+$. ^{1}H NMR ($CDCl_3$) δ 3.78 (s, 2H), 3.96 (s, 3H), 6.14 (d, *J* = 4.0 Hz, 1H), 6.69 (d, *J* = 4.0 Hz, 1H), 7.24 (dd, *J* = 4.5, 8.1 Hz, 2H), 7.71 (d, *J* = 8.0 Hz, 2H). Isotope incorporaton 3.9 d/mol, atom% abundance 98.7%.

Methyl 2-(5-(4-[^{14}C]cyanobenzoyl)-1-methyl-1*H*-pyrrol-2-yl)acetate (8c)
TMEDA (16.0 µL, 0.10 mmol), Zn([^{14}C]CN)$_2$ (30.1 mCi, 38.0 mg, 0.31 mmol), $Pd_2(dba)_3$ (1.30 mg, 1.30 µmol) and Xantphos (3.02 mg, 5.22 µmol) were added to **7** (200 mg, 0.52 mmol) in DMF (1.2 ml) and sealed into a microwave tube. The reaction mixture was heated to 160°C for 200 s in the microwave reactor then cooled to room temperature, filtered and washed through with DCM. The crude product was purified by flash silica chromatography, elution gradient 0 to 40% EtOAc in heptane to yield **8c** (106 mg, 21.1 mCi, 0.38 mmol, 70% radiochemical yield) as a white solid. LCMS (ESI) *m/z* 285 $[M+H]^+$.

Methyl 2-(1-methyl-5-(4-[^{14}C]methylbenzoyl)pyrrol-2-yl)acetate (9c)
8c (106 mg, 21.1 mCi, 0.37 mmol) and 10% Pd/C (63.5 mg, 0.06 mmol) were suspended in methanol (18 ml) and stirred under hydrogen gas for 5 h. The reaction mixture was filtered and evaporated to afford crude product which was purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.1% NH_3) and acetonitrile as eluents to yield **9c** (49.1 mg, 10 mCi, 0.18 mmol, 48% radiochemical yield) as a white solid. LCMS (ESI) *m/z* 274 $[M+H]^+$.

2-[1-Methyl-5-[4-[^{14}C]methylbenzoyl]-1*H*-pyrrol-2-yl]acetic acid ([^{14}C]tolmetin, 1c)
9c (49.0 mg, 10 mCi, 0.18 mmol) was dissolved in water (650 µL) and THF (1.45 ml) and 1M NaOH (0.22 ml, 0.22 mmol) added. The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated and acidified with 1M HCl to pH 6 then extracted with DCM (2 x 10 ml), dried with $MgSO_4$, filtered and evaporated to yield **1c** (7.37 mCi, 34.9 mg, 0.14 mmol, 74% radiochemical yield). Radiochemical purity >98% by HPLC. LCMS (ESI) *m/z* 260 $[M+H]^+$. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H), 3.77 (s, 2H), 3.96 (s, 3H), 6.14 (d, *J* = 4.0 Hz, 1H), 6.68 (d, *J* = 4.0 Hz, 1H), 7.24 (d, *J* = 7.8 Hz, 2H), 7.71 (d, *J* = 8.0 Hz, 2H). Specific activity, by mass spectrometry: 55.6 mCi/mmol, gravimetric: 211 µCi/mg.

Methyl 2-(5-(4-[^{3}H]methylbenzoyl)-1-methyl-1*H*-pyrrol-2-yl)acetate (9d)
8a (1.4 mg, 4.96 µmol) and 10% Pd/C (1.2 mg) were mixed in methanol (0.2 ml) in a 1.3 ml flask, which was fitted to the tritium manifold. The mixture was freeze-pump-thaw degassed and then stirred under tritium gas (1800 mCi, 167 mbar) for 3 hours. Palladium was removed by filtration through a PTFE filter, washing through with ethanol (5 ml). Labile tritium was remove by lyophilisation and the residue was dissolved in ethanol (6 ml) to give crude **9d** (59.1 mCi) which was used without further purification. LCMS (ESI) *m/z* 274 $[M+H]^+$.

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2-(1-Methyl-5-(4-[^{3}H]methylbenzoyl)-1*H*-pyrrol-2-yl)acetic acid ([^{3}H]tolmetin, 1d)
A portion (4 ml, 39 mCi) of the crude **9d** solution was purified by preparative HPLC (Waters XBridge Prep C_{18} OBD column, 5µ silica, 19 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 0.1% NH_3) and acetonitrile as eluents. Fractions containing the desired compound were combined and 1 M NaOH (100 µl, 0.10 mmol) was added and the mixture was kept at -20°C in the dark for 18 h. The solution was freeze-dried then purified by reverse-phase preparative HPLC (Waters XBridge Prep C_{18} OBD column, 5µ silica, 19 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 0.1% TFA) and acetonitrile as eluents. Fractions containing product were combined and freeze-dried then re-dissolved in acetonitrile / water (1:1, 3 ml) to give a solution of **1d** (6.3 mCi, 16% radiochemical yield). Radiochemical purity >98% by HPLC. LCMS (ESI) *m/z* 260 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.42 (s, 2H), 3.78 (s, 2H), 3.96 (s, 3H), 6.15 (d, *J* = 3.6 Hz, 1H), 6.69 (d, *J* = 4.2 Hz, 1H), 7.24 (d, *J* = 7.4 Hz, 2H), 7.71 (d, *J* = 7.7 Hz, 2H). ^{3}H NMR (640 MHz, $CDCl_3$) δ 2.32 - 2.43 (m). Specific activity by mass spectrometry: 20.5 Ci/mmol.

Synthesis of Celecoxib Isotopologues

4,4,4-Trifluoro-1-(4-iodophenyl)butane-1,3-dione (12)
Sodium hydride (0.358 g, 8.94 mmol) 60% in mineral oil was added portionwise to a solution of 1-(4-iodophenyl)ethanone **10** (1.85 g, 7.53 mmol) in THF (20 ml) at 0°C, under nitrogen. The resulting solution was stirred at 0°C for 0.5 h then ethyl 2,2,2-trifluoroacetate **11** (1.27 g, 8.94 mmol) was added and the solution stirred at room temperature for 3 h. The reaction mixture was cooled to 0°C and quenched with 1M HCl (10 ml) then extracted with EtOAc (2 x 50 ml) and the combined organic layers were washed with water (50 ml), dried with $MgSO_4$, filtered and evaporated to afford **12** (2.56 g, 7.48 mmol, 99%) as a brown oil. ^{1}H NMR (DMSO-d_6) δ 6.97 (s, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.95 (d, *J* = 8.4 Hz, 2H).

4-(5-(4-Iodophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzenesulfonamide (14)
A suspension of 4-hydrazinylbenzenesulfonamide hydrochloride **13** (1.84 g, 8.23 mmol) and **12** (2.56 g, 7.48 mmol) in ethanol (17 ml) was heated to reflux for 23 h. The reaction mixture was evaporated and the solid was then redissolved in EtOAc (50 ml) and washed sequentially with water (50 ml) and brine (50 ml). The organic layer was dried with $MgSO_4$, filtered and evaporated and the crude product purified by flash silica chromatography, elution gradient 20 to 60% EtOAc in heptane to afford **14** (3.06 g, 6.19 mmol, 83%) as a cream foam. LCMS (ESI) *m/z* 494 $[M+H]^+$. ^{1}H NMR (DMSO-d_6) δ 7.12 (d, *J* = 8.4 Hz, 2H), 7.27 (s, 1H), 7.51 (s, 2H), 7.56 (d, *J* = 8.6 Hz, 2H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.89 (d, *J* = 8.6 Hz, 2H).

4-(5-(4-Cyanophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzenesulfonamide (15a)
TMEDA (16.0 µL, 0.11 mmol), $Zn(CN)_2$ (25.7 mg, 0.22 mmol), $Pd_2(dba)_3$ (0.835 mg, 0.91 µmol) and Xantphos (2.11 mg, 3.65 µmol) were added to **14** (180 mg, 0.36 mmol) in DMF (0.84 ml) and sealed into a microwave tube. The reaction was heated to 160°C for 200 s in the microwave reactor and cooled to room temperature. The reaction was incomplete so additional reagents were added: TMEDA (16.0 µL, 0.11 mmol), $Zn(CN)_2$ (25.7 mg, 0.22 mmol), Xantphos (4.22 mg, 7.30 µmol) and $Pd_2(dba)_3$ (1.67 mg, 1.82 µmol) and the mixture was heated at 160°C for an additional 200 s in the microwave reactor. The reaction mixture was cooled, filtered and the microwave tube and compound were washed through with DCM. The resulting mixture was evaporated to afford crude product which was purified by flash silica chromatography, elution gradient 0 to 60% EtOAc in heptane to afford **15a** (74.3 mg, 0.189 mmol, 52% based on **14**, 43% based on $Zn(CN)_2$) as a cream solid. LCMS (ESI) *m/z*

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391 $[M+H]^+$. ^{1}H NMR (DMSO-d_6) δ 7.41 (s, 1H), 7.51 - 7.55 (m, 4H), 7.57 (d, *J* = 8.7 Hz, 2H), 7.89 (d, *J* = 8.7 Hz, 2H), 7.92 (d, *J* = 8.5 Hz, 2H).

4-(5-(*p*-Tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzenesulfonamide (celecoxib, 2a)
15a (30.0 mg, 0.08 mmol) and 10% Pd/C (13.0 mg, 0.01 mmol) were mixed in methanol (5 ml) and stirred under hydrogen gas for 16 h. The reaction mixture was filtered and evaporated to dryness to afford **2a** (14.9 mg, 0.039 mmol, 51%) as a white solid. HPLC purity >98% (UV detection at 254 nm). LCMS (ESI) *m/z* 382 $[M+H]^+$. ^{1}H NMR (DMSO-d_6) δ 2.32 (s, 3H), 7.18 (s, 1H), 7.19 - 7.23 (m, 4H), 7.36 (br s, 2H), 7.52 (d, *J* = 8.6 Hz, 2H), 7.86 (d, *J* = 8.6 Hz, 2H).

4-(5-(4-[^{13}C,^{15}N]Cyanophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzenesulfonamide (15b)
TMEDA (16.0 µL, 0.11 mmol), Zn([^{13}C,^{15}N]CN)$_2$ (26.6 mg, 0.22 mmol), $Pd_2(dba)_3$ (1.67 mg, 1.82 µmol) and Xantphos (4.22 mg, 7.30 µmol) were added to **14** (180 mg, 0.36 mmol) in DMF (0.84 ml) and sealed into a microwave tube. The reaction was heated to 160°C for 8 min in the microwave reactor and cooled to room temperature. The reaction mixture was filtered and the microwave tube and compound were washed through with DCM. The resulting mixture was evaporated to afford crude product which was purified by flash silica chromatography, elution gradient 10 to 60% EtOAc in heptane to afford **15b** (101 mg, 0.256 mmol, 70% based on **14**, 58% based on Zn([^{13}C,^{15}N]CN)$_2$) as a cream solid. LCMS (ES-) *m/z* 393 $[M-H]^-$. ^{1}H NMR (DMSO-d_6) δ 7.41 (s, 1H), 7.51 - 7.55 (m, 4H), 7.57 (d, *J* = 8.5 Hz, 2H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.92 (dd, *J* = 5.2, 8.4 Hz, 2H).

4-[5-([^{13}C,^{2}H$_3$]Methyl)phenyl]-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzenesulfonamide ([^{13}C,^{2}H$_3$]celecoxib, 2b)
15b (30 mg, 0.08 mmol) and 10% Pd/C (13.0 mg, 0.01 mmol) were mixed in d_4-methanol (5 ml) and stirred under deuterium gas for 16 h. The reaction mixture was filtered and evaporated to dryness to afford **2b** (27.2 mg, 0.071 mmol, 93%) as a white solid. LCMS (ESI) *m/z* 286 $[M+H]^+$. HPLC purity >98% (UV detection at 254 nm). ^{1}H NMR (DMSO-d_6) δ 7.18 - 7.25 (m, 5H), 7.51 (s, 2H), 7.54 (d, *J* = 8.7 Hz, 2H), 7.87 (d, *J* = 8.7 Hz, 2H). Isotope incorporaton 3.9 d/mol, atom% abundance 96.1%.

4-(5-(4-[^{14}C]Cyanophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzenesulfonamide (15c)
TMEDA (10.6 µl, 0.07 mmol), Zn([^{14}C]CN)$_2$ (20.1 mCi, 25.4 mg, 0.21 mmol), $Pd_2(dba)_3$ (1.60 mg, 1.74 µmol) and Xantphos (4.04 mg, 6.97 µmol) were added to **14** (172 mg, 0.35 mmol) in DMF (0.81 ml) and sealed into a microwave tube. The reaction was heated to 160°C for 8 min in the microwave reactor and cooled to room temperature. The reaction mixture was filtered and the microwave tube and compound were washed through with DCM. The resulting mixture was evaporated to afford crude product which was purified by flash silica chromatography, elution gradient 10 to 60% EtOAc in heptane to afford **15c** (100 mg, 14.2 mCi, 0.254 mmol, 71% radiochemical yield) as a cream solid. LCMS (ESI) *m/z* 395 $[M+H]^+$.

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4-[5-([^{14}C]Methyl)phenyl]-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzenesulfonamide ([^{14}C]celecoxib, 2c)

15c (100 mg, 0.25 mmol, 14.2 mCi) and 10% Pd/C (43.2 mg, 0.04 mmol) in methanol (17 ml) were mixed under hydrogen gas for 16 h. The reaction mixture was filtered and then evaporated to dryness to afford crude product which was purified by preparative HPLC using decreasingly polar mixtures of water (containing 0.1% NH_3) and acetonitrile as eluents to afford **2c** (48.6 mg, 7.08 mCi, 0.127 mmol, 50% radiochemical yield) as a white foam. Radiochemical purity >99% by HPLC. LCMS (ESI) *m/z* 384 $[M+H]^+$. ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H), 7.21 (dt, *J* = 6.3, 12.6 Hz, 5H), 7.49 (s, 2H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.87 (d, *J* = 8.5 Hz, 2H). Specific activity, by mass spectrometry: 55.8 mCi/mmol, gravimetric: 146 μCi/mg.

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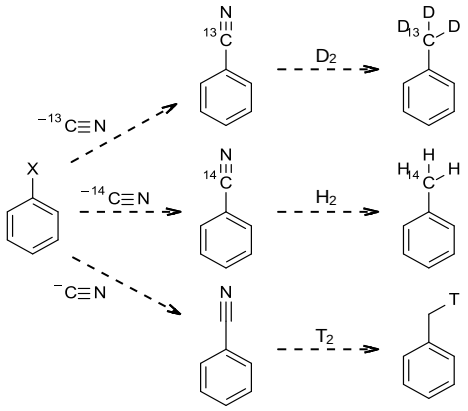

Scheme 1 Proposed labelling of methyl group with different isotopes by cyanation then reduction

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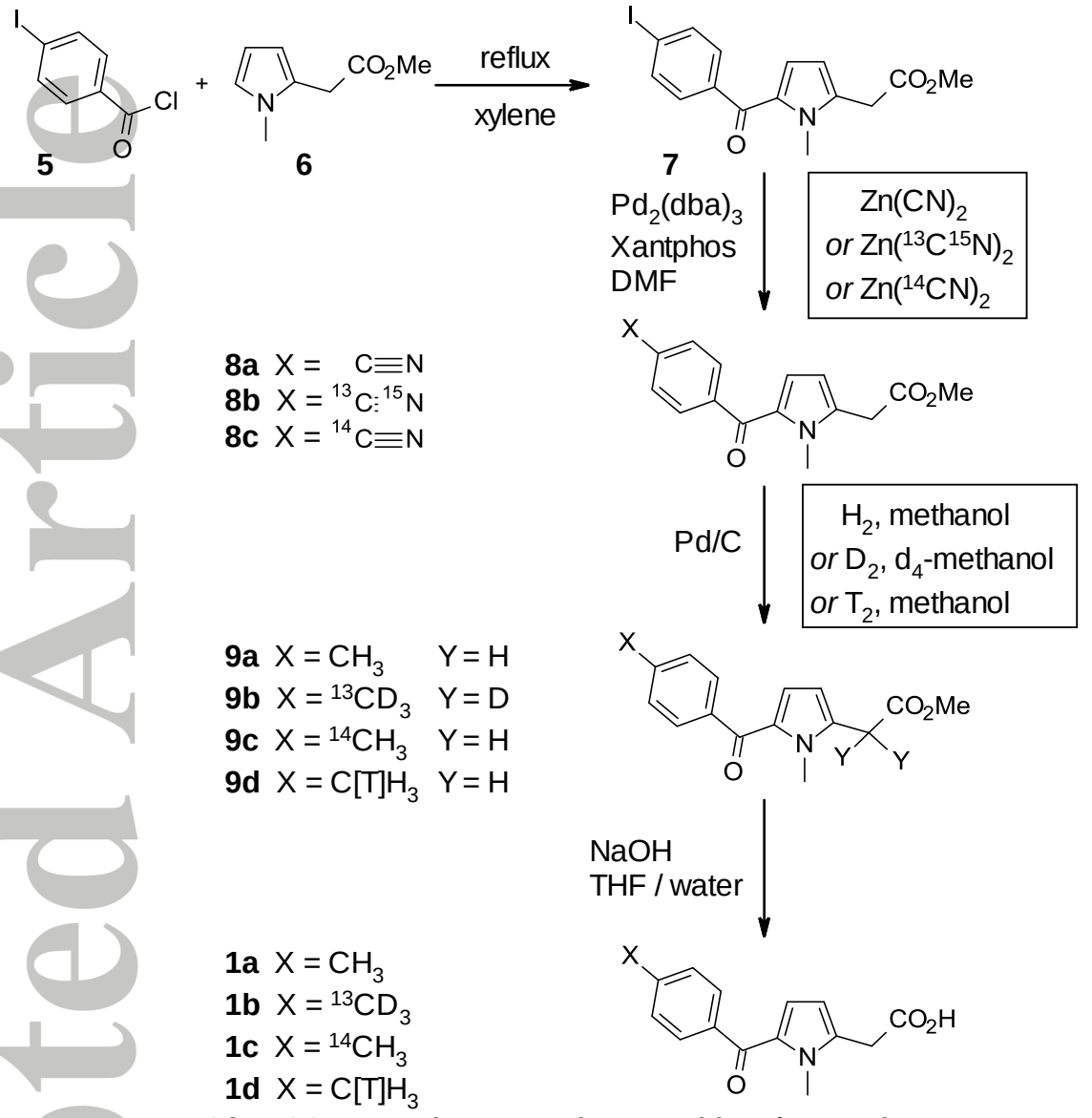

Scheme 2 Common synthetic route to tolmetin **1a** and three of its isotopologues

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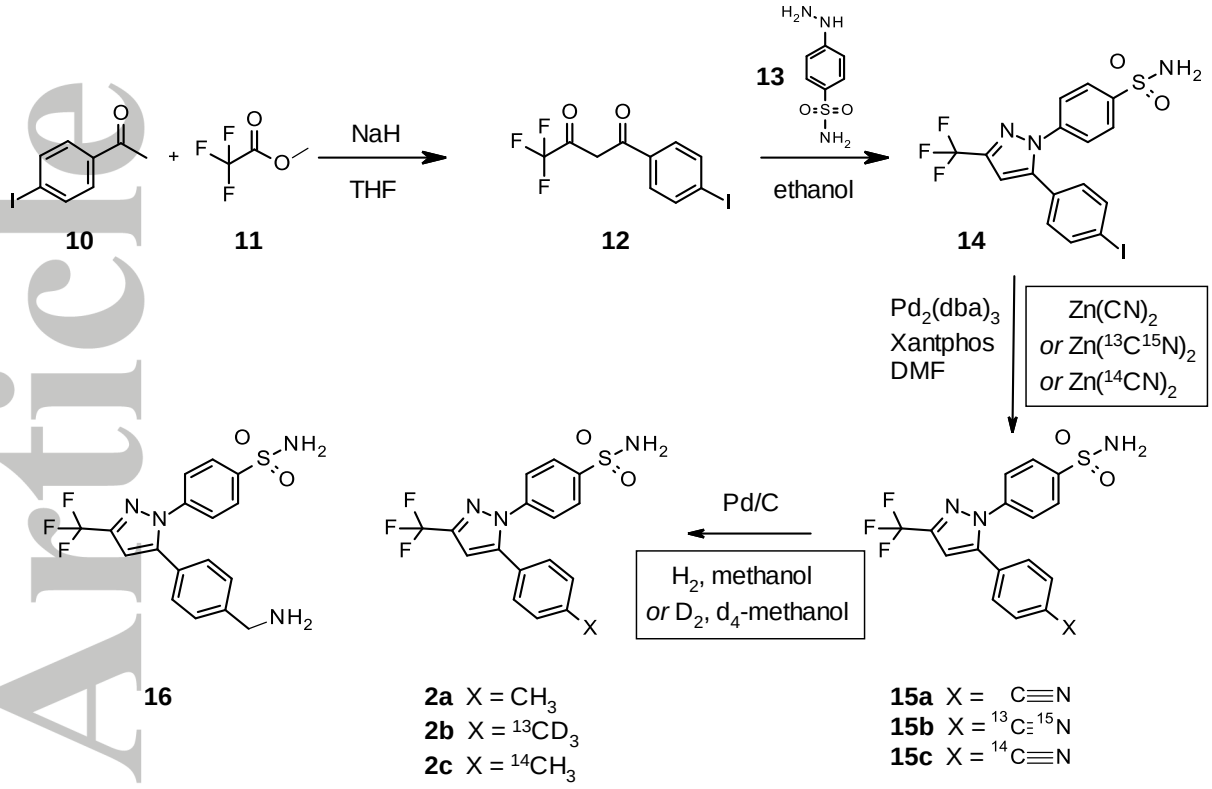

Scheme 3 Common synthetic route to celecoxib **2a** and two of its isotopologues

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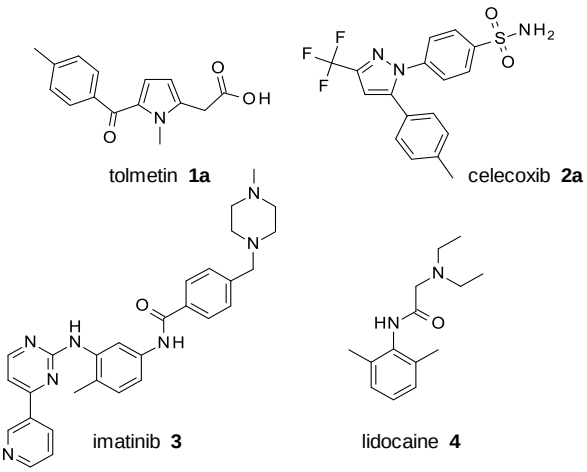

Figure 1 Important marketed drugs containing aryl methyl groups

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