Carbon-14 Labelled Nitrogen Heterocycles; The Syntheses of Three Phosphodiesterase Inhibitors.

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Summary

The syntheses of three heterocyclic phosphodiesterase inhibitors are described from a common radiolabelled precursor, namely 2-propoxybenzo[*cyano*-^{14}C]nitrile. Conversion of the nitrile to the corresponding methyl ketone or amidine allows elaboration of the heterocycles radiolabelled within the ring systems.

Keywords: phosphodiesterase inhibitor, 2-propoxybenz[^{14}C]amidine, [6-^{14}C]2-pyridone, [2-^{14}C]6-purinone, [2-^{14}C]pyrimido[4,5-d]pyrimidin-4-one.

Introduction

Drug metabolism studies with carbon-14 labelled compounds play a crucial role in the development of new pharmaceuticals. Critically, to obtain maximum information from these studies, the radiolabel must be in a metabolically stable position. This generally entails synthesising the target with a carbon-14 label in the core of the molecule e.g. within a heterocyclic ring. Recently we faced this problem with a series of three phosphodiesterase inhibitors each containing a different heterocyclic ring, but commonly substituted with a 2-propoxyphenyl moiety. We chose to prepare all three from the same radiolabelled precursor, 2-propoxybenzo[*cyano*-^{14}C]nitrile, and to build the heterocycles around the nitrile carbon. These syntheses are reported herein.

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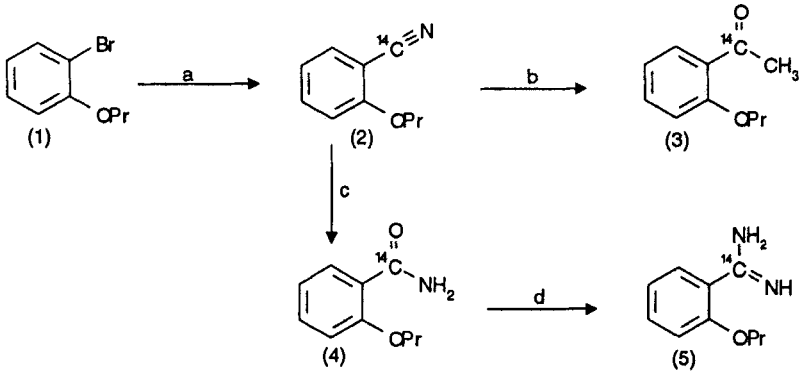
Discussion

The structures of the three target phosphodiesterase inhibitors (8), (11) and (13) are shown in the Schemes. Each contain a different heterocycle, a 2-pyridone in (8), SK&F 96679, a pyrimidol[4,5-d]pyrimidin-4-one in (11), SK&F 97792 and a 6-purinone in (13), SK&F 96231. We felt it expedient to prepare all three compounds from a common radiolabelled precursor as this would reduce cost, synthetic effort and, crucially, as all three were required simultaneously, the time required to complete the work. We chose the readily prepared 2-propoxybenzo[*cyano*-^{14}C]nitrile. A labelled nitrile is a very potent functional group as it can be easily converted into a wide variety of other functionality, e.g. acid *via* hydrolysis, amine *via* reduction, etc. For our purposes conversion to the [^{14}C]acetophenone (3) (Scheme 1) would allow elaboration of the radiolabelled 2-pyridone heterocycle by condensation of a 1,3 dicarbonyl compound and cyano acetamide[1] (Scheme 2). Furthermore, the amidine (5), readily accessible by aminolysis of the nitrile[2] or the imidate(4)[3] derived from the corresponding amide (Scheme 1), is suitably functionalised for elaboration of the pyrimido[4,5-d]pyrimidone and purinone ring systems of (11) and (13) respectively (Scheme 3).

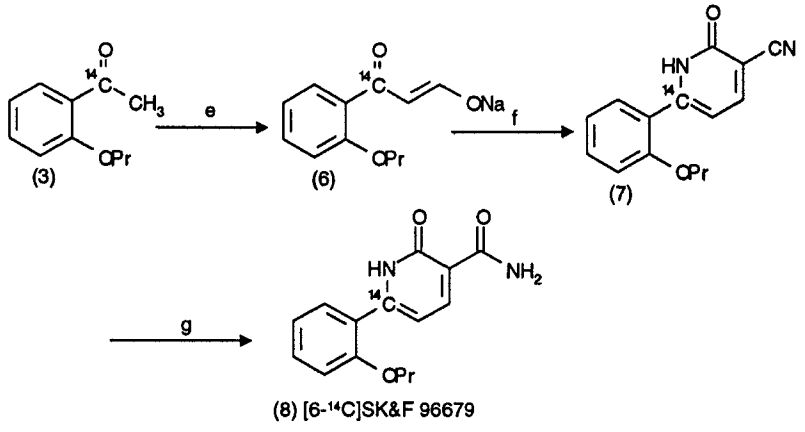
Scheme 1



a; Cu[^{14}C]CN , NMP (94%) , b; CH_3MgBr (72%) , c; H_2O_2, NaOH (79%), d; $Et_3O^+BF_4^-$, NH_3 (99%).

Treatment of 2-propoxybromobenzene[4] with Cu[^{14}C]CN[5] in N-methylpyrrolidin-2-one at 190°C gave 2-propoxybenzo[*cyano*-^{14}C]nitrile (2) in 94% yield. Reaction of a benzene solution of (2) with a six fold excess of ethereal methyl Grignard furnished the required [^{14}C]acetophenone (3) in 58.3% yield. The [^{14}C]amidine (5) was prepared[6] by the three step sequence of hydration (H_2O_2/NaOH), imidate formation and aminolysis, under standard conditions, in 78% overall yield.

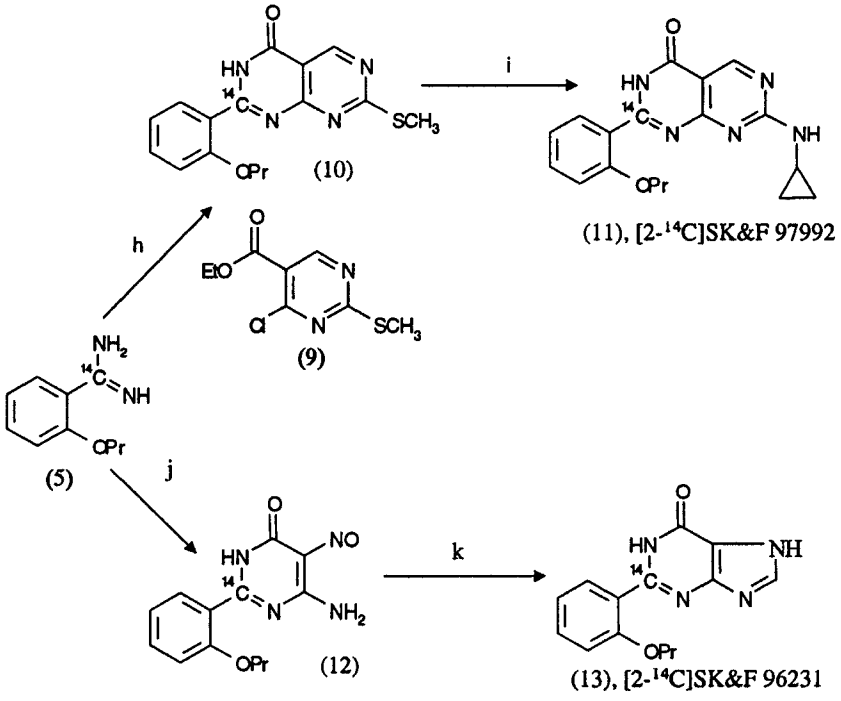
Scheme 2



e; NaOMe, HCO_2Et (100% based on recovered (3)), f; $CNCH_2CONH_2$, piperidine acetate (22.5%), g; H_2O_2, NaOH (84%)

Aldol condensation of 2-propoxy[*carbonyl*-^{14}C]acetophenone (3) with ethyl formate in diethyl ether, mediated by sodium methoxide, gave the enolate (6) which was isolated in aqueous solution and used directly. Unreacted 2-propoxy[*carbonyl*-^{14}C]acetophenone (3) (31%) was recovered at this stage.

Scheme 3



h;Et_3N,CH_3CN(19%), i; cyclopropylamine, EtOH, 110°C, (59%)
j; NaOEt, EtOCOC(CN)NOH (54%) k; $Na_2S_2O_4$, HCO_2H (61%)

Reaction of the enolate (6) with cyanoacetamide for 18 hours at reflux, catalysed by piperidine acetate, furnished the [6-^{14}C]pyridone (7) in 22.5% yield (38.8% based on recovered (3)). A further 11% of 2-propoxy[*carbonyl*-^{14}C]acetophenone (3) was isolated also, presumably arising *via* a retroaldol reaction. Increased reaction times or the use of alternative bases gave no improvement in yield. The synthesis of [6-^{14}C]SK&F 96679 was completed by hydration of the nitrile function (H_2O_2/NaOH) in 84% yield.

An amidine functionality is a versatile and long established template for the generation of 1,3 diazines[7]. Carbon-14 labelled 1,3 diazines have been prepared by this general route on a number of occasions[8], although the amidine carbon itself has been rarely labelled[9]. Condensation of an amidine with a β-keto ester (or equivalent) is a classical method for the preparation of 4-hydroxypyrimidines described by Pinner as long ago as 1889[10]. By modification of this chemistry, reaction of 2-propoxybenz[^{14}C]amidine (5) with ethyl 4-chloro-2-methylthio-5-pyrimidine carboxylate, the [2-^{14}C]pyrimido[4,5-d]pyrimidone ring system of SK&F 97992 (11) was generated in one step in modest yield (19%). Displacement of the thiomethyl group with cyclopropylamine completed the synthesis.

Reaction of amidines with β-keto nitriles is a common method for preparation of aminopyrimidones[11]. Consequently, treatment of 2-propoxybenz[^{14}C]amidine (5) with ethyl cyanoglyoxylate-2-oxime in the presence of sodium ethoxide smoothly furnished the required 4-amino-5-nitroso-2-(2-propoxyphenyl)-[2-^{14}C]pyrimidin-6-one, establishing the 4,5 substitution pattern necessary for formation of the 5-membered ring. Thus, reduction of the nitroso function with sodium dithionite gave the corresponding diamine, isolated as its hydrosulfate salt, which on treatment with formic acid[12] cyclised to the [2-^{14}C]6-purinone[13] (13) SK&F 96231 in 61% overall yield.

In conclusion, we have demonstrated that from a simple carbon-14 precursor three different nitrogen heterocycles can be rapidly prepared. The yields as they stand are modest but the shortness of the syntheses largely compensates for this. This strategy should be applicable to many other carbon-14 labelled heterocyclic systems.

Experimental

Potassium [^{14}C]cyanide was supplied by ICI-Cambridge Research Biochemicals, Billingham. Radiochemical purities were determined by TLC using a Berthold 2832 Linear Analyser, integration by Berthold Chroma software and by HPLC using Beckman 171 radioisotope detector, integration by System Gold software. Quantification of radioactivity was by use of a Beckman LS 6800 scintillation counter.

2-Propoxybenzo[*cyano*-^{14}C]nitrile (2)

2-Propoxybromobenzene(1) (344mg, 1.60mmol), N-methylpyrrolidin-2-one (1.5ml) and cuprous[^{14}C]cyanide (143.3mg, 1.60mmol, 43.5mCi) were stirred at room temperature until thoroughly mixed, and heated at 190°C, under nitrogen, for 1.5h and allowed to cool. A saturated aqueous solution of potassium cyanide (3ml) was added, the mixture stirred overnight, and extracted with diethyl ether. The combined organics were washed with water, dried over magnesium sulfate, filtered and evaporated to give the title compound (2)(241.7mg, 40.9mCi, 94% radiochemical yield).

2-Propoxy[*carbonyl*-^{14}C]acetophenone (3)

2-Propoxybenzo[*cyano*-^{14}C]nitrile (2) (853.3mg, 3.74mmol, 109.45mCi) prepared as above on a larger scale) was dissolved in dry benzene (10ml) and an ethereal solution of methyl magnesium bromide (3.34ml, 10.0mmol, 3.0M solution) added dropwise. The mixture was stirred at ambient temperature overnight when a further 3.34ml of Grignard reagent was added. The solution was stirred for a further 4h., quenched with water and 1N HCl. The layers were separated, and the aqueous layer extracted thoroughly with ether. The combined organics were dried over magnesium sulfate, filtered and evaporated. The residue was purified by column chromatography, (silica, dichloromethane) to furnish the title compound (3) (450.2mg, 63.478mCi) (58.3%) as a colourless crystalline solid.

3-Cyano-6-(2-propoxyphenyl)-2-(1H)-[6-^{14}C]pyridone (7)

To a stirred solution of anhydrous methanol (0.219ml, 5.39mmol) in anhydrous ether (7.5ml) was added, in portions, sodium hydride (50% dispersion in oil, 259mg, 5.39mmol) and the resultant suspension stirred for 30min. The reaction mixture was cooled in an ice/salt bath and a solution of 2-propoxy[*carbonyl*-^{14}C]acetophenone (3) (795mg, 3.94mmol) and ethyl formate (0.432ml, 5.36mmol) in ether (4ml) added dropwise over ~10min. The cooling bath was removed and the reaction stirred at ambient temperature for 2.5h. The reaction was thoroughly extracted with water (the ether layer contained 252.3mg, 31.7% of 2-propoxy[*carbonyl*-^{14}C]acetophenone (3)). To the combined aqueous extracts were added, piperidine acetate (128mg, 0.88mol), cyanoacetamide (452mg, 5.39mmol) and the solution was heated at reflux for 20h. On cooling, the pH was adjusted to ~4 (with acetic acid) and the solution allowed to stand for 48h., whereupon a brick red gummy precipitate formed, which on trituration with ethanol, furnished 3-cyano-6-(2-propoxyphenyl)-2-(1H)-[6-^{14}C]pyridone (7) (255.0mg, 22.5%, 38.8% based on recovered starting material). The ethanol layer contained a further 114.1mg of 2-propoxy[*carbonyl*-^{14}C]acetophenone (3).

[6-^{14}C]SK&F 96679, 3-acetamido-6-(2-propoxyphenyl)-2-(1H)-[6-^{14}C]pyridone (8).

3-Cyano-6-(2-propoxyphenyl)-2-(1H)-[6-^{14}C]pyridone (7) (220.8mg) was suspended in methanol/water (3:1v/v, 9.2ml) containing 5M sodium hydroxide (0.14ml) and 30% hydrogen peroxide (1.1ml). The mixture was heated at reflux for 19h. The pH of the cooled solution was adjusted to 3 and the precipitated SK&F 96679 filtered off. This crude product was recrystallised from ethanol furnishing 196.1mg (84%) of specific activity 24.5μCi/mg and of radiochemical purity of 98.8% (as assessed by HPLC μ-Bondapak C18 eluted with 0.09M H_3PO_4(pH=2.5)/acetonitrile 20-70% over 30min. at 1ml/min.).

2-Propoxy[*carbonyl*-^{14}C]benzamide (4)

2-Propoxybenzo[*cyano*-^{14}C]nitrile (2) (241.7mg, 1.50mmol) was stirred at 60°C for 18h in methanol/water (3:1 v/v, 5ml) containing 30% hydrogen peroxide (0.61ml) and 5M sodium hydroxide (0.072ml). The methanol was removed under vacuum and the residue extracted thoroughly with ethyl acetate. The combined extracts were dried over magnesium sulfate, filtered and evaporated to furnish the title compound (4) (210.8mg, 79%).

2-Propoxybenz[^{14}C]amidine (5)

To 2-propoxy[*carbonyl*-^{14}C]benzamide (4) (202.8mg, 1.13mmol) in dichloromethane (2ml) was added triethyloxonium tetrafluoroborate (0.38ml of a 703mg/ml solution in dichloromethane) and the mixture stirred at ambient temperature for 42h, whereupon a further 0.38ml was added in two portions and reaction continued for a further 24h. The solvent was removed and the residue dissolved in ethanol (4ml). Ammonia gas was bubbled through the solution until saturated and the reaction stirred at ambient temperature for 96h. The solvent was evaporated, and the residue dissolved in 5M sodium hydroxide and extracted with dichloromethane. The combined organics were washed with water, dried over magnesium sulfate, filtered and evaporated to dryness to give the title compound (5) (200mg, 99%).

7-Methylthio-2-(2-propoxyphenyl)-[2-^{14}C]pyrimido[4,5-d]pyrimidin-4-[3H]-one (10).

An ice cooled solution of 2-propoxybenz[^{14}C]amidine (5) (220mg, 1.12mmol) in anhydrous acetonitrile (2ml) was added to an ice cooled solution of ethyl 4-chloro-2-methylthio-5-pyrimidine carboxylate (9) (524mg, 2.25mmol) in acetonitrile (3ml), followed by triethylamine (0.313ml, 2.25mmol). The mixture was stirred under nitrogen, maintaining cooling for 1h., allowed to reach ambient temperature and stirred for a further 18h. The resultant white precipitate was filtered, washed with acetonitrile, water and dried by azeotroping with ethanol.

This preparation furnished the title compound (67mg, 0.2mmol) of >97% radiochemical purity by TLC (silica, ethyl acetate:methanol:concentrated ammonium hydroxide 5:1:1 by vol.).

7-Cyclopropylamino-2-(2-propoxyphenyl)-[2-^{14}C]pyrimido[4,5-d]pyrimidin-4-[3H]-one, SK&F 97992 (11).

7-Methythio-2-(2-propoxyphenyl)-[2-^{14}C]pyrimido[4,5-d]pyrimidin-4-[3H]-one (10) (67mg, 0.2mmol) was dissolved in ethanol (1ml) containing cyclopropylamine (0.071ml, 1.02mmol) and stirred in a sealed vessel at 110°C for 18h. Further aliquots (2 x 0.071ml) of cyclopropylamine were added over the next 77h reaction. The solvent was removed and the residue crystallised twice from ethanol to yield the title compound (11) (40.8mg, 59%) at a specific activity of 73.1μCi/mg and radiochemical purity of 98.4% (as assessed by HPLC μ-Bondapak C18 eluted with 0.09M H_3PO_4(pH=2.5)/acetonitrile 20-70% over 30min. at 1ml/min.).

4-Amino-5-nitroso-2-(2-propoxyphenyl)-[2-^{14}C]pyrimidin-6-one (12).

2-Propoxybenz[^{14}C]amidine (5) (55mg, 0.31mmol) was stirred in ethanol containing 1.24mmol of sodium ethoxide at ambient temperature for 20min., whereupon ethyl cyanoglyoxalate-2-oxime (53mg, 0.37mmol) was added and the mixture heated at reflux for 1h. Water (3ml) was added and the pH of the solution adjusted to 6. On stirring for 30min. a precipitate formed which was filtered, washed with water and dried to furnish the title compound (12) (45mg, 54%).

2-(2-Propoxyphenyl)-6-[2-^{14}C]purinone, SK&F 96231 (13)

4-Amino-5-nitroso-2-(2-propoxyphenyl)-[2-^{14}C]pyrimidin-6-one (12) (45mg, 0.17mmol) was stirred with sodium bicarbonate (31mg, 0.37mmol) in 50% aqueous acetonitrile at 70°C. Sodium dithionite (64mg, 0.37mmol) was added in portions and the solution extracted with chloroform. The combined organics were washed with water, dried over magnesium sulfate, filtered and evaporated to near dryness, whereupon ethanol (1ml) and sulfuric acid (0.16ml of a 1g in 10ml ethanol solution) were added. The solvent was removed and the residue dried under vacuum. The dried diamine sulfate salt was dissolved in formic acid (0.5ml) and heated at reflux for 1.5h. On cooling water was added and the mixture stirred until the crude SK&F 96231 precipitated (27.2mg, 60.8%). The crude product was purified by HPLC (10μ silica, dichloromethane/(methanol:ammonia 10:1v/v) (93:7v/v), to give the title compound (17.2mg) of specific activity 93.4μCimg and radiochemical purity of >99% (μ-Bondapak C18 eluted with 0.09M H_3PO_4(pH=2.5)/acetonitrile 20-70% over 30min. at 1ml/min.).

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