PREPARATION OF ^{14}C- AND ^{18}O-LABELED 2-[2-METHOXY-4-(METHYLSULFINYL)PHENYL]-1H-IMIDAZO[4,5-c]-PYRIDINE HYDROCHLORIDE (LY175326), A CARDIOTONIC WITH INOTROPIC AND VASODILATOR ACTIVITIES

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SUMMARY

Two different forms of ^{14}C-labeled 2-methoxy-4-(methylthio)benzoic acid were prepared and employed in the synthesis of ^{14}C-labeled 2-[2-methoxy-4-(methylsulfinyl)phenyl]-1H-imidazo-[4,5-c]pyridine hydrochloride (LY175326), a cardiotonic with inotropic and vasodilator activities that is currently in clinical trials. The synthetic procedures described in this report allowed the introduction of the ^{14}C-label in the antepenultimate step. Additionally, an ^{18}O-labeled form of LY175326 was synthesized to facilitate kinetic analysis of the formation of its sulfide and sulfone metabolites.

Key Words: Carbon-14, Oxygen-18, LY175326, positive inotrope, cardiotonic

INTRODUCTION

The development of noncatecholamine, nonglycoside cardiotonics with combined inotropic and vasodilator activities for the chronic management of congestive heart failure has been one of the major goals of cardiovascular drug research programs during the past decade. Sulmazole (1),[1] a 2-phenylimidazo[4,5-b]pyridine, was one of the earliest examples from this new class of cardiotonics to enter clinical trials, and several reports have indicated that this prototypical drug is effective in patients with refractory congestive heart failure.[2,3] We discovered that LY175326 (2), the 2-phenylimidazo[4,5-c]pyridine positional isomer of sulmazole, is 10-fold more potent than sulmazole both *in vitro* and *in vivo*. For example, the

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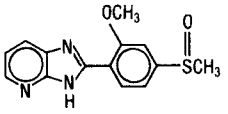
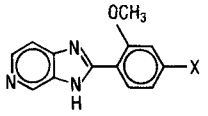




Sulmazole, 1

X = $S(O)CH_3$; LY175326; 2

X = SCH_3; LY137150; 3

X = $S(O)_2CH_3$; LY163252; 4

inotropic ED_{50}'s following i.v. administration of LY175326 or sulmazole to pentobarbital anesthetized dogs were 30 and 300 μg/kg, respectively.[4-7] Moreover, there are marked differences in the physicochemical characteristics, metabolism and disposition, and molecular pharmacologies of LY175326 and sulmazole.

We predictably[8] discovered[9] that 2-[2-methoxy-4-(methylthio)-phenyl]-1H-imidazo[4,5-c]pyridine (3, LY137150) and 2-[2-methoxy-4-(methylsulfonyl)phenyl]-1H-imidazo[4,5-c]pyridine (4, LY163252) were metabolic products following administration of LY175326 to rats or dogs. Both metabolites exhibited inotropic activity. Following i.v. administration to pentobarbital anesthetized dogs, the ED_{50}'s of 3 and 4 were 200 and 20 μg/kg, respectively. Because of the potency of these metabolites, a study of the kinetics for their bioformation was of paramount importance.

This report describes the preparation of LY175326 and its metabolites in two different ^{14}C-labeled forms to enable studies on the metabolism and disposition of this promising cardiotonic. We also detail the preparation of LY175326 with the sulfoxide moiety bearing an ^{18}O-label to facilitate experiments on the metabolically-mediated sulfur redox chemistry.

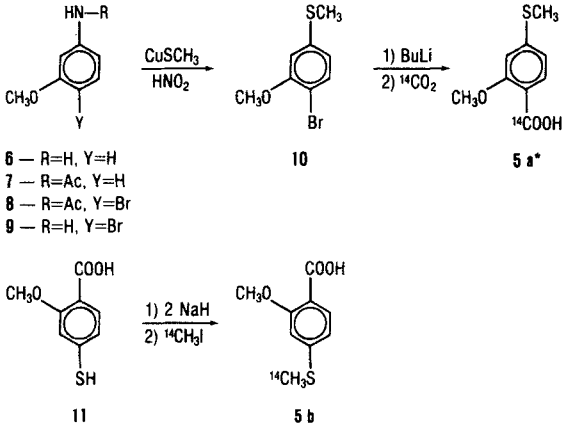
RESULTS AND DISCUSSION

Zipp and co-workers[10] employed [carboxy-^{14}C]-2-methoxy-4-(methylthio)benzoic acid (5a[11], Scheme 1) to prepare ^{14}C-sulmazole bearing the label in the metabolically stable 2-position of the imidazole ring. They introduced the label by reaction of ^{14}C-labeled cyanide with the diazonium ion derived from 2-methoxy-4-nitroaniline; 6 subsequent steps were required to transform the product to 5a in only 12% radiochemical yield. Similarly, we desired to label the imidazo[4,5-c]pyridine LY175326 in the 2-position of the imidazole ring using intermediate 5a; however, we employed a different route to 5a which allowed the introduction of the ^{14}C-label in the final step of its synthesis (Scheme 1). The key intermediate in our synthesis of 5a was 10, 2-bromo-5-(methylthio)anisole (Scheme 1). While a conceptually simple a priori approach to 10 would be bromination of 3-(methylthio)anisole, bromination of this compound is nonregioselective and produces 10 as well as the undesired regioisomer 4-bromo-3-(methylthio)anisole in a 35:65 ratio.[12,13] Intermediate 10 was regioselectively prepared on a large scale by acetylation[14] of 3-aminoanisole (6) with acetic anhydride in methylene chloride giving 7 (81%), followed by treatment with bromine in methylene chloride to provide 8.[15] Hydrolysis with 5N sodium hydroxide in methanol provided 9 in 50% yield (from 7); ^{1}H-NMR and HPLC analyses indicated this material was regiochemically pure. The diazonium ion of 9 was prepared with nitrous acid, and then reacted with methylthiocopper according to the method of Baleja[16] to provide 10 in 34% yield after purification by flash chromatography. Intermediate 9 was also converted to 10 by reaction of the diazonium ion with potassium ethyl xanthate, hydrolysis, and then methylation of the thiol anion with iodomethane; the overall yield for this more lengthy route was 33%.

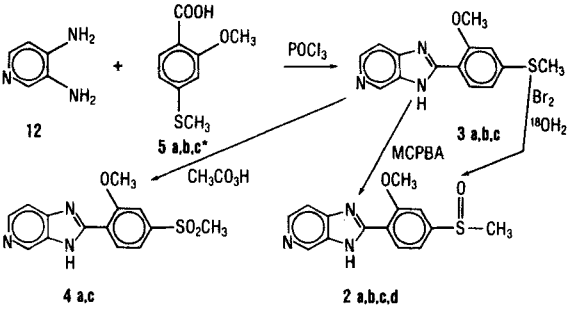
Halogen-metal exchange using butyllithium, followed by reaction of the resulting aryllithium reagent with ^{14}C-labeled carbon dioxide, transformed 10 to [carboxy-^{14}C]-labeled 5a in 70% chemical and radiochemical yield.

We were also interested in preparing ^{14}C-LY175326 labeled in the methylsulfinyl moiety to provide a sensitive method of determining whether metabolically-derived LY137150 (3) is further metabolized by S-demethylation.[17] The intermediate for this isotopomer of LY175326 was 5b, readily prepared from 4-mercapto-2-methoxybenzoic acid (11)[4] using ^{14}C-labeled iodomethane (Scheme 1); the chemical and radiochemical yields were 84%.

Scheme 1: Syntheses of ^{14}C-labeled Benzoic Acids



Scheme 2: Syntheses of ^{14}C- and ^{18}O-labeled Imidazopyridines



* The letters denote the following labels: a— in the benzylic carbon; b— in the SCH_3 carbon; c— unlabeled; d— in the sulfoxide oxygen.

Reaction of 5a or 5b with 3,4-diaminopyridine in refluxing phosphorus oxychloride[4] provided ^{14}C-labeled 3a or 3b (67% yield for each reaction). Each isotopomer was then carefully oxidized with 3-chloroperbenzoic acid at -40 to -50°C in methanol to provide 2a or 2b (LY175326); reaction at higher temperatures led to inordinate quantities of sulfone 4 and starting material (Scheme 2). The yields in the oxidation step were 64 and 50% for the [imidazole-^{14}C]- and [methyl-^{14}C-sulfinyl]-labeled compounds, respectively; the radiochemical purities were in excess of 97%. Oxidation of 3a (LY137150) with peracetic acid in methanol at 25-40°C provided ^{14}C-labeled sulfone 4a (LY163252) in 43% yield with a radiochemical purity of 97.3%.

Finally, we prepared the [methylsulfinyl-^{18}O]-isotopomer of LY175326 to facilitate kinetic analysis of the metabolic conversion of LY175326 to sulfide 3 and sulfone 4; we were particularly interested in determining whether the conversion of LY175326 to 3 is a reversible process as has been documented for sulindac and other sulfur containing drugs.[8] ^{18}O-Labeled LY175326 was prepared in 61% chemical yield by reaction of sulfide 3c with bromine and ^{18}O-labeled water in the presence of potassium carbonate[18] (Scheme 2). The material obtained was 86.7% ^{18}O-labeled which represented a 10.5% isotopic dilution during the course of the reaction. Pharmacokinetic experiments employing ^{18}O-labeled LY175326 and sensitive GC-MS analytical methods will provide a means of studying the kinetics and reversibility of the metabolically-mediated sulfur redox chemistry.[19,20]

CONCLUSIONS

In this report we have described the regioselective synthesis of 2-bromo-5-(methylthio)anisole and the efficient preparation of [methyl-^{14}C-thio]- and [carboxy-^{14}C]-labeled 2-methoxy-4-(methylthio)-benzoic acid in 84 and 70% radiochemical yields, respectively; this represents a dramatic improvement over the literature method which

produced [carboxy-^{14}C]-labeled material in only 12% radiochemical yield following 6 radiochemical reactions. These two ^{14}C-labeled benzoic acids were then employed in the efficient preparation of LY175326 and its metabolites, 3 and 4, in radiolabeled form. Finally, [methylsulfinyl-^{18}O]-labeled LY175326 (2d) was prepared in good yield. These labeled materials have permitted biochemical, metabolic, and pharmacokinetic studies of LY175326 which will be reported in due course.[9]

EXPERIMENTAL

Methods

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are not corrected. Proton magnetic resonance (^{1}H-NMR) spectra were taken on a Bruker WM270 spectrometer. Chemical shifts are reported in ppm downfield from a tetramethylsilane internal standard (δ scale). The ^{1}H-NMR data are presented in the form: (solvent in which spectra were taken), δ value of signal (peak multiplicity, integrated number of protons, coupling constant (if any), and assignment). Mass spectra were recorded from a Varian MAT CH-5 spectrometer, at the ionization voltage expressed in parentheses. Only the peaks of high relative intensity or of diagnostic importance are presented in the form: m/e (intensity relative to base peak). Microanalytical data were provided by the Physical Chemistry Department of the Lilly Research Laboratories.

Except where noted, a standard procedure was used for product isolation. This involved quenching by addition to water, filtration or exhaustive extraction with a solvent (washing of extract with aqueous solutions, on occasion), drying over an anhydrous salt, and evaporation of solvent under reduced pressure. Particular solvents, aqueous washes (if needed), and drying agents are mentioned in parentheses after the phrase "product isolation."

Radiochemical purity was measured by autoradiography employing E. Merck Silica Gel 60 F-254 TLC plates and Kodak X-ray film BB-5, and by HPLC performed on a Waters 6000A chromatograph using a 4.5mm x 25 cm Alltech Spherisorb S-5-005 ODS column eluted with 1% ammonium acetate in 80% H_2O:20% THF (w/v) at 2500 psi and at a flow rate of 1 ml/min; the detector was a Waters 440 operated at 315nm. For the HPLC determinations, equal fractions from the column were collected in vials containing PCS scintillation fluid (Amersham), and the radioactivity was measured in a Packard Model 3375 Liquid Scintillation Spectrometer.

Syntheses

3-Acetamidoanisole (7) - 3-Aminoanisole (99.7 g, 0.809 mole) was dissolved in 200 mL methylene chloride and the solution was cooled to 0°C. Acetic anhydride (113 ml, 1.20 mole) was added in a dropwise fashion and the reaction was then warmed to room temperature and stirred for 16 hours. The solvent was removed under reduced pressure. Product isolation (ether, 5N sodium hydroxide, water, brine, Na_2SO_4) followed by recrystallization from ether/ethyl acetate/hexane provided 108.5 g (81%) of 7 as white crystals: mp 79-80°C; ^{1}H-NMR ($CDCl_3$) δ 2.17 (s, 3H, $-COCH_3$), 3.80 (s, 3H, $-OCH_3$), 6.64-7.28 (m, 4H, ArH); mass spectrum (70eV) m/e (rel intensity) 165 (42, M^+), 122 (100).

Anal. Calcd for $C_9H_{11}NO_2$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.33; H, 6.62; N, 8.37.

5-Amino-2-bromoanisole (9) - Bromine (30.8 mL, 598 mmol) was added dropwise to a solution of 3-acetamidoanisole 7 (98.7 g, 598 mmol) in 1500 mL methylene chloride at 0°C. The heterogeneous reaction was warmed to room temperature and stirred for 4 hours. The mixture was cooled to 10°C, filtered and dried at ca. 40°C to afford 137.4 g of 8. As judged by TLC analysis, some hydrolysis of the acetamido moiety inadvertently occurred during the drying.

5N sodium hydroxide (640 mL, 3200 mmol) was added to a solution of the crude 5-acetamido-2-bromoanisole (8) in 1200 mL methanol. After heating to reflux for 8 hours, the solvents were removed under reduced pressure. Product isolation (ethyl acetate, water, brine, Na_2SO_4) and recrystallization from ether/hexane yielded 46.9 g of product 9 as white crystals with mp 95-97°C. The mother liquors were concentrated and chromatographed by preparative HPLC (0 to 25% ethyl acetate in hexane gradient) to afford an additional 13.4 g of homogeneous material. The overall yield for the 2 steps was 50%: ^{1}H-NMR ($CDCl_3$) δ 3.82 (s, 3H, $-OCH_3$), 6.16-6.24 (m, 2H, ArH ortho to $-OCH_3$ and $-NH_2$), 7.24 (d, 1H, J=8.6 Hz, ArH ortho to Br); mass spectrum (70eV) m/e (rel intensity) 203 (99, M^+), 201 (100, M^+).

Anal. Calcd for C_7H_8BrNO: C, 41.61; H, 3.99; N, 6.93. Found: C, 41.75; H, 3.96; N, 6.85.

2-Bromo-5-(methylthio)anisole (10) - A solution of sodium nitrite (1.5 g, 21 mmol) in 20 mL water was added dropwise to a mixture of 5-amino-2-bromoanisole (4.04 g, 20 mmol) and 3.6 mL concentrated sulfuric acid in 45 mL water at 0°C. The resulting homogeneous solution was added dropwise to a mixture of methylthiocopper[16] (15.5 g, 140 mmol) in 100 mL water at 0°C. The mixture was warmed to room temperature and stirred for 30 minutes. Product isolation (ethyl acetate, water, filtration through celite, 1N hydrochloric acid, 1N sodium hydroxide, water, brine, Na_2SO_4) and flash chromatography (0 to 3% ethyl acetate in hexane gradient) afforded 1.56 g (34%) of product as a colorless oil: ^{1}H-NMR ($CDCl_3$) δ 2.50 (s, 3H, $-SCH_3$), 3.92 (s, 3H, $-OCH_3$), 6.71 (d, 1H, J=6.5 Hz, ArH ortho to $-SCH_3$), 6.78 (s, 1H, ArH ortho to $-OCH_3$) 7.43 (d, 1H, J=6.5 Hz, ArH ortho to -Br); mass spectrum (70eV) m/e (rel intensity) 234 (100, M^+), 232 (98, M^+).

Anal. Calcd for C_8H_9BrOS: C, 41.22; H, 3.89. Found: C, 41.19; H, 3.81.

[Carboxy-^{14}C]-2-Methoxy-4-(methylthio)benzoic acid (5a) - n-Butyllithium (6.25 mL of a 1.6 N solution in hexane, 10 mmol) was added dropwise to a solution of 2-bromo-5-(methylthio)anisole (2.33 g, 10 mmol) in 30 mL THF at -70 to -78°C. After stirring 30 minutes at this temperature, the reaction flask was immersed in liquid nitrogen. ^{14}C-Labeled carbon dioxide was prepared by the dropwise addition of 10 mL 85% phosphoric acid to an intimate mixture of $Ba^{14}CO_3$ (Amersham, 0.575g, 2.762 mmol, 100 mCi, sp. act. = 36.2 mCi/mmol) and unlabeled $BaCO_3$ (1.43 g, 7.23 mmol); the resulting CO_2 was vacuum transferred continuously to the aryllithium reagent.

Fifteen minutes after the phosphoric acid addition was complete, the stopcock to the aryllithium reagent flask was closed, the liquid nitrogen bath was removed, and the reaction was warmed to room temperature and stirred for 1 hour. The reaction was reimmersed in liquid nitrogen and reevacuated to remove traces of labeled CO_2. The stopcock was closed, and the flask was allowed to warm to room temperature overnight. Water (20 mL) was added and the THF was removed in vacuo. The resulting aqueous solution was extracted with ethyl acetate (discarded). The aqueous layer was acidified with concentrated hydrochloric acid, and product isolation (ethyl acetate, water, brine, Na_2SO_4) afforded 1.38 g (70%) of product as a tan solid. TLC (methylene chloride: methanol, 15:1; R_f = 0.43) indicated the product was homogeneous.

2-Methoxy-4-(methyl-[^{14}C]-thio)benzoic acid (5b) - Sodium hydride (0.80 g of a 60% dispersion in oil, 20 mmol) was added in portions to a solution of 4-mercapto-2-methoxybenzoic acid[4] (1.84 g, 10 mmol) in 20 mL DMF; the temperature was maintained at approximately 5°C with an ice bath. After hydrogen evolution ceased, the reaction was allowed to warm to room temperature, stirred for 0.25 hour, and then frozen in liquid nitrogen. ^{14}C-Iodomethane (Amersham, 50 mCi,

0.893 mmol; sp. act. = 56.0 mCi/mmol) was vacuum transferred to the reaction flask, the stopcock to the flask was closed, and then the reaction was warmed to room temperature and stirred for an additional 30 minutes. The reaction flask was then frozen in liquid nitrogen and evacuated. The stopcock was closed and the flask was then warmed to room temperature. Unlabeled iodomethane (1.293 g, 9.107 mmol) in 5 mL DMF was added in a dropwise fashion and then the reaction was stirred overnight at room temperature. Solvents were removed in vacuo and the residue was dissolved in water and extracted with ethyl acetate (discarded). The aqueous layer was acidified with concentrated hydrochloric acid. Product isolation (ethyl acetate, water, brine, charcoal, Na_2SO_4) afforded 1.66 g (84%) of product. TLC analysis (methylene chloride: methanol; 9:1; R_f = 0.50) indicated nearly homogeneous material containing only traces of lower R_f impurities.

2-[2-Methoxy-4-(methylthio)phenyl]-2-[^{14}C]-1H-imidazo[4,5-c]pyridine ([imidazole-^{14}C]-LY137150, 3a) - An intimate mixture of [carboxy-^{14}C]-2-methoxy-4-(methylthio)benzoic acid 5a (1.38 g, 6.97 mmol), unlabeled acid[4] (1.195 g, 6.04 mmol), and 3,4-diaminopyridine 12 (1.42 g, 13 mmol) was added in small portions to 30 mL refluxing phosphorous oxychloride and the reaction was refluxed 4.5 hours. The solvent was removed under reduced pressure and the resulting foam was dissolved in approximately 20 mL of 2N hydrochloric acid; further dilution with 2N hydrochloric acid to a total volume of 90 mL led to the separation of an oil. Methanol was added to achieve homogeneity and then the volume of the solution was reduced in vacuo by 50%. The yellow crystals which formed were filtered, suspended in 50 mL water, and the heterogeneous mixture was treated with 1N sodium hydroxide until a pH of 8-9 was obtained. The free base was filtered and dried under reduced pressure for 4 hours at 50°C to afford 1.94 g (7.12 mmol,

55%) of 3a. TLC analysis (methylene chloride: methanol; 9:1; R_f = 0.37) revealed a single product containing only trace amounts of starting acid.

The filtrates were concentrated and chromatographed over 40 g silica gel using a 0 to 20% methanol in methylene chloride gradient. The resulting homogeneous 3a was dissolved in methanol. Concentrated hydrochloric acid (2 mL) was added, and the methanol was removed under reduced pressure. Ten mL water was added and the product precipitated as yellow crystals. Filtration and recrystallization from 20 mL ethanol afforded 0.505 g of product (total yield, calculated as free base, = 67%) with a specific activity of 4.665 mCi/mmol. Radiochemical purity as assessed by TLC and HPLC was 98.8 or 99.1%, respectively.

2-[2-Methoxy-4-(methylsulfinyl)phenyl]-2-[^{14}C]-1H-imidazo[4,5-c]-pyridine hydrochloride ([imidazole-^{14}C]-LY175326, 2a) - 3-Chloroperbenzoic acid (0.796 g of 80-85% material; approximately 3.69 mmol) in 20 mL methanol was added in a dropwise fashion over 1 hour to a vigorously stirred solution of the free base 3a (1.0 g, 3.64 mmol) in 13 mL methanol at -40 to -50°C. The reaction was stirred for an additional 4 hours at -40 to -50°C and then concentrated *in vacuo* to a foam. The material was dissolved in 10 mL methanol, 4 g of silica gel was added, and the solvent was removed *in vacuo*. This material was applied to a column containing 40 g silica gel and eluted with a 0 - 10% methanol in methylene chloride gradient. This chromatography was repeated twice. The resulting product was dissolved in 20 mL methanol, 1 mL concentrated hydrochloric acid was added, and the solvent was removed under reduced pressure. Recrystallization from ethanol gave 0.76 g (64%) of product with a specific activity of 4.92 mCi/mmol. Analysis by TLC indicated the following radiochemical composition: LY175326 (sulfoxide) - 97.7%; LY163252 (sulfone) - 0.4%; LY137150 (sulfide) - 1.3%. The R_f's in

methylene chloride: methanol (85:15) were 0.30, 0.50, and 0.53, respectively. As indicated by HPLC analysis, this material was 97.2% radiochemically pure LY175326.

2-[2-Methoxy-4-(methylsulfonyl)phenyl]-2-[^{14}C]-1H-imidazo[4,5-c]-pyridine ([imidazole-^{14}C]-LY163252, 4a) - A solution of peracetic acid (1.45 g, 7.62 mmol) in 3.5 mL acetic acid was added in a dropwise fashion to a solution of [imidazole-^{14}C]-LY137150 (0.94 g, 3.47 mmol, sp. act. = 4.66 mCi/mmol) and concentrated hydrochloric acid (0.30 mL) in 9 mL acetic acid. The reaction was stirred for 2 hours at room temperature, whereupon 28 mL diethylether was added over a 15 minute period. The mixture was cooled and stirred for 1.5 hours at 0° and then the product was filtered. This material was dissolved in 5 mL methanol, 5 g silica gel was added, and the solvent was removed *in vacuo*. This mixture was applied to a 30 g silica gel column and then eluted with a 2-10% methanol in methylene chloride gradient. The resulting product was dissolved in methanol, 1 mL concentrated hydrochloric acid was added, and the solvents were removed under reduced pressure. Recrystallization from methanol:ethanol (1:1) afforded 0.503 g (43%) of product 4a with specific activity of 4.65 mCi/mmol and radiochemical purity (HPLC) of 97.3%. Starting material 3a represented 2.3% of this material.

2-[2-Methoxy-4-(methyl-[^{14}C]-thio)phenyl]-1H-imidazo[4,5-c]pyridine ([methyl-^{14}C-thio]-LY137150, 3b) - A mixture of 2-methoxy-4-(methyl-[^{14}C]-thio)benzoic acid (1.66 g, 8.38 mmol) and 3,4-diaminopyridine (0.933 g, 8.38 mmol) in 25 mL pyridine was heated to 80°C. Phosphorus oxychloride (3.86 g, 25.15 mmol) was added dropwise at a rate which maintained the temperature below 110°C. After the addition was complete, the reaction was allowed to cool to 85°C and maintained at this temperature for 6 hours. The reaction was cooled to room temperature and water (2.1 mL) was added carefully to decompose excess

phosphorus oxychloride. Approximately one-half the solvent was removed in vacuo and 20 mL of 5N sodium hydroxide was added. After stirring at room temperature for 2 hours, the precipitate was filtered, washed with water, and dried to afford 1.52 g (67%) of 3b. Concentrated hydrochloric acid (2 mL) was added to 400 mg of the crude sulfide in methanol. Removal of solvents in vacuo and recrystallization from ethanol provided 321 mg of product. Radiochemical purity as indicated by HPLC and autoradiography was 98.8 or 98.5%, respectively.

2-[2-Methoxy-4-(methyl-[^{14}C]-sulfinyl)phenyl]-1H-imidazo[4,5-c]-pyridine([methyl-^{14}C-sulfinyl]-LY175326, 2b) - A solution of 3-chloroperbenzoic acid (0.637 g of an 80-85% sample, ca. 2.952 mmol) in 16 mL methanol was added in a dropwise fashion over 1 hour to a solution of the sulfide 3b (800 mg, 2.952 mmol) in 10 mL methanol at -40 to -50°C. After stirring at this temperature for 4 hours the solvent was removed in vacuo, and the residue was dissolved in methanol. Silica gel (2 g) was added, solvent was removed in vacuo, and this material was applied to a column packed with 30 g silica in methylene chloride. Elution with a 2-8% methanol in methylene chloride gradient provided 424 mg (50%) of 2b . This was dissolved in 5 mL methanol, 1 mL concentrated hydrochloric acid was added, and the solvents were removed in vacuo. Recrystallization from ethanol afforded 385 mg (40%) of product with a specific activity of 5.70 mCi/mmol and a radiochemical purity of 97.2% (HPLC) or 96.7% (autoradiography).

2-[2-Methoxy-4-(methylsulfinyl-[^{18}O]-)phenyl]-1H-imidazo[4,5-c]-pyridine ([methylsulfinyl-^{18}O]-LY175326, 2d) - Bromine (94.6 μL, 1.84 mmol) was added dropwise to a vigorously stirred mixture of potassium carbonate (254.5 mg, 1.84 mmol), unlabeled LY137150 (500

mg, 1.84 mmol), and 1 mL of 97.25 atom percent ^{18}O-labeled water (50 mmol, MSD isotopes) in 100 mL of methylene chloride at 0°C.[18] Fifteen minutes following completion of the addition, the solvent was removed in vacuo. Preparative TLC (four 20x20 cm plates developed with methylene chloride:methanol (9:1), 3 developments) afforded 320 mg (61%) of product. Recrystallization from DMF containing 1 equivalent concentrated hydrochloric acid provided 230 mg (43%) of 2d with ^1H-NMR spectral data identical to those of unlabeled material. Mass spectral analysis indicated this sample was 86.7% ^{18}O-sulfoxide: ^1H-NMR (DMSO-d_6) δ 2.8 (s, 3H, $-SCH_3$), 4.08 (s, 3H, $-OCH_3$), 7.36-8.56 (m, 5H, ArH), 9.34 (s, 1H, 4-CH); mass spectrum (70eV) m/e (rel intensity) 289 (12, M^+), 36 (100).

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