JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 883–892.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.727

Research Article

Synthesis of [^{14}C]ABT-770, matrix metalloproteinase inhibitor (MMPI), labelled in the phenoxy ring

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Summary

The MMPI [^{14}C]ABT-770 **(1)**, *N*-[*(1S)*-1-[(4,4-Dimethyl-2,5-dioxo-1-imidazolidinyl)methyl]]-2-[[4′-(trifluoromethoxy)[1,1′-biphenyl]-4-yl]oxy]ethyl]-*N*-hydroxyformamide was synthesized in 8 steps using 4-bromophenol-UL-^{14}C **(10)** as a starting material. The Carbon-14 label was introduced in one of the metabolically stable biphenyl rings. The key sequence of the synthesis was a three-step one-pot reaction in which the hydantoin moiety was introduced, the imine oxidized and further hydrolyzed to get the penultimate precursor to [^{14}C]ABT-770 **(1)** in 56% yield. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: matrix metalloproteinase inhibitors; 4-bromophenol-UL-^{14}C; ABT-770; trifluoromethoxy; retrohydroxamate

Introduction

The matrix metalloproteinases (MMPs) are a family of enzymes containing zinc that degrade extra cellular matrix.[1] It is now known that the mammalian MMP family consists of at least 20 different enzymes.[2] An increase in MMP activity is associated with cancer and inflammatory diseases such as arthritis and multiple sclerosis.[1,2]

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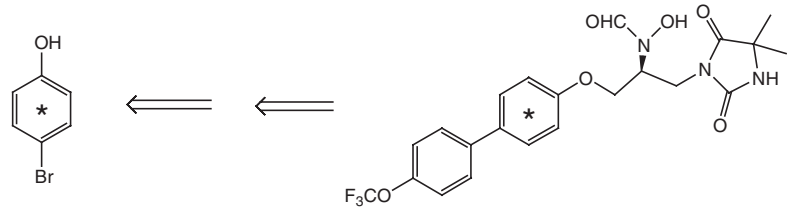
Received 31 January 2003
Revised 10 April 2003
Accepted 6 May 2003

Therefore a variety of compounds have been synthesized as potential therapeutic agents, acting by inhibition of matrix metalloproteinase activity. They are known as matrix metalloproteinase inhibitors (MMPIs). Our discovery group selected MMPI ABT-770 as a novel and potent antitumor agent because of its potency, selectivity, bioavailability and efficacy.[3]

It was shown that ABT-770 is highly selective for MMP-2 (gelatinase A) and exhibits desirable pharmacokinetic properties such as long half-life and high oral bioavailability in multiple species.[3] For preclinical development of this compound it was necessary to have a metabolically stable ^{14}C-labeled form of ABT-770 to be used in animal metabolism studies. Selection of the carbon-14 label in the phenoxymethylene ring was based upon earlier *in vivo* and *in vitro* metabolism studies with non-radioactive ABT-770. These results showed that most of the metabolism occurred in the hydroxamic acid portion of the molecule by formation of formamide and amine.[4] By taking advantage of the procedure used by our process research group[5] and by using readily available 4-bromophenol- UL-^{14}C **(10)**, a radiosynthetic strategy for [^{14}C]ABT-770 **(1)** was developed, as outlined in Scheme 1. The carbon-14 label was introduced in one of the biphenyl rings using 4-bromophenol-UL-^{14}C **(10)**. In the key sequence of this synthesis, a three-step, one-pot reaction was carried out to obtain 3-[*(2S)*-3-(4-bromophenoxy)-2-(hydroxyamino)propyl]-5,5-dimethyl-1,3-diazolidine-2,4-dione[bromophenylUL-^{14}C] **(8)** which was further elaborated to complete the synthesis of [^{14}C]ABT-770 **(1)**.

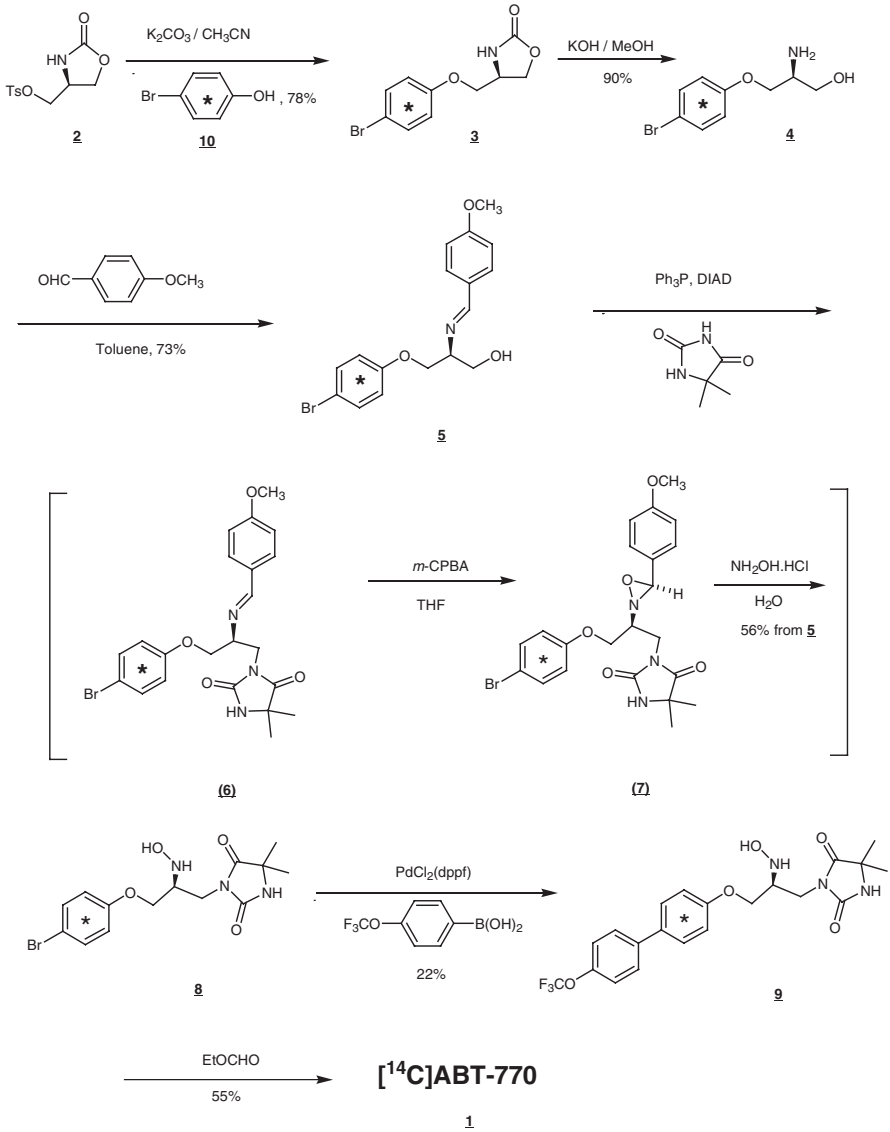


10 **[^{14}C]ABT-770 (1)**

Results and discussion

[^{14}C]ABT-770 **(1)** was synthesized using 4-bromophenol-UL-^{14}C **(10)** as radioactive starting material. Thus the alkylation of (L)-serine oxazolidinone tosylate (made by the Abbott process chemistry research group by following the procedure in Sibi *et al.*),[6] **2** with 1 molar

equivalent of 4-bromophenol-UL-^{14}C **(10)** and potassium carbonate gave only 50% conversion of **2** to **3**. To complete the reaction another 1 molar equivalent of potassium carbonate and **2** in MeCN were added to give the displacement product (L)-serine oxazolidinone *p*-bromophenyl ether [bromophenyl-UL-^{14}C] **(3)** in a 78% yield. This was followed by the hydrolysis of the oxazolidone ring by using potassium hydroxide in methanol to yield *(L)*-2-amino-3-hydroxypropyl (*p*-bromophenyl) ether [bromophenyl-UL-^{14}C] **(4)** in 90% yield. Initially **3** was not soluble in methanolic KOH but as the reaction progressed it formed a homogeneous mixture. Condensation of **4** with *p*-anisaldehyde in toluene at elevated temperature occurred readily to give 73% yield of iminoalcohol **5** as a key intermediate. Because of degradation observed during silica gel TLC analysis, imino alcohol **5** was characterized only by ^{1}H NMR spectroscopy and was used in the next step without purification. The iminoalcohol [bromophenyl-UL-^{14}C] **5** thus obtained was subjected to the key transformation which involved a three steps, one-pot reaction sequence to yield *N*-hydroxyl amine [bromophenyl-UL-^{14}C] **8**. The sequence involved coupling 5,5-dimethyl hydantoin with iminoalcohol [bromophenyl-UL-^{14}C] **5** by using triphenyl phosphine in the presence of diisopropyl azodicarboxylate (DIAD) under Mitsunobu conditions to yield imine [bromophenyl-UL-^{14}C] **6**. This product was further subjected to *m*-chloroperbenzoic acid (*m*-CPBA) oxidation to yield oxaziridine [bromophenyl-UL-^{14}C] **7**. The formation of diastereomers was indicated by observation of three radiochemical peaks in the HPLC trace. Hydrolysis of oxaziridine [bromophenyl-UL-^{14}C] **7** was accomplished with excess aqueous *N*-hydroxylamine hydrochloride to form the *N*-hydroxide [bromophenyl-UL-^{14}C] **8**. The overall radiochemical yield for these three steps was 56%, starting from **5**. During the transformation of **5** to **8** a number of byproducts were formed, as observed in the HPLC trace. These byproducts were not analyzed for their identity. Instead, HPLC analysis was utilized to monitor consumption of starting material. Suzuki coupling of *N*-hydroxide [bromophenyl-UL-^{14}C] **8** with 4-(trifluoromethoxy)phenyl boronic acid (made by process research group by using bromo-4-(trifluoromethoxy)benzene, *n*-BuLi, and triisopropyl borate) in the presence of $PdCl_2$(dppf) and K_3PO_4 gave trifluoromethoxybiphenyl ether [phenoxy-UL-^{14}C] **(9)**. The yield for this reaction was 22%. After silica gel column purification the product obtained was only 40% pure. It was speculated that this low yield was due to instability on silica gel, so **9** was subjected immediately to formylation. In the final step, formylation



Scheme 1.

of **9** was carried out in presence of excess of ethyl formate to yield [^{14}C]ABT-770 **(1)**. The overall yield for 8 steps was 3.5%.

Experimental

All chemicals and solvents were reagent grade or better and purchased from commercial suppliers. They were used without further purification.

4-bromophenol-UL-^{14}C was purchased from American Radiolabelled Chemicals, St. Louis, MO. (L)-serine oxazolidinone tosylate **(2)**[6] was obtained from the Abbott process chemistry group. The identity of intermediates was confirmed by comparison with authentic samples supplied by the Abbott process chemistry group. Liquid scintillation counting was performed on a LKB-Wallac 1214 Rack Beta 'Excel' counter. TLC plates were coated with Kieselgel 60 (0.25 mm, Merck) and were scanned for radioactivity with a Radiomatic RS chromatograph. The ^{1}H-NMR spectrum of **5** was recorded at 500 MHz and chemical shifts are reported in ppm (δ). [^{14}C]ABT-770 was analyzed using a Hitachi L-7000 Series analytical HPLC system consisting of an auto-sampler, a pump, a variable wavelength UV detector, and a Flow-One/Beta Model A-500 liquid scintillation radioactivity flow detector (Packard Instruments, 0.5 ml flow cell, 3:1 ratio of Packard Ultima-Flo M scintillant to effluent). A Perkin Elmer 250 analytical HPLC system and an Applied Biosystems UV detector were used to analyze **6**, **7**, **8** and **9** along with the same liquid scintillation radioactivity flow detector as above. Preparative HPLC was performed using a Shimadzu system consisting of a Shimadzu SIL-10A autosampler and two Shimadzu LC-8A pumps. Peak detection and chromatograms were obtained with a Shimadzu SPD-10A VP variable wavelength UV detector set at 260 nm and Shimadzu Ezchrom software. Fractions of the [^{14}C]ABT-770 **(1)** peak were collected using a Shimadzu FRC-10A fraction collector. An Eppindorf CH-30 column heater was used to maintain columns at 30°C and 35°C.

(4*R*)-4-*[(4-Bromophenoxy)methyl]-1,3-oxazolidin-2-one[bromophenyl-UL-^{14}C]* (**3**)

To a solution of (L)-serine oxazolidinone tosylate (**2**, 0.600 g, 2.4 mmol) in dry CH_3CN (4 ml) was added anhydrous K_2CO_3 (0.33 g, 2.4 mmol) and 4-bromophenol-UL-^{14}C (100 mCi, 2.0 mmol) under N_2. The reaction mixture was stirred at 80°C for 15 h. TLC analysis (silica gel, 5% MeOH and 1% NH_4OH in CH_2Cl_2) showed 50% product formation. Additional K_2CO_3 (0.330 g, 2.4 mmol), CH_3CN (2 ml) and compound **2** (0.600 g, 2.4 mmol) were added and heating was continued at 80°C for 4 h. The reaction mixture was then cooled to room temperature and diluted with water (10 ml). The aqueous layer was extracted with CH_2Cl_2 (3 × 10 ml) and the combined organic layers were successively washed with 1N NaOH (3 ml) and a brine solution (25 ml).

The organic layers were concentrated on a rotary evaporator to yield (*4R*)-4-[(4-bromophenoxy)methyl]-1,3-oxazolidin-2-one [bromophenyl-UL-^{14}C] **(3)** (78 mCi). TLC analysis (silica gel, 5% MeOH and 1% NH_4OH in CH_2Cl_2) indicated the compound ($R_f \sim 0.3$) was 97% pure.

(2S)-2-Amino-3-(4-bromophenoxy)propan-1-ol[bromophenyl-UL-^{14}C] **(4)**

Compound **3** (78 mCi, 1.56 mmol) was mixed with 2.5 M 90% methanolic KOH solution (2.5 ml) and heated at 65°C for 4 h and 75°C overnight. The solvent was removed using a rotary evaporator, and the residue was transferred into a separatory funnel using H_2O (20 ml). The aqueous solution was extracted with CH_2Cl_2 (3×20 ml), the combined organic layers were washed with brine solution (25 ml), dried over anhydrous Na_2SO_4, and filtered. The solvent was removed using a rotary evaporator to yield *(2S)*-2-amino-3-(4-bromophenoxy)propan-1-ol[bromophenyl-UL-^{14}C] **(4)** (70 mCi). TLC analysis (silica gel, 5% MeOH and 1% NH_4OH in CH_2Cl_2) indicated the compound ($R_f \sim 0.2$) was 98% pure.

(3E)(2S)-3-Aza-2-[(4-bromophenoxy)methyl]-4-(4-methoxyphenyl)-but-3-en-1-ol [bromophenyl-UL-^{14}C] **(5)**

(2S)-2-Amino-3-(4-bromophenoxy)-1-propanol [bromophenol-UL-^{14}C] (**4**, 70 mCi, 1.4 mmol) and *p*-anisaldehyde (0.23 g, 1.7 mmol) were dissolved in toluene (7 ml). The reaction flask was attached to a Dean-Stark trap containing a few beads of 4 Å molecular sieves. The reaction mixture was heated at 125°C for 4 h, cooled to room temperature and dried in *vacuo* to give a sticky solid, which upon trituration with pentane (2 ml) provided **5** (51 mCi). ^{1}H NMR (500 MHz, DMSO-d6) ppm (δ) 3.51 (dd, $J = 9.85$, 6.96 Hz, 1 H) 3.62 (m, 1 H) 3.69 (m, 1 H) 3.80 (s, 3 H) 4.05 (dd, $J = 9.85$, 7.54 Hz, 1 H) 4.22 (dd, $J = 9.85$, 4.06 Hz, 1 H) 4.77 (s, 1 H) 6.90 (d, $J = 8.69$ Hz, 2 H) 6.99 (d, $J = 8.69$ Hz, 2 H) 7.41 (d, $J = 8.69$ Hz, 2 H) 7.69 (d, $J = 8.69$ Hz, 2 H) 8.29 (s, 1 H). As judged by ^{1}H NMR analysis, the pentane wash also contained more of the desired product **5** (19 mCi).

3-{*(3E)(2S)-3-Aza-2-[(4-bromophenoxy)methyl]-4-(4-methoxyphenyl)but-3-enyl*}*-5,5-dimethyl-1,3-diazolidine-2,4-one[bromophenyl-UL-^{14}C]* (**6**)

A THF (4 ml) solution of **5** (50 mCi, 1 mmol), triphenylphosphine (0.41 g, 1.57 mmol), and 5,5-dimethylhydantoin (0.22 g, 1.7 mmol) was

cooled to 0°C under a nitrogen atmosphere. A solution of 95% diisopropyl azodicarboxylate (0.318 g, 1.5 mmol) was slowly added over 10 min. The reaction was stirred at room temperature for 3.5 h. HPLC analysis (Zorbax SB C8 column, 4.6 × 250 mm, 5 μm, UV at 230 nm, flow rate = 1 ml/min, at 30°C, 20% CH_3CN/0.1% H_3PO_4 to 60% CH_3CN/0.1% H_3PO_4 over 15 min, hold at this concentration for 10 min, RT = 8.3 min) at this time showed complete consumption of **5**. The product was used directly in the next step.

3-{2-[(3R)-3-(4-Methoxyphenyl)(1,2-oxaziridin-2-yl)(2S)-3-(4-bromophenoxy) propyl-5,5-dimethyl-1,3-diazolidine-2,4-dione [bromophenyl-UL-^{14}C] (**7**)

The reaction mixture containing **6** from the previous step was cooled to –78°C and to this was added a solution of *m*CPBA (0.703 g, 4.0 mmol) in THF (1.5 ml). The reaction mixture was warmed to 0°C and stirred at that temperature for 1.5 h. The reaction progress was monitored by HPLC (Zorbax SB C8 column, 4.6 × 250 mm, 5 μm, UV at 230 nm, flow rate = 1 ml/min, at 30°C, 20% CH_3CN/0.1% H_3PO_4 to 60% CH_3CN/0.1% H_3PO_4 over 50 min, hold at this concentration for 10 min RT = 20.8, 21.9, 22.7 min). Reactant **6** was completely consumed, and three new peaks appeared, suggesting formation of a mixture of diastereomers of the desired product. The crude product was used directly in the next step.

3-[(2S)-3-(4-Bromophenoxy)-2-(hydroxyamino)propyl]-5,5-dimethyl-1,3-diazolidine-2,4-dione [bromophenyl-UL-^{14}C] (**8**)

The mixture containing **7** from the previous step was cooled to 0°C and aqueous solution (1.5 ml) of hydroxylamine hydrochloride (0.73 g, 10.6 mmol) was added. The reaction mixture was warmed to room temperature and was stirred at that temperature for 15 h. HPLC analysis (Zorbax SB C8 column, 4.6 × 250 mm, 5 μm, UV at 230 nm, flow rate = 1 ml/min, at 30°C, 20% CH_3CN/0.1% H_3PO_4 to 60% CH_3CN/0.1% H_3PO_4 over 50 min, hold at this concentration for 10 min, RT = 10.5 min) of the organic layer showed complete disappearance of the three peaks of **7**. After cooling the reaction mixture to 0°C, a saturated solution of K_2CO_3 was added until pH 8.0 (5 ml), and the solution was extracted with EtOAc (2 × 40 ml). The combined organic layers were washed with saturated $NaHCO_3$ (10 ml) and a brine solution (25 ml). The solvent was evaporated using a rotary evaporator,

and the crude product obtained was purified by silica gel column chromatography using 5% MeOH in CH_2Cl_2 as an eluent followed by preparative silica gel TLC using 5% MeOH in CH_2Cl_2 as eluent to yield greater than 95% pure **8** (28 mCi). TLC analysis (silica gel, 5% MeOH : CH_2Cl_2) $R_f \sim 0.2$.

3-(2S)-2-(Hydroxyamino)-3-{4-[4-(trifluoromethoxy)phenyl]phenoxy} propyl)-5,5-dimethyl-1,3-diazolidine-2,4-dione[phenoxymethylene-UL-^{14}C] (**9**)

To a slurry of compound **8** (25 mCi, 0.5 mmol) in 1:1 toluene : water (2.5 ml each) was added 4-(trifluoromethoxy)phenylboronic acid (0.199 g, 0.96 mmol) and K_3PO_4 (0.573 g, 2.7 mmol). Nitrogen was sparged through the mixture for 20 min. [1,1′-bis(Diphenylphosphino)-ferrocene]-dichloropalladium (II) complex with dichloromethane [$PdCl_2$(dppf)] (0.0082 g, 0.01 mmol) was added and the reaction mixture was heated at 69°C for 15 h. The reaction mixture was diluted with H_2O and extracted with EtOAc (3×25 ml). The combined organic layers were washed with brine solution, and the solvent evaporated to give an oily residue, which was triturated with 5% CH_2Cl_2/pentane (2 ml) to provide a solid. Attempts to purify the crude product on a silica gel column using 5% MeOH and CH_2Cl_2 gave 18 mCi containing > 40% of **9** as indicated by HPLC analysis. (Zorbax SB C8 column, 4.6×250 mm, 5 μm, UV at 210 nm, flow rate = 1.5 ml/min, at 35°C, 20% CH_3CN/0.1% H_3PO_4 to 60% CH_3CN/0.1% H_3PO_4 over 15 min, hold at this concentration for 10 min, RT = 10.9 min).

[^{14}C] *ABT-770* (**1**)

A solution containing 40% of **9** (18 mCi) and EtOCHO (4 ml) was heated at 65°C for 15 h. Excess EtOCHO was removed on a rotary evaporator to give an oil, which was transformed into a solid residue after trituration with 5% CH_2Cl_2/pentane (2 ml). The crude [^{14}C]ABT-770 was dissolved in a solution of 0.1% TFA/CH_3CN (1:1) and purified by HPLC using a YMC Basic (250 mm × 20 mm i.d.) column. The analyte was eluted at a flow rate of 20 ml/min with a mobile phase consisting of 50% H_2O and CH_3CN containing 0.1% trifluoroacetic acid. Peaks were detected with the UV detector set at 260 nm.Fractions containing [^{14}C]ABT-770 were pooled together and partially evaporated on a rotary evaporator. The aqueous portion was made basic with

Na_2CO_3 and **1** was extracted with CH_2Cl_2 (25 ml). The solvent was evaporated on a rotary evaporator and the residue obtained was dissolved in ethanol to give 3.95 mCi of [^{14}C]ABT-770 **(1)** with 96% radiochemical purity (22% yield).

Determination of purity

High performance liquid chromatography (HPLC)

[^{14}C]ABT-770 **(1)** was analyzed by reverse phase HPLC and compared to authentic ABT–770. Each sample was injected onto a YMC-Pack basic 5 μ (250 × 4.6 mm i.d.) column and eluted at 1 ml/min with mobile phase consisting of 50% 0.1% trifluoroacetic acid and 50% acetonitrile. Peaks were detected with a UV detector at 260 nm and a liquid scintillation radioactivity flow detector. Greater than 95% of radioactivity corresponded to the UV peak of ABT-770 at 9.20 min. An assay of effluent showed that greater than 98% of the activity was recovered from the column. The specific activity was determined to be 48.1 mCi/mmol (99.9 μCi/mg) by measuring mass and radioactivity concentrations of the material in a given solution. The concentration of the radioactivity was determined by liquid scintillation counting of an accurately measured aliquot. The mass concentration was measured by comparing the HPLC UV peak area of an accurately measured aliquot to a standard curve fitting a straight line equation (correlation coefficient of 0.998) generated by injecting a standard solution of ABT-770 and measuring the resulting peak areas.

Conclusion

An efficient eight step synthetic sequence starting with 4-bromophenol-UL-^{14}C (**10**) was developed and executed for the preparation of [^{14}C]ABT-770 **(1)**. A three-step, one-pot reaction sequence was the unique feature of the synthetic scheme. This method gave the [^{14}C]ABT-770 **(1)** compound in a reasonable radiochemical yield with high specific activity, which was suitable for use in *in vivo* and *in vitro* studies.

Acknowledgements

The author would like to gratefully acknowledge the process and discovery chemistry groups for starting materials, intermediate samples

and experimental details, Samuel B. Thomas for analytical support and Tom Pagano for ^{1}H-NMR analyses. It is a pleasure to acknowledge the radiochemistry group and Stanley A. Roberts, Director, Drug Metabolism and Preclinical Kinetics, for their helpful suggestions during the preparation of this manuscript.

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