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Research Article

Synthesis of carbon-14 labelled gemifloxacin

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Summary

A new antibacterial agent gemifloxacin was labelled with carbon-14 for studies of pharmacokinetics and metabolism, the label was located in position 3 of the quinolone ring system. The overall radiochemical yield of the 14-step synthesis, starting from [2-^{14}C]sodium acetate was 16.6%, and the radiochemical purity 97.5%.

Key Words: ^{14}C-labelling; LB20304a; factive; Gemifloxacin

Introduction

Gemifloxacin[1] (7-(3-aminomethyl-4-methyloxyiminopyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid methanesulfonate, or SB 265805, or LB20304a, or Factive™) is a fluoroquinolone antibacterial agent which possesses an enhanced affinity for topoisomerase IV. Compared to earlier quinolones, gemifloxacin has an improved spectrum of antibacterial activity notably against Gram-positive bacteria. It also displays superior activity against methicillin-resistant *Staphylococcus aureus* (MRSA), key respiratory tract pathogens such as *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and has great potential to become a once-daily drug of choice for the treatment of respiratory tract infection. Urinary tract pathogens including *Escherichia coli* and *Proteus mirabilis* are also very susceptible towards Gemifloxacin. To facilitate the pharmacokinetic and metabolic studies of gemifloxacin, we

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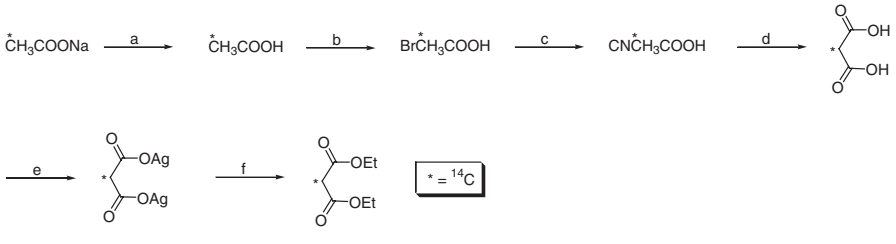
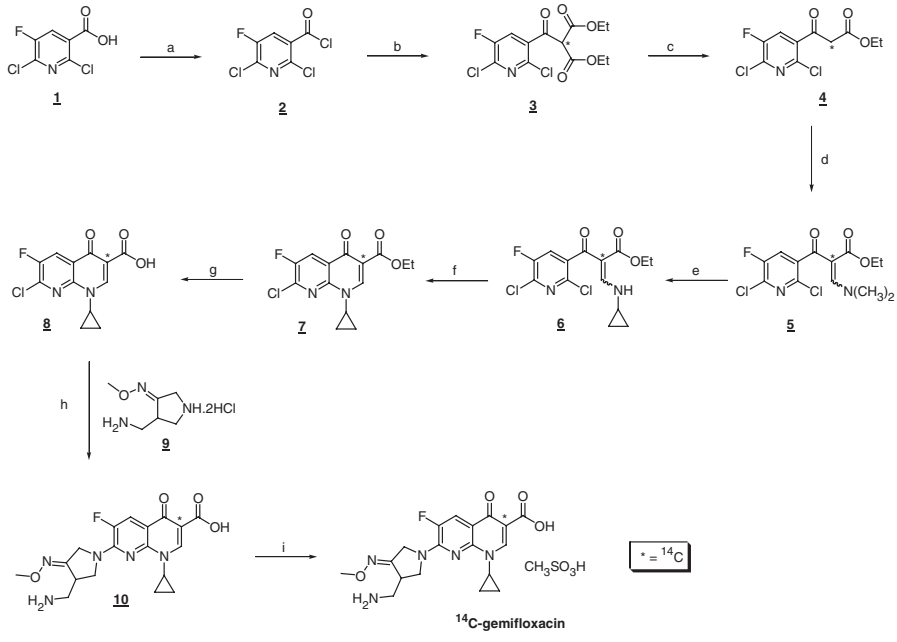
required the ^{14}C-labelled compound. In order that the carbon-14 label be in a metabolically stable position, a radiosynthesis was desired to insert the label in position 3 of the heterocyclic quinolone ring system.

Results and discussion

Several routes for preparing carbon-14 labelled quinolone compounds in different positions are known. [4-^{14}C]quinolone carboxylic acids were obtained from substituted [carbonyl-^{14}C]benzoyl derivatives.[2,3] Use of [2-^{14}C]malonates as the starting material, led to labelling of position 3 of quinolone compounds.[4–6] Labelled substituents were introduced at the nitrogen (the position 1 or position 7) of the quinolone compounds by substitution reactions.[7,8] Gemifloxacin was labelled at position 3 of the quinolone system starting from 2,6-chloro-5-fluoro-nicotinoyl chloride and [2-^{14}C]diethyl malonate as shown in Schemes 1 and 2. The starting material, [2-^{14}C]diethyl malonate, was prepared in six steps from [2-^{14}C]sodium acetate in an overall 62.7% yield (Scheme 1). Enolization of [2-^{14}C]diethyl malonate with magnesium ethoxide in diethyl ether at room temperature and subsequent reaction with nicotinoyl chloride 2 gave the acylated diethyl malonate 3 in 89% yield. Compound 3 was converted to the keto propionic acid ethyl ester 4 by hydrolysis and decarboxylation in the presence of a catalytic amount of *p*-toluenesulfonic acid in a combined yield of 54% after chromatographic purification. In this reaction, we found prolonged reaction time caused double decarboxylation to result in the increase of acetophenone type by-product. After the formation of the dimethylamino acrylic acid ethyl ester 5 from the reaction of propionic acid ethyl ester 4 with *N,N*-dimethylformamide dimethyl acetal and acetic anhydride at room temperature, treatment of cyclopropylamine displaced the dimethylamine group to give the acrylic acid ethyl ester 6 in 94% yield. Two reaction conditions were employed for the synthesis of 5: (1) use of triethyl orthoformate/acetic anhydride, and (2) use of *N,N*-dimethylformamide dimethyl acetal/acetic anhydride. The latter reagent



Scheme 1. Synthesis of [2-^{14}C]diethyl malonate: (a) HCl gas; (b) red P, AcCl, Br_2, 95°C, 6 h; (c) 30% K_2CO_3, NaCN, H_2O, 95°C, 1 h; (d) 5 N NaOH, 95°C, 2 h; (e) 2 N NaOH, $AgNO_3$, H_2O, rt, 1 h; (f) EtI, 95°C, 3 h



Scheme 2. Synthesis of ^{14}C-gemifloxacin: (a) $SOCl_2$, cat. DMF, benzene, 110°C, 3 h; (b) [2-^{14}C]diethyl malonate, magnesium ethoxide, diethyl ether, rt, 2 h; (c) *p*-TsOH, H_2O, 100–120°C, 3 h; (d) DMFDA, Ac_2O, CH_2Cl_2, rt, 2 h; (e) cyclopropyl amine, EtOH, rt, 1 h; (f) K_2CO_3, DMF, 90°C, 2 h; (g) c-HCl, AcOH, 120°C, 4 h; (h) DBU, CH_3CN, rt, 22 h; (i) CH_3SO_3H, CH_2Cl_2/EtOH(7/3), 0°C, 1 h

afforded 6 in higher yield (68 vs 94%). Intramolecular ring closure reaction of 6 to the carboxylic acid ethyl ester 7 was carried out in anhydrous DMF at 90°C in the presence of anhydrous K_2CO_3. 7 was obtained in 83% yield after purification on silica gel. Hydrolysis of 7 was performed using hydrochloric acid and acetic acid at 120°C to afford the carboxylic acid 8 in a quantitative yield. Coupling of 8 with the pyrrolidine 9 was carried out in anhydrous acetonitrile at room temperature using DBU to give the carboxylic acid 10 in high yield (88%). Finally, ^{14}C-gemifloxacin was obtained by salt formation of 10 with methanesulfonic acid in dichloromethane/ethanol at 0°C in 81% yield. The overall radiochemical yield of ^{14}C-gemifloxacin from [2-^{14}C]sodium acetate in a 14-step sequence was 16.6%, and the radiochemical purity 97.5%.

Experimental

General

Reagents and solvents were purchased from Aldrich or Fluka and used without further purification. [2-^{14}C]Diethyl malonate was synthesized from

[2-^{14}C]sodium acetate (Perkin Elmer Life Sciences). Radioactivity was measured by a Tri-carb 2100TR liquid scintillation counter (Packard) using ULTIMA FLO M (Packard) as a liquid scintillation cocktail. High performance liquid chromatography (HPLC) was performed using a 1100 series (Agilent) instrument. Radiochemical purity (RCP) was determined either by an automatic TLC-linear analyzer Tracemaster 20 (EG&G Berthold) or by a HPLC radioactivity monitor LB506C-1 (EG&G Berthold) equipped with a pump, LB 5035 (EG&G Berthold) and with Flo-scint II (Packard) as the liquid scintillation cocktail. The HPLC was run on a C_{18} μ-Bondapak column (Millipore-Waters). All reactions were monitored by TLC (silica gel 60 F_{254} plate, Merck), ultraviolet light, automatic TLC-linear analyzer Tracemaster 20, X-ray film (Konica) were used in TLC visualization. For column chromatography, we employed silica gel 60 (230–400 mesh; ASTM, Merck). All labelled materials were identified by chromatographic comparison with the corresponding authentic unlabelled samples. ^{1}H NMR spectra were recorded on a Varian Unity 300 spectrometer; the chemical shifts are reported in parts per million (ppm) relative to TMS in $CDCl_3$ or DMSO-d_6. Electron impact mass spectra (EI-MS) were obtained on a LCQ DECA XP Plus LC-Mass spectrometer (Thermo Finnigan). Elemental analyses were performed on an EA 1110 elemental analyzer (CE Instruments). Unlabelled samples, prepared by the same synthetic procedure, were used for mass spectra and elemental analyses.

2-(2,6-Dichloro-5-fluoro-pyridine-3-carbonyl)-[2-^{14}C]malonic acid diethyl ester (3)

To a stirred solution of 2,6-dichloro-5-fluoro nicotinic acid (529 mg, 2.52 mmol) in benzene (4 ml) were added thionyl chloride (0.276 ml, 3.78 mmol) and a catalytic amount of DMF. The mixture was refluxed at 110°C for 3 h, concentrated, and azeotroped with benzene (5 ml × 3) to give 2,6-dichloro-5-fluoro nicotinoyl chloride. To a stirred solution of [2-^{14}C]diethyl malonate (366 mg, 2.29 mmol, 4.96 GBq) in diethyl ether (6 ml) at room temperature was added magnesium ethoxide (314 mg, 2.74 mmol) and then the mixture was stirred at room temperature for 2 h and concentrated. To the residue in diethyl ether (4 ml) was added 2,6-dichloro-5-fluoro nicotinoyl chloride in diethyl ether (3 ml) at 0°C and then the mixture was stirred at room temperature for 1 h. After quenching by 10% aqueous citric acid (5 ml) at 0°C, the aqueous layer was extracted with benzene (5 ml × 3). The combined organic layers were washed with brine (3 ml), dried (Na_2SO_4), filtered, and concentrated to give the malonic acid diethyl ester 3 (976 mg, 2.77 mmol, 4.41 GBq, yield: 89%) as an oil, which had a specific activity of 1.59 GBq/mmol: TLC RCP >95%, R_f = 0.39, silica gel, hexane/ethyl acetate = 4/1).

3-(2,6-Dichloro-5-fluoro-pyridine-3-yl)-3-oxo-[2-^{14}C]propionic acid ethyl ester (4)

A mixture of 3 (976 mg, 2.77 mmol, 4.41 GBq) and *p*-toluenesulfonic acid (8 mg) in H_2O (2.3 ml) was stirred at 100–120°C for 3 h. The reaction mixture was extracted with ethyl acetate (6 ml × 2) and the combined organic layer was dried ($MgSO_4$), filtered, and concentrated. The residue in ethanol (1 ml) was sonicated, left to stand in a refrigerator for 1 h, filtered, washed with cold hexane (1 ml), and dried *in vacuo* for 15 h to give the propionic acid ethyl ester 4 (364 mg, 1.30 mmol, 2.04 GBq, yield: 46%) as a white solid. A filtrate was purified by chromatography (SiO_2, hexane/ethyl acetate = 9/1) to give additional 4 (60 mg, 0.214 mmol, 336 MBq, yield: 8%) as a white solid. The combined 4 had a specific activity of 1.57 GBq/mmol: TLC RCP > 95%, R_f = 0.45, silica gel, hexane/ethyl acetate = 4/1; ^{1}H NMR ($CDCl_3$/TMS) δ 12.57 (s, 0.6 H, OH), 7.85 (d, J = 7.5 Hz, 1 H, pyridine CH), 5.83 (s, 0.6 H, CH), 4.30 (q, J = 7.2 Hz, 1.2 H, CH_2), 4.20 (q, J = 7.2 Hz, 0.8 H, CH_2), 4.09 (s, 0.8 H, CH_2) 1.35 (t, J = 7.2 Hz, 1.8 H, CH_3), 1.26 (t, J = 7.2 Hz, 1.2 H, CH_3). EI-MS m/z 583 $[2M + Na]^+$; 302 $[M + Na]^+$. Anal. Calcd for $C_{10}H_8Cl_2FNO_3$: C, 42.38; H, 2.86; N, 4.86. Found: C, 42.53; H, 2.88; N, 4.91.

3-Cyclopropylamino-2-(2,6-dichloro-5-fluoro-pyridine-3-carbonyl)-[2-^{14}C] acrylic acid ethyl ester (6)

To a stirred solution of 4 (424 mg, 1.51 mmol, 2.37 GBq) in dichloromethane (4 ml) at 0°C were added *N,N*-dimethylformamide dimethyl acetal (360 mg, 3.02 mmol) and acetic anhydride (308 mg, 3.02 mmol) and then the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated and azeotroped with toluene (4 ml) to give 5. To a solution of 5 in ethanol (4 ml) at 0°C was added cyclopropyl amine (104 mg, 1.82 mmol) and then the mixture was stirred at room temperature for 1 h and concentrated. The residue was purified (SiO_2, hexane/ethyl acetate = 10/1) to give 6 (495 mg, 1.43 mmol, 2.23 GBq, yield: 94%) as a white solid, which had a specific activity of 1.56 GBq/mmol: TLC RCP > 95%, R_f = 0.13, silica gel, hexane/ethyl acetate = 4/1; ^{1}H NMR ($CDCl_3$/TMS) δ 8.36 (d, J = 14.4 Hz, 0.2 H, pyridine CH), 8.28 (d, J = 14.4 Hz, 0.8 H, pyridine CH), 7.39 (d, J = 7.2 Hz, 0.2 H, CH), 7.32 (d, J = 7.2 Hz, 0.8 H, CH), 4.03 (q, J = 7.2 Hz, 1.6 H, CH_2), 3.96 (q, J = 7.2 Hz, 0.4 H, CH_2), 3.02 (m, 1 H, CH_2CHCH_2), 1.04 (t, J = 7.2 Hz, 2.4 H, CH_3), 0.98–0.84 (m, 4.6 H, $CH_3 + CH_2CHCH_2$). EI-MS m/z 347 $[M + H]^+$. Anal. Calcd for $C_{14}H_{13}Cl_2FN_2O_3$: C, 48.13; H, 3.75; N, 7.93. Found: C, 48.18; H, 3.78; N, 7.91.

7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-[^{14}C]carboxylic acid ethyl ester (7)

A stirred supension of 6 (495 mg, 1.43 mmol, 2.23 GBq) and potassium carbonate (216 mg, 1.56 mmol) in DMF (7 ml) was stirred at 90°C for 2 h and then the reaction mixture was diluted with H_2O (10 ml) and extracted with ethyl acetate (10 ml × 3). The combined organic layer was washed with brine (10 ml), dried ($MgSO_4$), concentrated, and purified (SiO_2, hexane/ethyl acetate = 10:1 → neat ethyl acetate) to give 7 (272 mg, 0.875 mmol, 1.86 GBq, yield: 83%) as a white solid, which had a specific activity of 2.11 GBq/mmol: TLC RCP > 95%, R_f = 0.50, silica gel, hexane/ethyl acetate = 1/1; ^{1}H NMR ($CDCl_3$/TMS) δ 8.65 (s, 1 H, CH), 8.43 (d, J = 7.8 Hz, 1 H, pyridine CH), 4.40 (q, J = 7.2 Hz, 2 H, CH_2), 3.65 (m, 1 H, CH_2CHCH_2), 1.41 (t, J = 7.2 Hz, 3 H, CH_3), 1.35–1.02 (m, 4 H, CH_2CHCH_2). EI-MS m/z 311 $[M+H]^+$. Anal. Calcd for $C_{14}H_{12}ClFN_2O_3$: C, 53.37; H, 3.82; N, 8.79. Found: C, 53.40; H, 3.81; N, 8.79.

7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-[^{14}C]carboxylic acid (8)

A mixture of 7 (272 mg, 0.875 mmol, 1.86 GBq) and concentrated HCl (3.0 ml) in acetic acid (3.0 ml) was stirred at 120°C for 4 h. The reaction mixture was diluted with water (7 ml) and stirred at 0°C for 2 h. The resulting solid was filtered, washed with water (5 ml) and hexane (5 ml), and dried (P_2O_5) *in vacuo* for 15 h to give 8 (251 mg, 0.888 mmol, yield: quantitative) as a white solid. TLC RCP > 95%, R_f = 0.1–0.35, silica gel, chloroform/methanol = 10/1; ^{1}H NMR ($CDCl_3$/TMS) δ 8.93 (s, 1 H, CH), 8.48 (d, J = 6.9 Hz, 1 H, pyridine CH), 3.81 (m, 1 H, CH_2CHCH_2), 1.43–1.10 (m, 4 H, CH_2CHCH_2). EI-MS m/z 283 $[M+H]^+$. Anal. Calcd for $C_{12}H_8ClFN_2O_3$: C, 49.45; H, 2.97; N, 9.07. Found: C, 49.46; H, 2.93; N, 9.09.

7-(3-Aminomethyl-4-methoxyiminopyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-[^{14}C]carboxylic acid (10)

To a stirred suspension of 8 (251 mg, 0.888 mmol), unlabelled compound 8 (420 mg, 1.49 mmol), and pyrrolidine 9 (646 mg, 2.99 mmol) in acetonitrile (14 ml) was added DBU (1.23 g, 8.08 mmol) at room temperature and then the reaction mixture was stirred at room temperature for 22 h and diluted with water (10 ml). After stirring for 20 min, the resulting slurry was filtered, washed with ethanol/water (4/1, 10 ml) and ethanol (15 ml), and dried (P_2O_5) *in vacuo* for 15 h to give 10 (810 mg, 2.08 mmol, yield: 87%) as a pale brown solid. TLC RCP > 97%, R_f = 0.34, silica gel, CH_2Cl_2/EtOH/NH_4OH/CH_3CN = 4/4/2/1; ^{1}H NMR (DMSO-d_6/TMS) δ 8.56 (s, 1 H, CH), δ 7.99 (d, J = 12.6 Hz, 1 H, pyridine CH), 4.55 (s, 2 H, pyrrolidine CH_2), 4.17 (m, 1 H,

pyrrolidine CH_2), 3.93 (m, 1 H, pyrrolidine CH_2), 3.85 (s, 3 H, OCH_3), 3.72 (m, 1 H, CH_2CHCH_2), 3.04 (m, 1 H, pyrrolidine CH), 2.86 (m, 1 H, pyrrolidine CH_2), 2.74 (m, 1 H, pyrrolidine CH_2), 1.03–1.21 (m, 4 H, CH_2CHCH_2). EI-MS m/z 390 $[M+H]^+$. Anal. Calcd for $C_{18}H_{22}FN_5O_4$: C, 48.98; H, 5.20; N, 15.68. Found: C, 48.95; H, 5.22; N, 15.70.

7-(3-Aminomethyl-4-methoxyiminopyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-[^{14}C]carboxylic acid methanesulfonate (^{14}C-gemifloxacin)

To a stirred solution of 10 (810 mg, 2.08 mmol) in dichloromethane/ethanol (7/3, 33 ml) was added methanesulfonic acid (190 mg, 1.98 mmol) at 0°C and then the mixture was stirred at 0°C for 1 h. The resulting solid was filtered, washed with cold dichloromethane (3 ml), and dried (P_2O_5) *in vacuo* for 15 h to give ^{14}C-gemifloxacin (704 mg, 1.45 mmol, yield: 70%). A filtrate was concentrated up to 9 ml and the resulting solid was filtered, washed with cold dichloromethane (3 ml), and dried (P_2O_5) *in vacuo* for 15 h to give ^{14}C-gemifloxacin (114 mg, 0.235 mmol, yield: 11%). The specific activity of the combined ^{14}C-gemifloxacin (818 mg, 1.68 mmol) was measured as 784 MBq/mmol: radio-HPLC RCP 97.5%, column μ-Bondapak C_{18} (3.9 × 150 mm), Eluant A = 0.1% CF_3COOH in H_2O and B = 0.1% CF_3COOH in CH_3CN, Gradient: 0 → 5 min; A/B = 80/20, 5 → 18 min; A/B = 80/20 → 5/95, 18 → 20 min; A/B = 5/95, 20 → 25 min; A/B = 5/95 → 80/20, Flow rate 1 ml/min, Detector UV 272 nm, Temperature 30°C, Retention time = 15.1 min, TLC RCP > 97.9%, R_f = 0.32, silica gel, CH_2Cl_2/EtOH/NH_4OH/CH_3CN = 4/4/2/1; ^{1}H NMR (DMSO-d_6/TMS) δ 8.59 (s, 1 H, CH), 8.05 (d, J = 12.9 Hz, 1 H, pyridine CH), 4.57 (s, 2 H, pyrrolidine CH_2), 4.37 (m, 1 H, pyrrolidine CH_2), 3.90 (s, 3 H, OCH_3), 3.84 (m, 1 H, pyrrolidine CH_2), 3.71 (m, 1 H, CH_2CHCH_2), 3.41 (m, 1 H, pyrrolidine CH), 3.31 (s, 3 H, NH_3^+), 3.23–3.10 (m, 2 H, pyrrolidine CH_2), 2.33 (s, 3 H, SO_3CH_3), 1.24–1.04 (m, 4 H, CH_2CHCH_2). EI-MS m/z 390 $[M+H]^+$. Anal. Calcd for $C_{19}H_{24}FN_5O_7S$: C, 42.53; H, 5.39; N, 13.05; S, 5.73. Found: C, 42.47; H, 5.38; N, 13.01; S, 5.74.

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