Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 795-805 (2000)

Synthesis of Carbon-14 Labelled Moxifloxacin Hydrochloride

D. Seidel [1], M. Conrad [1], P. Brehmer [1], K. Mohrs [1] and U. Petersen [2]

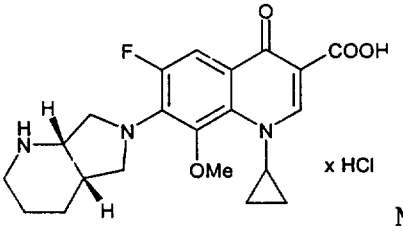
Bayer AG, Pharmaceutical Business Group, Wuppertal, Germany [1], Central Research, Leverkusen, Germany [2]

SUMMARY

For studies of pharmacokinetics and metabolism of the new antiinfective moxifloxacin, the corresponding carbon-14 labelled substance was required. Starting from the appropriately substituted benzoic acid chloride and diethyl [2-^{14}C]malonate, a 7-step synthesis and the final crystallisation of the hydrochloride led to [^{14}C]moxifloxacin hydrochloride, labelled in position 3 of the quinolone ring system. Two radiosyntheses were performed leading to 0.108 g (0.340 GBq) of the labelled product in the first and 0.729 g (2.143 GBq) of the labelled product in the second synthesis.

INTRODUCTION

Moxifloxacin is a new enantiomerically pure 8-methoxyquinolone with the S,S-piperidinopyrrolidine substituent in the 7-position of the quinolone ring. It demonstrates a good tolerability with a broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria, anaerobes and atypical organisms (1).



Moxifloxacin hydrochloride

Copyright © 2000 John Wiley & Sons, Ltd.

Received 22 December 1999
Revised 7 February 2000
Accepted 9 March 2000

For studies of pharmacokinetics and drug metabolism of moxifloxacin the substance with a metabolically stable carbon-14 label was required. Therefore a radiosynthesis was planned locating the label in the heterocyclic quinolone ring.

RESULTS AND DISCUSSION

For preparing carbon-14 labelled quinolone carboxylic acids several different synthetic routes are described in the literature. Starting from appropriately substituted [carbonyl-^{14}C]benzoyl derivatives, [4-^{14}C]quinolone carboxylic acids were obtained (2, 3). Precursors such as [2-^{14}C]malonates led to the labelling of position 3 of the final quinolones (4, 5). By substitution reactions labelled substituents were introduced at the nitrogen (position 1) (6, 7) or at position 7 of the quinolone system (8). Because of the special substitution pattern of the quinolone drug substances, uniformly labelled aromatics are not suitable precursors for appropriate radiosyntheses.

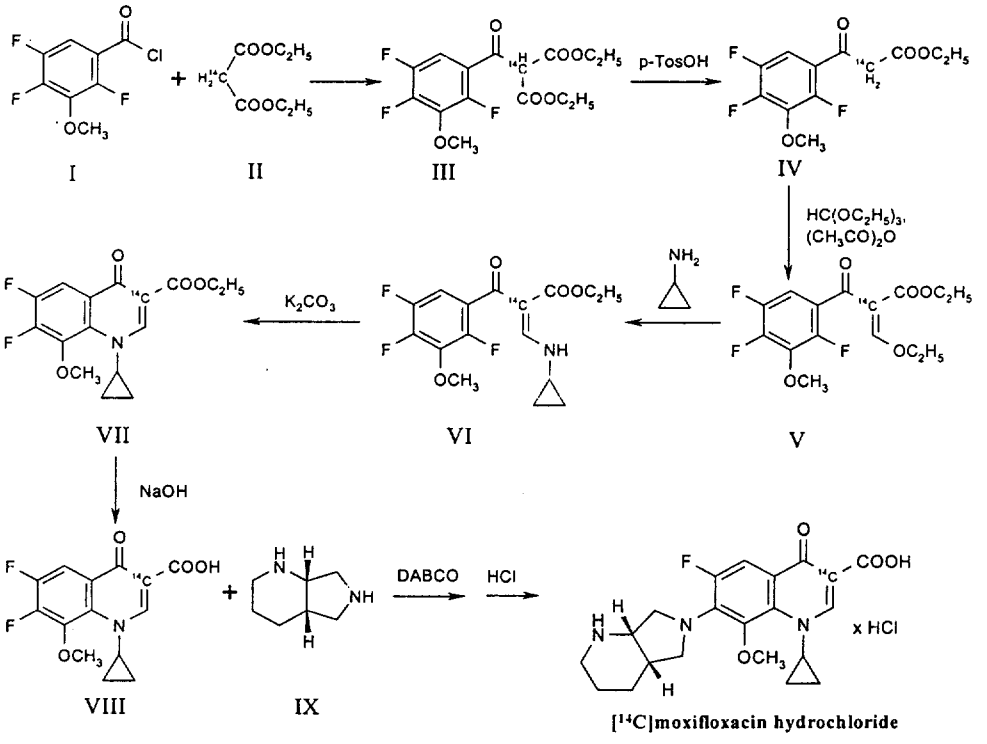
The present labelling synthesis shown in the reaction scheme started from commercially available diethyl [2-^{14}C]malonate (II) and 2,4,5-trifluoro-3-methoxybenzoyl chloride (I), an intermediate of the synthesis for non-labelled product (9).

We synthesised the carbon-14 labelled moxifloxacin hydrochloride twice. The synthetic pathway was kept in the second synthesis, but some steps were modified to obtain better yields.

In the first step the carbon-14 labelled starting substance diethyl [2-^{14}C]malonate (II) was acylated with the appropriately substituted benzoic acid chloride (I). In both radiosyntheses hydrolysis and decarboxylation with p-toluenesulfonic acid yielded the substituted ethyl oxo-phenyl-propionate IV. It is important to note that an optimised reaction time had to be found because longer time led to 2,4,5-trifluoro-3-methoxy-acetophenone as the product of a twofold hydrolysis and decarboxylation. Approximately 10 % of unreacted compound III together with the equal amount of acetophenone had to be accepted for an optimal yield of the desired product IV.

Copyright © 2000 John Wiley & Sons, Ltd.

REACTION SCHEME



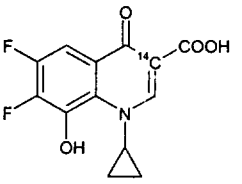
After formation of the ethoxy-benzoyl-[2-^{14}C]acrylate V and subsequently substitution of the ethoxy group by cyclopropylamine, a first purification by crystallisation of the intermediate VI was possible.

In the first radiosynthesis the ring closure to the quinolone system was performed using sodium fluoride in boiling DMF. In the repeated synthesis the reaction was carried out in N-methylpyrrolidinone with potassium carbonate at 80 to 100 °C. Also the next step of ester hydrolysis was optimized to milder conditions using sodium hydroxide in methanol instead of sulphuric acid in boiling glacial acetic acid.

[^{14}C]Moxifloxacin hydrochloride was prepared by introduction of S,S-2,8-diazabicyclo[4.3.0]nonane (IX) in position 7 of the quinolone ring and crystallisation as the hydrochloride.

Copyright © 2000 John Wiley & Sons, Ltd.

Unexpectedly, HPLC analysis of the solid showed 30 % (radiodetection) of a by-product. By LC/MS and comparison with a reference compound it could be characterised as 1-cyclo-propylamino-6,7-difluoro-8-hydroxy-4-oxo-1,4-dihydro[3-^{14}C]-quinoline carboxylic acid X, a demethylation product of VIII.



X

The by-product was removed using the different solubility in water compared with moxifloxacin hydrochloride.

The first radiosynthesis yielded 4 % (0.108 g with a total radioactivity of 0.340 GBq) of the theory related to the carbon-14 labelled diethyl malonate. The result of the optimised second radiosynthesis was an overall yield of 14.5 % (0.729 g with a total radioactivity of 2.143 GBq).

The following experimental section reports the details of the second radiosynthesis.

EXPERIMENTAL

General methods

The ^{1}H NMR spectrum was recorded with Bruker DRX 400 nuclear magnetic resonance spectrometer. Radiochemical purities of the labelled intermediates were determined with the GC system HP 5890 equipped with radioactivity detector FHT 7000. HPLC analyses of the final product were performed on a HP 1050 Series II. Radioactivity and UV signals (radioactivity detector Ramona® 92) were recorded by the work station of the chromatcgraph. The radioactivity signal was digitised by the A/D-converter HP 35900 and evaluated by the work station. Radiochemical counting was performed on a Liquid Scintillation Analyzer TRI-CARB® 2500 TR using Ultima Gold™ cocktail. Mass spectrometric analysis was performed on a PE/Sciex API III mass spectrometer with MacIntosh Quadra® 900.

Copyright © 2000 John Wiley & Sons, Ltd.

Diethyl 2-(2,4,5-trifluoro-3-methoxy-benzoyl)-[2-^{14}C]malonate (III)

The diethyl [2-^{14}C]malonate (II) was delivered as a solution in acetonitrile by Amersham International plc (product no. CFQ 9261) with a specific activity of 2 GBq/mmol, a concentration of 1.48 MBq/ml (0.74 mmol/ml respectively 118 mg/ml, as calculated from the specific radioactivity), and a total radioactivity of 14.8 GBq (according to the certificate). The chemical purity was $\geq$ 98 % and the radiochemical purity was $\geq$ 95 %, as established by GC.

Non-labelled diethyl malonate (577 mg, 3.6 mmol) was added to the labelled compound. The resulting specific activity was calculated to be 1.345 GBq/mmol.

This solution was added dropwise to a mixture of 1.153 g (12.1 mmol) magnesium chloride (water content $\leq$ 1.5 %) and 3 ml of dry acetonitrile within 15 minutes at 0 °C under an argon atmosphere. At the end the dosing syringe was rinsed with 2 ml of acetonitrile. The mixture was stirred for 10 minutes at 0 °C. Then 3.37 ml (24.2 mmol) of triethylamine was dropped to the mixture within 10 minutes. After stirring for 20 minutes at 0 °C 2.638 g (11.7 mmol) of 2,4,5-trifluoro-3-methoxy-benzoyl chloride (I) in of 2.5 ml acetonitrile was added within 10 minutes. The mixture was stirred for a further 2 hours at a temperature of 0 °C and overnight at room temperature.

For work up 16 ml of 2 M hydrochloric acid and subsequently 10 ml of water were added at 0 °C and the resulting solution was stirred for 5 minutes without further cooling. For the extraction of the product 20 ml of dichloromethane was added and stirring was continued. The dichloromethane was siphoned off and the extraction was repeated twice each with 10 ml of dichloromethane. The combined organic layers were washed five times each with 10 ml of water and dried over sodium sulphate. The dry solution was filtered over quartz wool and evaporated to dryness.

Yield: 4.158 g of III (as an oily residue), chemical purity (GC): $\geq$ 98 %, radiochemical purity (GC): $\geq$ 99 % (ROI).

The crude product was used without further purification in the next chemical step.

Ethyl 3-oxo-3-(2,4,5-trifluoro-3-methoxy-phenyl)-[2-^{14}C]propionate (IV)

To 4.158 g of compound III a solution of 60 mg of p-toluenesulfonic acid in 25 ml of water was added. The mixture was heated to reflux (bath temperature 120 °C) and

Copyright © 2000 John Wiley & Sons, Ltd.

stirred for 2 hours. Analysis of the reaction mixture by GC gave 12.7 % of the educt. So reflux was continued for a further 45 minutes. After cooling to room temperature 8 ml of saturated sodium bicarbonate solution was added and the product was extracted with 15 ml of dichloromethane. The extraction of the aqueous phase was repeated twice each with 10 ml of dichloromethane. The combined organic layers were washed with saturated sodium bicarbonate solution and subsequently twice with water. Then the organic layer was dried over sodium sulphate and evaporated to dryness under reduced pressure. The residue solidified during storage in a refrigerator overnight.

Yield: 3.223 g of compound IV, chemical purity (GC): $\geq$ 83 %, radiochemical purity (GC): $\geq$ 78 % (ROI).

The crude product was used without further purification in the next chemical step.

Ethyl 3-ethoxy-2-(2,4,5-trifluoro-3-methoxy-benzoyl)-[2-^{14}C]acrylate (V)

A mixture of 3.223 g of compound IV, 2.83 ml (17 mmol) of triethyl orthoformate and 8.5 ml of acetic anhydride was stirred for 4 hours at 140 °C. After cooling the volatiles were removed by distillation in vacuo (bath temperature 50 °C) and the desired product was obtained as an oily residue.

Yield: 3.826 g of compound V (as a brownish oily residue), chemical purity (GC): $\geq$ 91 %, radiochemical purity (GC): $\geq$ 92 %.

The crude product was used without further purification in the next chemical step.

Ethyl 3-cyclopropylamino-2-(2,4,5-trifluoro-3-methoxy-benzoyl)-[2-^{14}C]acrylate (VI)

The total amount of compound V (3.826 g) was dissolved in 40 ml of cyclohexane. The solution was cooled to 0 °C and 1.04 ml = 857 mg (15 mmol) of cyclopropylamine was added. After completion of the addition a small amount of non-labelled ethyl 3-cyclopropylamino-2-(2,4,5-trifluoro-3-methoxy-benzoyl)-acrylate was added as seed crystals. The mixture was cooled to 0 °C, and crystallisation of the product began. The suspension was stirred for 1 hour at 0 °C and overnight at room temperature. Most of the solid dissolved during this time. For purification the solution was decanted from a small amount of insoluble

Copyright © 2000 John Wiley & Sons, Ltd.

substance. Then 20 ml of petroleum benzine and an additional small amount of seed crystals were added. After stirring for 2 hours at 0 °C the solid was filtered off, washed with petroleum benzine and dried.

Yield: 1.674 g of compound VI (first fraction, light beige crystals), chemical purity (GC): ≥ 99 %.

To yield a second product fraction the volume of the mother liquor was reduced to approximately 10 ml by evaporation under reduced pressure. Then 5 ml of petroleum benzine and a small amount of seed crystals were added. In order to separate the non-crystalline substance, the supernatant was decanted and the residue was dissolved in 10 ml of cyclohexane. After the addition of petroleum benzine the resulting supernatant was decanted once more. The remaining residue was dissolved in 12 ml of cyclohexane with warming and 6 ml of petroleum benzine was added. Then seed crystals were added and the mixture was cooled quickly to 0 °C. After stirring for 30 minutes at 0 °C the mixture was stored overnight in a refrigerator. The solid was filtered off, washed with petroleum benzine and dried.

Yield: 0.397 g of compound VI (second fraction, light brownish crystals), chemical purity (GC): ≥ 82 %.

Both fractions were combined and used in the next chemical step.

Ethyl 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydro-[3-^{14}C]quinoline-3-carboxylate (VII)

The total amount of VI (2.07 g) was dissolved in 25 ml of dry N-methyl-2-pyrrolidinone. Powdered potassium carbonate (630 mg) was added and the mixture was stirred for 2 hours at 80 °C. A reaction control by HPLC showed an educt/product ratio of 88 : 12. So stirring was continued for 30 minutes at 100 °C. After cooling to room temperature the product partically crystallised. For completion of the precipitation 50 ml of water was added in portions. The resulting suspension was cooled to 0 °C and a further 15 ml of water was added. After stirring for 30 minutes at 0 °C the colourless solid was filtered off, washed with water and dried in a desiccator over blue gel. Because of the use of an aqueous sodium hydroxide solution in the next chemical step the product was not dried completely.

Yield: 2.346 g of compound VII (with residual moisture).

The substance was reacted without further drying in the next chemical step.

Copyright © 2000 John Wiley & Sons, Ltd.

1-Cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydro-[3-^{14}C]quinoline-3-carboxylic acid (VIII)

The ethyl ester VII (2.346 g with residual moisture) was suspended in 30 ml of methanol. Sodium hydroxide solution (1 M, 12 ml) was added and the mixture was stirred for 45 minutes at 50 °C. Then the resulting clear solution was cooled to room temperature (the sodium salt of the desired acid partically precipitated) and 7 ml of 2 M hydrochloric acid was added to liberate and precipitate the quinolone carboxylic acid VIII. For better stirring water was added and the suspension was stored overnight in a refrigerator. The colourless product was filtered off, washed with water and dried in a desiccator over blue gel.

Yield: 1.591 g of compound VIII, chemical purity (HPLC) $\geq$ 98 %, radiochemical purity (HPLC) $\geq$ 99 %.

1-Cyclopropyl-7-(S,S-2,8-diazabicyclo[4.3.0]non-8-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydro[3-^{14}C]quinoline-3-carboxylic acid hydrochloride, [^{14}C]moxifloxacin hydrochloride

During the following steps daylight was excluded.

The quinolone carboxylic acid VIII (1.591 g, 5.39 mmol) was suspended in 15 ml of dry acetonitrile. The hydrogen fluoride trapping agent 1,4-diazabicyclo[2.2.2]octane (DABCO, 0.907 g, 8.1 mmol)) and subsequently a solution of 0.85 g (6.75 mmol) of S,S-2,8-diazabicyclo[4.3.0]nonane IX (purity 98.5 %, enantiomeric excess 99.7 %) in 2 ml of dry acetonitrile were added. The mixture was heated to reflux and stirred for 2 hours at this temperature. A reaction control by HPLC showed a product/educt ratio of 75 : 25 (radio-detection). So reflux was continued for a further 1.5 hours. Then the solvent was removed under reduced pressure and the dark brownish residue was dissolved in 20 ml of water. For extraction of the betain form of the desired product 20 ml of dichloromethane was added and the solution was adjusted to pH 5-6 with 2 M hydrochloric acid (4.2 ml). The organic layer was separated and the extraction was repeated three times each with 8 ml of dichloromethane. The combined organic layers were dried over sodium sulphate, filtered over quartz wool and evaporated to dryness under reduced pressure.

Copyright © 2000 John Wiley & Sons, Ltd.

A dark brownish residue of 1.949 g was obtained. By HPLC analysis the by-product X was estimated to be present at the 30 % level.

For the formation of the desired product (hydrochloride) the total amount of the betain form (including the by-product) was suspended in 12 ml of ethanol/1 M HCl = 85:15. Then 1.3 ml of 25 % hydrochloric acid was added and the mixture was heated to reflux. Ethanol/1 M HCl = 85:15 (18 ml) was added in portions under reflux until a clear solution was obtained. A solid crystallised during cooling to room temperature. After 2 hours in a refrigerator the crystals were filtered off, washed with ethanol/water = 85:15 and dried in a desiccator over blue gel. A first product fraction of 1.007 g was obtained. The content of the by-product X was not reduced by this procedure.

To yield a second fraction of product the volume of the mother liquor was reduced until a pulpy residue was observed. This residue was dissolved by addition of about 20 ml of ethanol. For product crystallisation the solution was stored overnight in a refrigerator, the resulting crystals were filtered off and treated in the same manner as the first material. This second product fraction (0.322 g) was free of the by-product.

Separation from the by-product:

The total amount of the first product fraction (1.007 g) was suspended in 30 ml of water. The solubility of moxifloxacin hydrochloride in water is known to be 24 mg/ml, whereas the by-product X is almost insoluble in water. The mixture was stirred for 30 minutes at room temperature and filtered off from the solid. The solid (by-product X, purity by HPLC = 96 %) was washed three times each with 5 ml of water and the filtrate was evaporated to dryness under reduced pressure. The purity of the obtained labelled product was determined as 97 % by HPLC.

Final purification:

The crude [^{14}C]moxifloxacin hydrochloride of the separation and the second product fraction (0.322 g) of the hydrochloride formation were recrystallised from 30 ml of ethanol/1 M HCl = 95:5. After storage for 5 hours in a refrigerator the crystallised product was filtered off, washed with ethanol and dried in a desiccator over blue gel.

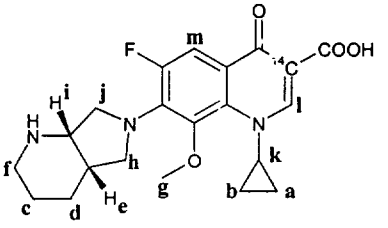
Yield: 0.729 g of [^{14}C]moxifloxacin hydrochloride (1.66 mmol), radiochemical purity (HPLC): $\geq$ 97 %, chemical purity (HPLC): $\geq$ 98 %.

Copyright © 2000 John Wiley & Sons, Ltd.

Determination of the specific radioactivity:

The specific radioactivity was determined by two different methods. A known amount of the labelled compound was weighed, dissolved and the total radioactivity was determined by LS counting. On the other hand the content of [^{14}C]moxifloxacin hydrochloride in a solution was determined by HPLC/UV quantification using non-labelled reference compound and the total radioactivity was also measured by LSC. By these two methods the specific activity was determined as 2.94 MBq/mg (1.286 GBq/mmol). As both methods gave similar values with a relative deviation of < 0.5 %, it is concluded, that the product crystallised free of water or solvent.

The total radioactivity of 2.143 GBq corresponds to a yield of 14.5 % of the theory, related to the starting diethyl [2-^{14}C]malonate.



NMR (DMSO-d_6) δ (ppm); 1.00 (m, 4H, **a+b**), 1.75 (m, 4H, **c+d**), 2.63 (s broad, 1H, **e**), 3.03 (m, 2H, **f**), 3.55 (s, 3H, **g**), 3.80 (multiplets, 6H, **h+i+j+k**), 7.68 (d, 1H, **m**), 8.65 (s, 1H, **l**), 9.70 (s broad, 1H, **NH**), 15.14 (s, 1H, **COOH**).

ACKNOWLEDGEMENT

We thank H. Schnorbus for performing the GC and HPLC analyses, Dr. P. Schmitt for performing the NMR analysis and Dr. W. Kanhai for performing the MS measurement. Thanks to Dr. P. Naab for providing the non-labelled intermediate IX.

REFERENCES

1. Dalhoff A., Petersen U. and Endermann R. – Chemotherapy 42 (1996) 410; Woodcook J.M., Andrews J.M., Boswell F.J., Brenwald N.P. and Wise R. – Antimicrobial Agents and Chemotherapy 41 (1997) 101; Boswell F.J., Andrews J.M and Wise R. - Antimicrobial Agents and Chemotherapy 41 (1997) 1377

Copyright © 2000 John Wiley & Sons, Ltd.

2. Kagemoto A., Negoro T., Nakao, M., Ochi T., Chiba K., Kataoka M. and Sekine Y. - Arzneim.-Forsch./Drug Res. 41 (1991) 744
3. Ekhato I.V. and Huang C.C. – J. Labelled Compd. Radiopharm. 33 (1993) 869
4. Yoshitake A., Makari Y. and Endo M. - J. Labelled Compd. Radiopharm. 10 (1974) 589
5. Harrison L.I., Schuppan D., Rohlfing S.R., Hansen A.R., Gerster J.F., Hansen C.S., Funk M.L. and Ober R.E. – Drug Metab. Dispos. 14 (1986) 555
6. Nagatsu Y. and Irikura T. - J. Labelled Compd. Radiopharm. 18 (1981) 1765
7. Kagemoto A., Negoro T., Nakao M., Sekine Y. and Hashimoto M. – Chemotherapy (Tokyo) 32 (1984) 147
8. Siefert H.-M., Maruhn D., Maul W., Foerster D. and Ritter W. - Arzneim.-Forsch./Drug Res. 36 (1986) 1496
9. Petersen U., Bremm K.-D., Dalhoff A. Endermann R., Heilmann W., Krebs A. et al. – Program and Abstracts of the Thirty-Sixth Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, 1996, Abstract F1

Copyright © 2000 John Wiley & Sons, Ltd.