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Research Article

A general method for the fluorine-18 labelling of fluoroquinolone antibiotics

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Summary

Fluoroquinolones are an important class of antibiotic agents with a broad spectrum of antibacterial activity. Labelling of fluoroquinolones with fluorine-18 is of interest for the performance of pharmacokinetic measurements and the visualization of bacterial infections in humans with positron emission tomography. A two-step radiosynthetic pathway to prepare fluorine-18-labelled ciprofloxacin (1-cyclopropyl-6-[^{18}F]fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic acid) has previously been developed. In the present work this approach was applied to the preparation of the structurally related compounds [^{18}F]norfloxacin (1-ethyl-6-[^{18}F]fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic acid) and [^{18}F]pefloxacin (1-ethyl-6-[^{18}F]fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-quinoline-3-carboxylic acid). The first step of the radiosynthesis consisted of a ^{18}F for ^{19}F exchange reaction on a 7-chloro-substituted precursor molecule, followed by coupling reactions with the amines piperazine or 1-methylpiperazine. Starting from 51–58 GBq of [^{18}F]fluoride 1.9–2.0 GBq of [^{18}F]norfloxacin or [^{18}F]pefloxacin, ready for intravenous injection, could be obtained in a synthesis time of 130 min (3.5–3.8% overall radiochemical yield). Moreover, the preparation of [^{18}F]levofloxacin ((-)-(*S*)-9-[^{18}F]fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic

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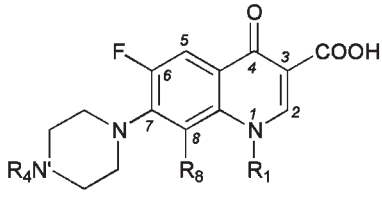
acid) was attempted but failed to afford the desired product in practical amounts.

Key Words: fluorine-18; PET; fluoroquinolone; norfloxacin; pefloxacin

Introduction

Fluoroquinolones are widely prescribed antibiotics that possess a broad spectrum of activity against Gram-negative and Gram-positive bacteria.[1] In recent years several fluoroquinolones (i.e. fleroxacin, trovafloxacin, lomefloxacin, levofloxacin and *N*-methyl ciprofloxacin) have been radiolabelled with the positron emitters fluorine-18 (^{18}F) or carbon-11 (^{11}C) and used for positron emission tomography (PET) studies in humans.[2–6] These studies were mainly aimed at measuring drug distribution within body tissues. Additionally, technetium-99m-labelled ciprofloxacin has been proposed as a single-photon emission tomography (SPET) imaging agent for the visualization of bacterial infections.[7,8]

The structure of most fluoroquinolones can be described by the general scheme depicted in Figure 1. [9] Whereas all representatives of this class of compounds are characterized by a carboxylic acid function in 3-, an oxo group in 4- and a fluoro substituent in 6-position, they differ with respect to the substituents in the 1-, 7- and 8-positions of the quinoline nucleus. Trovafloxacin ((1α,5α,6α)-7-(6-amino-3-azabicyclo[3.1.0]hex-3-yl)-1-(2,4-difluorophenyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthydrine-3-carboxylic acid) and lomefloxacin (1-ethyl-6,8-difluoro-7-(3-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid) are two fluoroquinolones that have previously been labelled with ^{18}F by direct nucleophilic ^{18}F for ^{19}F substitution on the



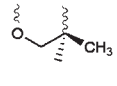
R_8	R_1	R_4'	compound
H	cyclopropyl	H	**1**, Ciprofloxacin
		CH_3	**2**, Levofloxacin
H	ethyl	H	**3**, Norfloxacin
H	ethyl	CH_3	**4**, Pefloxacin

Figure 1. General scheme for the structure of the fluoroquinolones discussed in the present work

unprotected target molecules themselves.[4,10] Both molecules possess several fluoro substituents and it has not been elucidated which of these has actually been substituted by ^{18}F. In our previous attempt to prepare ^{18}F-labelled ciprofloxacin (1-cyclopropyl-6-[^{18}F]fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic acid or [^{18}F]-**1**) we have observed that the molecule was inactivated for a direct nucleophilic exchange reaction. This prompted us to develop a novel two-step radiosynthetic approach towards [^{18}F]-**1** (Figure 2).[11] In the first step nucleophilic ^{18}F for ^{19}F exchange was performed on the 7-chloro derivative of ciprofloxacin (**5**) and the obtained ^{18}F-labelled intermediate [^{18}F]-**5** was subsequently transformed into the target molecule by a coupling reaction with piperazine.

The present work focused on applying the newly developed radiosynthetic approach to the ^{18}F labelling of other structurally related fluoroquinolones. Different commercially available halo derivatives of fluoroquinolones were first subjected to nucleophilic substitution reactions with [^{18}F]fluoride. The obtained ^{18}F-labelled molecules were then tested for their suitability to undergo coupling reactions with piperazine or 1-methylpiperazine.

Results and Discussion

Exchange reaction

For the previously described preparation of [^{18}F]ciprofloxacin ([^{18}F]-**1**) 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**5**) has been reacted with K[^{18}F]F-K_{222} for 40 min at 180°C to afford the ^{18}F-labelled derivative 7-chloro-1-cyclopropyl-6-[^{18}F]fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ([^{18}F]-**5**) in an average incorporation yield (based on K[^{18}F]F-K_{222}) of 23 $\pm$ 7% (Figure 2).[11]

In the present work, we performed exchange reactions with [^{18}F]fluoride on three different commercially available halo derivatives of fluoroquinolones (**6-8**, Figure 2). Table 1 gives an overview of the radiochemical yields obtained in these reactions.

The first compound was 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**6**), which was like compound **5** a precursor for ciprofloxacin (**1**) but differed from compound **5** with respect to a fluoro instead of a chloro substituent in the 7-position. Incorporation yields were determined by combined TLC and HPLC

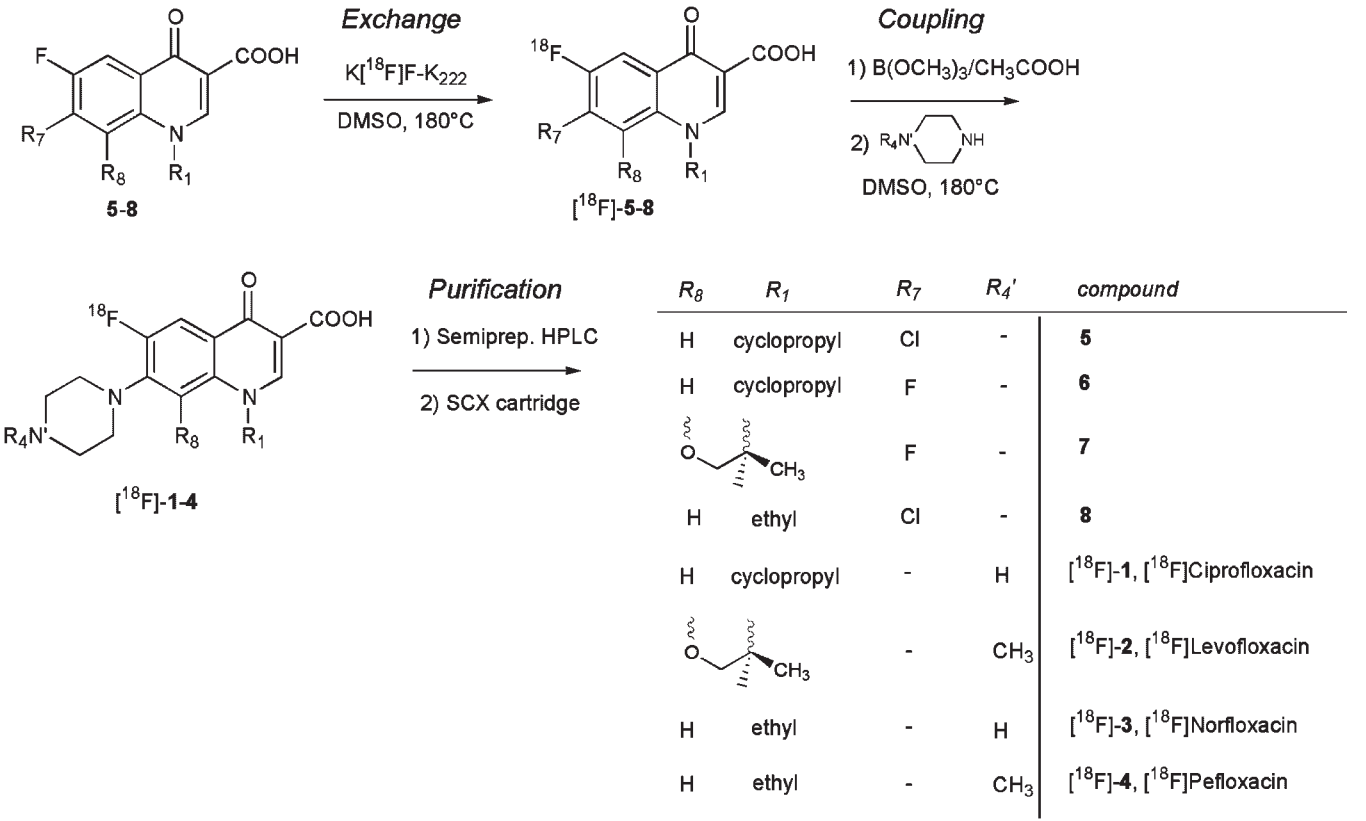


Figure 2. General scheme for the synthesis of the ^{18}F-labelled fluoroquinolones [^{18}F]ciprofloxacin ([^{18}F]-1), [^{18}F]levofloxacin ([^{18}F]-2), [^{18}F]norfloxacin ([^{18}F]-3) and [^{18}F]pefloxacin ([^{18}F]-4) via the ^{18}F-labelled halo derivatives [^{18}F]-5-8

Table 1. Incorporation yields (based on K[^{18}F]F-K_{222}) for the reaction of different halo derivatives of fluoroquinolones ([^{18}F]-5-8) with K[^{18}F]F-K_{222}

Compound	Conditions	Yield (%)	Number of experiments
[^{18}F]-**5**	180°C, 40 min	23 ± 7	11[11]
[^{18}F]-**6**	180°C, 20 min	78 ± 7	4
[^{18}F]-**7**	180°C, 20 min	59 ± 7	3
[^{18}F]-**8**	180°C, 40 min	29 ± 4	6

analysis, whereby TLC was used to estimate the amount of unreacted [^{18}F]fluoride in the reaction mixture. After 20 min heating at 180°C the ^{18}F-labelled compound [^{18}F]-**6** was obtained as the only radiolabelled product in an incorporation yield of 78 ± 7% ($n = 4$). This yield was about three-fold higher than for compound [^{18}F]-**5** (23%). Prolongation of the reaction time to 40 min did not further increase the yield. The remarkably higher electrophilicity of the aromatic ring in **6** as compared to **5** could probably be attributed to the stronger electron-withdrawing effect of fluorine compared to chlorine. The ^{18}F label in [^{18}F]-**6** could be located in either the 6- or 7-position of the aromatic ring.

The second compound tested for the exchange reaction was (-)-(*S*)-9,10-difluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (**7**), the 7-fluoro derivative of levofloxacin ((-)-(*S*)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid, **2**). Like compound **6** this derivative has two ortho-orientated fluoro substituents in the quinoline structure. Likewise, the [^{18}F]fluoride incorporation yield was significantly better than for the 6-fluoro-7-chloro-substituted derivative **5**. After 20 min heating at 180°C [^{18}F]-**7** was formed as the only radiolabelled product in a yield of 59 ± 7% (n = 3). Again, there were two possible positions for the ^{18}F label, namely the 6- and the 7-positions of the aromatic ring (according to the IUPAC nomenclature these positions should be referred to as 9 and 10, respectively).

The third compound was 7-chloro-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**8**), the 7-chloro derivative of norfloxacin (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic acid, **3**) and pefloxacin (1-ethyl-6-fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-quinoline-3-carboxylic acid, **4**). Compound **8** differs from **5** only with respect to an 1-ethyl instead of a cyclopropyl substituent. In the exchange reaction with K[^{18}F]F-K_{222} 7-chloro-1-ethyl-6-[^{18}F]fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid ([^{18}F]-**8**) was formed after 40 min heating at 180°C in a

yield of 29 ± 4% ($n = 6$). This yield was significantly lower than for the previous two compounds but in a similar range as the incorporation yield for [^{18}F]-**5** obtained in the synthesis of [^{18}F]ciprofloxacin ([^{18}F]-**1**). Four by-products, eluting each before [^{18}F]-**8** on the analytical reversed-phase HPLC column and each comprising less than 5% of the total radioactivity, were formed in the reaction. One of these could be suspected to be the product formed by ^{18}F substitution for chloro, by analogy to the previously published synthesis of [^{18}F]-**1**, where one of the by-products has been identified as the 7-[^{18}F]fluoro compound.[11]

Coupling reaction

For the previously reported synthesis of [^{18}F]ciprofloxacin ([^{18}F]-**1**) the ^{18}F-labelled 7-chloro derivative [^{18}F]-**5** was first transformed in situ into a boron complex by addition of trimethylborate ($B(OCH_3)_3$) and acetic acid to the reaction mixture and subsequently reacted with the amine piperazine for 40 min at 180°C to afford [^{18}F]-**1** in a conversion yield (based on [^{18}F]-**5**) of 49 ± 12% ($n = 8$).[11] Boron complex formation with $B(OCH_3)_3$ is based on a patent application by Ochi *et al*. and is believed to activate the molecule to nucleophilic attack at the 7-position.[12] The proposed structure of the boron complex of [^{18}F]-**5** is depicted in Figure 3. We have previously observed that omission of $B(OCH_3)_3$ resulted in about two-fold lower yields in the coupling reaction of [^{18}F]-**5** with piperazine.

In the present work the ^{18}F-labelled intermediates [^{18}F]-**6**-**8** were subjected to reactions with the amines piperazine or 1-methylpiperazine employing identical reaction conditions as for the synthesis of [^{18}F]ciprofloxacin (Figure 2).[11] Table 2 summarizes the conversion yields (based on the respective ^{18}F-labelled halo derivatives) obtained in these reactions. When compound [^{18}F]-**8** was reacted with piperazine or

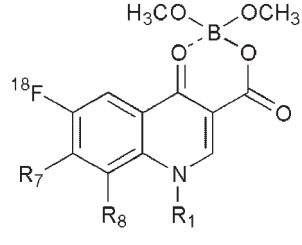


Figure 3. Proposed structure of the *in-situ* generated boron complexes of derivatives [^{18}F]-5-8. For substituents R_1, R_7 and R_8 refer to Figure 2

Table 2. Conversion yields (based on ([^{18}F]-5-8) for the coupling of different ^{18}F-labelled halo derivatives of fluoroquinolones ([^{18}F]-5-8) with piperazine (A) or 1-methylpiperazine (B) to yield the ^{18}F-labelled fluoroquinolones [^{18}F]-1-4

Compound	Amine	Yield (%)	Number of experiments
[^{18}F]-**5** → [^{18}F]-**1** or [^{18}F]ciprofloxacin	A	49 ± 12	8[11]
[^{18}F]-**6** → [^{18}F]-**1** or [^{18}F]ciprofloxacin	A	< 3	3
[^{18}F]-**7** → [^{18}F]-**2** or [^{18}F]levofloxacin	B	< 2	3
[^{18}F]-**8** → [^{18}F]-**3** or [^{18}F]norfloxacin	A	70 ± 14	4
[^{18}F]-**8** → [^{18}F]-**4** or [^{18}F]pefloxacin	B	53 ± 2	2

1-methylpiperazine for 40 min at 180°C the corresponding ^{18}F-labelled fluoroquinolones [^{18}F]norfloxacin ([^{18}F]-**3**) or [^{18}F]pefloxacin ([^{18}F]-**4**) were formed in conversion yields of 70 ± 14% ($n = 4$) and 53 ± 2% ($n = 2$), respectively. In both cases the starting compound [^{18}F]-**8** was almost quantitatively consumed (< 5% of total radioactivity). For both coupling reactions two to three unidentified by-products were observed, which eluted with retention times of about 14–15 min on the analytical reversed-phase column and comprised together about 15–25% of the total radioactivity.

When the ortho-difluoro-substituted compounds [^{18}F]-**6** and [^{18}F]-**7** were reacted with piperazine and 1-methylpiperazine, respectively, only very low amounts of the ^{18}F-labelled fluoroquinolones [^{18}F]ciprofloxacin ([^{18}F]-**1**) and [^{18}F]levofloxacin ([^{18}F]-**2**) were generated (< 2% of the total radioactivity). However, analysis of the UV track of the two-channel analytical HPLC system, revealed that at the same time considerable amounts of the respective unlabelled fluoroquinolones **1** and **2** were formed. Autoradiographic TLC analysis of the crude reaction mixture showed almost exclusively one radioactive spot with a retention factor of 0. A possible explanation for these observations and the failure to obtain a [^{18}F]fluoroquinolone by this approach was that the ^{18}F label was located for the two 6,7-difluoro-substituted compounds [^{18}F]-**6** and [^{18}F]-**7** in 7-position of the quinoline structure (i.e. the leaving group for amine substitution) instead of the desired 6-position (Figure 2). Hence, the coupling reaction with the amine might have led to a loss of the ^{18}F label and the generation of free [^{18}F]fluoride as the only radiolabelled product.

Purification and formulation

The ^{18}F-labelled fluoroquinolones [^{18}F]norfloxacin ([^{18}F]-**3**) and [^{18}F]pefloxacin ([^{18}F]-**4**) were purified by combined HPLC and solid-phase

extraction. On the semipreparative HPLC system the elution profiles of [^{18}F]-**3** and [^{18}F]-**4** were rather broad (from about 12 to 22 min), which corresponded to volumes of up to 30 ml. Figure 4 shows semi-preparative HPLC chromatograms for the purification of [^{18}F]-**3** and [^{18}F]-**4**. The product fractions from HPLC were passed on-line over a disposable strong cation exchange (SCX) cartridge. The respective [^{18}F]fluoroquinolones were almost quantitatively concentrated on the cartridge and subsequently eluted with aqueous sodium hydroxide solution (3 ml) into a sterile vial containing phosphate buffer for adjustment of pH and osmolality. By this method the final product could be obtained in a rather small volume of phosphate buffer (7 ml), which was well suited for administration of intravenous bolus injections in humans.

Preparative syntheses

Starting from 57.6 GBq [^{18}F]fluoride 2.0 GBq of [^{18}F]-**3**, readily formulated for intravenous injection, could be obtained in a total synthesis time of 130 min. In the case of [^{18}F]-**4** 50.5 GBq of [^{18}F]fluoride yielded 1.9 GBq of the product in an equally long synthesis time. The total radiochemical yields for [^{18}F]-**3** and [^{18}F]-**4** were 3.5% and 3.8%, respectively, which was slightly higher than

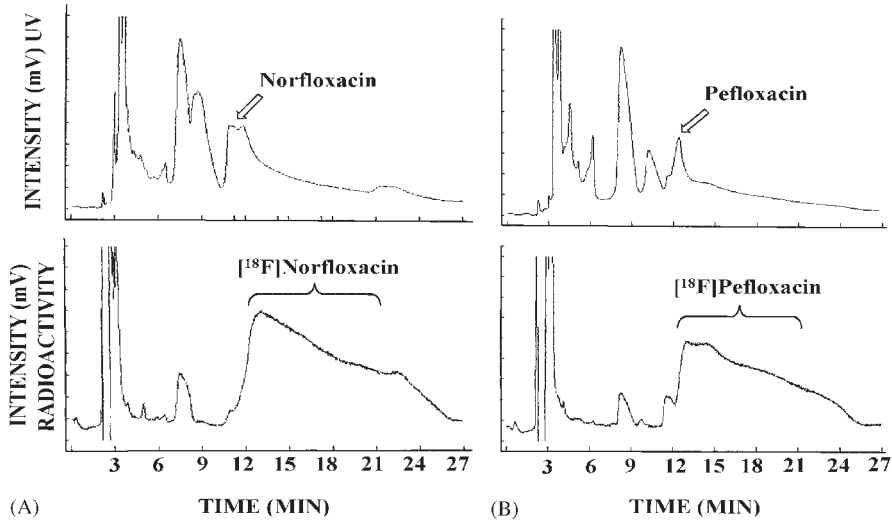


Figure 4. Semi-preparative HPLC chromatograms for the purification of [^{18}F]norfloxacin (A) and [^{18}F]pefloxacin (B) [column: Waters μBondapak C18 (300 mm × 7.8 mm, 10 μm); mobile phase: 10 mM aqueous H_3PO_4/EtOH 90/10; flow rate: 3–6 ml/min; UV detection at 380 nm].

previously reported for [^{18}F]ciprofloxacin (2.5%).[11] Both compounds co-eluted on analytical HPLC with the respective unlabelled reference molecules. The radiochemical purity of both products was better than 99% and the chemical purity exceeded 97%. The specific radioactivity at the end of synthesis was 909 MBq/μmol for [^{18}F]-**3** and 339 MBq/μmol for [^{18}F]-**4**, respectively. These values were considerably lower than those usually obtained in no-carrier-added syntheses. However, for the prospective applications of these compounds (pharmacokinetic measurements[2] and/or infection imaging) high specific radioactivity is not mandatory. The pH of the product solution ranged from 5.0 to 5.5 and the osmolality was 230-240 mosm/kg. Moreover, analysis of the formulated product with the European Pharmacopoeia semiquantitative TLC assay revealed that the Kryptofix 2.2.2 content was below the limit of detection of the assay (<10 μg/ml).[13]

Synthesis of other ^{18}F-labelled fluoroquinolones

The choice of compounds prepared in the present work was mainly based on the commercial availability of the intermediates needed for the preparation. The synthesised [^{18}F]fluoroquinolones belong to the first generation of fluoroquinolones. The preparation of newer-generation ^{18}F-labelled fluoroquinolones, such as gatifloxacin (1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid) or moxifloxacin (1-cyclopropyl-7-(*S*,*S*-2,8-diazabicyclo[4.3.0]non-8-yl)-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid) by the present pathway should be possible in principle. However, since the corresponding 6-fluoro-7-chloro substituted precursor molecules and/or cyclic amines were commercially not available, their preparation was not attempted.

Experimental

General

Chemicals. Ciprofloxacin hydrochloride was provided by Bayer AG (Wuppertal, Germany). 7-Chloro-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid was purchased from ABCR GmbH (Karlsruhe, Germany). Pefloxacin (1-ethyl-6-fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-quinoline-3-carboxylic acid) methane sulfonate dihydrate was purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany). All other chemicals were obtained from Sigma-Aldrich

Chemie GmbH (Schnelldorf, Germany) or Merck (Darmstadt, Germany) and used without further purification.

Aqueous [^{18}F]fluoride was produced in a General Electrics PETtrace cyclotron (General Electrics, USA) via the ^{18}O(p,n)^{18}F nuclear reaction by irradiation of a 1.5 ml water target containing 95.9% enriched [^{18}O]water (Hyox^{18}, Rotem Industries, Beer Sheva, Israel) with a 16.5 MeV proton beam. Typically, a 60 min irradiation with a beam current of 35 μA yielded 60–65 GBq of [^{18}F]fluoride.

Analytical procedures. Analytical high performance liquid chromatography (HPLC) was performed on a system consisting of a Rheodyne 7725i injector (Rheodyne, Rohnert Park, USA) equipped with a Rheodyne 20 μl sample loop, a Merck Hitachi D-6000 interface (Hitachi High Technologies, San Jose, USA) and a Merck Hitachi L-6200A pump. For detection a Merck Hitachi L-4000 UV detector (wavelength: 280 nm) in series with a Packard Radiomatic Flo-one Beta Flow scintillation analyser (PerkinElmer Life Sciences Inc., Boston, USA) were employed. A Waters μBondapak C18 column (300 mm × 3.9 mm, 10 μm) (Waters Corporation, Milford, USA) was eluted with a mixture of 10 mM aqueous phosphoric acid (A) and absolute ethanol (B) at a flow rate of 2 ml/min. The following binary gradient time programs were used: 'Exchange': 0–4 min, (A:B, v:v) 70:30 isocratic; 4–9 min, (A:B) 70:30–40:60; 9–14 min, (A:B) 40:60 isocratic; 14–15 min, (A:B) 40:60–70:30; 15–17 min, (A:B) 70:30 isocratic. 'Coupling': 0–5 min, (A:B, v:v) 87:13 isocratic; 5–10 min, (A:B) 87:13–75:25; 10–12 min, (A:B) 75:25–50:50; 12–17 min, (A:B) 50:50 isocratic; 17–18 min, (A:B) 50:50–87:13; 18–20 min, (A:B) 87:13 isocratic.

Data were collected on the Merck Hitachi D-7000 chromatography data station software.

The specific radioactivity of ^{18}F-labelled fluoroquinolones was estimated by comparing the UV absorption on analytical HPLC (method 'Coupling') of known amounts of the unlabelled reference molecules with that of aliquots of the formulated tracer solutions.

For thin layer chromatography (TLC) analysis Merck silica gel 60 F_{254} TLC aluminium sheets (layer thickness: 0.2 mm) were used with dichloromethane/methanol/trifluoroacetic acid 70/30/1 (v/v/v) as mobile phase. UV detection was performed using a standard UV lamp at a wavelength of 350 nm. For analysis of radioactive spots a Berthold digital autoradiograph LB 286-20 (Berthold Australia Pty Ltd., Bundoora, Australia) was used.

The analysis of the Kryptofix 2.2.2 content in the formulated [^{18}F]fluoroquinolone preparations was performed according to the European Pharmacopoeia TLC assay (silica gel 60 F_{254} sheets, methanol/aqueous ammonia 90/10 v/v, detection: iodine chamber).

Osmolality (in milliosmol per kilogram, mosm/kg) of the [^{18}F]fluoroquinolone preparations was determined using a Vapro Vapor Pressure Osmometer 5520 (Wescor Inc., Logan, USA). The pH was measured with a WTW pH 526 pH meter (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany).

Radiochemistry

General procedure for the nucleophilic exchange reaction on halo derivatives of fluoroquinolones. Aqueous [^{18}F]fluoride ion from the cyclotron target was inserted into a 3 ml Wheaton V-vial (Wheaton Science Products, Millville, USA) containing acetonitrile (0.1 ml), Kryptofix 2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8] hexacosane, 12.0 mg, 32.7 μmol) and potassium carbonate (4.0 mg, 28.9 μmol). The mixture was brought to dryness at 180°C under a stream of nitrogen. The vial was then cooled in an ethanol bath for 30 s, acetonitrile (0.7 ml) was added and the mixture concentrated to dryness at 180°C under a stream of nitrogen. To the dried K[^{18}F]F-K_{222} complex the halo-substituted fluoroquinolone derivative (20–25 μmol) dissolved in dimethyl sulfoxide (DMSO, 0.3 ml) was added and the resulting solution stirred at 180°C for 40 min. An aliquot of the reaction mixture was analysed by HPLC and TLC : HPLC (method 'Exchange'): Retention time (R_t) ([^{18}F]-**6**) 8.5–9.0 min; R_t ([^{18}F]-**7** and [^{18}F]-**8**) 9.5–10.0 min; TLC: Retention factor (R_f) ([^{18}F]-**6**-**8**) 0.50–0.60; R_f ([^{18}F]fluoride) 0.0.

General procedure for the coupling reaction between ^{18}F-labelled halo derivatives of fluoroquinolones and piperazine derivatives. After the exchange the reaction solution was cooled in an ethanol bath for 1 min. Then a mixture of trimethylborate ($B(OCH_3)_3$, 20 μl, 178 μmol) and acetic acid (20 μl, 350 μmol) in DMSO (0.1 ml) was added and the solution stirred at room temperature for 2 min. Then the piperazine derivative (250–350 μmol) dissolved in DMSO (0.2 ml) was added and the reaction mixture stirred at 180°C for 40 min. The vial was then cooled in an ethanol bath for 1 min and mobile phase for semi-preparative HPLC (0.9 ml) was added. An aliquot of the solution was analysed by HPLC and TLC:

HPLC (method 'Coupling'): R_t ([^{18}F]-**6-8**) 16.0–17.0 min; R_t ([^{18}F]-**1**) 6.0–6.5 min; R_t ([^{18}F]-**2**) 5.5–6.0 min; R_t ([^{18}F]-**3**) 5.5–6.0 min; R_t ([^{18}F]-**4**) 6.5–7.0 min; TLC: R_f ([^{18}F]-**1-4**) 0.20–0.30.

General procedure for the purification and formulation of ^{18}F-labelled fluoroquinolones. The purification was performed on a semi-preparative HPLC system consisting of a Rheodyne 7010 titanium injector equipped with a Rheodyne 2 ml sample loop mounted on a Besta motor valve (Besta-Technik GmbH, Wilhelmsfeld, Germany), a Jasco 880-PU HPLC pump (Jasco Corporation, Tokyo, Japan) and a Jasco 875-UV detector (wavelength: 380 nm) in series with a Berthold LB508 C-1 radioactivity detector (Berthold Australia Pty Ltd., Bundoora, Australia). A Waters μBondapak C18 column (300 mm × 7.8 mm, 10 μm) was isocratically eluted with a mixture of 10 mM aqueous phosphoric acid and absolute ethanol (90/10, v/v) at a flow rate of 6 ml/min. Data were collected on an Axxiom Chromatography 747 chromatography data system (Axxiom Chromatography Inc., Moorpark, USA).

The crude product was siphoned from the reaction vial into the sample loop via standard tubing and a disposable 5 ml syringe and injected on to the HPLC system. The product fraction (about 30 ml) was passed on-line over a strong cation exchange cartridge (Isolute 100 mg SCX, International Sorbent Technology Ltd., Hengoed, UK), whereby the flow rate was reduced from 6 to 3 ml/min. Prior to use the cartridge had been pre-washed with absolute ethanol (5 ml) and mobile phase for semi-preparative HPLC (10 ml). The radiolabelled fluoroquinolone was retained on the cartridge. The cartridge was dried with an air stream and eluted with 0.1 M aqueous sodium hydroxide solution (3.0 ml) into a sterile 11 ml TechneVial (Mallinckrodt Medical B.V., Petten, The Netherlands), which contained 0.20 M aqueous phosphate buffer (5.0 ml, pH 3.0–3.5).

The product mixture was then homogenized and filtered through a vented Millex-GS filter (0.22 μm, Millipore Corporation, Bedford, USA) into a sterile 11 ml TechneVial. An aliquot of the formulated product solution was analysed by HPLC (method 'Coupling') and TLC (retention times and retention factors see above).

Conclusion

A two-step radiosynthetic approach initially developed for the synthesis of [^{18}F]ciprofloxacin was applied to the preparation of [^{18}F]norfloxacin

and [^{18}F]pefloxacin. These compounds were obtained in sufficiently high radioactivity (about 2 GBq at the end of synthesis) for PET studies in humans. The obtained results suggested that 6-fluoro-7-chloro-substituted precursor molecules were better-suited substrates for the present synthetic method than 6,7-difluoro-substituted compounds. Although the latter gave higher yields in the initial exchange steps they failed to afford practical amounts of the corresponding [^{18}F]fluoroquinolones in the subsequent coupling reactions.

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