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Research Article

Synthesis of an ^{18}F-fluorobenzoate idarubicin derivative as new potential PET radiotracer to predict chemotherapy resistance

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Summary

Anthracyclines are among the most widely used antineoplastic agents in current clinical practice. Nevertheless, chemoresistance, which results in failure to eradicate the tumor, is often observed after administration of anthracyclines, and no assay system has yet been found to accurately predict tumor resistance to those antitumor agents. We sought to prepare an F-18 labeled derivative of idarubicin, a 4-demethoxy-daunorubicin analogue, to use in helping to assess physiologic resistance to anthracyclines *in vivo*. Two different synthetic pathways, which required the preparation of the key intermediate [^{18}F]fluorobenzoic acid ([^{18}F]FBA), are advanced to label idarubicin with F-18 on its primary amine. The first approach yielded the desired [^{18}F]fluorobenzoate idarubicin derivative in two steps from [^{18}F]FBA, while the second strategy consisted of a direct acylation of idarubicin by treatment with [^{18}F]FBA in presence of diethyl cyanophosphonate. Although the first method led to fewer byproducts, it required more time to obtain the HPLC-purified radiopharmaceutical (100 min vs 90 min) and resulted in lower radiochemical yields (8–25% vs 25–39% decay corrected from starting fluoride). Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: chemotherapy; anthracyclines; fluorine-18; positron emission tomography

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Introduction

Several radiolabeled chemotherapeutics have been advanced in the past to allow *in vivo* prediction of therapeutic efficacy prior to treatment.[1–4] Positron emitter labeled derivatives of antitumor agents commonly used in clinical practice have been examined in this context.[5–8] We have recently explored the possibility of predicting breast cancer therapy *in vivo* with fluorine-18 labeled analogs of paclitaxel and cyclophosphamide.[9,10] Anthracycline glycosides are among the most widely used antineoplastic agents in current clinical practice. Doxorubicin and daunorubicin are natural glycosides whose antitumor activity against a relatively broad spectrum of human cancers was demonstrated in the 1960s, while epirubicin and idarubicin (4-demethoxy-daunorubicin analogue, **1**) are semisynthetic analogues endowed with different pharmacological and toxicological profiles (Figure 1).[11] Despite their extensive clinical use, their specific mechanism of action has never been well established, but anthracyclines are considered likely to interact with topoisomerase II by stabilization of the ternary DNA–enzyme–drug complex, which hinders the relegation of the DNA strands and ultimately induces irreversible DNA breaks.[12] This interaction between the drug and the enzyme leads to delay in cell cycle progression and eventually apoptosis.

Idarubicin has been reported to be a useful alternative to other anthracyclines due to its therapeutic efficacy against a wide range of human tumors and its reduced cardiotoxicity.[11] Nevertheless, chemoresistance, which results in failure to eradicate the tumor, is often observed after administration of idarubicin. However, no assay system has yet been found to accurately predict tumor resistance to anthracyclines. Therefore, the opportunity to radiolabel idarubicin with a positron emitter would provide an experimental tool to assess drug concentration in the tumor tissue and to monitor anthracycline based therapy. Previous studies reported an iodine-124 labeled doxorubicin derivative and a carbon-11 analogue of daunorubicin. However, so far those imaging agents have not been extensively evaluated for clinical purposes.[10,13–17]

We sought to prepare an F-18 labeled derivative of idarubicin to use in helping to assess physiologic resistance to anthracyclines *in vivo*, and thus to

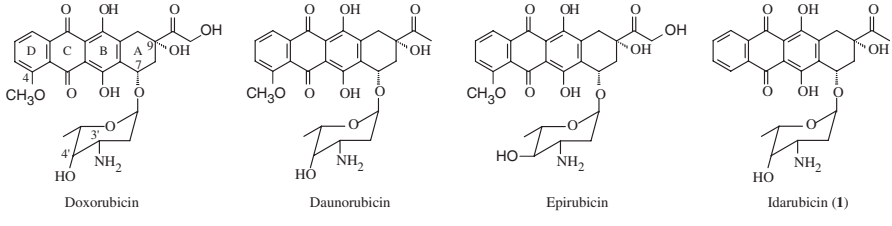


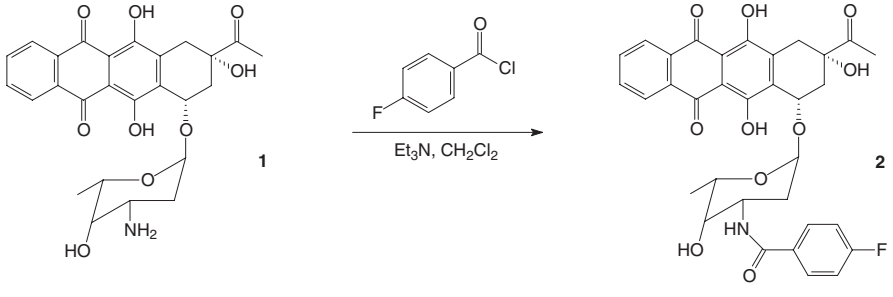
Figure 1. Molecular structures of the clinically active anthracyclines

prevent those patients who would not respond to idarubicin from suffering toxicities of that chemotherapy regimen without clinical benefit, and possibly delay the initiation of a more effective regimen. We herein report the synthesis of (8S,10S)-9-acetyl-7-[[3-[(4-fluorobenzoyl)amino]-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl]oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-5,12-naphthacenedione (**2**), as well as the radiolabeling of idarubicin on its primary amine from [^{18}F]fluorobenzoic acid.

Results and discussion

Chemistry

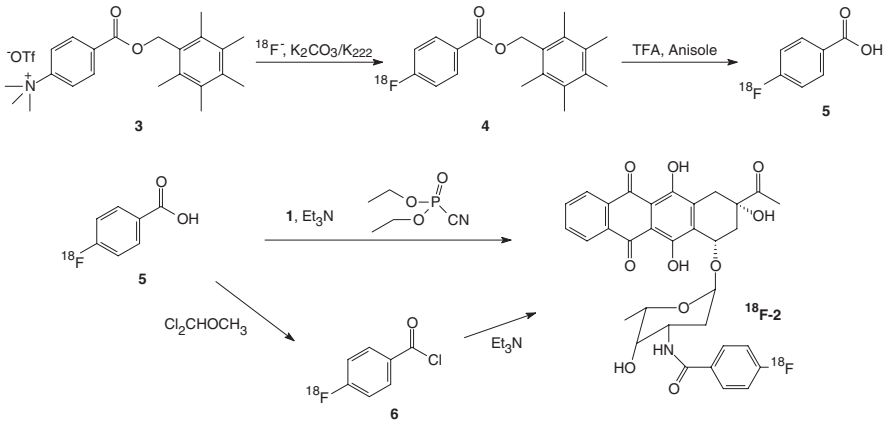
We synthesized the fluorobenzoate idarubicin derivative **2** by treatment of idarubicin hydrochloride **1** with 4-[^{19}F]fluorobenzoyl chloride in presence of triethylamine in CH_3CN. The reaction afforded the desired amide **2** in 96% yield (Scheme 1). The regioselectivity of the coupling was assigned from the signals in the ^{1}H NMR. The amide proton gives a characteristic broad singlet at 5.26 ppm, and two additional groups of two aromatic protons corresponding to the fluorobenzoate substituent were observed. Identity of the product was also confirmed by mass spectrometry.



Scheme 1. Synthesis of the cold reference 2, (8S,10S)-9-acetyl-7-[[3-[(4-fluorobenzoyl)amino]-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl]oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-5,12-naphthacenedione

Radiochemistry

Two different synthetic pathways, which required the preparation of the key intermediate [^{18}F]fluorobenzoic acid ([^{18}F]FBA; **5**) are advanced to label idarubicin with the positron emitter F-18 on its primary amine (Scheme 2). Preparation of [^{18}F]FBA was achieved in two steps from the pentamethylbenzyl 4-trimethylammoniumbenzoate precursor: (1) incorporation of [^{18}F]fluoride into the activated trimethylammonium triflate salt, followed by



Scheme 2. Synthesis of [^{18}F]fluorobenzoate idarubicin derivative ([^{18}F]-2)

Table 1. Preparation of [^{18}F]fluorobenzoic acid ([^{18}F]FBA; 5)

	Solvent	Temperature	Time (min)	RCY (%) (decay corrected)
Step 1	CH_3CN	110°C	10	13–27 ($n = 6$)
	DMSO	140–150°C	10	54–59 ($n = 4$)
	DMSO	140–150°C	15	30–42 ($n = 3$)
Step 2	TFA	RT	2	50–77 ($n = 10$)

(2) cleavage of the ester. A pentamethylbenzyl 4-trimethylammoniumbenzoate triflate salt (**3**) was chosen to prepare [^{18}F]FBA, since it was extensively used in our laboratory to synthesize an F-18 analog of paclitaxel, as described earlier by Kiesewetter *et al.*[18]

The first radiochemical step consisted of the introduction of the fluorine-18 using a no-carrier-added nucleophilic substitution with K[^{18}F]F-K_{222} (K_{222}: Kryptofix [2.2.2]; 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) and was initially performed in acetonitrile (non-stirred sealed V-vial placed in a heating block at 110°C for 10 min), leading to relatively low radiochemical yields (Table 1). However, the multi-step radiosynthesis approaches proposed here to radiolabel idarubicin require optimized reaction conditions, high yields for each step and short-time reactions compatible with the short half-life of the radioisotope concerned ($t_{1/2} = 109.8$ min). Therefore, we investigated whether we could improve the nucleophilic F-18 exchange reaction with the trimethyl ammonium triflate precursor, by changing the reaction conditions (solvent, temperature, and time). Incorporation of fluorine-18 into the trimethylammoniumbenzoate precursor was significantly increased by using DMSO instead of CH_3CN, since the reaction could be performed at higher temperature (Table 1).

Radiochemical yields were, with respect to initial [^{18}F]fluoride: 54–59% decay corrected when the reaction mixture was heated in DMSO for 10 min at 140–150°C vs 13–27% in CH_3CN at 110°C. However, a longer reaction time led to a decreased yield, probably due to a rapid degradation of the fluorinated compound **4** in hot DMSO.

Treatment of **4** in trifluoroacetic acid (TFA) resulted in the cleavage of the ester to yield [^{18}F]FBA. Although the hydrolysis was quantitative and occurred cleanly in 2 min at room temperature, we observed loss of activity during the subsequent evaporation of the TFA. Therefore, the decay corrected radiochemical yields reported for this step were only 50–77% (Table 1). Typically, starting from a 1.6–1.8 GBq (42–49 mCi) aliquot of a cyclotron [^{18}F]F^- batch, 0.5 GBq (13–14 mCi) of [^{18}F]FBA (**5**) could be obtained in 50–55 min.

Two different strategies were elaborated to radiolabel idarubicin from [^{18}F]fluorobenzoic acid. The first approach allowed us to obtain the final F-18 labeled compound in two steps from [^{18}F]FBA and was developed to mimic the synthesis of the unlabeled compound **2**. [^{18}F]FBA was converted into its corresponding acyl chloride **6** by treatment with α,α-dichloromethyl methyl ether. Subsequent coupling of 4-[^{18}F]fluorobenzoyl chloride (**6**) with idarubicin in presence of triethylamine afforded, after HPLC purification, [^{18}F]-**2** in 8–25% (decay corrected and based on starting aliquot of cyclotron-produce [^{18}F]fluoride, $n = 5$). The average total synthesis time was about 100 min and identity of the new radiopharmaceutical was confirmed by comparing its HPLC mobility upon co-injection with authentic non-radioactive analogue. The second strategy provided the desired [^{18}F]fluorobenzoate idarubicin derivative by direct acylation, in only one step from [^{18}F]FBA. Idarubicin was treated with [^{18}F]fluorobenzoic acid ([^{18}F]FBA; **5**) in presence of triethylamine and diethyl cyanophosphonate at 110°C for 10 min. According to this procedure, pure [^{18}F]-**2** was obtained in about 90 min and the radiochemical yields, based on starting [^{18}F]fluoride, were 25–39% (decay corrected, $n = 5$).

Although the first method had the general advantage of leading to fewer radioactive byproducts, as seen by HPLC, it required more time and resulted in significantly lower radiochemical yields. The labeling intermediate used in the first approach, 4-[^{18}F]fluorobenzoyl chloride, is known to be volatile and therefore loss of activity occurred by co-evaporation of **6** during the removal of the excess of chlorinating reagent. Furthermore, the high sensitivity of 4-[^{18}F]fluorobenzoyl chloride (**6**) to hydrolysis could impair the efficiency of the coupling reaction, resulting in relatively low radiochemical yields obtained with the two-step procedure. Also, we observed that the additional radiochemical step included in the first method significantly increased our radiation exposure.

Experimental

General

All reagents, including anhydrous solvents, were purchased from Aldrich, Sigma and Acros, and were used without further purifications. The pentamethylbenzyl 4-trimethylammoniumbenzoate triflate precursor (**3**) was synthesized as previously described by Lang *et al.*[19] Analytical thin layer chromatography (TLC) was performed on Sigma–Aldrich silica gel pre-coated plastic sheets with fluorescent indicator (UV 254). Visualization was achieved with short-wave ultraviolet light. Column chromatography was performed using silica gel (60–200 mesh). High performance liquid chromatography (HPLC) was carried out on an Agilent 1100 Series system. HPLC eluates were monitored for their UV absorbance at 254 nm, and their radioactivity content by connecting the outlet of the UV-photometer to a NaI detector. The recorded data were processed by an Agilent ChemStation software system. ^{18}F-activity was also measured with an ion chamber Capintec CRC11. The HPLC column used for the characterization of the product against standards and for the purification of radioactive compounds was a Phenomenex Luna-C18 (5 µm, 250 mm × 10 mm) column, operated at a flow rate of 2 ml/min (linear gradient over 20 min of 50–95% CH_3CN in water containing 0.5% AcOH followed by 10 min isocratic elution with 95% CH_3CN). ^{1}H NMR spectrum was taken in chloroform-d on a Bruker AM360/Wb spectrometer (at 360 MHz) using Me_4Si as an internal standard, and selected proton resonances are reported. Chemical shifts are expressed in ppm (δ) relative to the standard and coupling constants (*J*) in Hz. Mass spectrum was obtained on MALDI-TOF instrument (ABI DE STR).

Chemistry

*Synthesis of (8S,10S)-9-acetyl-7-[[3-[(4-fluorobenzoyl)amino]-2,3,6-trideoxy-α-*L*-lyxo-hexopyranosyl]oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-5,12-naphthacenedione (**2**).* Idarubicin hydrochloride (**1**) (10 mg, 19 µmol) in CH_3CN (3 ml) was treated with 4-fluorobenzoyl chloride (2.5 µl, 21 µmol) and triethylamine (3 µl, 22 µmol) at room temperature for 30 min. The reaction mixture was concentrated, poured onto a column of silica and was eluted with CH_2Cl_2/MeOH (19/1). Evaporation of the fractions containing the expected product led to **2** (11.5 mg, 96%) as a red solid. ^{1}H NMR (360 MHz, $CDCl_3$): δ 13.60 (s, 1 H), 13.33 (s, 1 H), 8.34 (m, 2 H), 7.83 (m, 2 H), 7.72 (m, 2 H), 7.06 (t, J = 8.4 Hz, 2 H), 6.49 (d, J = 8.3 Hz, 1 H), 5.52 (d, J = 3.6 Hz, 1 H), 5.26 (brs, 1 H), 4.45–4.25 (m, 2 H), 3.74 (brs, 1 H), 3.29 (d, J = 18.7 Hz, 1 H), 2.98 (d, J = 18.7 Hz, 1 H), 2.44 (s, 3 H), 2.36 (m, 1 H), 2.14 (dd, J = 4.1 and 15.1 Hz, 1 H), 2.04 (dd, J = 5 and 14 Hz, 1 H), 1.87 (dt, J = 4.3 and 13 Hz, 1 H), 1.32 (d, J = 6.5 Hz, 3 H). MS (MALDI-TOF) m/z 642.17 $[M + Na]^+$. HRMS

(MALDI-TOF) m/z: $[M+Na]^+$ calculated for $C_{33}H_{30}NO_{10}FNa^+$, 642.1746; found, 642.1772.

Radiochemistry

Preparation of K[^{18}F]F-K$_{222}$ complex. A 5 ml V-vial was loaded with 0.5 ml of a CH_3CN/H_2O solution of potassium carbonate (0.5 mg; 3 μmol) and Kryptofix [2.2.2] (K_{222}; 2.25 mg; 6 μmol). Fluorine-18 in water was added and the V-vial was gently heated at 110°C. Water was removed under a stream of argon by azeotropic distillation with acetonitrile (3 × 0.5 ml) to give the no-carrier-added K[^{18}F]F-K_{222} complex as a white semi-solid residue.

General procedure for preparation of fluorine-18-labeled 4-fluorobenzoic acid. A solution of pentamethylbenzyl 4-trimethylammoniumbenzoate triflate (**3**, 3 mg; 6 μmol) in anhydrous acetonitrile or dimethyl sulfoxide (0.3 ml) was added into the vial containing dry cryptate (K[^{18}F]F-K_{222}) and heated for 10 min, respectively at 110°C and 140–150°C (Scheme 2, Table 1). After cooling the acetonitrile solution was diluted with ether (0.2 ml) and transferred onto a silica Sep-Pak. The V-vial was subsequently washed twice with ether (0.3 ml), used to elute **4** from the short silica gel column. If DMSO was used instead of CH_3CN, the reaction mixture was poured into water (8 ml), and the aqueous solution was passed through a C18 Sep-Pak. Compound **4** was then eluted through the column with ether (2.5 ml). The ethereal fraction containing **4** was subsequently evaporated under a stream of argon. Anisole (5 μl) was added and the intermediate **4** was treated with trifluoroacetic acid (100 μl) for 2 min at room temperature. Finally, the volatiles were evaporated under an argon stream to yield [^{18}F]fluorobenzoic acid ([^{18}F]FBA; **5**). Typically, 0.5 GBq (13–14 mCi) of [^{18}F]FBA (**5**) could be obtained in 50–55 min starting from 1.6–1.8 GBq (42–49 mCi) aliquot of a cyclotron-produced [^{18}F]F- batch (overall radiochemical yields, based on starting [^{18}F]fluoride: 39–45% decay corrected).

*Preparation of the fluorine-18-labeled fluorobenzoate idarubicin derivative ([^{18}F]-**2**) – Two steps approach*. [^{18}F]fluorobenzoic acid was treated with α,α-dichloromethyl methyl ether (50 μl, 0.55 mmol) at 110°C for 5 min. The vial was then cooled in an ice bath (2–3 min) and the excess of chlorinating reagent was evaporated under an argon stream while the reaction vessel was maintained in the ice bath. A solution of idarubicin (1 mg, 2 μmol) in CH_3CN (0.4 ml) and iPr_2NEt (5 μl) were added to the residue and the solution was stirred at room temperature for 10 min.The solution was diluted with water containing 0.5% AcOH (0.4 ml) and the reaction mixture was injected onto a reverse phase HPLC column (Phenomenex Luna C-18, 10 × 250 mm, 5 μm). Retention time of the fluorine-18-labeled fluorobenzoate idarubicin derivative

[^{18}F]-**2** was 21 min vs 5 min for the starting material **1** under the same elution conditions. Typically, 0.07–0.2 GBq (2–6 mCi) of pure [^{18}F]-**2** could be obtained in 45–50 min starting from 0.5 GBq (13–14 mCi) of [^{18}F]fluorobenzoic acid (overall radiochemical yields, based on starting [^{18}F]fluorobenzoic acid: 21–57% decay corrected). Specific activity, determined by using on-line measurements of radioactivity and UV absorption, was >37 GBq/μmol (>1000 Ci/mmol).

*Preparation of the fluorine-18-labeled fluorobenzoate idarubicin derivative ([^{18}F]-**2**) – One step approach.* Idarubicin (1 mg, 2 μmol) in CH_3CN (0.4 ml), diethyl cyanophosphonate (1 mg, 6 μmol) in CH_3CN (50 μl), and iPr_2NEt (10 μl) were added to the dried [^{18}F]fluorobenzoic acid. The mixture was heated at 110°C for 10 min and then cooled in a water bath for 1–2 min. The solution was diluted with 450 μl water containing 0.5% AcOH, and loaded onto a Phenomenex Luna C-18 column (10×250 mm, 5 μm). Typically, 0.3–0.4 GBq (7–10 mCi) of pure [^{18}F]-**2** could be obtained in 35–40 min starting from 0.5 GBq (13–14 mCi) of [^{18}F]fluorobenzoic acid (overall radiochemical yields, based on starting [^{18}F]fluorobenzoic acid: 63–87% decay corrected). Specific activity was estimated >37 GBq/μmol (>1000 Ci/mmol).

*Formulation of the [^{18}F]fluorobenzoate idarubicin derivative ([^{18}F]-**2**) and quality control.* HPLC fractions (4–5 ml) containing the ^{18}F labeled product were collected and solvents were evaporated under reduced pressure. [^{18}F]-**2** was dissolved in ethanol and the solution was then diluted to the proper dosage with sterile physiological saline, so that the injection dose contained no more than 10% of alcohol.

As demonstrated by HPLC analysis (Phenomenex Luna C-18 column, 10×250 mm, 5 μm), radiolabeled idarubicin [^{18}F]-**2** obtained by both approaches co-eluted with authentic synthesized unlabeled reference **2**. The radiopharmaceutical was found to be $>95\%$ chemically and radiochemically pure.

Conclusion

In conclusion, this report describes two synthetic strategies for the production of a new F-18 labeled anthracycline for *in vivo* prediction of physiologic chemoresistance to therapy. Conditions for radiosynthesis are suitable for preparation of the compound in quantities and times practical for use as a PET radiopharmaceutical. However, considering the radiochemical yields, time and radiation exposure, the direct acylation of idarubicin with [^{18}F]fluorobenzoic acid ([^{18}F]FBA) appears preferable as a more robust procedure for routine clinical production of the radiolabeled fluorobenzoate idarubicin derivative. Future studies will be aimed at determining how useful this imaging agent may be in assessing the biodistribution of idarubicin, measuring drug concentration in tumor tissue, and monitoring therapy *in vivo*.

Acknowledgements

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