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Research Article

Novel synthesis of [^{18}F]-fluorobenzene and pyridinecarbohydrazide-folates as potential PET radiopharmaceuticals

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Summary

In an attempt to visualize folate receptors that over-express on many cancers, [^{18}F]-fluorobenzene and pyridine carbohydrazide-folates were synthesized using two different synthetic approaches starting from nucleophilic displacement reactions on ethyl-trimethylammonium-benzoate and pyridine carboxylate precursors. The intermediates ethyl [^{18}F]-fluorinated benzene and pyridine esters were reacted with hydrazine to produce the [^{18}F]-fluorobenzene and pyridine carbohydrazides followed by coupling with NHS-folate **11** in the first approach. Whereas hydrazide-folate **5** was reacted with 2,5-dioxoazolidinyl [^{18}F]-fluorobenzenecarboxylate in the second approach. Based on starting [^{18}F]-fluoride, radiochemical yields and synthesis times were found to be around 80% (45 min) and 35% (80 min) for the first and the second approaches, respectively. The first synthetic approach holds considerable promise as a rapid and simple method for the radiofluorination of folic acid with high radiochemical yield and short time.

Key Words: ^{18}F-fluorination; ^{18}F-fluorobenzene; ^{18}F-fluoropyridine; ^{18}F-fluorohydrazide; ^{18}F-fluorofolic acid

Introduction

Advancement of scintigraphic tumor imaging is highly determined by development of more tumor-specific radiotracers. Receptor-targeted radiotracers have shown promises in improvement of the specificity and sensitivity

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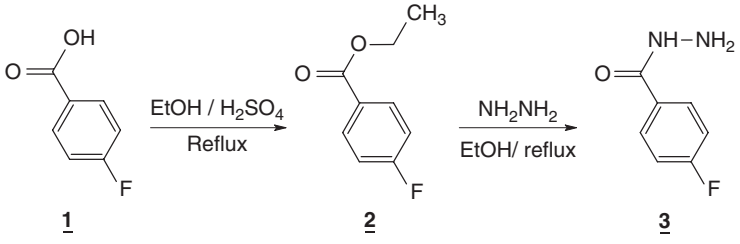
of nuclear medicine imaging procedures. Membrane-folic acid receptor is a glycosylphospstidylinositol protein that overexpressed in approximately 100% of serious ovarian adenocarcinomasa and various epithelial cancers including cervical, colorectal and renal cancers.[1,2] Meanwhile, this receptor is highly restricted in most normal tissues which make these tumors as excellent candidates for molecular targeting through the folate receptor system. The vitamin folic acid has been mainly conjugated with macromolecules and low molecular weight chelates covalently via its gamma carboxylate of the glutamic acid fragment.[3–5] These conjugates have considerable success for delivering various bioactive agents to folate-receptor-positive tumor cells. In addition, folate-receptor has been successfully targeted with folate–chelate conjugates through alpha carboxylate.[6] In the last decade considerable number of radiolabeled folate–chelate conjugates were synthesized and evaluated for tumor imaging. These include ^{67}Ga–deferoxamine–folate (^{67}Ga–DF–folate)[5,7,8] and ^{111}In–diethylenetriamine pentaacitic acid (^{111}In–DTPA–folate).[9,10] These radiolabeled folate conjugates appeared to be feasible in targeting tumor folate receptors *in vivo*. However, ^{111}In–DTPA–folate provided tumor-selective radionuclide delivery *in vivo* which has led to the initial clinical study for imaging of ovarian malignancy and differentiation between malignant and benign ovarian masses.[11] Due to its favorable physical properties and availability, numerous ^{99m}Tc–folate conjugates have also been synthesized and biologically evaluated.[12–15] For example, the folate conjugates of ^{99m}Tc–6–hydrazinonicotinamidohydrazido (HYNIC), ^{99m}Tc–ethylenedicysteine (EC), ^{99m}Tc–EC20 and ^{99m}Tc–DTPA have shown capability of imaging folate receptor positive tumors. With the increased use of positron emission tomography (PET), there has been great interest in the development of positron emitters radiopharmaceuticals for earlier detection and characterization of cancer, molecular assessment of treatment effects and more fundamental understanding of the disease process.[16] Recently, Ga–DF–folate was radiolabeled with two positron-emitting isotopes of gallium (^{66}Ga 9.5 h half-life and ^{68}Ga 68 min half-life) in high radiochemical purity.[17] Imaging of folate-receptor-positive tumor-bearing mouse with ^{66}Ga–DF–folate was feasible, however, high positron (4.15 MeV) and gamma energies (1.04, 2.75 MeV) of this radionuclide may hamper its application in humans. On the other hand ^{18}F rather than ^{66}Ga or ^{68}Ga represent the ideal nuclear and chemical characteristics for PET diagnostic imaging applications. These include relatively long half-life (110 min), production in Curie quantities, low positron energy (0.64 MeV) which result in low radiation doses and short range (2.3 mm) in tissues, insignificant sterical changes and physiological properties when introduced to metabolic substrates.[16] As part of our ongoing research effort to develop prosthetic precursors for radiofluorination of bioactive molecules, we here

report the synthesis of [^{18}F]-fluorobenzene and pyridine carbohydrazide-folates using two different synthetic approaches.

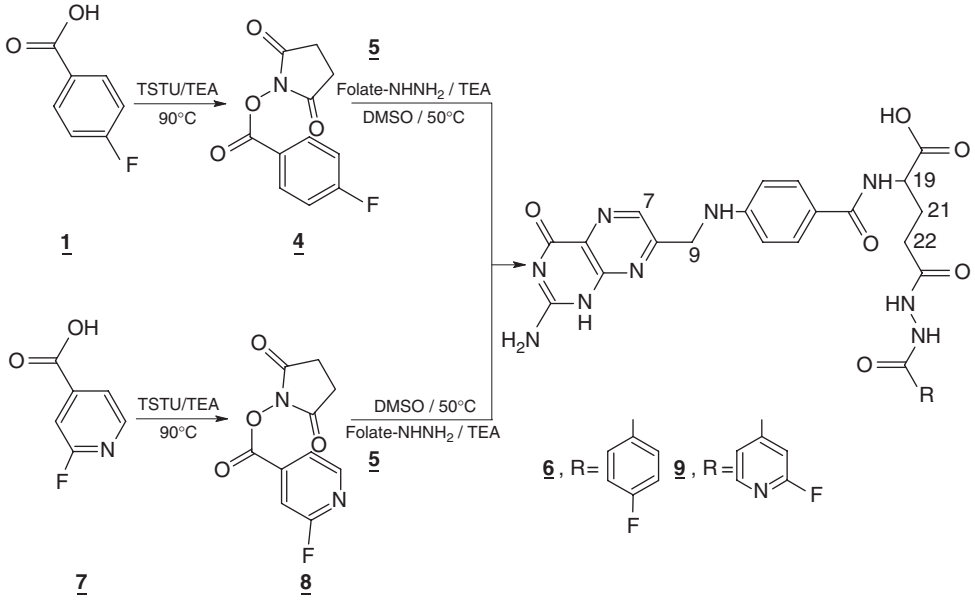
Results and discussion

Chemistry

The reference 4-fluorobenzenecarbohydrazide **3** was synthesized as outlined in Scheme 1. Scheme 2 was used to synthesize the reference compounds 2-[(4-{[(2-amino-4-oxohydropteridin-7yl)methyl]amino}phenyl)carbonylamino]-4-{*N*-[(4-fluorophenyl)carbonylamino]carbamoyl}butanoic acid: (4-fluorobenzenecarbohydrazide-folate, **6**) and 2-[(4-{[(2-amino-4-oxohydropteridin-7yl)-



Scheme 1. Synthesis of the reference 4-fluorobenzene-1-carbohydrazide (3)



Scheme 2. Synthesis of the reference fluorobenzene and pyridinecarbohydrazide-folates (6, 9)

methyl]amino}phenyl)carbonylamino]-4-{*N*-[(2-fluoro (4-pyridyl))carbonylamino]carbamoyl} butanoic acid: (2-fluoropyridine-4-carbohydrazide-folate, **9**). These compounds were characterized by physical, chromatographic and spectral data and were in agreement with the anticipated structures (see the supporting information).

Compound **3** was obtained by converting the acid **1** to the ethyl ester **2** followed by addition of hydrazine hydrate. After subjection to preparative thin-layer chromatography (TLC), yellowish crystals were separated in 57% yield. This method was a modification of the previously published procedure.[18] The calculated molecular mass for $C_7H_7FN_2O$ was 154.14 which was in agreement with the found ES-MS $[M + 1]^+ = 155$.

Since, derivatization of the folate at the γ-carboxyl moiety revealed that its affinity for the folate receptors remains unaltered and comparable to folic acid.[5] The compound 2-[(4-{[(2-amino-4-oxohydropteridin-7yl)methyl] amino}phenyl)carbonylamino]butanoic acid: (hydrazide-folate (γ), **5**) was prepared to serve as a linkage between folic acid and fluorinated compounds. This was confirmed by ^{1}H NMR spectroscopy as protons on the ethylene group of the γ moiety shifted to lower field. The calculated molecular mass for high-pressure liquid chromatography (HPLC)-purified hydrazide-folate (γ) **5** was 455.43. This was in agreement with the attained ES-MS $[M + 1]^+ = 456$, indicating the desired 1:1 conjugation ratio. Chemical purity was found to be greater than 98% as determined by analytical HPLC.

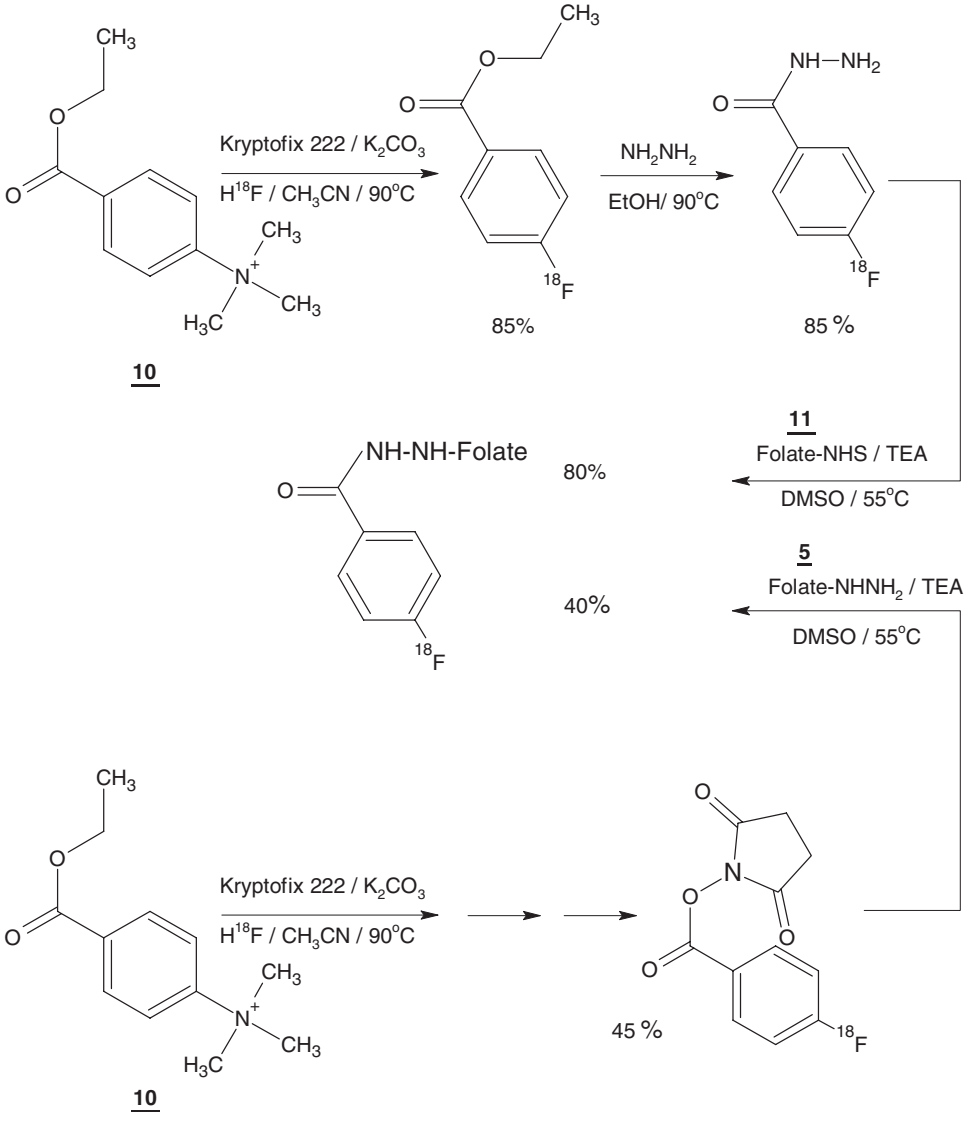
The amide-linked compound **6** was obtained as a yellow powder by conjugation of 2,5-dioxoazolidinyl 4-fluorobenzoate **4** with the hydrazide-folate (γ) **5**. The overall yield was greater than 90%. The calculated molecular mass for $C_{26}H_{24}FN_9O_6$ was 577.52 and was in agreement with the found ES-MS $[M + 1]^+ = 578$ as well as the observed MS fragmentation pattern. The base peak $m/z = 209$, corresponding to the γ moiety of the conjugated folate (–CH_2–CH_2–CO–NH–NH–Ar–F).

The intermediate 2,5-dioxoazolidinyl 2-fluoropyridine-4-carboxylate **8** was obtained as white crystals by the activation of the acid **7** using *O*-(*N*-succinimidyl)-tetramethyluronium tetrafluoroborate (TSTU). Chemical yield was 47% and the calculated molecular mass for $C_{10}H_7FN_2O_4$ was 238.17. This was in agreement with the found ES-MS $[M + 1]^+ = 239$.

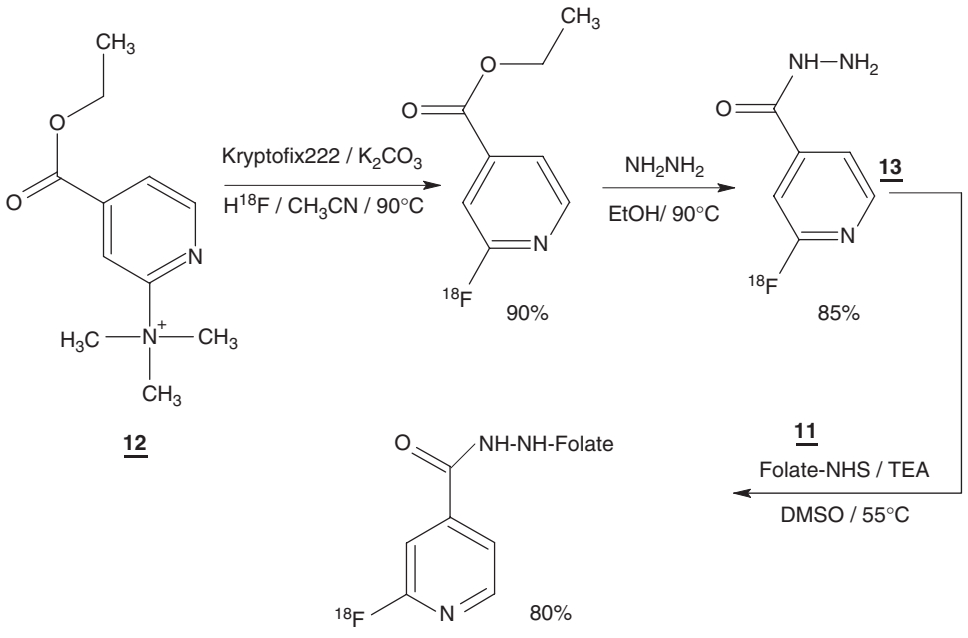
Similarly, compound **9** was obtained as a yellow powder when compound **8** was conjugated with hydrazide-folate (γ) **5** in basic condition. Chemical yield was greater than 90% and the found ES-MS $[M + 1]^+ = 579$ (calculated 578.51). The base peak $m/z = 211$, correspond to the γ moiety of the conjugated folate (–CH_2–CH_2–CO–NH–NH–Ar–F). Chemical purity of γ-isomers of both reference compounds **6** and **9** as quantified by HPLC were found to be greater than 98%.

Radiochemistry

The synthetic approaches for preparation of [^{18}F]-**6** and [^{18}F]-**9** (Schemes 3 and 4) entailed several sequence of reactions. The key precursors 4-*N*,*N*,*N*-trimethylammonium ethylbenzoate and pyridine carboxylate triflates **10** and **12** were treated using classical catalyzed nucleophilic no-carrier-added radiofluoride described by Amartey *et al.*[18] and Haka *et al.*[19] The intermediates ethyl 4-[^{18}F]-fluorobenzoate and 2-[^{18}F]-fluoro-4-pyridine



Scheme 3. Radiosynthetic approaches for preparation of 4–[^{18}F]-fluorobenzene-carbohydrazide-folate ([^{18}F]-6)



Scheme 4. Radiosynthesis of 2–[^{18}F]-fluoropyridine-4-carbohydrazide-folate ([^{18}F]-9)

carboxylate were extracted by ether and followed by passing through Sep-Pak silica cartridge to remove polar impurities before conversion to either the corresponding acids or hydrazides. In the first approach, the radiofluorinated ethyl benzene and pyridine substrates were reacted with 5 µl of hydrazine hydrate to give the corresponding 4-[^{18}F]-fluorobenzenecarbohydrazide ([^{18}F]-**3**) and 2-[^{18}F]-fluoropyridine-4-carbohydrazide ([^{18}F]-**13**) in almost quantitative yield (> 98%) for the former and higher than 90% for the latter. These results were determined by HPLC and confirmed by TLC silica gel (TLC-SG). The R_f-values of [^{18}F]-**3** and [^{18}F]-**13** were 0.6 and 0.2, respectively, in TLC-SG using ethyl acetate as a mobile phase, whereas retention times as determined by HPLC found to be 9.7 and 3.5 min, respectively. A typical radiochromatograms are shown in Figure 1. The [^{18}F]-**3** and [^{18}F]-**13** intermediates were reacted with 2-[(4-{[(2-amino-4-oxohydropteridin-7yl)methyl]amino}-4-[(2,5-dioxoazolidinyl)oxycarbonyl]butanoic acid (NHS-folate **11**) in DMSO at pH 9 and reaction mixtures were then purified using Sep-Pak silica cartridge followed by elution with saline to give the [^{18}F]-**6** and [^{18}F]-**9** in excellent yields ready for biological studies. The overall radiochemical yields for both were greater than 80% (based on starting [^{18}F]-fluoride) with total synthesis time of approximately 45 min. Radiochemical purities of the [^{18}F]-**6** and [^{18}F]-**9** were always greater than 97% as determined by HPLC and confirmed by TLC-SG.

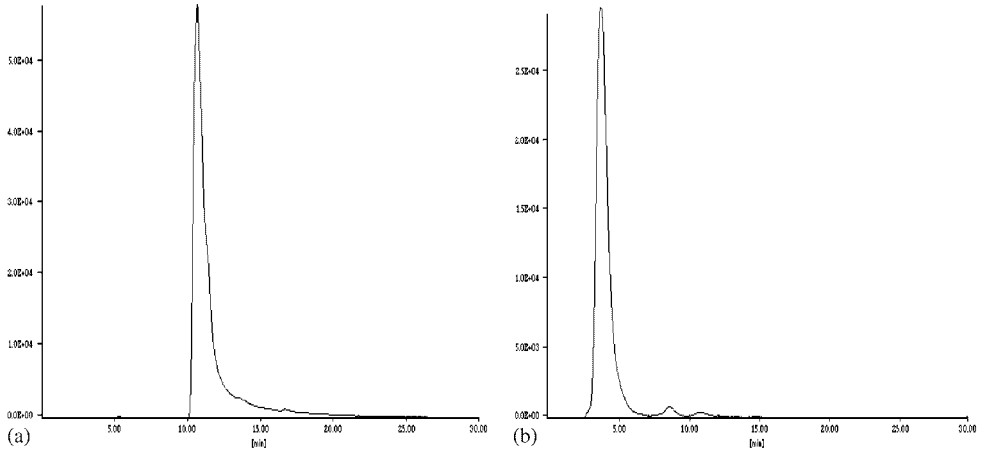


Figure 1. HPLC chromatograms of [^{18}F]-fluorobenzene and pyridine carbohydrazide intermediates, [^{18}F]-3 (A) and [^{18}F]-13 (B)

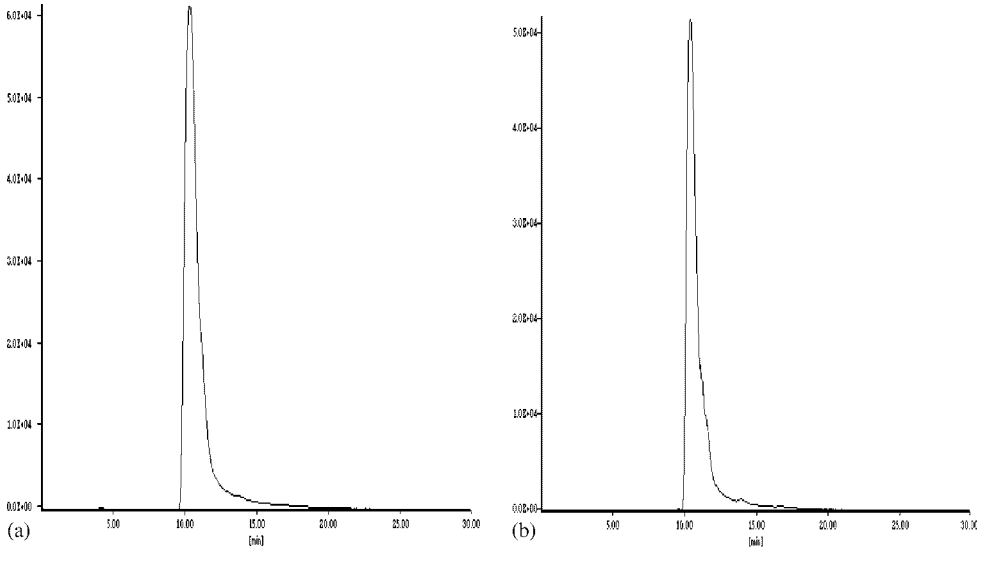


Figure 2. HPLC chromatograms of [^{18}F]-fluorobenzene and pyridine carbohydrazide-folates, [^{18}F]-6 (A) and [^{18}F]-9 (B)

Retention times for both [^{18}F]-**6** and [^{18}F]-**9** were 9.5 and 10.6 min, respectively, as shown in the radiochromatograms in Figure 2.

In the second approach, the resulted ethyl 4-[^{18}F]-fluorobenzoate ([^{18}F]-**2**) was converted to the corresponding acid then activated with TSTU to form the intermediate [^{18}F]-**4**. This prosthetic intermediate was used to label the hydrazide-folate (γ) **5** in DMSO to give the folate conjugate [^{18}F]-**6**. Work up

of this product by Sep-Pak silica column gave consistently [^{18}F]-**6** in radiochemical purity greater than 97%. However, the overall yield was nearly 35% with total synthesis time of about 85 min.

Although, we have started with the purified hydrazide-folate (γ) **5** which was coupled with [^{18}F]-**4** in the second approach. Yet, the final product ([^{18}F]-**6**) of this approach was found to be identical to what have been obtained in the first approach. In addition, the absence of α [^{18}F]-folate in the radioactivity chromatogram (7–8 min) implies that whether pure γ folate or a mixture of α and γ folate conjugates are used for radiofluorination, radiochemical purity of the [^{18}F]-fluorofolates (γ) were always better than 97% and other impurities ($<3\%$) represent either 4-[^{18}F]-fluorobenzoic acid or 2-[^{18}F]-fluoropyridine-4-carboxylic acid. That could be attributed to readily availability and less steric hindrance of the gamma moiety compare to the alpha ones. In addition, the measured specific activity for both [^{18}F]-**6** and [^{18}F]-**9** were >300 mCi/μmol. Hence, the radiofluorinated folate conjugates would be suitable for biochemical studies such as radioligand binding assays.

The first synthetic approach in comparison with the second one appears to be advantageous and holds considerable promise in the synthesis of [^{18}F]-fluorofolate conjugates (γ) in high radiochemical yield ($>80\%$, based on starting [^{18}F]-fluoride), shorter synthesis time (45 min), less laborious way and amenable for automation.

Moreover, the calculated partition coefficient for [^{18}F]-**6** and [^{18}F]-**9** were found 0.38 ± 0.02 and 0.14 ± 0.01, respectively, representing a low lipophilicity of these compounds.

Experimental

The chemicals used in the study were all analytical reagent grade purchased from Aldrich and were used without further purification unless stated. Acetonitrile was kept over molecular sieves. Sep-Pak cartridges were purchased from Waters-Millipore. TLC-SG sheets were purchased from Gelman Sciences Inc. HPLC analysis was carried out on Econosil C-18 reversed phase columns (semipreparative, 250 mm × 10 mm or analytical, 250 mm × 4.6 mm). The solvent system used for the latter was non-linear gradient (eluant A: water with 0.1 TFA; eluant B: acetonitrile/water, 3/1 v/v with 0.1% TFA; gradient: 0–90% B, 90–90% B and 90–10% B over 10 min each at flow rate of 1.5 ml/min). A Jasco chromatographic system equipped with a variable wavelength ultraviolet monitor and in tandem with a Canberra flow through radioactivity detector was used. Ultraviolet absorption was monitored at 254 nm. Chromatograms were acquired and analyzed using BORWIN software. Melting points were determined on a Thomas_Hoover Unimelt capillary melting point apparatus. Elemental analyses were performed

by ResTech, Riyadh. Mass spectroscopy was run on Quattra electrospry mass spectrometer (ES-MS). NMR was run on JEOL (400 MHz) Spectrometer.

4-Fluorobenzenecarbohydrazide **3**

Compounds **2**, **4**, **10** and **12** in Schemes 1–4 were all synthesized utilizing the methods reported by Amartey *et al.*,[18] Haka *et al.*[19] and Wester *et al.*[20] Compound **2**, (230 mg, 1.37 mmol) was placed in 25 ml flask containing ethanol (3 ml) followed by the addition of hydrazine mono-hydrate (100 µl, 2.05 mmol). Mixture was refluxed for over-night then excess of hydrazine and volatile components were removed under reduced pressure. The yellowish residue was re-solublilized in acetone (2 ml) and subjected to preparative TLC. The desire product **3** was separated as yellow crystals (57% yield) using ethylacetate/methanol (1/1) as eluant before drying under vacuum. Melting point = 121–123°C and the found ES-MS $[M+1]^+ = 155$. Analysis calculated: C, 54.90; H, 4.61; N, 18.29. Found: C, 54.44; H, 4.23; N, 17.95. ^{1}H NMR ($CDCl_3$): δ 8.06–8.12 (m, 2H, H1), δ 7.10–7.17 (m, 2H, H2), δ 1.25 (s, 2H, NH_2).

2-[(4-{[(2-Amino-4-oxohydropteridin-7yl)methyl]amino}phenyl)carbonylamino]butanoic acid: hydrazide-folate (γ) **5**

Starting from the folic acid and via the 2-[(4-{[(2-amino-4-oxohydropteridin-7yl)methyl]amino}-4-[(2,5-dioxoazolidinyl)oxycarbonyl]butanoic acid (NHS-folate **11**) route, hydrazide-folate **5** was prepared and purified using methods reported by Wang *et al.*[9] and Guo *et al.*[13] The γ-isomer of hydrazide-folate was separated from the α-isomer by HPLC on semipreparative C-18 column using linear gradient (eluant A: water with 0.05% TFA; eluant B: acetonitrile; gradient: 0–15% B over 40 min at flow rate of 5 ml/min). The elution time for hydrazide-folate (γ) and hydrazide-folate (α) were 26 and 34 min, respectively. The fraction containing hydrazide-folate (γ) was lyophilized and stored at −15°C. Melting point ∼250°C (decomposed) and the attained ES-MS $[M+1]^+ = 456$. ^{1}H NMR (DMSO): δ 8.64 (s, 1H, H7), δ 7.59 (d, 2H, Ar), δ 6.63 (d, H2, Ar), δ 4.47 (d, 2H, H9), δ 4.33 (dd, 1H, H19), δ 2.20 (m, 2H, H22), δ 1.90 (m, 2H, H21).

2-[(4-{[(2-Amino-4-oxohydropteridin-7yl)methyl]amino}phenyl)carbonylamino]-4-{N-[(4-fluorophenyl)carbonylamino]carbamoyl}butanoic acid 4-fluorobenzenecarbohydrazide-folates **6**

The hydrazide-folate (γ) (20 mg, 44 µmol) was dissolved in DMSO (2 ml) followed by the addition of triethylamine (TEA) (11 µl, 66 µmol). 2,5-dioxoazolidinyl 4-fluorobenzoate **4** (31 mg, 132 µmol) was then added and mixture stirred while shielded from light at 50°C for 3 h. The product was precipitated by addition of acetonitrile (2 ml), centrifuged and then washed

several times with diethyl ether before drying into yellow powder under vacuum and storing at −15°C (83% yield). Melting point = ∼199°C (decomposed) and the found ES-MS $[M+1]^+ = 578$. Analysis calculated: C, 54.17; H, 4.20; N, 21.87. Found: C, 54.51; H, 4.32; N, 21.21. ^{1}H NMR (DMSO): δ 8.65 (s, 1H, H7), δ 8.09 (m, 2H, Ar–F), δ 7.65 (d, 2H, Ar), δ 6.65 (d, H2, Ar), δ 4.47 (d, 2H, H9), δ 4.30 (dd, 1H, H19), δ 2.05 (m, 2H, H22), δ 1.91 (m, 2H, H21).

2,5-Dioxoazolidinyl 2-fluoropyridine-4-carboxylate **8**

To a mixture of 2-fluoropyridine-4-carboxylic acid **7** (50 mg, 0.35 mmol) and TEA (100 µl, 0.71 mmol) dissolved in acetonitrile (300 µl), *O*-(*N*-succinimidyl)-TSTU (128 mg, 0.43 mmol) was added and heated in a heating block for 1 h at 90°C. The reaction mixture was diluted with hexane/ethyl acetate 8/2 v/v (1 ml) and passed through a Sep-Pak silica cartridge. Then the product eluted by hexane/ethyl acetate 8/2 v/v (10 ml). After solvent was evaporated to dryness, white crystals separated and dried under vacuum (47% yield). Melting point = 121–123°C and the found ES-MS $[M+1]^+ = 239$. Analysis calculated: C, 50.64; H, 2.97; N, 11.81. Found: C, 50.11; H, 2.52; N, 11.99. ^{1}H NMR ($CDCl_3$): δ 10.2 (d, 1H, H6), δ 9.34 (d, 1H, H5), δ 9.23 (s, 1H, H3), δ 3.0 (s, 4H, NHS).

2-[(4-{[(2-Amino-4-oxohydropteridin-7yl)methyl]amino}phenyl)carbonylamino]-4-{N-[(2-fluoro(4-pyridyl))carbonylamino]carbamoyl}butanoic acid: 2-fluoropyridine-4-carbohydrazide-folates **9**

The hydrazide-folate (γ) (10 mg, 22 µmol) was dissolved in DMSO (1 ml) followed by the addition of TEA (6 µl, 33 µmol). To this mixture, compound **8** (15 mg, 65 µmol) was added and stirred while shielded from light at 50°C for 3 h. The product was precipitated by addition of acetonitrile (2 ml), centrifuged, washed several times with diethyl ether and dried into yellow powder under vacuum then stored at −15°C (92% yield). Melting point ∼186°C (decomposed) and the found ES-MS $[M+1]^+ = 579$. Analysis calculated: C, 51.99; H, 4.01; N, 24.25. Found: C, 51.33; H, 4.21; N, 24.01. ^{1}H NMR (DMSO): δ 9.82 (d, 2H, Ar–F), δ 8.94 (d, 2H, Ar–F), δ 8.88 (s, 2H, Ar–F), δ 8.66 (s, 1H, H7), δ 7.64 (d, 2H, Ar), δ 6.66 (d, H2, Ar), δ 4.47 (d, 2H, H9), δ 4.34 (dd, 1H, H19), δ 2.06 (m, 2H, H22), δ 1.90 (m, 2H, H21).

Radiosynthesis

*2-[(4-{[(2-amino-4-oxohydropteridin-7yl)methyl]amino}phenyl)carbonylamino]-4-{N-[(4-[^{18}F]-fluorophenyl)carbonylamino]carbamoyl}butanoic acid: 4-[^{18}F]-fluorobenzenecarbohydrazide-folate ([^{18}F]-**6**). First approach*: Aqueous [^{18}F]-fluoride was produced by the ^{18}O (p,n) ^{18}F reaction. The flouride activity (2–10 mCi, 74–370 MBq) was trapped in Kryptofix 2.2.2 (5 mg) and

potassium carbonate (1 mg) in acetonitrile/water solution (950/50 μl), dried by azeotropic distillation with aliquots of acetonitrile. The solid residue was resolubilized in CH_3CN (0.2 ml) containing the precursor ethyl 4-(*N*,*N*,*N*-trimethylammonium)-1-benzoate triflate **10** (5 mg). The reaction mixture was heated in capped 2 ml reaction-vial at 90°C for 5 min. The intermediate ethyl 4-[^{18}F]-fluorobenzoate was extracted with ether (2 × 1 ml) and passed through Sep-Pak silica cartridge. Ether layer was dried with steady stream of nitrogen, then residue resolubilized with absolute ethanol (500 μl) followed by the addition of hydrazine hydrate (5 μl). Reaction mixture was heated in capped 2 ml reaction-vial at 90°C for 5 min. The excess solvent and hydrazine were evaporated with steady stream of nitrogen. To the dried activity was added TEA (2 μl, 14 μmol) followed by addition of NHS-folate **11** (50 μg, 0.1 μmol) in DMSO (100 μl). After 15 min at 55°C, the reaction mixture was diluted with diethyl ether (1 ml), passed through a Sep-Pak silica cartridge, washed several times with diethyl ether and dried. The product [^{18}F]-**6** was then eluted by saline (1 ml).

Second approach. The 2,5-dioxoazolidinyl 4-[^{18}F]-fluorobenzoate ([^{18}F]-**4**) was prepared adopting procedures reported by Wester *et al.* [20] Briefly, the precursor **10** was radiofluorinated and ethyl 4-[^{18}F]-fluorobenzoate intermediate was converted to corresponding acid ([^{18}F]-**1**) by heating in the presence of NaOH. This was followed by activation of the acid to the [^{18}F]-**4** using the TSTU. HPLC purified [^{18}F]-**4** was dried and resolubilized in DMSO (100 μl) containing hydrazide-folate (γ) **5** (50 μg, 0.11 μmol) and TEA (2 μl, 14 μmol). The reaction mixture was heated at 55°C for 15 min then diluted with diethyl ether (1 ml), passed through a Sep-Pak silica cartridge, washed several times with diethyl ether and dried. Finally, the product [^{18}F]-**6** was eluted by saline (1 ml).

*2-[(4-{[(2-amino-4-oxohydropteridin-7yl)methyl]amino}phenyl)carbonylamino]-4-{N-[(2-[^{18}F]-fluoro(4-pyridyl))carbonylamino]carbamoyl}butanoic acid 2-[^{18}F]-fluoropyridine-4-carbohydrazide-folate([^{18}F]-**9**)*. The desire compound [^{18}F]-**9** was synthesized following the procedure mentioned above for the preparation of [^{18}F]-**6**. The prosthetic 2-[^{18}F]-fluoropyridine-4-carbohydrazide **13** was synthesized adopting the method reported by Amartey *et al.*[18] Briefly, the ethyl 2-(*N*,*N*,*N*-trimethylammonium)-4-pyridine carboxylate triflate **12** was radiofluorinated ([^{18}F]KF, Kryptofix, acetonitrile) at 90°C for 2 min to obtain ethyl 2-[^{18}F]-fluoro-4-pyridine carboxylate. This was converted to the intermediate **13**, by heating at 90°C with hydrazine hydrate (5 μl) in absolute ethanol (500 μl) for 5 min. After solvents evaporation, TEA (2 μl, 11 μmol) and NHS-folate **11** (50 μg, 0.1 μmol) in DMSO (100 μl) were added and heated at 55°C for 15 min. Finally, reaction mixture was diluted with diethyl ether, passed through a Sep-Pak silica cartridge, washed several times

with diethyl ether and dried. The product [^{18}F]-**9** was then eluted by saline (1 ml).

Partition coefficient

One hundred microliters each of [^{18}F]-**6** and [^{18}F]-**9** were added into test tubes containing 1 ml of each *n*-octanol and buffered water (pH = 7.3). The tubes were shaken for 1 min. After partial separation of the phases by gravity, 0.7 ml of each phase was transferred to separate tubes and centrifuged at 5000 rpm for 5 min. Duplicate 0.2 ml aliquots of each phase will be taken for γ-radioactivity measurement and the partition coefficient will be determined by the function: Partition coefficient = Log_{10} (counts in *n*-octanol layer/counts in aqueous layer).

Conclusion

We have developed a synthetic schemes for the production of [^{18}F]-fluorobenzene and pyridine carbohydrazide-folates ([^{18}F]-**6**, [^{18}F]-**9**) as a new PET radiopharmaceuticals. The overall radiochemical yields for both [^{18}F]-**6** and [^{18}F]-**9** were greater than 80% (based on starting [^{18}F]-fluoride) with total synthesis time of approximately 45 min. Radiochemical purities of both tracers were always greater than 97% without HPLC purification which make this method amenable for automation. *In vitro* and *in vivo* characterization of these radiofluorinated folates are currently in progress.

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