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Synthesis and preliminary biological evaluation of O^6-[4-(2-[^{18}F]fluoroethoxymethyl)benzyl]guanine as a novel potential PET probe for the DNA repair protein O^6-alkylguanine-DNA alkyltransferase in cancer chemotherapy

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Abstract—A novel fluorine-18-labeled O^6-benzylguanine (O^6-BG) derivative, O^6-[4-(2-[^{18}F]fluoroethoxymethyl)benzyl]guanine (O^6-[^{18}F]FEMBG, [^{18}F]**1**), has been synthesized for evaluation as a potential positron emission tomography (PET) probe for the DNA repair protein O^6-alkylguanine-DNA alkyltransferase (AGT) in cancer chemotherapy. The appropriate radiolabeling precursor $N^{2,9}$-bis(*p*-anisyldiphenylmethyl)-O^6-[4-(hydroxymethyl)benzyl]guanine (**6**) and reference standard O^6-[4-(2-fluoroethoxymethyl)benzyl]guanine (O^6-FEMBG, **1**) were synthesized from 1,4-benzenedimethanol and 2-amino-6-chloropurine in four or six steps, respectively, with moderate to excellent chemical yields. The target tracer O^6-[^{18}F]FEMBG was prepared in 20–35% radiochemical yields by reaction of MTr-protected precursor **6** with [^{18}F]fluoroethyl bromide followed by quick deprotection reaction and purification with a simplified Silica Sep-Pak method. Total synthesis time was 60–70 min from the end of bombardment. Radiochemical purity of the formulated product was >95%, with a specific radioactivity of >1.0 Ci/μmol at the end of synthesis. The activity of unlabeled O^6-FEMBG was evaluated via an in vitro AGT oligonucleotide assay. Preliminary findings from biological assay indicate that the synthesized analogue has similarly strong inhibiting effect on AGT in comparison with O^6-BG and O^6-4-fluorobenzylguanine (O^6-FBG). The results warrant further in vivo evaluation of O^6-[^{18}F]FEMBG as a new potential PET probe for AGT.

1. Introduction

Alkylating agents are extensively used in the chemotherapy of various cancers. The cytotoxicity of these agents stems from their ability to alkylate DNA guanine residues at their O^6-position. The effectiveness of the alkylating agents is limited by tumor overexpression of the DNA repair protein O^6-alkylguanine-DNA alkyltransferase (AGT), also commonly referred to as O^6-methylguanine-DNA methyltransferase (MGMT), which removes cytotoxic O^6-alkylguanine adducts. AGT transfers alkyl groups such as methyl, ethyl, or benzyl from the O^6-position of the guanine residues in DNA to the cysteine-145 residue in its active site in a suicidal manner, whereby the AGT molecule is irreversibly inactivated, the alkylated protein is then rapidly degraded, and its recovery requires de novo protein synthesis.[1–3] The ability of tumor cells to be resistant to the toxic effects of alkylating agents is thus dependent on cellular AGT levels. Inactivation of AGT by administration of direct substrates such as O^6-benzylguanine (O^6-BG) has been shown to increase the cytotoxicity of alkylators and the chemotherapeutic efficacy of alkylating agents. Therefore, it is highly imperative to detect the AGT levels in tumor cells. The overexpression of AGT in cancers provides a target for the development of medical probes.

Positron emission tomography (PET) is a non-invasive biomedical imaging technique. O^6-BG derivatives with suitable positron emitting radionuclides attached to the benzyl ring are potentially useful in the non-invasive imaging of the DNA repair protein AGT. The efforts of

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our group have been to develop novel positron labeled O^6-BG derivatives for PET to monitor the AGT in cancers and its response to chemotherapy. A series of carbon-11 labeled O^6-BG derivatives as shown in Figure 1 have been synthesized and evaluated in this laboratory.[4–7] However, the short half-life ($t_{1/2}$) of the radionuclide carbon-11, 20.4 min, limits the imaging protocol to a maximum of about 90 min post-injection. If imaging were to extend past 90 min, more complete information could be obtained regarding the concentration and localization of AGT levels in cancer tissue. It is attractive, therefore, to pursue analogues of O^6-BG, which can be labeled with the longer-lived radionuclide fluorine-18 ($t_{1/2}$ 110 min). It is hoped that fluorine-18 labeled O^6-BG analogues, which permit imaging of up to 5 h post-injection, will result in a better match between the pharmacokinetics of binding and the physical decay of the label. To this end, we turn our efforts toward the development of fluorine-18 labeled O^6-BG derivatives. A potentially useful fluorine-18 labeled O^6-BG derivative, O^6-(4-[^{18}F]fluorobenzyl)guanine (O^6-[^{18}F]FBG) as shown in Figure 1, has been described in the literature.[1] It is expected that the methodology with [^{18}F]fluoride ([^{18}F]F^-) for the radiolabeling of O^6-[^{18}F]FBG will not be applicable to all O^6-BG derivatives, as specific compound may require specific labeling method. Therefore, we designed and synthesized a novel fluorine-18 labeled O^6-BG derivative, O^6-[4-(2-[^{18}F]fluoroethoxymethyl)benzyl]guanine (O^6-[^{18}F]FEMBG, [^{18}F]**1**), and performed the radiolabeling of the precursor using 2-[^{18}F]fluoroethyl bromide ([^{18}F]FEBr)[8,9] within a multipurpose fluorine-18 radiosynthesis system. The biological activity of new unlabeled O^6-BG derivative O^6-[4-(2-fluoroethoxymethyl)benzyl]guanine (O^6-FEMBG, **1**) was evaluated via an in vitro AGT oligonucleotide assay.

2. Results and discussion

2.1. Chemistry and radiochemistry

The synthetic approach for the monomethoxytrityl (MTr-) protected precursor $N^{2,9}$-bis(*p*-anisyldiphenylmethyl)-O^6-[4-(hydroxymethyl)benzyl]guanine (**6**), and reference standard O^6-[4-(2-fluoroethoxymethyl)benzyl]guanine (O^6-FEMBG, **1**) is shown in Scheme 1. 1,4-Benzenedimethanol was converted to its monosodium alkoxide, which was reacted with 2-amino-6-chloropurine (**2**) to give O^6-[4-(hydroxymethyl)benzyl]guanine (**3**) in 59% yield.[10] Oxidation of compound **3** with pyridinium chlorochromate (PCC) provided O^6-(4-formylbenzyl)guanine (**4**) in 35% yield.[10] Protection of N^2 and N^9 positions of the guanine moiety in compound **4** with monomethoxytrityl group using monomethoxytrityl chloride (MTrCl)[11–14] afforded $N^{2,9}$-bis(*p*-anisyldiphenylmethyl)-O^6-(4- formylbenzyl)guanine (**5**) in 43% yield. Reduction of compound **5** with sodium

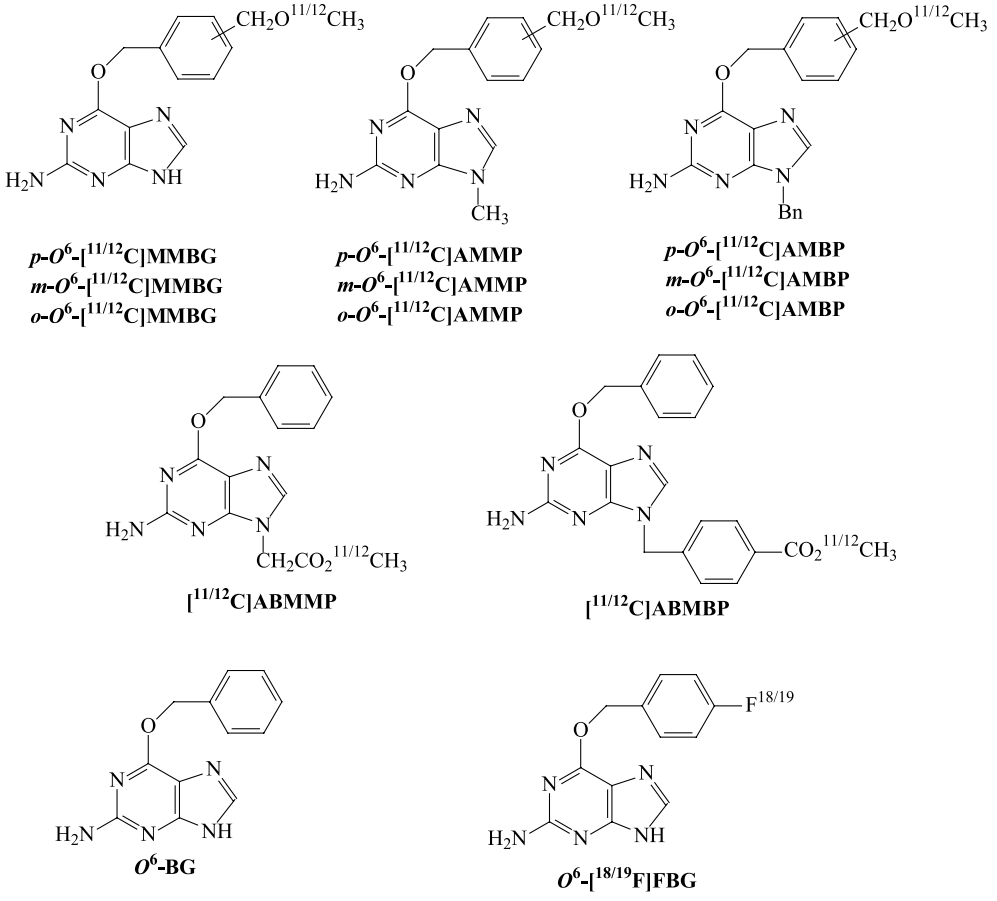
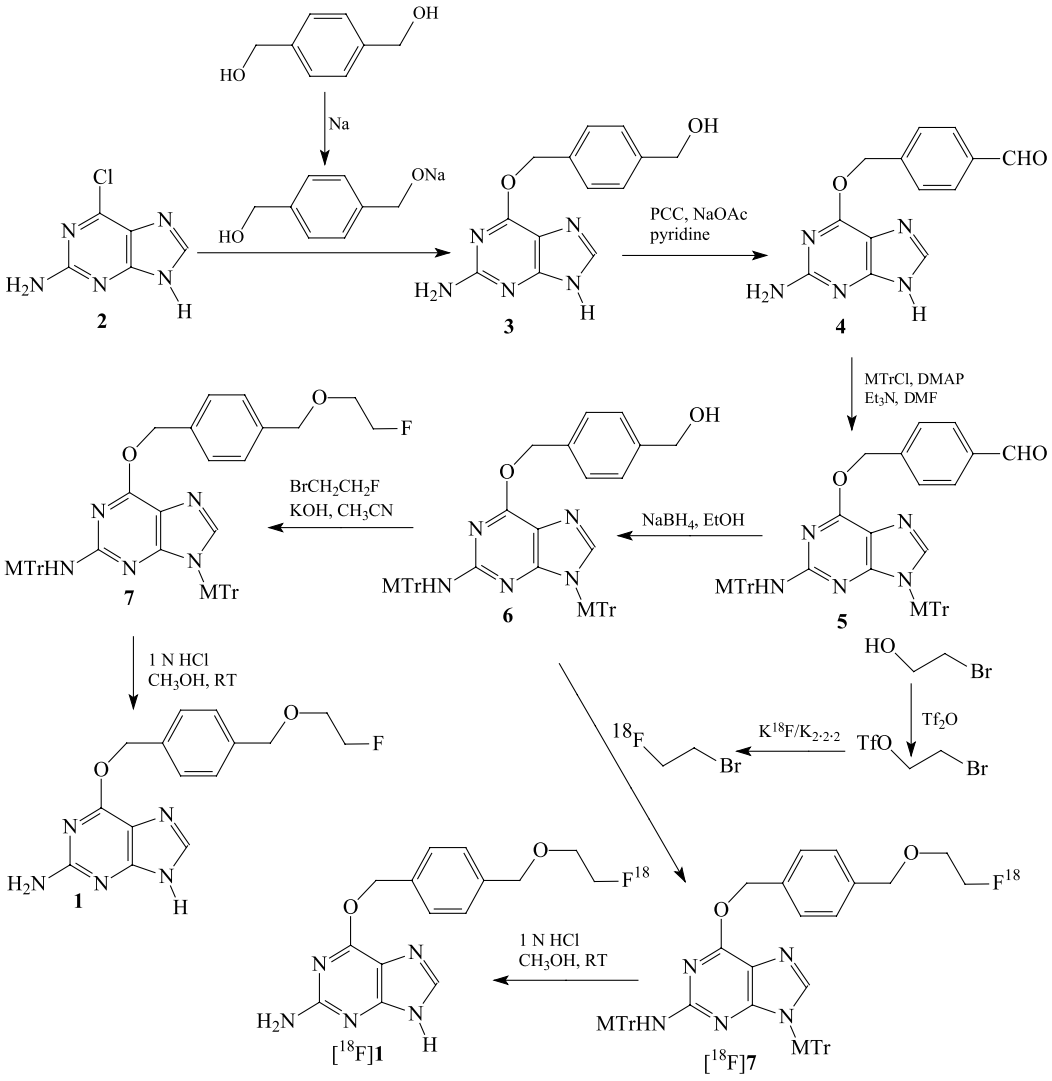


Figure 1. Chemical structures of O^6-BG, carbon-11 labeled O^6-BG derivatives, and O^6-[^{18}F]FBG.



Scheme 1. Synthetic approach for O^6-FEMBG (**1**) and O^6-[^{18}F]FEMBG ([^{18}F]**1**).

borohydride ($NaBH_4$) gave the MTr-protected precursor **6** in 96% yield. Compound **6** was alkylated with 1-bromo-2-fluoroethane under basic condition to produce $N^{2,9}$-bis(*p*-anisyldiphenylmethyl)-O^6-[4-(2- fluoroethoxymethyl)benzyl]guanine (**7**) in 66% yield. Deprotection of compound **7** with 1 N HCl furnished the reference standard O^6-FEMBG (**1**) in 66% yield. The overall chemical yield for the synthesis of precursor **6** was 8.5% in four steps. The overall chemical yield for the reference standard **1** was 5.1% in six steps. The chemical purity of precursor **6** and standard sample **1** was >95% measured by HPLC method.

The synthetic approach for the target tracer O^6-[4-(2-[^{18}F]fluoroethoxymethyl)benzyl]guanine (O^6-[^{18}F]FEMBG, [^{18}F]**1**) is also shown in Scheme 1. 2-Bromoethanol was reacted with trifluoromethane sulfonic anhydride to give 2-bromoethyl triflate in 47% yield.[15] Nucleophilic substitution of 2-bromoethyl triflate with [^{18}F]KF/Kryptofix 2.2.2 in CH_3CN provided the radiolabeled reagent [^{18}F]FEBr, which was distilled under a nitrogen flow at 130 °C for 5 min and bubbled into a reaction vessel containing the precursor **6** in CH_3CN. After the distillation of [^{18}F]FEBr was completed, the reaction mixture of precursor **6** with [^{18}F]FEBr was heated at 120 °C for 10–15 min to form a radiolabeled intermediate $N^{2,9}$-bis(*p*-anisyldiphenylmethyl)-O^6-[4-(2- [^{18}F]fluoroethoxymethyl)benzyl]guanine ([^{18}F]**7**). The radiolabeling reaction was monitored by analytical radio-HPLC method using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 × 250 mm; 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 $KHPO_4^-$ mobile phase, 1.5 mL/min flow rate, and UV (240 nm) and γ-ray (NaI) flow detectors. Retention times (t_Rs) in the analytical HPLC system for [^{18}F]FEBr, **6** and [^{18}F]**7** are 2.31, 10.15, and 13.38 min, respectively. The radiolabeling mixture was passed through a Silica Sep-Pak column to remove unreacted [^{18}F]FEBr. The large polarity difference between **6** and [^{18}F]**7**, and unreacted [^{18}F]FEBr permitted the use of a simple solid-phase extraction (SPE) technique[11–13,16] for fast isolation of **6** and [^{18}F]**7** from the radiolabeling reaction mixture. The Sep-Pak was first eluted with 15% MeOH/CH_2Cl_2 and the solvent was evaporated under high vacuum (0.1–1.0 mmHg) to give a mixture of **6** and [^{18}F]**7**. As the remaining unreacted [^{18}F]FEBr may affect the deprotection reaction

of [^{18}F]**7** through the interaction with N^2 and N^9 positions of the guanine moiety in compound [^{18}F]**7**, they had to be removed prior to deprotection of [^{18}F]**7**. The existence of the [^{18}F]FEBr would also affect the purification of labeled product from its mixture with precursor and the quality control (QC) of the tracer production.[17] Less than 10% of [^{18}F]FEBr was usually left unreacted, and the unreacted [^{18}F]FEBr was stayed in the Sep-Pak column. Then, the [^{18}F]FEBr remainder was removed by washing the column with some additional more polar solvent, AcOH/EtOH/H_2O combination (2:8:90). Compound [^{18}F]**7** was treated with 1 N HCl at 80 °C for 5–10 min and the reaction mixture was neutralized with 6 N NaOH to provide the target tracer [^{18}F]**1**. To simplify the synthetic procedure, the final reaction mixture was purified with SPE method instead of HPLC, which makes it amenable to automation. The crude product was passed through another Silica Sep-Pak column to remove the precursor **6** and radioactive byproducts with ethanol. The large polarity difference between [^{18}F]**1** and the precursor **6** and radioactive byproducts permitted the use of SPE technique for fast purification of radiotracer [^{18}F]**1**. The radiochemically pure compound [^{18}F]**1** was isolated from the Sep-Pak using a 2:8:90 HOAc/EtOH/H_2O eluant and the combined product containing fraction was treated with 2 M NaOH and 150 mM NaH_2PO_4 mixed solution to adjust pH to 5.5–7.0. The overall radiochemical yield of [^{18}F]**1** was 20–35% (n = 5), and the synthesis time was 60–70 min (n = 5) from the end of bombardment (EOB). Chemical purity, radiochemical purity, and specific radioactivity were determined by analytical HPLC. Retention times in the analytical HPLC system were: t_R**6** = 10.15 min, t_R**7** = 13.38 min, t_R**1** = 2.66 min, and t_R[^{18}F]**1** = 2.66 min. The radiochemical purity of target radiotracer [^{18}F]**1** was >96%, and the chemical purity was ~93%. The specific radioactivity of radiotracer [^{18}F]**1** was 1.0–1.3 Ci/μmol at the end of synthesis (EOS).

Overall, the three-step radiolabeling procedure including the production of radiolabeled precursor [^{18}F]FEBr, the radiolabeling of the precursor **6** with [^{18}F]FEBr to form the radiolabeled intermediate [^{18}F]**7**, and the deprotection of [^{18}F]**7** to give the target tracer [^{18}F]**1** were combined into a multipurpose fluorine-18 radiosynthesis system. This prototype device can perform three-step ^{18}F-labeling reactions in three separate reaction vials. Purification capabilities include a novel chromatography methodology with a simplified dual Silica Sep-Pak method[11–13,16] and direct distillation. Organic solvents can be removed when necessary by conventional roto-evaporation, and the product recovered for formulation and direct aseptic delivery to the final product vial. Automated cleaning routines have also been developed to prepare the device for subsequent syntheses and to minimize radiation exposure to production personnel. In comparison with the fluorine-18 radiolabeling method reported in the literature,[1] our improved method has several advantages such as higher radiochemical yield and specific radioactivity, shorter purification time, and more automatic operating processes.

2.2. In vitro O^6-methylguanine-DNA methyltransferase oligonucleotide assay

Human AGT is encoded by the MGMT gene.[18] The cellular MGMT specific activity of O^6-FEMBG was evaluated using breast cancer MCF-7 cells in comparison with the parent compound O^6-BG and compound O^6-4-fluorobenzylguanine (O^6-FBG),[5–7] via a modified technique of an in vitro MGMT oligonucleotide assay

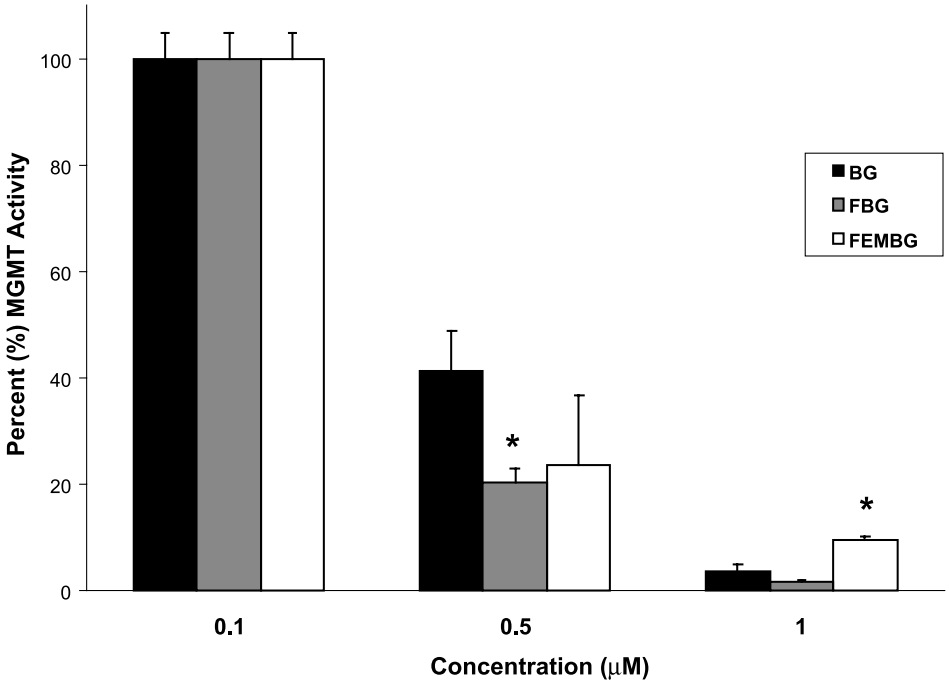


Figure 2. Percent MGMT activity of O^6-FEMBG (FEMBG) in comparison with O^6-BG (BG) and O^6-FBG (FBG) in different concentrations. *FGB was slightly but significantly more effective than BG at 0.5 μM, and FEMBG was slightly but significantly less effective that BG at 1.0 μM.

Table 1. MGMT specific activity of O^6-FEMBG in comparison with O^6-BG and O^6-FBG in fmol O^6-methylguanine (O^6-MeG) removed/mg of protein

Dose (μM)	O^6-BG[a]	O^6-FBG[a]	O^6-FEMBG[a]
0.1	4500 ± 575	4500 ± 575	4500 ± 575
0.5	1868 ± 328	918 ± 118*	1071 ± 225
1	166 ± 65	76 ± 23	432 ± 33*
10	<50 ± 5	<50 ± 5	<50 ± 5
50	<50 ± 5	<50 ± 5	<50 ± 5
100	<50 ± 5	<50 ± 5	<50 ± 5

[a] MGMT specific activity (fmol O^6-MeG removed/mg of protein).
* $p < 0.01$ compared to respective O^6-BG treatment.

developed in our laboratory.[19] The MGMT assay was originally described by Wu et al.[20] Both reference compounds O^6-BG and O^6-FBG were previously prepared in this laboratory.[4] The new compound O^6-FEMBG proved to be a potent AGT inhibitor similar to the two reference compounds. The results are summarized in Figure 2 and Table 1. The data are reported as IC_{90} values because therapeutic efficacy of O^6-BG and O^6-BG related compounds require >90% inhibition of MGMT. In this case, the IC_{90} value of each compound is also more precisely represented by the data than the IC_{50} value. The relative differences among the compounds, however, appear to be similar based on either IC_{90} or IC_{50} values. The respective IC_{90} values for O^6-BG (BG) and O^6-FBG (FBG) are between 0.5 and 1.0 μM, whereas the IC_{90} value for O^6-FEMBG (FEMBG) is ~1.0 μM. As seen in the bar graph and the table, O^6-FBG was slightly but significantly more effective than O^6-BG at 0.5 μM, and O^6-FEMBG was slightly but significantly less effective than O^6-BG at 1.0 μM. In summary, O^6-FBG and O^6-FEMBG appear to be similar in efficacy compared to O^6-BG at inhibiting cellular MGMT activity in MCF-7 cells.

3. Conclusion

The synthetic procedures that provide the MTr-protected precursor, reference standard O^6-FEMBG, and fluorine-18 labeled O^6-BG derivative O^6-[^{18}F]FEMBG have been well developed. A novel fluorine-18-radiolabeling methodology within a multiuse ^{18}F-radiosynthesis module for the preparation of fluorine-18 tracer and a novel chromatography methodology with a simplified dual Silica Sep-Pak method for the purification of fluorine-18 tracer have also been developed. Preliminary findings from in vitro biological assay indicate that the synthesized analogue O^6-FEMBG has an inhibitory effect on AGT (MGMT) similar to that of O^6-BG or O^6-FBG. The chemistry and in vitro biological results warrant further in vivo evaluation of O^6-[^{18}F]FEMBG as a new potential PET probe for AGT in cancers.

4. Experimental

4.1. General

All commercial reagents and solvents were used without further purification unless otherwise specified. Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker QE 400 NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (δ) relative to the internal standard TMS (δ 0.0). Low-resolution mass spectra (LRMS) were obtained using a Bruker Biflex III MALDI-TOF mass spectrometer, and high-resolution mass spectra (HRMS) were obtained using a Kratos MS80 mass spectrometer, in the Department of Chemistry at Indiana University. Chromatographic solvent proportions are expressed on a volume:volume basis. Thin-layer chromatography was run using Analtech silica gel GF uniplates (5×10 cm^2). Plates were visualized by UV light. Normal phase flash chromatography was carried out on EM Science silica gel 60 (230–400 mesh) with a forced flow of the indicated solvent system in the proportions described below. All moisture-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source.

Analytical HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6×250 mm; 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 $KHPO_4^-$ (buffer solution) mobile phase; flow rate 1.5 mL/min; and UV (240 nm) and γ-ray (NaI) flow detectors. Semi-prep SiO_2 Sep-Pak type cartridge was obtained from Waters Corporation, Milford, MA. Sterile Millex-GS 0.22 μm vented filter unit was obtained from Millipore Corporation, Bedford, MA.

4.2. O^6-[4-(Hydroxymethyl)benzyl]guanine (3)

To a 250 mL two-necked flask, 1,4-benzenedimethanol (20 g, 144.75 mmol) was added, and then heated to 130 °C until it was completely melted. Under nitrogen, sodium (0.60 g, 26.10 mmol) was added in two portions. When gas evolution ceased and all sodium disappeared, the temperature was lowered to 115 °C, and 2-amino-6-chloropurine, **2** (2.171 g, 12.80 mmol) was added. The mixture was stirred at 115 °C for 24 h. While it was still hot, the mixture was poured into a beaker containing 600 mL water with constant stirring. It was stirred until the solution was cooled to room temperature. Undissolved solid was filtered off, and the filtrate was neutralized with glacial acetic acid. The resultant precipitate was collected by filtration and crystallized from 1:1 MeOH/H_2O to give compound **3** (2.063 g, 59%) as an off-white solid, mp 226–229 °C (lit.[8] 229–231 °C dec). ^{1}H NMR (DMSO-d_6, ppm) δ 12.42 (s, 1H, NH, D_2O exchangeable), 7.80 (s, 1H, 8-CH), 7.44 (d, 2H, $J = 6.62$ Hz, phenyl), 7.32 (d, 2H, $J = 6.62$ Hz, phenyl), 6.29 (s, 2H, NH_2, D_2O exchangeable), 5.45 (s, 2H, CH_2), 5.20 (s, 1H, OH, D_2O exchangeable), 4.49 (s, 2H, CH_2).

4.3. O^6-(4-Formylbenzyl)guanine (4)

To a 250 mL two-necked flask, compound **3** (1.00 g, 3.69 mmol), sodium acetate (0.11 g, 1.34 mmol), pyridinium chlorochromate (PCC, 1.21 g, 5.61 mmol), and

dry pyridine (15 mL), were added. The mixture was stirred overnight under nitrogen. To the dark brown mixture methanol (15 mL) was added. After stirring for 2 h, the solvents were removed. Methanol (60 mL) was added to the solid residue to extract the product, then silica gel was added to absorb the mixture and dried under vacuum. The dry silica gel bearing the reaction mixture was transferred to the top of a silica gel column eluted with 30:1 MeCN/H_2O to give compound **4** (0.35 g, 35%) as an off-white solid, mp > 247 °C dec, (lit.[8] >247 °C dec). ^{1}H NMR (DMSO-d_6, ppm) δ 12.58 (br s, 1H, NH, D_2O exchangeable), 10.00 (s, 1H, CHO), 7.91 (d, 2H, J = 8.09 Hz, phenyl), 7.82 (s, 1H, 8-CH), 7.71 (d, 2H, J = 8.08 Hz, phenyl), 6.32 (s, 2H, NH_2, D_2O exchangeable), 5.60 (s, 2H, CH_2).

4.4. $N^{2,9}$-Bis(*p*-anisyldiphenylmethyl)-O^6-(4-formylbenzyl)guanine (5)

To a 100 mL two-necked flask equipped with a condenser, compound **4** (0.29 g, 1.08 mmol), MTrCl (1.00 g, 3.23 mmol), 4-(dimethylamino)pyridine (DMAP, 0.029 g, 0.24 mmol), DMF (25 mL), and triethylamine (1.2 mL) were added. The solution was stirred at 50–60 °C for 4 h. The solution was transferred to a separation funnel by the aid of EtOAc, and washed twice with brine. The organic layers were combined, dried over $MgSO_4$, and evaporated to dryness. The residue was dissolved in CH_2Cl_2, transferred to the top of a silica gel column, and eluted with 3:1 hexane/EtOAc to give compound **5** (0.38 g, 43%) as a white solid, mp 80 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ 10.00 (s, 1H, CHO), 7.85 (d, 2H, J = 7.36 Hz, O^6-phenyl), 7.57 (s, 1H, 8-CH), 6.62-7.40 (m, 30H, MTr-phenyls), 6.11 (s, 1H, NH), 4.98 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3). LRMS (EI, *m*/*e*): 273 (100%), 813 (M^+, 0.4%). HRMS (EI, *m*/*e*): calcd for $C_{53}H_{43}N_5O_4$, 813.3315; found 813.3314.

4.5. $N^{2,9}$-Bis(*p*-anisyldiphenylmethyl)-O^6-[4-(hydroxymethyl)benzyl]guanine (6)

To a solution of compound **5** (0.38 g, 0.46 mmol) in ethanol (15 mL) $NaBH_4$ (0.045 g, 1.19 mmol) was added over three portions at 0 °C. The mixture was warmed to room temperature in a period of 3 h. The solution was absorbed by silica gel, which was dried in vacuum and transferred to the top of a silica gel column. The column was eluted with 1:1 hexane/EtOAc to give compound **6** (0.36 g, 96%) as a white solid, mp 110 °C (dec). ^{1}H NMR (DMSO-d_6, ppm) δ 7.52 (s, 1H, 8-CH), 6.65–7.32 (m, 32H, phenyls), 6.08 (s, 1H, NH), 5.19 (t, 1H, J = 5.88 Hz, OH), 4.82 (s, 2H, CH_2), 4.47 (d, 2H, J = 5.15 Hz, CH_2), 3.72 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3). LRMS (CI, *m*/*e*): 274 (100%), 815 (M^+, 0.2%). HRMS (CI, *m*/*e*): calcd for $C_{53}H_{45}N_5O_4$, 815.3472; found 815.3448.

4.6. $N^{2,9}$-Bis(*p*-anisyldiphenylmethyl)-O^6-[4-(2-fluoroethoxymethyl)benzyl]guanine (7)

To a solution of compound **6** (0.20 g, 0.25 mmol) and 1-bromo-2-fluoroethane (0.4 mL, 5.37 mmol) in acetonitrile (10 mL), KOH (0.60 g, 10.69 mmol) was added. The mixture was stirred at 80 °C overnight. To the cooled reaction, saturated NH_4Cl solution was then added. The reaction mixture was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated to dryness. The residue was dissolved in 2:1 hexane/EtOAc and subjected to column chromatography eluted with 2:1 hexane/EtOAc to give compound **7** (0.14 g, 66%) as a white solid, mp 90 °C (dec). ^{1}H NMR ($CDCl_3$, ppm) δ 7.49 (s, 1H, 8-CH), 6.95–7.35 (m, 28H, MTr-phenyls), 6.78 (d, 2H, J = 8.82 Hz, O^6-phenyl), 6.66 (d, 2H, J = 8.82 Hz, O^6-phenyl), 5.69 (s, 1H, NH), 4.74 (s, 2H, benzyl CH_2), 4.57 (d of t, 2H, J_1 = 47.80 Hz, J_2 = 3.68 Hz, CH_2CH_2F), 4.57 (s, 2H, benzyl CH_2), 3.79 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.68 (d of t, 2H, J_1 = 29.41 Hz, J_2 = 3.68 Hz, CH_2CH_2F). LRMS (ESI, *m*/*e*): 273 (100%), 862 [$(M+H)^+$, 19%]. HRMS (ESI, *m*/*e*): calcd for $C_{55}H_{49}FN_5O_4$, 862.3769; found, 862.3798.

4.7. O^6-[4-(2-Fluoroethoxymethyl)benzyl]guanine (1)

To a solution of compound **7** (0.14 g, 0.16 mmol) in methanol (9 mL), 1 N HCl (2 mL) was added. The reaction solution was stirred at room temperature for 20 min and then neutralized with 1 N NaOH. Silica gel was added to absorb the solution, dried under vacuum, and transferred to the top of a silica gel column eluted with 2:1 EtOAc/hexane, then 30:1 MeCN/H_2O to give compound **1** (0.047 g, 91%) as a white solid, mp 144–146 °C. ^{1}H NMR (DMSO-d_6, ppm) δ 12.48 9 (s, 1H, NH, D_2O exchangeable), 7.79 (s, 1H, 8-CH), 7.48 (d, 2H, J = 5.88 Hz, phenyl), 7.35 (d, 2H, J = 5.88 Hz, phenyl), 6.30 (s, 2H, NH_2, D_2O exchangeable), 5.46 (s, 2H, benzyl CH_2), 4.55 (d, 2H, J = 47.06 Hz, CH_2CH_2F), 4.52 (s, 2H, benzyl CH_2), 3.66 (d, 2H, J = 31.62 Hz, CH_2CH_2F). LRMS (CI, *m*/*e*): 318 [$(M+H)^+$, 100%]. HRMS (CI, *m*/*e*): calcd for $C_{15}H_{17}FN_5O_2$ 318.1366; found 318.1359.

4.8. 2-Bromoethyl triflate

To a 100 mL two-necked flask, pyridine (1.7 mL, 21.02 mmol) and dry CH_2Cl_2 (20 mL) were added. The flask was cooled in an ice–salt bath. Then trifluoromethane sulfonic anhydride (3.4 mL, 20.21 mmol) was added. After 5 min, 2-bromoethanol (1.42 mL, 20.03 mmol) was added. The reaction mixture was stirred for 1.5 h, during which time it was gradually warmed to room temperature. The reaction mixture was then filtered, and the solid was washed with 1:1 CH_2Cl_2/hexane. The filtrate was passed through a short silica gel column eluted with 1:1 CH_2Cl_2/hexane. The solvent was removed, and the residue was distilled under vacuum to give 2-bromoethyl triflate (2.44 g, 47%) as a colorless oil. ^{1}H NMR ($CDCl_3$, ppm) δ 4.75 (t, 2H, J = 6.62 Hz, CH_2), 3.62 (t, 2H, J = 6.62 Hz, CH_2).

4.9. O^6-[4-(2-[^{18}F]Fluoroethoxymethyl)benzyl]guanine (O^6-[^{18}F]FEMBG, [^{18}F]1)

No-carrier-added (NCA) aqueous $H^{18}F$ (0.5 mL) prepared by $^{18}O(p,n)^{18}F$ nuclear reaction in a RDS-112

cyclotron on an enriched $H_2{}^{18}O$ water (95+%) target was added to a Pyrex vessel, which contains K_2CO_3 (4 mg, in 0.2 mL H_2O) and Kryptofix 2.2.2 (10 mg, in 0.5 mL CH_3CN). Azeotropic distillation at 120 °C for 15 min with HPLC grade CH_3CN (3 × 1 mL) under a nitrogen stream efficiently removed water to form anhydrous [^{18}F]KF/Kryptofix 2.2.2 complex. 2-Bromoethyl triflate (10–20 mg, dissolved in 0.5 mL CH_3CN) was introduced to this complex. The reaction mixture was sealed and heated at 120 °C for 10–15 min to produce 2-[^{18}F]fluoroethyl bromide ([^{18}F]FEBr) and was subsequently allowed to cool down and connect to another reaction vessel containing the precursor **6** (3–5 mg, dissolved in 0.5 mL CH_3CN), which was air-cooled at −15 to −20 °C with a Venturi cooling device powered with 100 psi compressed air. Then, the reaction vessel containing [^{18}F]FEBr was heated to 130 °C to distill [^{18}F]FEBr, under a nitrogen stream, into the solution of precursor **6**. When radioactivity reached a maximum, the reaction mixture was heated at 120 °C for 10–15 min, and subsequently allowed to cool down. The radiolabeling mixture was passed through a Silica Sep-Pak cartridge. The Sep-Pak column was first eluted with 15% MeOH/CH_2Cl_2 (3.5 mL), and the fractions were passed onto a rotatory evaporator. Then, the Sep-Pak column was eluted with 2:8:90 AcOH/EtOH/H_2O to remove the unreacted [^{18}F]FEBr. The organic solvent in 15% MeOH/CH_2Cl_2 fractions was removed by evaporation under high vacuum (0.1–1.0 mmHg). The residue was acidified with 1 N HCl (0.6 mL) and heated for 10 min at 80 °C to give the target tracer O^6-[^{18}F]FEMBG ([^{18}F]**1**). The content was neutralized with 6 N NaOH (0.1 mL), diluted with ethanol (3 mL), and evaporated under vacuum. The crude product was passed through another Silica Sep-Pak cartridge with the aid of ethanol. The Sep-Pak was eluted with EtOH to remove the radioactive byproducts and unreacted precursor **6**. The radiochemically pure product [^{18}F]**1** was eluted from the Sep-Pak with 2 : 8 : 90 AcOH/EtOH/H_2O and adjusted pH to 5.5–7.0 by addition of a 2 M NaOH and 150 mM NaH_2PO_4 mixed solution. The solution was sterile-filtered through a 0.22 μm cellulose acetate membrane and collected into a sterile vial. The overall radiochemical yield of [^{18}F]**1** was 20–35%, and the synthesis time was 60–70 min from EOB. Retention times in the analytical HPLC system were: t_R**6** = 10.15 min, t_R[^{18}F]KF = 1.88 min, t_R[^{18}F]FEBr = 2.31 min, t_R[^{18}F]**7** = 13.38 min, and t_R[^{18}F]**1** = 2.66 min.

4.10. In vitro MGMT oligonucleotide assay

MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine calf serum. Measurement of cellular MGMT specific activity was performed by a modification[19] of the assay described by Wu et al.[20] Control and drug-treated cells were washed in cold PBS, pH 7.4 and resuspended in the cold assay buffer (Tris–HCl, pH 8, 5% glycerol, 1 mM DTT, 1 mM EDTA). The cells were sonicated in 400 μL of the assay buffer for 5 s on ice. The lysate was pelleted by centrifugation for 5 min, at 12,000 × g, at 4 °C. The protein concentrations were determined by the Pierce Coomassie plus protein assay (Pierce, Rockford, IL). Fifty micrograms of total cell protein was then reacted with 0.2 pmol of 5′-Hex-labeled 18mer oligo in the assay buffer for 2 h, at 37 °C. The reaction was terminated by two phenol:chloroform extractions, one chloroform:isoamyl alcohol extraction, and then ethanol-precipitated in the presence of 1 μg carrier glycogen. Following precipitation, the precipitate was washed with 70% ethanol, dried under vacuum, and reacted with 3 units of PvuII (Promega, Madison, WI) in a total volume of 20 μL for 2 h at 37 °C. The reaction was terminated by the addition of 10 μL of gel loading buffer (96% formamide, 1 mM EDTA, pH 8) and heating at 90 °C for 2 min. Samples (O^6-BG, O^6-FBG and O^6-FEMBG) were chilled on ice and loaded directly on to the gel. The samples were electrophoresed through a 1.5 mm, 20% acrylamide, 7 M urea gel, at 300 V, for approximately 30 min. The gels were then placed on a FMBIO II fluorescent scanner (MiraiBio, South San Francisco, CA) and quantitated using the calculated fluorescent absorbance. MGMT specific activity (fmol of O^6-methylguanine removed/mg of protein) was calculated according to the following equation:

$$(\text{fluorescent units of 10 base pair fragment} / \text{fluorescent units of 18 base pair fragment}) \times (200\ \text{fmol/mg of protein})$$

4.11. Statistical analysis

O^6-BG, O^6-FBG, and O^6-FEMBG treatments were separately compared with control treatment by Student's *t*-test to determine the statistical significance of differences. Differences were considered significant at $p < 0.01$ unless otherwise stated. All data are presented as the mean ± standard deviation (SD) from at least three independent measurements.

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References and notes

1. Vaidyanathan, G.; Affleck, D. J.; Cavazos, C. M.; Johnson, S. P.; Shankar, S.; Friedman, H. S.; Colvin, M. O.; Zalutsky, M. R. *Bioconjugate Chem.* **2000**, *11*, 868.
2. Vaidyanathan, G.; Affleck, D. J.; Norman, J.; Welsh, P.; Liu, W.; Johnson, S. P.; Friedman, H. S.; Zalutsky, M. R. *Bioconjugate Chem.* **2004**, *15*, 402.
3. Kreklau, E. L.; Pollok, K. E.; Bailey, B. J.; Liu, N.; Hartwell, J. R.; Williams, D. A.; Erickson, L. C. *Mol. Cancer Ther.* **2003**, *2*, 1321.
4. Liu, X.; Zheng, Q.-H.; Hutchins, G. D.; Fei, X.; Erickson, L. C.; Miller, K. D.; Mock, B. H.; Glick-Wilson, B. E.;

Winkle, W. L.; Stone, K. L.; Carlson, K. A. *Synth. Commun.* **2003**, *33*, 941.
5. Zheng, Q.-H.; Liu, X.; Fei, X.; Wang, J.-Q.; Ohannesian, D. W.; Erickson, L. C.; Stone, K. L.; Martinez, T. D.; Miller, K. D.; Hutchins, G. D. *J. Label. Compd. Radiopharm.* **2002**, *45*, 1239.
6. Liu, X.; Zheng, Q.-H.; Fei, X.; Wang, J.-Q.; Ohannesian, D. W.; Erickson, L. C.; Stone, K. L.; Hutchins, G. D. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 641.
7. Zheng, Q.-H.; Liu, X.; Fei, X.; Wang, J.-Q.; Ohannesian, D. W.; Erickson, L. C.; Stone, K. L.; Hutchins, G. D. *Nucl. Med. Biol.* **2003**, *30*, 405.
8. Zhang, M.-R.; Tsuchiyama, A.; Haradahira, T.; Furutsuka, K.; Yoshida, Y.; Kida, T.; Noguchi, J.; Irie, T.; Suzuki, K. *Nucl. Med. Biol.* **2002**, *29*, 463.
9. Zhang, M.-R.; Maeda, J.; Ogawa, M.; Noguchi, J.; Ito, T.; Yoshida, Y.; Okauchi, T.; Obayashi, S.; Suhara, T.; Suzuki, K. *J. Med. Chem.* **2004**, *47*, 2228.
10. Chae, M.-Y.; McDougall, M. G.; Dolan, M. E.; Swenn, K.; Pegg, A. E.; Moschel, R. C. *J. Med. Chem.* **1994**, *37*, 342.
11. Wang, J.-Q.; Zheng, Q.-H.; Fei, X.; Mock, B. H.; Hutchins, G. D. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3933.
12. Zheng, Q.-H.; Wang, J.-Q.; Liu, X.; Fei, X.; Mock, B. H.; Glick-Wilson, B. E.; Sullivan, M. L.; Raikwar, S. P.; Gardner, T. A.; Kao, C.; Hutchins, G. D. *Synth. Commun.* **2004**, *34*, 689.
13. Wang, J.-Q.; Zheng, Q.-H.; Fei, X.; Liu, X.; Gardner, T. A.; Kao, C.; Raikwar, S. P.; Glick-Wilson, B. E.; Sullivan, M. L.; Mock, B. H.; Hutchins, G. D. *Synth. Commun.* **2004**, *34*, 917.
14. Zheng, Q.-H.; Wang, J.-Q.; Fei, X.; Hutchins, G. D. *Synthesis* **2003**, 2785.
15. Chi, D. Y.; Kilbourn, M. R.; Katzenellenbogen, J. A.; Welch, M. J. *J. Org. Chem.* **1987**, *52*, 658.
16. Fei, X.; Wang, J.-Q.; Miller, K. D.; Sledge, G. W.; Hutchins, G. D.; Zheng, Q.-H. *Nucl. Med. Biol.* **2004**, *31*, 1033.
17. Mock, B. H.; Winkle, W.; Vavrek, M. T. *Nucl. Med. Biol.* **1997**, *24*, 193.
18. Pegg, A. E. *Cancer Res.* **1990**, *50*, 6119.
19. Kreklau, E. L.; Limp-Foster, M.; Liu, N.; Xu, Y.; Kelley, M. R.; Erickson, L. C. *Nucleic Acids Res.* **2001**, *29*, 2558.
20. Wu, R. S.; Hurst-Calderone, S.; Kohn, K. W. *Cancer Res.* **1987**, *47*, 6229.