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Synthesis and Preliminary Biological Evaluation of 6-*O*-[^{11}C]-[(methoxymethyl)benzyl]guanines, New Potential PET Breast Cancer Imaging Agents for the DNA Repair Protein AGT

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Abstract—Novel radiolabeled O^6-benzylguanine derivatives, 6-*O*-[^{11}C]-[(methoxymethyl)benzyl]guanines ([^{11}C]*p*-O^6-MMBG, **1a**; [^{11}C]*m*-O^6-MMBG, **1b**; ([^{11}C]*o*-O^6-MMBG, **1c**), have been synthesized for evaluation as new potential positron emission tomography (PET) breast cancer imaging agents for DNA repair protein, O^6-alkylguanine-DNA alkyltransferase (AGT).

Breast cancer is the most common cancer among women and the second leading cause of cancer death in women. Breast cancer is commonly treated by various combinations of surgery, radiation therapy, chemotherapy, hormone therapy and gene therapy.[1] In the diagnosis and treatment of breast cancer, positron emission tomography (PET) has become a clinically valuable and accepted new medical imaging tool.[2] PET coupled with radiopharmaceutical 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) is widely used in clinical settings to diagnose breast cancer.[3] FDG is the only PET breast cancer imaging agent used clinically at this point in time. However, FDG is not in all cases satisfactory. Only a limited number of PET studies using other radiotracers have been conducted to image breast cancer and monitor its response to treatment, due to the limited accessibility of radiotracers. Therefore, novel PET tracers for breast cancer imaging are needed. Radiotracer development is a key area for advancement of research and clinical applications of PET in cancer detection and treatment.[4]

Elevated levels of the DNA repair protein, O^6-alkylguanine-DNA alkyltransferase (AGT), are known to result in resistance of human gliomas to nitrosoureas.[5] In breast tumors, resistance to chemotherapeutic drugs that alkylate the O^6 position of guanine correlates with the levels of the AGT. AGT is a suicide protein as it reverts alkylator cytotoxicity by transferring the O^6-alkyl group from the modified DNA guanine to cysteine-145 in its active site in an irreversible, stoichiometric process, thereby restoring a normal guanine at the site of the modified base.[6] However, the resultant alkylated AGT is inactivated for subsequent dealkylations. Therefore, the ability of tumor cells to be resistant to toxic effects of alkylating agents is dependent on cellular AGT levels; the higher the AGT content, the less effective will be tumor killing by alkylator therapy.[7] Breast tumor cells frequently express high levels of AGT. AGT plays a critical role in protecting breast cancer cells from the cytotoxic effects of chemotherapeutic drugs that alkylate the O^6 position of guanine.[8] The inactivation of AGT by administration of substrates such as O^6-benzylguanine (O^6-BG) has been shown to increase the cytotoxicity of alkylators in human tumor cell lines and xenografts.[9,10] O^6-BG provides a means to effectively inactivate the AGT protein and increase the chemotherapeutic effectiveness of alkylating agents.[11]

The overexpression of AGT in breast tumors[12] indicates that AGT is a suitable target for the development of PET breast tumor imaging agent to detect, image and monitor breast cancer and its response to chemotherapy. Using

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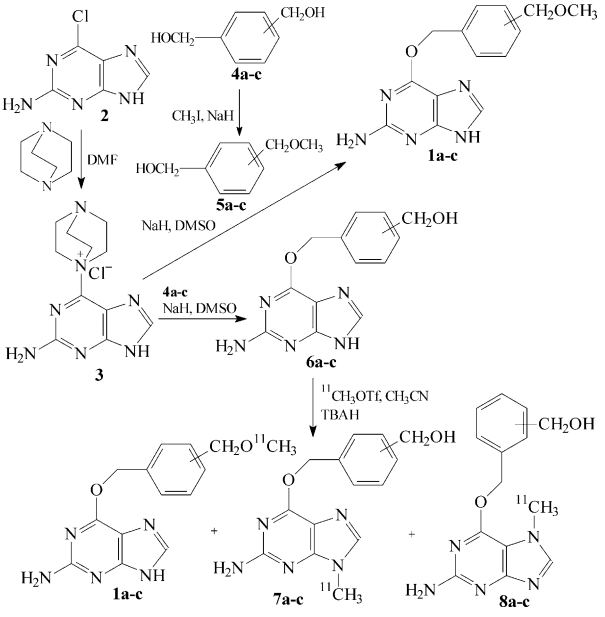
PET with a positron emitting radionuclide carbon-11 or fluorine-18 labeled O^6-BG analogue, in which the O^6-benzyl moiety binds selectively to AGT, may prove to be a useful tool for monitoring AGT levels in breast tumor tissues and for evaluating the effectiveness of drug and genetic strategies to down-regulate AGT in breast tumors and/or up-regulate its expression in chemotherapy sensitive tissues, such as bone marrow.

O^6-BG is a well-known low-molecular-weight inhibitor of mammalian AGT.[13] The application of O^6-BG and a number of alkylating agents in combination cancer chemotherapy is currently being employed in clinical trials. Numerous O^6-BG derivatives and their related compounds have been synthesized and tested for their ability to inhibit AGT activity, which include those derivatives modified at the 2-amino group, 7-nitrogen, 9-nitrogen, and the *para* position of the O^6-benzyl group.[14–16] Our objective was to develop radiolabeled O^6-BG analogues as PET imaging agents for the AGT in breast cancer as well as in other cancers. The radiochemistry strategy was to synthesize guanine derivatives amendable to labeling at the O^6-benzyl group. Here we report the synthesis, radiolabeling and preliminary biological evaluation of completely novel O^6-BG derivatives, 6-*O*-[^{11}C]-[(methoxymethyl)benzyl]guanines ([^{11}C]*p*-O^6-MMBG, **1a**; [^{11}C]*m*-O^6-MMBG, **1b**; [^{11}C]*o*-O^6-MMBG, **1c**).

The synthetic approach for the radiolabeled **1a–c** and unlabeled **1a–c** is shown in Scheme 1.

The commercially available starting material 2-amino-6-chloropurine (ACP, **2**) was reacted with 1,4-diazabicyclo[2.2.2]octane (DABCO) to provide the important intermediate quaternary amine 1-(2-amino-9H-purin-6-yl)-4-aza-1-azoniabicyclo[2.2.2]octane chloride (DABCO-purine, **3**) at a yield of 90%.[17] The crude product **3**, without further purification, was reacted with the sodium alkoxide of 4-, 3-, or 2-methoxymethylbenzyl alcohol (**5a–c**), which was prepared from benzene 1,4-, 1,3-, or 1,2-dimethanol (**4a–c**) with 60–80% yields, to give the corresponding standard samples unlabeled **1a–c** with 59–62% yields.



Scheme 1. Synthesis of **1a–c**.

Compound **3** was reacted with the sodium alkoxide of **4a–c** to provide the corresponding precursors, *p*-, *m*-, or *o*- substituted 6-*O*-[(hydroxymethyl)benzyl]guanine (*p*-O^6-HMBG, **6a**;[18] *m*-O^6-HMBG, **6b**; *o*-O^6-HMBG, **6c**). The overall chemical yields of **6a–c** from ACP were moderate (47–52%).

Compounds **5a–c**, **6a–c** and **1a–c** have analytical data such as mp, ^{1}HNMR and MS in agreement with the indicated structures.[19]

The precursors **6a–c** were labeled by the [^{11}C]methyl triflate[20] through *O*-[^{11}C]methylation of the hydroxymethyl position under basic conditions.[21,22] The tracers were isolated by the semi-preparative reversed phase high performance liquid chromatography (HPLC) purification procedure, which employed a Prodigy (Phenomenex) 5 μm C-18 column, 10 × 250 mm; 3:1:3 CH_3CN:MeOH:20 mM, pH 6.7 $KHPO_4^-$ mobile phase, 5.0 mL/min flow rate, and UV (240 nm) and γ-ray (NaI) flow detectors, to produce radiochemically pure target compounds radiolabeled **1a–c** (35–70 mCi) with 10–20% radiochemical yields, based on $^{11}CO_2$, decay corrected to end of bombardment (EOB). There are three positions in precursors **6a–c** including hydroxymethyl position at O^6 benzyl group, N^7 and N^9 positions at purine ring that could be labeled by the [^{11}C]methyl triflate to produce desired labeled products **1a–c** and undesired N^9-[^{11}C]methylated products **7a–c** and N^7-[^{11}C]methylated products **8a–c**. The radiochemical yields of desired products **1a–c** were low due to the undesired products **7a–c** and **8a–c**. The *O*-[^{11}C]methylation radiolabeling reaction was carried out under basic conditions which employed organic base tetrabutylammonium hydroxide (TBAH, 1 M solution in methanol), the ratio of labeled products **1a–c** and **7a–c**, **8a–c** was affected by the amount of base TBAH, more base TBAH was used, more products **7a–c** and **8a–c** were produced, the suitable amount of base TBAH for the reaction was 2–4 μL. The ranking of radiochemical yields was **1a–c** > **7a–c** > **8a–c**. Chemical purity, radiochemical purity, and specific radioactivity were determined by the analytical HPLC methods, which employed a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 × 250 mm; 3:1:3 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 in the analytical HPLC system were: RT**6a** = 2.95 min, RT**6b** = 2.82 min, RT**6c** = 2.87 min; RT**1a** = 3.22 min, RT**1b** = 3.16 min, RT**1c** = 3.10 min. Retention times in the semi-preparative HPLC system were: RT**6a** = 3.24 min, RT**6b** = 3.04 min, RT**6c** = 3.10 min; RT**1a** = 3.58 min, RT**1b** = 3.67 min, RT**1c** = 3.64 min. The chemical purities of the precursors **6a**, **6b**, and **6c**, and the standard samples **1a**, **1b**, and **1c** were >95%. The radiochemical purities of the target radio-

tracers **1a–c** were >99%, and the chemical purities of the target radiotracers **1a–c** were >90%. The average ($n = 5$–8) specific radioactivity of the target radiotracers **1a–c** was 0.6–0.8 Ci/μmol at end-of-synthesis (EOS).

The affinities of the unlabeled standard samples **1a–c** were evaluated via an in vitro AGT oligonucleotide assay using breast cancer MCF-7 and MDA-MB-435 cells.[23,24] The three compounds **1a–c** proved to be fairly potent AGT inhibitors in comparison with the parent compound O^6-BG, which was synthesized in our laboratory.[25] The concentration giving ≥90% inhibition of AGT activity in breast cancer MCF-7 cell extracts were as follows: O^6-BG = 1 μM, **1a** = ≥1 μM, **1b** = ~10 μM, **1c** = >50 μM (Table 1). The ranking is therefore O^6-BG ≥ **1a** > **1b** > **1c**. The results show that the modified compound **1a** exhibits strong inhibitory effectiveness on AGT greater than or equal to the parent compound O^6-BG; and the modified compounds **1b** and **1c** are fairly potent AGT inhibitors too albeit not as effective as the parent compound O^6-BG. The potentiation effect order of the substituted O^6-BG analogues is *para* > *meta* > *ortho*,[26,27] most likely because the steric effect at the *ortho*-position plays a more important role than the electronic effect. Preliminary findings from in vitro biological assay of the unlabeled **1a**, **1b**, and **1c** warrant further in vivo evaluation of the radiolabeled **1a**, **1b**, and **1c**.

In summary, the synthetic procedures that provide novel radiolabeled O^6-BG derivatives **1a**, **1b**, and **1c** have been developed. Preliminary findings from biological assay indicate the synthesized analogues have similar strong inhibitory effectiveness on AGT in comparison with the O^6-BG. These results warrant further evaluation of these radiotracers as new potential PET breast cancer imaging agents for the DNA repair protein AGT in vivo.

Table 1. AGT-inhibitory activity of O^6-BG, *p*-O^6-MMBG (**1a**), *m*-O^6-MMBG (**1b**), and *o*-O^6-MMBG (**1c**), in fmol O^6-methylguanine (O^6-MeG) removed/mg of protein

	Fmol O^6-MeG removed/mg of protein ± SD ($n = 3$)
MCF-7 control	2599 ± 167
MDA-MB-435 (methylation repair deficient)	33 ± 28
MCF-7 O^6-BG 0.1 μM	2389 ± 28
MCF-7 O^6-BG 1 μM	164 ± 93
MCF-7 O^6-BG 10 μM	50 ± 16
MCF-7 O^6-BG 50 μM	19 ± 26
MCF-7 **1a** 0.1 μM	457 ± 293
MCF-7 **1a** 1 μM	292 ± 286
MCF-7 **1a** 10 μM	35 ± 41
MCF-7 **1a** 50 μM	58 ± 76
MCF-7 **1b** 0.1 μM	1644 ± 738
MCF-7 **1b** 1 μM	1137 ± 427
MCF-7 **1b** 10 μM	21 ± 18
MCF-7 **1b** 50 μM	15 ± 18
MCF-7 **1c** 0.1 μM	2345 ± 100
MCF-7 **1c** 1 μM	2299 ± 210
MCF-7 **1c** 10 μM	2489 ± 261
MCF-7 **1c** 50 μM	660 ± 54

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19. **5a–c**, colorless oil. **5a**, ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.85 (s, br, 1H, $ArCH_2\underline{OH}$, exchange with D_2O), 3.34 (s, 3H, OCH_3), 4.41 (s, 2H, $Ar\underline{CH_2}O$), 4.57 (s, br, 2H, $Ar\underline{CH_2}OH$), 7.28 (s, 4H, ArH). **5b**, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.22 (s, br, 1H, $ArCH_2\underline{OH}$, exchange with D_2O), 3.35 (s, 3H, OCH_3), 4.42 (s, 2H, $Ar\underline{CH_2}O$), 4.58 (s, br, 2H, $Ar\underline{CH_2}OH$), 7.23–7.31 (m, 4H, ArH). **5c**, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.39 (s, 3H, OCH_3), 3.64 (s, br, 1H, $ArCH_2\underline{OH}$, exchange with D_2O), 4.53 (s, 2H, $Ar\underline{CH_2}O$), 4.62 (s, br, 2H, $Ar\underline{CH_2}OH$), 7.28–7.37 (m,

4H, ArH). **6a**, a white solid, mp 226–229 °C (lit.,[18] 229–231 °C dec.). ^{1}H NMR (DMSO-d_6): δ 4.50–4.53 (d, J = 9.5 Hz, 2H, $\underline{CH_2}OH$), 5.24 (br, s, 1H, OH, exchanges with D_2O), 5.50 (s, 2H, $Ar\underline{CH_2}$), 6.32 (s, 2H, NH_2, exchanges with D_2O), 7.29–7.51 (m, 4H, ArH), 7.85 (s, 1H, 8-H), 12.65 (br, s, 1H, >NH, exchanges with D_2O). **6b**, a white solid, mp 192–194 °C. ^{1}H NMR (DMSO-d_6): δ 4.53–4.56 (d, J = 9.5 Hz, 2H, $\underline{CH_2}OH$), 5.24 (br, s, 1H, OH, exchanges with D_2O), 5.49 (s, 2H, $Ar\underline{CH_2}$), 6.31 (s, 2H, NH_2, exchanges with D_2O), 7.30–7.34 (m, 1H, Ar-5-H), 7.36–7.38 (m, 2H, Ar-4-H and Ar-6-H), 7.47 (s, 1H, Ar-2-H), 7.85 (s, 1H, 8-H), 12.48 (br, s, 1H, >NH, exchanges with D_2O). HRMS (CI, CH_4) calcd for $C_{13}H_{13}N_5O_2$ 271.1069, found 271.1065. **6c**, a white solid, mp 198–202 °C dec. ^{1}H NMR (DMSO-d_6): δ 4.66–4.69 (d, J = 9.3 Hz, 2H, $\underline{CH_2}OH$), 5.24 (br, s, 1H, OH, exchanges with D_2O), 5.54 (s, 2H, $Ar\underline{CH_2}$), 6.32 (s, 2H, NH_2, exchanges with D_2O), 7.27–7.39 (m, 2H, Ar-4-H, Ar-5-H), 7.49–7.51 (d, J = 7.4 Hz, 2H, Ar-3-H and Ar-6-H), 7.84 (s, 1H, 8-H), 12.48 (br, s, 1H, >NH, exchanges with D_2O). HRMS (CI, CH_4) calcd for $C_{13}H_{13}N_5O_2$ 271.1069, found 271.1060. **1a**, a white solid, mp 163–165 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 3.36 (s, 3H, OCH_3), 4.44 (s, 2H, CH_2O), 4.90 (s, 2H, NH_2, exchange with D_2O), 5.53 (s, 2H, O^6–CH_2), 7.32–7.34 (d, 2H, J = 8.1 Hz, ArH), 7.48–7.51 (d, 2H, J = 8.1 Hz, ArH), 7.84 (s, 1H, 8-H). HRMS (CI, CH_4): calcd for $C_{14}H_{15}N_5O_2$ 285.1226, found 285.1222. **1b**, a white solid, mp 190–192 °C (dec.); 100–110 °C (sinter). ^{1}H NMR (300 MHz, CD_3OD): δ 3.37 (s, 3H, OCH_3), 4.64 (s, 2H, CH_2O), 4.86 (s, br, 2H, NH_2, exchange with D_2O), 5.63 (s, 2H, O^6–CH_2), 7.31–7.40 (m, 3H, ArH), 7.56–7.59 (m, 1H, ArH), 7.83 (s, 1H, 8-H). HRMS (CI, CH_4): calcd for $C_{14}H_{15}N_5O_2$ 285.1226, found 285.1234. **1c**, a white solid, mp 204–205 °C (dec.). ^{1}H NMR (300 MHz, CD_3OD): δ 3.36 (s, 3H, OCH_3), 4.46 (s, 2H, CH_2O), 4.90 (s, br, 2H, NH_2, exchange with D_2O), 5.55 (s, 2H, O^6–CH_2), 7.28–7.49 (m, 4H, ArH), 7.84 (s, 1H, 8-H). HRMS (CI, CH_4): calcd for $C_{14}H_{15}N_5O_2$ 285.1226, found 285.1221.

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22. Typical experimental procedure for the radiosynthesis of [^{11}C]*p*-O^6-MMBG, **1a**; [^{11}C]*m*-O^6-MMBG, **1b**; [^{11}C]*o*-O^6-MMBG, **1c**. The precursor (*p*-O^6-HMBG, **6a**; *m*-O^6-HMBG, **6b**; or *o*-O^6-HMBG, **6c**) (0.6–1.0 mg) was dissolved in CH_3CN (300 μL). To this solution was added TBAH (2–4 μL, 1 M solution in methanol). The mixture was transferred to a small volume, three-neck reaction tube. [^{11}C]methyl triflate was passed into the air-cooled reaction tube at −15 to −20 °C, which was generated by a Venturi cooling device powered with 100 psi compressed air, until radioactivity reached a maximum (~3 min), then the reaction tube was heated at 70–80 °C for 3 min. The contents of the reaction tube were diluted with 0.1 M $NaHCO_3$ (1 mL) and 1:1 CH_3CN:H_2O (0.6 mL), and injected onto the preparative HPLC column. The product fraction was collected, the solvent was removed by rotatory evaporation, and the final product **1a**, **1b**, or **1c** was formulated in saline containing 5% ethanol (1–3 mL), sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane and collected into a sterile vial. Total radioactivity was assayed and total volume was noted. The overall synthesis and formulation time was 30–40 min EOB. The decay corrected yields of the target radiotracers **1a**–**c**, from $^{11}CO_2$, were 10–20%.

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