Synthesis of Carbon-11 Labeled Triphenylacetamides as Novel Potential PET Melanoma Cancer Imaging Agents

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Abstract: New carbon-11 labeled triphenylacetamides (TPAs), N-(4-[11C]methoxyphenyl)-2,2,2-triphenylacetamide ([11C]MTA) and 3-phenyl-(R)-2-(2,2,2-triphenylacetamino)propionic acid [11C]methyl ester ([11C]PAME), were designed and synthesized as potential positron emission tomography (PET) melanoma cancer imaging agents. The single crystal structure of the potent anti-melanoma agent, N-(4-methoxyphenyl)-2,2,2-triphenylacetamide (MTA) is reported.

Key words: triphenylacetamides, N-(4-[11C]methoxyphenyl)-2,2,2-triphenylacetamide, 3-phenyl-(R)-2-(2,2,2-triphenylacetamino)propionic acid [11C]methyl ester, positron emission tomography, melanoma cancer, imaging agents

Triphenylacetamides (TPAs) are small molecules that have recently been developed as potential anti-melanoma agents by Dothager et al. Some of these TPAs were found to potent ly induce apoptosis in melanoma cells through G1 cell cycle arrest and dramatically reduce the level of active nuclear factor k-B (NFkB) in the cell.1 We are interested in the development of new cancer imaging agents. TPAs labeled with carbon-11 may enable non-invasive monitoring of cancer proliferation and apoptosis in melanoma cells and NFkB, and melanoma cancer response to chemotherapy using positron emission tomography (PET) imaging technique. To further develop potential therapeutic drugs as diagnostic agents, we designed and synthesized carbon-11 labeled TPAs as novel potential PET melanoma cancer imaging agents.

The synthesis of reference standards and desmethylated precursors, N-(4-methoxyphenyl)-2,2,2-triphenylacetamide (MTA, 1a), 3-phenyl-(R)-2-(2,2,2-triphenylacetamino)propionic acid methyl ester (PAME, 1b), N-(4-hydroxyphenyl)-2,2,2-triphenylacetamide (2a), and 3-phenyl-(R)-2-(2,2,2-triphenylacetamino)propionic acid (2b), was performed using a modification of the literature procedure.1 The synthetic approach is outlined in Scheme 1. Commercially available starting material, triphenylacetic acid, was reacted with thionyl chloride to afford triphenylacetyl chloride (3), which was reacted with corresponding amines p-anisidine (a) and o-phenylalanine methyl ester (b) to give reference standards TPAs, MTA 1a and PAME 1b, respectively. Demethyla tion of MTA with aluminum trichloride and ethanethiol2 yielded desmethylated phenol precursor 2a. The hydrolysis of methyl ester PAME under basic conditions gave carboxylic acid precursor 2b. The overall chemical yields for MTA and PAME were 92% and 90% (two steps), while 2a and 2b formed in 83% and 86% (three steps), respectively.

The synthesis of target radiotracers N-(4-[11C]methoxyphenyl)-2,2,2-triphenylacetamide ([11C]MTA, [11C]1a) and 3-phenyl-(R)-2-(2,2,2-triphenylacetamino)propionic acid [11C]methyl ester ([11C]PAME, [11C]1b) is shown in Scheme 2. [11C]MTA was synthesized through O-[11C]methylation of phenol precursor 2a using [11C]meth yl triflate ([11C]CH3OTf)3 and purified by HPLC4 in 30–35% radiochemical yields, based on 11CO2, decay corrected to end of bombardment (EOB), 30–35 minutes overall synthesis and formulation time from EOB, >99% radiochemical purity, and 1.5–1.8 Ci/μmol specific radioactivity at end of synthesis (EOS). [11C]PAME was synthesized through O-[11C]methylation of carboxylic acid precursor 2b using [11C]meth yl triflate ([11C]CH3OTf)5 and purified by HPLC5 in 40–45% radiochemical yields, based on 11CO2, decay corrected to EOB, 20-25 minutes overall synthesis and formulation time from EOB, >95% radiochemical purity, and 1.0–1.5 Ci/μmol specific radioactivity at EOS. The method for determining the specific radioactivity of radiotracers is an automated measurement using HPLC, which has been developed in our previous work.6,7

Compounds 2a and 2b are new TPAs, synthesized for the first time in this laboratory. Compounds 1a and 1b have been reported to be potent anti-melanoma agents of TPAs, and the reported IC50 values in melanoma cell lines (normal human bone marrow) UACC-62, B16-F10, SK-MEL-5, and bone marrow for 1a are 0.69 μM, 0.60 μM, 0.83 μM, and 4.77 μM, respectively, while those for 1b are 0.62 μM, 0.80 μM, 0.57 μM, and 7.00 μM, respectively.1 To better understand the structure of the new PET tracers, the structure of compound 1a was determined by X-ray crystallography. Compound 1a was obtained as air-stable, colorless crystals by slow evaporation from a solution of 1a in acetone. We report the first single crystal structure of compound 1a, and the perspective view of the asymmetric unit 1a with one solvent molecule (acetone) at

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ellipsoid of 30% (anisotropic picture) is shown in Figure 1.

In summary, the synthetic procedures that provided both the phenol and carboxylic acid precursors, reference standards MTA and PAME, and target tracers $[^{11}\text{C}]$TMA and $[^{11}\text{C}]$PAME have been well developed. We have reported the first single crystal structure of the potent compound MTA. The results obtained for in vitro data warrant further in vivo evaluation of the new tracers $[^{11}\text{C}]$MTA and $[^{11}\text{C}]$PAME as potential PET melanoma cancer imaging agents.

All commercial reagents and solvents were used without further purification unless otherwise specified. The $^{11}\text{C}$CH$_3$OTf was synthesized according to a literature procedure. $^1$H NMR spectra were recorded on a Bruker QE 300 NMR spectrometer using TMS as an internal standard. LRMS were obtained using a Bruker Biflex III MALDI-TOF mass spectrometer, and HRMS were obtained using a Kratos MS80 mass spectrometer, in the Department of Chemistry at Indiana University. TLCs were 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; MeCN–MeOH–KHPO$_4$– (20 mM, pH 6.7; buffer solution), 3:1:3 mobile phase, flow rate 1.5 mL/min, and UV (254 nm) and γ-ray (NaI) flow detectors. Semi-preparative HPLC was performed using a Prodigy (Phenomenex) 5 mm C-18 column, 10 × 250 mm; MeCN–MeOH–KHPO$_4$– (20 mM, pH 6.7) 3:1:3 mobile phase, 5.0 mL/min flow rate, UV (254 nm) and γ-ray (NaI) flow detectors. Semi-prep C-18 guard cartridge column (1 × 1 cm) was obtained from E. S. Industries, Berlin, NJ, with part number 300121-C18-BD 10µ. Sterile vented Millex-GS 0.22 µm filter unit was obtained from Millipore Corporation, Bedford, MA.

**Triphenylacetyl Chloride (3)**

Triphenylacetyl acid (4.5 g, 0.016 mol) was mixed with SOCl$_2$ (30 mL, excess) and DMF (two drops) was added, the resulting mixture was heated at reflux for 3 h. Excess SOCl$_2$ was removed by evaporation under reduced pressure. Residual SOCl$_2$ was removed by co-evaporation with anhyd benzene (40 mL) to afford compound 4 as a white solid in ca. 100% yield; mp 125–127 °C.

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 7.28–7.37 (m, 15 H, Ph).
N-(4-Methoxyphenyl)-2,2,2-triphenylacetamide (1a) and 3-Phenyl-(R)-2-(2,2,2-triphenylacetamido)propionic Acid Methyl Ester (1b); General Procedure

To a cold solution (~5 to 0 °C) of p-anisidine (a) or D-phenylalnine methyl ester (b) (5.4 mmol) and Et3N (2.5 mL) in CH2Cl2 (50 mL) was added a solution of compound 3 (5 mmol) in CH2Cl2 (6 mL) over 30 min. The reaction mixture was washed at 0 °C for 2 h and then at r.t. for 3 h. The mixture was adjusted to 6 by the addition of aq HCl (2 N). The mixture was concentrated, extracted with CH2Cl2 (2 × 50 mL), and washed with brine (50 mL). The organic phase was dried over Na2SO4 and concentrated under reduced pressure to afford compound 2b as a white solid in 95% yield; mp 76–78 °C; Rf = 0.62 (MeOH–CH2Cl2, 1:9).

1H NMR (300 MHz, CDCl3): δ = 2.90 (dd, J = 8.45 Hz, 1 H, CHH), 3.19 (dd, J = 13.97, 4.78 Hz, 1 H, CHH), 4.89 (dt, J = 8.45, 4.78 Hz, 1 H, CH), 6.17 (d, J = 6.62 Hz, 1 H, NH), 6.90 (dd, J = 6.98, 1.35 Hz, 2 H, Ph), 7.10 (dd, J = 6.20, 3.30 Hz, 2 H, Ph), 7.17–7.25 (m, 16 H, Ph).

HRMS (CI): m/z (%) = 436 (M+ + H, 88), 243 (100).

Tracer [11C]1a

Phenol precursor 2a (0.3 mg) was dissolved in MeCN (400 μL). To this solution was added a 6 N aq solution of NaOH (2 μL). The mixture was transferred to a small volume, three-neck reaction tube. [11C]CH3OTf, produced from bubbling 11CO2 through 11CH4, and [11C]CH3Br were passed into the air-cooled reaction tube at −15 °C to −20 °C, which was generated by a Venturi cooling device powered with 100 psi of compressed air, until the radioactivity in solution reached a maximum (ca. 3 min); then the reaction tube was heated at 70–80 °C for 2 min. The contents of the reaction tube were diluted with 0.1 M NaHCO3 (0.8 mL) and MeCN–MeOH–KHPO4 (3:1:3, 1 mL), and injected onto the preparative HPLC column. The product fraction was collected and the solvent was removed by rotary evaporation under vacuum. The final product [11C]1a was formulated in saline, sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane, and collected in a sterile vial. Total radioactivity was assayed and the total volume noted. The overall synthesis, purification, and formulation time was ca. 30 min from EOB.

Analytical HPLC: precursor 2a rt 2.36 min, MTA 1a rt 3.75 min, [11C]1a rt 3.75 min.
Preparative HPLC: precursor 2a t_R 2.85 min, MTA 1a t_R 5.32 min, [^{11}C]1a t_R 5.32 min.

Tracer [^{11}C]1b

Carboxylic acid precursor 2b (0.3 mg) was dissolved in MeCN (400 µL). To this solution was added 6 N NaOH (2–3 µL). The mixture was transferred to a small volume, three-neck reaction tube. \(^{11}\text{CH}_3\text{OTf}\) 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 the radioactivity reached a maximum (ca. 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 NaHCO₃ (1 mL). This solution was passed onto a C-18 cartridge by gas pressure. The cartridge was washed with H₂O (2 × 3 mL), and the aqueous washing was discarded. The product was eluted from the column with EtOH (2 × 3 mL), and the eluent was concentrated on a rotary evaporator at high vacuum. The labeled product [^{11}C]1b was formulated with 50 mM NaH₂PO₄, whose volume was dependent upon the use of the labeled product [^{11}C]1b in tissue biodistribution studies (ca. 6 mL, 3 × 2 mL) or in micro-PET imaging studies (1–3 mL), sterile-filtered through a sterile vented Millex-GS 0.22 µm cellulose acetate membrane, and collected in a sterile vial. Total radioactivity was assayed and total volume noted. The overall synthesis time was ca. 20 min.

Analytical HPLC: precursor 2b t_R 2.18 min, PAME 1b t_R 4.04 min, and [^{11}C]1b t_R 4.04 min.

X-ray Crystallography

The crystallographic measurements were carried out on a Siemens P4 diffractometer with graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) and 12 kW rotating generator. The data were collected at 110 K. The structure was solved and refined using the programs SHELXS-97 (Sheldrick, 1997) and SHELXL (Sheldrick 1997). The program X-Seed (Barbour, 1999) was used as an interface to the SHELX programs. X-ray coordinates have been deposited with the Cambridge Crystallographic Data Centre (CCDC) for small molecules and the deposition number is CCDC 294956.

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References