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Research Article

Synthesis of MMP inhibitor radiotracer [^{11}C]CGS 25966, a new potential PET tumor imaging agent

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Summary

[^{11}C]CGS 25966, a novel radiolabeled matrix metalloproteinase (MMP) inhibitor, has been synthesized for evaluation as new potential positron emission tomography (PET) tumor imaging agent. The precursor was labeled by [^{11}C]methyl triflate through O-[^{11}C]methylation method at the hydroxyl position of phenol under basic conditions and isolated by HPLC purification to produce pure target compound in 15–25% radiochemical yield, based on $^{11}CO_2$, decay corrected to end of bombardment. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: matrix metalloproteinase inhibitor; tumor imaging agent; radiotracer; carbon-11; positron emission tomography; [^{11}C]CGS 25966

Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes. MMP family of enzymes plays a critical role in cancer

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invasion, metastasis, and angiogenesis.[1–3] The overexpression of MMPs is implicated in tumor growth and metastasis, which provides a potential target for tumor imaging by medical imaging technique positron emission tomography (PET).[4,5] MMP inhibitors (MMPIs) can significantly reduce the growth rate of both primary and secondary tumors and can block the process of metastasis.[6] MMPIs are being actively studied as therapeutic agents.[7–14] MMPI radiotracers labeled with carbon-11 or fluorine-18 may enable non-invasive monitoring of cancer MMP levels and cancer response to MMPI therapy using PET imaging techniques.

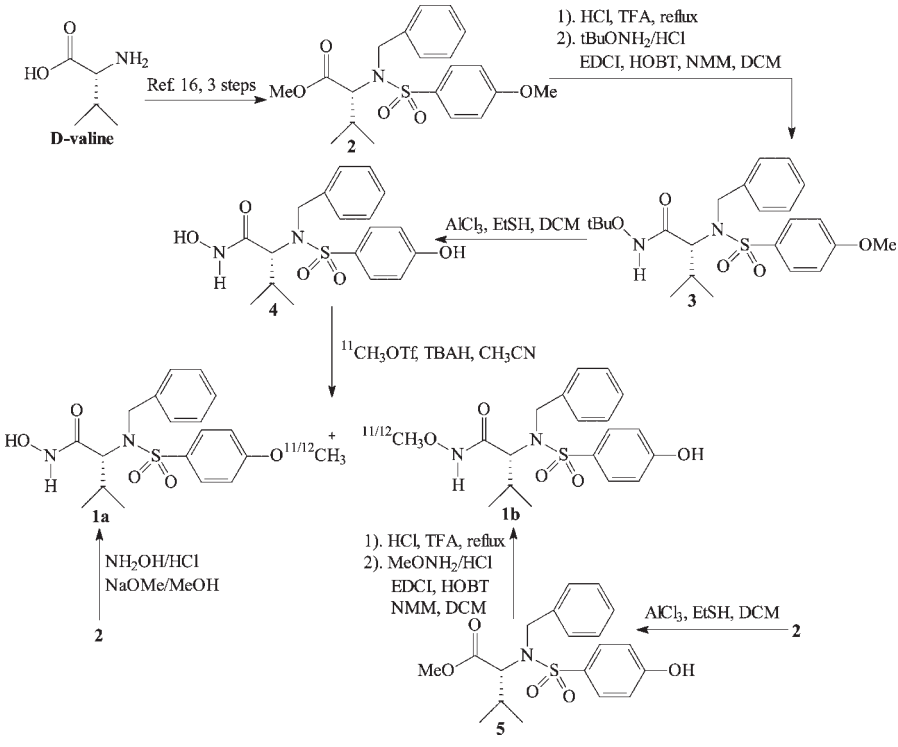
In our previous work,[15–17] the carbon-11 labeling was focused on the labeling at the aminohydroxyl position of hydroxamic acid CGS 27023A[12] to prepare methylated CGS 27023A analogs. In this ongoing study, the carbon-11 labeling was focused on the labeling at the methoxyphenyl position of hydroxamic acid CGS 25966.[18,19] Here we report the synthesis of [^{11}C]CGS 25966, an analog of [^{11}C]CGS 27023A.

Results and discussion

CGS 25966 is a potent MMP inhibitor for several MMP subtypes such as MMP-1 (K_i 43 nM), MMP-2 (K_i 11 nM), MMP-8 (K_i 13 nM), MMP-9 (K_i 27 nM), and MMP-12 (macrophage metalloelastase, MME; IC_{50}, 4.9 nM).[18,19] New MMPI radiotracer [^{11}C]CGS 29566 was synthesized as shown in Scheme 1.

The key intermediate *N*-benzyl ester (**2**) was synthesized in our previous work[16] from commercially available starting material D-valine in three steps. **2** was hydrolyzed under acidic conditions with hydrochloride and trifluoroacetic acid (TFA) into its carboxylic acid and followed by coupling with O-(*tert*-butyl)hydroxylamine hydrochloride in the presence of *N*-[(dimethylamino)propyl]-*N*-ethylcarbodiimide hydrochloride (EDCI), 1-hydroxybenzotrizole (HOBT) and *N*-methylmorphorine (NMM) to give *t*-butylated hydroxamic acid (**3**).[12,15,16] Treatment of **3** with aluminum chloride and ethanethiol in dichloromethane (DCM) unexpectedly removed two protecting groups methyl and *t*-butyl to give the O-desmethylated precursor of CGS 25966 (**4**).

The *N*-benzyl ester (**2**) was reacted directly with hydroxylamine hydrochloride to produce the standard sample unlabeled hydroxamic acid CGS 25966 (**1a**), which has been described in our previous work.[16]



Scheme 1. Synthesis of [$^{11/12}$C]CGS 25966 and [11/12C]CGS 25966 Isomer

The treatment of **2** with aluminum chloride and ethanethiol in dichloromethane (DCM) gave the compound (**5**), which was hydrolyzed into its carboxylic acid followed by coupling with methoxylamine hydrochloride in the presence of EDCI, HOBT and NMM to provide the standard sample unlabeled CGS 25966 isomer (**1b**).

The O-desmethylated precursor of CGS 25966 (**4**) was labeled by [^{11}C]methyl triflate[20,21] through O-[^{11}C]methylation method[22–24] at hydroxyl position of phenol and/or aminohydroxyl position under basic conditions and isolated by the reversed high performance liquid chromatography (HPLC) purification to produce pure desired target compound [^{11}C]CGS 25966 (**1a**) in 15–25% radiochemical yields based on $^{11}CO_2$, decay corrected to end of bombardment (EOB), and undesired labeled compound [^{11}C]CGS 25966 isomer (**1b**) in 20–30% radiochemical yields. Chemical purity, radiochemical purity, and specific radioactivity were determined by analytical HPLC method. The chemical purities of precursor **4** and standard samples **1a** and **1b**

were >95%, the radiochemical purities of target radiotracer **1a** and side product radiotracer **1b** were >99%, and the chemical purities of radiotracers **1a** and **1b** were ~95% (**1a**) and ~97% (**1b**). The average ($n = 5$–8) specific radioactivity of radiotracers **1a-b** was 0.6–0.8 Ci/μmol at end-of-synthesis (EOS).

Conclusion

The facile synthetic procedure that provide MMP inhibitor radiotracer [^{11}C]CGS 25966 (**1a**) and [^{11}C]CGS 25966 isomer (**1b**) has been developed. The published *in vitro* data indicate CGS 25966 has strong inhibitory effectiveness for several MMP subtypes. Our results combined with the literature results warrant further evaluation of [^{11}C]CGS 25966 as a new potential PET tumor imaging agent *in vivo*.

Experimental

All commercial reagents and solvents were used without further purification unless otherwise specified. The [^{11}C]methyl triflate was made according to a literature procedure[20]. 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 300 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 internal standard TMS (δ 0.0). The low resolution mass spectra were obtained using a Bruker Biflex III MALDI-Tof mass spectrometer, and the high resolution mass measurements 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 ($5 \times 10\,cm^2$). 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, $4.6 \times 250\,mm^2$; 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. Preparative HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 10 × 250 mm^2; 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. Sterile vented Millex-GS 0.22 μM vented filter unit was obtained from Millipore Corporation, Bedford, MA.

*N-(tert-Butyoxy)-2(R)-[[(4-methoxyphenyl)sulfonyl]benzylamino]-3-methylbutanamide (**3**)*

A mixture of ester **2** (0.73 g, 1.80 mmol) in TFA (3 ml) and concentrated HCl (4 ml) was refluxed overnight. The solvent was removed under vacuum. The residue was dissolved in DCM (20 ml). Into this solution were added EDCI (0.45 g, 2.30 mmol), HOBT (0.36 g, 2.70 mmol) and NMM (1.0 ml). After the resulting solution was stirred at room temperature (r.t.) for 1 h, O-(*tert*-butyl)hydroxylamine hydrochloride (*t*BuONH_2/HCl) (0.38 g, 3.03 mmol) was added. The reaction mixture was stirred at r.t. for 1 day. The solvent was removed by evaporation. The residue was diluted by EtOAc (200 ml), washed by brine (20 ml × 2) and dried over Na_2SO_4. The evaporation of the solution gave the crude product, which was purified by flash chromatography (1:5 EtOAc:Hexane) to give a white solid **3** (0.65 g, 78%), TLC R_f 0.50 (1:5 EtOAc:Hexane), m.p. 134–135°C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.73–0.75 (d, J = 6.6 Hz, 3 H, $\underline{CH_3}CH$), 0.82–0.84 (d, J = 6.6 Hz, 3 H, $\underline{CH_3}CH$), 1.22 (s, 9 H, *t*Bu), 1.96–2.10 (m, 1 H, $(CH_3)_2\underline{CH}$), 3.80 (s, 3 H, $PhO\underline{CH_3}$), 3.84–3.87 (d, J = 11.1 Hz, 1 H, CHN), 4.45–4.50 (d, J = 15.4 Hz, 1 H, $N\underline{CH_a}Ph$), 4.60–4.65 (d, J = 15.1 Hz, 1 H, $N\underline{CH_b}Ph$), 6.89–6.92 (d, J = 8.8 Hz, 2 H, $\underline{H\text{-}Ph}OMe$), 7.20-7.23 (m, 3 H, H-Ph), 7.36–7.40 (m, 2 H, H-Ph), 7.66-7.70 (d, J = 8.8 Hz, 2 H, $\underline{H\text{-}Ph}OMe$).

*N-Hydroxy-2(R)-[[(4-hydroxyphenyl)sulfonyl]benzylamino]-3-methylbutanamide (**4**)*

A mixture of ethanethiol (1 ml) and DCM (2 ml) was cooled to 0°C. Aluminum trichloride (0.14 g, 1.05 mmol) was added, and the mixture was warmed to r.t. Compound **3** (0.10 g, 0.22 mmol) was added into the above solution. The reaction mixture was stirred at r.t. for 3 h, and then quenched with cool water (1 ml). The mixture was diluted with EtOAc (150 ml), and washed with brine and dried over Na_2SO_4. The

evaporation gave the crude product, which was purified by flash chromatography (1:10 MeOH:EtOAc) to give a white solid **4** (66 mg, 78%), TLC R_f 0.30 (1:10 MeOH:EtOAc), m.p. 157–158°C. ^{1}H NMR (300 MHz, DMSO-d): 0.67–0.69 (d, J = 6.6 Hz, 3 H, $\underline{CH_3}CH$), 0.75–0.77 (d, J = 6.6 Hz, 3 H, $\underline{CH_3}CH$), 1.86–1.98 (m, 1 H, $(CH_3)_2\underline{CH}$), 3.79–3.83 (d, J = 11.1 Hz, 1 H, CHN), 4.55–4.60 (d, J = 16.1 Hz, 1 H, $N\underline{CH_a}Ph$), 4.67–4.72 (d, J = 16.1 Hz, 1 H, $N\underline{CH_b}Ph$), 6.73–6.76 (d, J = 8.8 Hz, 2 H, $\underline{H\text{-}Ph}OH$), 7.19–7.20 (m, 3 H, H-Ph), 7.36–7.39 (m, 2 H, H-Ph), 7.45–7.47 (d, J = 8.8 Hz, 2 H, $\underline{H\text{-}Ph}OH$). MS: *m/z* 378 ($M^+$).

*Methyl-2(R)-[[(4-hydroxyphenyl)sulfonyl]benzylamino]-3-methyl-butanoate (***5***)*

A mixture of ethanethiol (3 ml) and DCM (4 ml) was cooled to 0°C. Aluminum trichloride (0.73 g, 5.47 mmol) was added, and the mixture was warmed to r.t. Compound **2** (0.74 g, 1.82 mmol) was added into the above solution. The reaction mixture was stirred at r.t. for 1 h, and then quenched with cool water (1 ml). The mixture was diluted with EtOAc (200 ml), and washed with brine and dried over Na_2SO_4. The evaporation gave the crude product, which was purified by flash chromatography (1:2 EtOAc:Hexane) to give a white solid **5** (0.48 g, 67%), TLC R_f 0.40 (1:2 EtOAc:Hexane), m.p. 144–146°C. ^{1}H NMR (300 MHz, CD_3OD): 0.76–0.78 (d, J = 6.6 Hz, 3 H, $\underline{CH_3}CH$), 0.80–0.82 (d, J = 6.6 Hz, 3 H, $\underline{CH_3}CH$), 1.90–1.98 (m, 1 H, $(CH_3)_2\underline{CH}$), 3.41 (s, 3 H, CO_2CH_3), 4.11–4.15 (d, J = 10.2 Hz, 1 H, CHN), 4.52–4.57 (d, J = 15.5 Hz, 1 H. $N\underline{CH_a}Ph$), 4.64–4.69 (d, J = 15.5 Hz, 1 H, $N\underline{CH_b}Ph$), 6.84–6.87 (d, J = 8.8 Hz, 2 H, $\underline{H\text{-}Ph}OH$), 7.23–7.29 (m, 3 H, H-Ph), 7.36–7.40 (m, 2 H, H-Ph), 7.61–7.64 (d, J = 8.8 Hz, 2 H, $\underline{H\text{-}Ph}OH$).

N-Methoxyl-2(R)-[[(4-hydroxyphenyl)sulfonyl]benzylamino]-3-methylbutanamide (CGS 25966 isomer, **1***b*)

A mixture of compound **5** (0.28 g, 0.74 mmol) in TFA (2 ml) and concentrated HCl (3 ml) was refluxed overnight. After the solvent was removed under vacuum, the residue was dissolved in DCM (10 ml). Into this solution was added EDCI (0.21 g, 1.11 mmol), HOBT (0.16 g, 1.19 mmol) and NMM (1.0 ml). After the reaction solution was stirred at r.t. for 1 h, methoxylamine hydrochloride ($MeONH_2$/HCl) (0.10 g, 1.48 mmol) was added. The reaction mixture was stirred at r.t. for 1 day. The solvent was removed by evaporation. The residue was diluted by

EtOAc (200 ml), washed by brine (20 ml × 2) and dried over Na_2SO4. The evaporation of the solution gave the crude product, which was purified by flash chromatography (1:3 EtOAc:Hexane) to give a white solid **1b** (0.11 g, 39%), TLC R_f 0.30 (1:3 EtOAc:Hexane), m.p. 164–167°C. ^{1}H NMR (300 MHz, CD_3OD): 0.61–0.63 (d, $J = 6.6$ Hz, 3 H. $\underline{CH_3}CH$), 0.69-0.71 (d, $J = 6.6$ Hz, 3 H, $\underline{CH_3}CH$), 1.98–2.11 (m, 1 H, $(CH_3)_2\underline{CH}$), 3.41 (s, 3 H, $\underline{CH_3}ONH$), 3.62–3.66 (d, $J = 11.0$ Hz, 1 H, CHN), 4.68 (bs, 2 H, $N\underline{CH_2}Ph$), 6.67–6.70 (d, $J = 8.8$ Hz, 2 H, H-PhOH), 7.10–7.14 (m, 3 H, H-Ph), 7.29–7.31 (m, 2 H, H-Ph), 7.43–7.45 (d, $J = 8.1$ Hz, 2 H, H-PhOH). MS: *m/z* 393 (MH^+).

*[^{11}C]CGS 25966 (1a) and [^{11}C]CGS 25966 isomer (**1b**)*

Precursor **4** (0.6–1.0 mg) was dissolved in CH_3CN (300 µl). To this solution was added TBAH (2–8 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 in solution 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** was formulated in saline containing 5% ethanol, 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 (1–3 ml) was noted. The overall synthesis and formulation time was 30–40 min EOB. The undesired labeled compound **1b** was also isolated by the preparative HPLC purification from radiolabeling reaction mixture. Retention times in the analytical HPLC system were: RT**4** = 4.72 min, RT**1a** = 7.34 min, RT**1b** = 6.40 min. Retention times in the preparative HPLC system were: RT**4** = 5.85 min, RT**1a** = 9.34 min, RT**1b** = 8.06 min.

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