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Synthesis of (*E*)-4-[4,4-Dimethyl-2,5-dioxo-3-{1′-(^{125}I)iodo-1′-propen-3′-yl}-1-imidazolidinyl]-2-trifluoromethylbenzonitrile: A Potential Radioligand for the Androgen Receptor

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SUMMARY

The synthesis of a novel radioiodinated nonsteroidal androgen receptor ligand (*E*)-4-[4,4-dimethyl-2,5-dioxo-3-{1′-(^{125}I)iodo-1′-propen-3′-yl}-1-imidazolidinyl]-2-trifluoromethylbenzonitrile ([^{125}I]**1**) is described. *In vitro* competitive binding studies demonstrated that **1** bound to the rat androgen receptor with high affinity (K_i = 13 nM). No-carrier-added [^{125}I]**1** was synthesized from the corresponding tri-*n*-butyltin precursor by an oxidative radioiododestannylation reaction with Na[^{125}I]iodide and 3% aqueous hydrogen peroxide as oxidant. Subsequent HPLC purification provided [^{125}I]**1** in 86-92% (average = 90%; n = 5) isolated radiochemical yields having >99% radiochemical and chemical purity and a specific activity range of 1574-1835 Ci/mmol (average = 1733 Ci/mmol; n = 3).

Key Words: androgen receptor, iodine-125, iododestannylation, prostate cancer, RU 58841, single-photon emission computed tomography.

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INTRODUCTION

Most prostate tumors and metastases express androgen receptors (AR) (1). Thus, radiolabeled androgen receptor ligands are of potential interest for prostate tumor imaging with positron emission tomography (PET) or single-photon emission computed tomography (SPECT) (2,3). To date, the majority of radioligands developed for this purpose have focused on either steroid (testosterone, dihydrotestosterone) or synthetic steroid [mibolerone, metribolone (R1881)] derivatives (4-7). Successful PET imaging of prostate and prostate tumor metastases has been recently reported in a preliminary clinical study with a fluorine-18-labeled derivative of dihydrotestosterone (8).

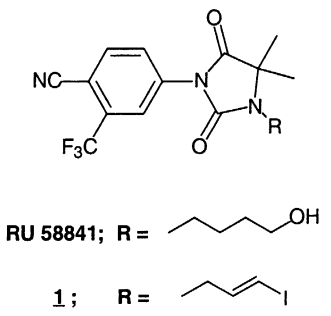


Figure 1. Structures of androgen receptor ligands

The antiandrogen, 4-[4,4-dimethyl-2,5-dioxo-3-(4′-hydroxybutyl)-1-imidazolidinyl]-2-trifluoromethylbenzonitrile (RU 58841) (Figure 1), is a prototype of a new series of nonsteroidal androgen receptor ligands reported to have high AR affinity (K_a = 1.1 nM) and selectivity (9). The ease of synthesis and structural modification of RU 58841, as compared to steroid-based AR ligands, prompted us to select it as a lead for development of a SPECT AR radioligand. Towards this goal, we recently conducted a structure-activity evaluation of a series of RU 58841 derivatives that were functionalized at the hydantoin *N*3 position with prosthetic groups suitable for radioiodination (10). Among the compounds evaluated, the *E*-isomer of the *N*-iodoallyl derivative (**1**) (Figure 1), was found to display high

binding affinity (K_i = 13 ± 2 nM) towards the rat AR in *in vitro* competitive binding assays. We describe herein, the synthesis of [^{125}I]**1** for evaluation of its utility as an AR radioligand.

EXPERIMENTAL

Melting points were determined in open capillary tubes using a Thomas Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained in $CDCl_3$ with a Bruker WM-360 (360 MHz) instrument using tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) are reported in parts per million (ppm) downfield from TMS. NMR peak splitting patterns are designated as follows: s, singlet; d, doublet; dd, doublet doublet; t, triplet; dt, doublet triplet; m, multiplet. Mass spectra were obtained with a Finnigan 4021 GCMS/DS (low resolution) or a UG70-250-S (high resolution) instrument. Na[^{125}I]iodide was obtained from Nordion Ltd, Ontario, Canada as a no-carrier-added solution in aqueous 0.1 N NaOH (pH = 10-12). All chemical reagents were obtained from Aldrich Chemical Co., Milwaukee, WI. The key synthetic intermediates, 4-[4,4-dimethyl-2,5-dioxo-1-imidazolidinyl]-2-trifluoromethylbenzonitrile (**2**) and (*E*)-1-chloro-3-(tri-n-butylstannyl)-2-propene were synthesized as previously described (9,11). Flash chromatography was performed using E. Merck Kieselgel 60 (230-400 mesh) by the method of Still *et al.*(12).

In vitro competitive receptor binding assays were conducted by a commercial laboratory (MDS Panlabs, Bothell, WA) using [^{3}H]mibolerone as radioligand and rat prostate cytosol as the AR source (13,14).

Radio-TLC analysis was performed on either Whatman K6F silica gel glass-backed TLC plates (20 cm, 250 μ) with hexane:EtOAc (7:4) as mobile phase (System A) or Whatman KC18F reversed-phase glass-backed plates (20 cm, 250 μ) with 95% EtOH:0.2 M aqueous NH_4OAc (7:3) as mobile phase (System B). The labeled compound was co-spotted with the authentic unlabeled compound prior to plate development. TLC plates were scanned for radioactivity using a Berthold Model LB 2832 TLC-Linear Analyzer equipped with a Model LB 500 Data Acquisition System.

The R_f values for **1** and NaI were 0.30 and 0.0, respectively, in System A and 0.37 and 0.81, respectively, in System B.

HPLC analysis and purification were performed on a cyano analytical column (3.9 x 75 mm, 4-μm particle, Nova-Pak CN HP, Waters, Milford, MA) eluted with 95% EtOH:0.1 M aqueous NH_4OAc (pH = 6.8) [35:65, v/v] at 1.2 mL per minute. UV absorbance was monitored at 254 nm and radioactivity in the column effluent was monitored with a Beckman 170 radiation detector. All chromatographic procedures were conducted at ambient temperature.

Radioactivity measurements were made with a Capintec CRC-12 radioisotope dose-calibrator. Specific activity estimates were determined from a standard curve relating mass to UV absorbance peak area.

(*E*)-4-[4,4-Dimethyl-2,5-dioxo-3-{1′-(tri-n-butylstannyl)-1′-propen-3′-yl}-1-imidazolidinyl]-2-trifluoromethylbenzonitrile (3)

A solution of the precursor (**2**) (0.3 g; 1.0 mmol) in dry DMF (2 mL) was treated with NaH (0.036 g, 1.5 mmol) and stirred at ambient temperature for 15 min under argon. The resulting clear yellow solution was treated dropwise with a solution of (*E*)-1-chloro-3-(tri-n-butylstannyl)-2-propene (11) (0.44 g, 1.2 mmol) in dry DMF (2 mL) and stirred a further 3 h at ambient temperature. The reaction mixture was diluted with H_2O (50 mL), extracted with Et_2O (2 × 50 mL) and the combined organic extracts washed with saturated brine (50 mL), H_2O (50 mL) and dried (Na_2SO_4). Removal of volatiles and flash chromatography of the residue on silica gel [gradient elution with 100% hexane to 10% EtOAc in hexane] provided 0.26 g (41%) of **3** as a yellow viscous oil: ^{1}H NMR ($CDCl_3$): δ 8.17 (d, 1H, *J* = 1.8 Hz, Ar-*H*2), 8.02 (dd, 1H, J = 8.5 Hz, 1.8 Hz, Ar-*H*6), 7.92 (d, 1H, J = 8.5 Hz, Ar-*H*5), 6.28 (d, 1H, J = 18.9 Hz, C=C*H*Sn), 5.99 (dt, 1H, J = 18.9 Hz, J = 5.6 Hz, C*H*=CHSn), 4.10 (d, 2H, J = 5.6 Hz, NCH_2), 1.53 (s, 6H, (CH_3)$_2$), 1.51-1.41 (m, 6H, SnCH_2), 1.35-1.25 (m, 6H, SnCH$_2$CH_2), 0.96-0.78 (m, 15H, SnCH$_2$CH$_2$CH_2CH_3); High resolution MS (CI with CH_4 and NH_3): m/z 628.2195 ($C_{28}H_{41}N_3O_2F_3{}^{120}Sn$ [M + H]$^+$ requires 628.2173).

(*E*)-4-[4,4-Dimethyl-2,5-dioxo-3-{1′-iodo-1′-propen-3′-yl}-1-imidazolidinyl]-2-trifluoromethylbenzonitrile (1)

A solution of **3** (0.12 g, 0.19 mmol) in $CHCl_3$ (2 mL) was treated dropwise with a solution I_2 (0.05 g, 0.2 mmol) in $CHCl_3$ (5 mL) and stirred at ambient temperature. TLC analysis (30% EtOAc in hexane) indicated completeness of reaction after 1 h. The reaction mixture was concentrated by rotoevaporation and the residue was flash chromatographed on silica gel (30% EtOAc in hexane) to give 0.08 g (91%) of **1** as a white solid: mp 108-109 °C [EtOAc:hexane (1:5)]; ^{1}H NMR ($CDCl_3$): δ 8.14 (d, 1H, J = 1.8 Hz, Ar-*H*2), 7.97 (dd, 1H, J = 8.5 Hz, 1.9 Hz, Ar-*H*6), 7.93 (d, 1H, J = 8.5 Hz, Ar-*H*5), 6.61 (dt, 1H, J = 14.6 Hz, 6.1 Hz, CH_2CH=CHI), 6.54 (d, 1H, J = 14.6 Hz, CH_2CH=C*H*I), 3.99 (d, 2H, J = 5.8 Hz, NCH_2), 1.54 (s, 6H, $(CH_3)_2$); High resolution MS (CI with NH_3): m/z 481.0326 ($C_{16}H_{17}N_4O_2F_3I$ $[M + NH_4]^+$ requires 481.0348).

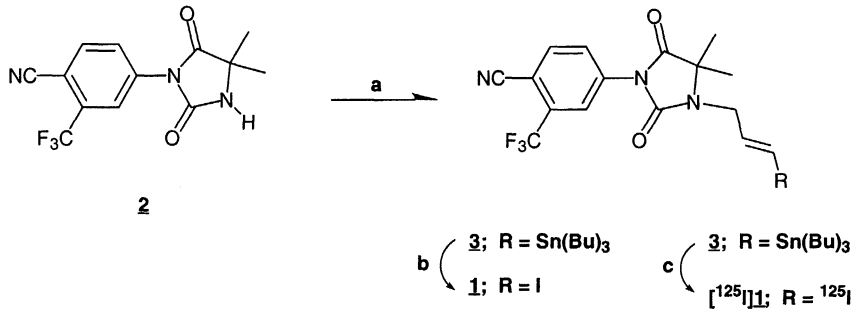
(*E*)-4-[4,4-Dimethyl-2,5-dioxo-3-{1′-(^{125}I)iodo-1′-propen-3′-yl}-1-imidazolidinyl]-2-trifluoromethylbenzonitrile ([^{125}I]1)

A polypropylene V-vial (Sarstedt) containing a solution of **3** (50 μg, 80 nmol) in absolute EtOH (50 μL) was treated with 50 μL of 0.1 N aqueous HCl followed by 2.5 mCi of Na^{125}I in 0.1 N aqueous NaOH (5 μL). The reaction was initiated by the addition of 50 μL of freshly prepared aqueous H_2O_2 (3% wt/vol.). The vial was capped, shaken periodically for 20 min at ambient temperature and the reaction quenched by addition of 100 μL of aqueous $NaHSO_3$ (12 mg/mL). Radio-TLC analysis of the crude product mixture (Systems A and B) showed >98% radiochemical purity. The reaction mixture was treated with 1 mL of aqueous saturated $NaHCO_3$, extracted with EtOAc (3 × 1 mL) and the combined organic layers dried (Na_2SO_4). The solvent was removed using a nitrogen flow, the crude product formulated in 95% EtOH:0.1 M aqueous NH_4OAc (1:4) and purified by injection on an HPLC column as described. UV and radioactivity traces were monitored during HPLC purification. The column effluent was filtered during

purification via a sterile 0.2 μm alumina filter (Anotop 10, Whatman) connected directly to the HPLC outlet line. The pure product (2.3 mCi) was isolated in a 2.1 mL volume and diluted with 0.9% normal saline to provide an injectable solution. HPLC analysis of purified [^{125}I]**1** showed the same retention time as **1** (t_R = 12.9 min). The chemical and radiochemical purity was >99% as determined by radio-TLC and radio-HPLC analysis and the radiochemical yield and specific activity was 92% and 1790 Ci/mmole, respectively. The purified, labeled product (stored at 4 °C) was stable for up to 10 days (less than 2% radiolytic decomposition by radio-TLC and radio-HPLC analysis).

RESULTS AND DISCUSSION

The synthesis of the iodoallyl derivative **1** was carried out as outlined in Scheme 1. Treatment of **2** with NaH and subsequent alkylation with the *E*-isomer of



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Reagents: (a) 1. NaH/DMF, 2. *(E)*-$ClCH_2CH{=}CHSn(Bu)_3$; (b) I_2, $CHCl_3$; (c) $Na^{125}I$, 3% aq H_2O_2.

Scheme 1. Synthesis of **1** and [^{125}I]**1**

1-chloro-3-(tri-n-butylstannyl)-2-propene (11) provided the tri-n-butyltin product **3** in 41% yield. Iododestannylation of **3** was accomplished by treatment with I_2 in $CHCl_3$, which provided the iodoallyl derivative **1** in 91% yield. Assignment of the *E*-configuration for the vinyl protons in the products was confirmed by ^{1}H NMR analysis. Thus, a doublet centered at 6.28 ppm was observed for the proton geminal to the carbon bearing the tri-n-butyltin group in compound **3** with a characteristic

trans coupling constant of 18.9 Hz. Similarly, a doublet of triplets centered at 5.99 ppm was seen for the proton vicinal to the tri-n-butyltin group with coupling constants of 18.9 and 5.6 Hz, respectively. In addition, ^{1}H NMR analysis of iodo analog **1** showed a distinctive proton coupling constant of 14.6 Hz for the proton geminal to the iodine-bearing carbon (J = 14.6 Hz and 6.1 Hz for the vicinal proton) indicative of retention of configuration during iodination (11,15).

Radioiododestannylation of **3** using 3% aqueous hydrogen peroxide as oxidant according to the method of Goodman *et al.* (15) afforded [^{125}I]**1** in high radiochemical yield and purity (>96% as determined by radio-TLC analysis). The chromatographic behavior of **1** was investigated on cyano and C-18 stationary phases, respectively, under reversed-phase conditions (95% EtOH/aqueous NH_4OAc mixtures) to determine the optimum conditions for HPLC purification. In these systems, **1** displayed better elution characteristics (sharp peak shape and absence of tailing) on cyano as compared to C-18. Thus, HPLC purification of the crude product was conducted using a cyano column as described, which provided pure [^{125}I]**1** in high radiochemical and chemical purity (>99%). Under these conditions, [^{125}I]**1** is well resolved from the (tri-n-butyl)tin precursor **3** (retention time > 60 min). Typically, the radiolabeled product was collected in a 1.9-2.3 mL elution volume and diluted with 0.9% normal saline to provide a directly injectable solution containing 8% or less ethanol. The radiochemical and chemical purity of the final product was >99% as determined by radioanalytical HPLC and radio-TLC. The average isolated radiochemical yield was 86-92% (n = 5) and the average specific activity was 1733 Ci/mmole (n = 3).

CONCLUSION

A high-yield, no-carrier-added synthesis of a radioiodinated nonsteroidal AR ligand ([^{125}I]**1**), is described. The radioligand was obtained in high radiochemical yield (>86%) and specific activity (>1574 Ci/mmol) suitable for conducting *in vivo* studies in small animals. Studies are in progress to evaluate the utility of [^{125}I]**1** as an AR radioligand.

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