

Chiral dimethylamine flutamide derivatives—modeling, synthesis, androgen receptor affinities and carbon-11 labeling

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Abstract

Most prostate cancers are androgen dependent upon initial diagnosis. On the other hand, some very aggressive forms of prostate cancer were shown to have lost the expression of the androgen receptor (AR). Although the AR is routinely targeted in endocrine treatment, the clinical outcome remains suboptimal. Therefore, it is crucial to demonstrate the presence and activity of the AR in each case of prostate cancer, before and after treatment. While noninvasive positron emission tomography (PET) has the potential to determine AR expression of tumor cells *in vivo*, fully optimized PET imaging agents are not yet available. Based on molecular modeling, three novel derivatives of hydroxyflutamide (Compounds 1–3) were designed and synthesized. They contain an electron-rich group (dimethylamine) located on the methyl moiety, which may confer a better stability to the molecule *in vivo*. Compounds 1–3 have AR binding that is similar or higher than that of the currently used commercial drugs. An automated carbon-11 radiolabeling route was developed, and the compounds were successfully labeled with a 10–15% decay-corrected radiochemical yield, 99% radiochemical purity and a specific activity of 4Ci/ μ mol end of bombardment ($n=15$). These labeled biomarkers may facilitate the future quantitative molecular imaging of AR-positive prostate cancer using PET and may also allow for image-guided treatment of prostate cancer.

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1. Introduction

Prostate cancer is one of the most frequently diagnosed cancers and is the second-leading cause of death due to cancer in the male population of North America [1,2]. The standard tests for detecting prostate cancer include digital rectal examination and measurement of prostate-specific antigen (PSA) levels, with the ultimate examination being histopathology of prostate tissue obtained by ultrasound-guided biopsy. PSA is regulated at the transcriptional level by the androgen receptor (AR) through androgen response elements in the promoter region of the gene. However,

screening for prostate cancer using PSA levels does not provide any information regarding the molecular characteristics of this type of cancer.

The AR is a cytoplasmatic protein and is a member of the steroid/thyroid hormone receptor superfamily [3,4]. Upon androgen binding to its receptor, the AR migrates into the nucleus, binds to specific DNA sequences called androgen response elements and modulates the transcription of target genes [3].

Since approximately 80–90% of prostate cancers are androgen dependent at initial diagnosis, endocrine therapy of prostate cancer is directed toward the reduction of serum androgens and inhibition of the AR [5]. On the other hand, some very aggressive forms of prostate cancer were shown

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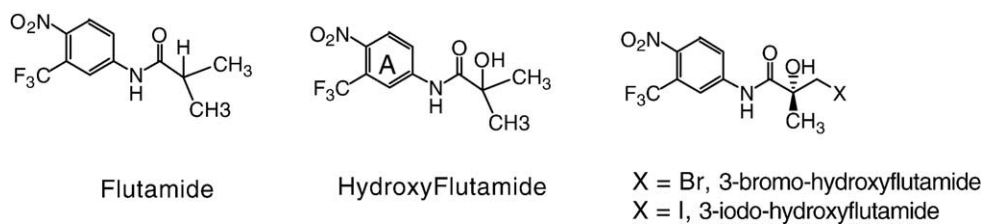


Fig. 1. Nonsteroidal AR ligands.

to have lost the expression of the AR and are insensitive to inhibition of the AR [5,6]. Therefore, it is crucial to determine the role of the AR on an individual basis, before initiating endocrine therapy and after completion, in order to evaluate treatment efficiency.

The actions of androgen hormones are mediated by the AR. Androgens such as testosterone and 5 α -dihydrotestosterone (5 α -DHT) are steroid hormones crucial for the expression of the male phenotype. They play a critical role during development and maintenance of male secondary characteristics [7].

AR ligands can be divided into two main chemical structural classes, steroidal and nonsteroidal, and into two different functional classes, androgenic and antiandrogenic [3].

The first nonsteroidal antiandrogens used for the treatment of prostate cancer have been anilide analogues, with flutamide being the first nonsteroidal antiandrogen used clinically [8]. Its active metabolite, 2-hydroxyflutamide, was shown to have a threefold order higher affinity toward the AR than the flutamide itself (Fig. 1). Bicalutamide is another antiandrogen drug used in clinical practice as a racemic mixture, although its *R*-enantiomer has a higher affinity toward the AR [4].

A novel group of nonsteroidal androgen ligands was developed by Dalton et al. [4]; these compounds contain a chiral center that was created by a substitution of a single hydrogen atom with bromine or iodine on one of the methyl groups of the hydroxyflutamide molecule (Fig. 1). Binding assays of these compounds toward the AR revealed that the binding affinities of the *R*-enantiomers are higher than *S*-enantiomers [3,4].

Since it has been established that the AR is a specific target for prostate cancer treatment and since its loss of expression has been observed in several aggressive prostate tumors, it is critical to determine the role of the AR in

individual patients using a noninvasive molecular imaging modality such as positron emission tomography (PET) in order to guide and monitor treatment.

Currently, imaging tools such as MRI and PET for diagnosing local recurrence and metastatic sites of prostate cancer, which are the most serious clinical challenges, are suboptimal [9–14]. In this field, the most advanced PET agent for AR visualization is an androgenic steroid derivative of 5 α -DHT, [¹⁸F]fluoro-dihydrotestosterone [15–17]. Although the metabolic rate of this labeled compound is rapid, fast tumor uptake and prolonged retention of radioactivity have been observed in human studies [15–17]. While androgenic steroid radiopharmaceuticals have high affinity to the AR, they often have inadequate AR selectivity and tend to bind to additional steroid receptors [15–17].

We report here on the design, synthesis, radiosynthesis and preliminary biological evaluation of novel nonsteroidal flutamide derivative androgen ligands: (*R*)-3-dimethyl-2-hydroxy-2-methyl-*N*-(4-nitro-3-trifluoromethyl-phenyl)-propionamide ([¹¹C]-1), (*R*)-3-dimethyl-2-hydroxy-2-methyl-*N*-(4-cyano-3-trifluoromethyl-phenyl)-propionamide ([¹¹C]-2) and (*R*)-3-dimethyl-2-hydroxy-2-methyl-*N*-(4-fluoro-3-trifluoromethyl-phenyl)-propionamide ([¹¹C]-3) (Fig. 2).

2. Materials and methods

2.1. General methods

All operations with air- and moisture-sensitive compounds were performed using the Schlenk techniques under argon atmosphere. All solvents were of analytical grade. Toluene and THF were distilled over sodium/benzophenone. Other solvents were purchased as anhydrous from Sigma-Aldrich (Tel Aviv, Israel), Fisher Scientific (Pittsburgh, PA,

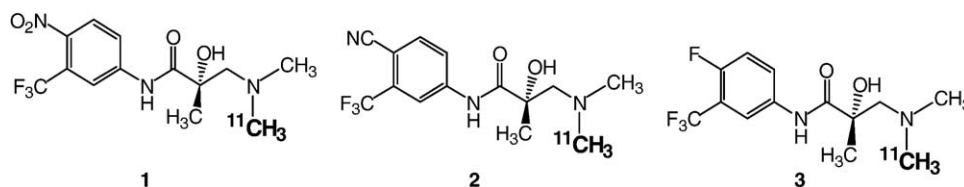


Fig. 2. Novel AR ligands labeled with carbon-11.

USA), Merck (Darmstadt, Germany) or J.T. Baker (New Jersey, USA).

^1H and ^{13}C NMR spectra were recorded on 200-, 300- or 400-MHz spectrometers in CDCl_3 or $\text{DMSO}-d_6$. ^1H and ^{13}C NMR signals are reported in parts per million. ^1H NMR signals are referenced to the residual proton (7.26 ppm for CDCl_3 or 2.50 ppm for $\text{DMSO}-d_6$) of deuterated solvent, and for ^{13}C NMR spectra, the signal of CDCl_3 (77.16 ppm) or $\text{DMSO}-d_6$ (39.52 ppm) was used as a reference. Mass spectra were obtained on a spectrometer equipped with CI, EI and FAB probes and on a spectrometer equipped with ESI probe. HRMS results were obtained on MALDI-TOF and ESI mass spectrometers. IR spectra were recorded on FTIR spectrometer. Optical activity of the chiral molecules was measured in a polarimeter equipped with optical rotation 1 dc cell in CH_2Cl_2 or DMSO solutions. The progress of reactions was monitored by TLC (SiO_2) and visualized by UV light or developed with vanillin spray (1.0 g in 95.0 ml ethanol, 5.0 ml water and 1.0 ml concentrated H_2SO_4) or with iodine chamber. Flash chromatography was carried out on SiO_2 (0.04–0.063 mm).

Elemental analysis was performed at the Hebrew University Microanalysis Laboratory. Radiosyntheses were carried out on a $[^{11}\text{C}]\text{-CH}_3\text{I}$ module (GE, Munster, Germany). Specific radioactivities were determined by HPLC, using cold mass calibration curves. $[^{11}\text{C}]\text{-carbon dioxide}$ was produced by the $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ nuclear reaction on nitrogen containing 0.5% oxygen, using an 18/9 IBA-cyclotron. Bombardment was carried out for 20–30 min with a 16- to 18- μA beam of 16-MeV protons. At the end of bombardment (EOB), the target gas was delivered and trapped by a cryogenic trap in the $[^{11}\text{C}]\text{CH}_3\text{I}$ module.

HPLC was performed on a system with variable wavelength detector operating at 254 nm and with a radioactivity detector with a NaI crystal. Two systems were used: a reverse-phase system employing C-18 column [Bischoff Nucleosil 100-7-C18 reverse phase preparative column (7 μm , 250 \times 16 mm), flow rate of 8.5 ml/min and 33% $\text{CH}_3\text{CN}/67\%$ H_2O as eluent] and a reverse-phase system using a C-18 column (10 μm , 300 \times 3.9 mm) and 35% $\text{CH}_3\text{CN}/65\%$ H_2O as eluents with a flow rate of 1 ml/min for analysis of the formulated radiotracer.

2.2. Molecular modeling

The model complex of (*R*)-3-dimethyl-2-hydroxy-2-methyl-*N*-(4-nitro-3-trifluoromethyl-phenyl)-propionamide (**1**) with the AR was generated by modifying 3-bromohydroxyflutamide into Compound **1**, in the crystal structure of the complex of 3-bromohydroxyflutamide with the AR (2ax9 PDB code). This modification was carried out using DS Modeling 1.1 (Accelrys Software Inc., San Diego, CA). Compound **1**–AR-generated complex was energetically minimized using CHARMM (Accelrys Software Inc.). This procedure was repeated for generating the model complexes of the AR with (*R*)-3-dimethyl-*N*-(4-cyano-3-trifluoromethyl-phenyl)-2-hydroxy-2-methyl-propionamide

(**2**) and (*R*)-3-dimethyl-*N*-(4-fluoro-3-(trifluoromethyl)-phenyl)-2-hydroxy-2-methyl-propanamide (**3**).

2.3. Chemistry

2.3.1. (*R*)-3-Dimethyl-2-hydroxy-2-methyl-*N*-(4-nitro-3-trifluoromethyl-phenyl)-propionamide (**1**)

Dimethylamine (2 M in THF, 9 ml) was stirred and cooled in an ice bath. A solution of (*R*)-3-bromo-2-hydroxy-2-methyl-*N*-(4-nitro-3-(trifluoromethyl)phenyl) propanamide (**8a**) [18] (300 mg, 0.808 mmol) in dimethyl acetamide (5 ml) was added dropwise; the reaction temperature was raised to 40°C, and the mixture was stirred for 2 h. Then, ethyl acetate (30 ml) and saturated NaHCO_3 (30 ml) were added. The layers were separated, and the organic layer was washed with brine, dried (MgSO_4) and evaporated. The residual oil was purified by flash chromatography on silica gel ($\text{MeOH}-\text{CH}_2\text{Cl}_2$, 5:95) to give 160 mg (59%) of Compound **1** as yellow oil. MS (m/z) 336 (MH^+); ^1H NMR (300 MHz, CDCl_3): δ 9.50 (s, 1H), 8.1 (d, 1H, $J=1.5$ Hz), 8.0 (dd, 1H, $J_1=1.5$, $J_2=6.0$ Hz), 7.8 (d, 1H, $J=6.0$ Hz), 3.1 (d, 1H, $J=3.0$ Hz), 2.45 (d, 1H, $J=3.0$ Hz), 2.30 (s, 6H), 1.41 (s, 3H).

Elemental analysis $\text{C}_{13}\text{H}_{16}\text{F}_3\text{N}_3\text{O}_4 \cdot 2\text{CH}_3\text{OH}$: Calculated: C=45.1%, H=6.01%, N=10.52%. Found: C=45.19%, H=5.63%, N=10.09%.

2.3.2. (*R*)-3-Dimethyl-*N*-(4-cyano-3-trifluoromethyl-phenyl)-2-hydroxy-2-methyl-propionamide (**2**)

The synthesis of Compound **2** proceeded in the same manner as Compound **1**. Compound **8b** (300 mg, 0.854 mmol) was added to a cooled solution of dimethylamine (2 M) to give 120 mg (45%) of Compound **2** as a white solid. MS (m/z) 316 (MH^+). Mp. 81°C. ^1H NMR (300 MHz, CDCl_3): δ 9.47 (s, 1H), 8.01 (d, 1H, $J=1.5$ Hz), 7.93 (dd, 1H, $J_1=1.5$, $J_2=6.0$ Hz), 7.78 (d, 1H, $J=6.0$ Hz), 3.0 (d, 1H, $J=3.0$ Hz), 2.45 (d, 1H, $J=3.0$ Hz), 2.28 (s, 6H), 1.50 (s, 3H).

Elemental analysis $\text{C}_{14}\text{H}_{16}\text{F}_3\text{N}_3\text{O}_2 \cdot \text{CH}_3\text{OH}$: Calculated: C=51.85%, H=5.76%, N=12.1%. Found: C=51.87%, H=5.28%, N=12.96%.

2.3.3. (*R*)-3-Dimethyl-*N*-(4-fluoro-3-(trifluoromethyl)-phenyl)-2-hydroxy-2-methyl-propanamide (**3**)

The synthesis of Compound **3** proceeded in the same manner as Compound **1**. (*R*)-3-Bromo-2-hydroxy-2-methyl-*N*-(4-fluoro-3-(trifluoromethyl)phenyl) propanamide (**8c**) (300 mg, 0.872 mmol) was added to a cooled solution of dimethylamine (2 M) to give 140 mg (52%) of Compound **3** as a white oil. MS (m/z) 310 (MH^+); ^1H NMR (300 MHz, CDCl_3): δ 9.17 (s, 1H), 7.8 (d, 1H, $J=6$ Hz), 7.7 (dd, 1H, $J=9.0$ Hz), 7.12 (d, 1H, $J=6.0$ Hz), 3.1 (d, 1H, $J=3.0$ Hz), 2.41 (d, 1H, $J=3.0$ Hz), 2.30 (s, 6H), 1.40 (s, 3H).

Elemental analysis $\text{C}_{13}\text{H}_{16}\text{F}_4\text{N}_2\text{O}_2$: Calculated: C=50.65%, H=5.23%, N=9.09%, F=24.65%. Found: C=50.93%, H=5.41%, N=8.77%, F=24.17%.

2.3.4. (*R*)-1-(2-Methylacryloyl)pyrrolidine-2-carboxylic acid (**4**) [18]

A solution of methacryloyl chloride (32 ml, 443 mmol) in acetone (240 ml) was added dropwise at 0°C to a solution of D-proline (50.0 g, 434 mmol) in 2.0 M aqueous NaOH (240 ml) and acetone (240 ml). During the addition, the reaction pH was monitored and kept within a 10.3±0.3 range via simultaneous dropwise addition of 2.0 M aqueous NaOH. At the end of the addition, the reaction mixture was allowed to warm up to room temperature and stirred for additional 4 h. Following the evaporation of acetone, the mixture was acidified to pH 2 with concentrated HCl and NaCl added to saturation. The resulting solution was extracted with ethyl acetate (3×250 ml), and the combined organic extracts were dried over MgSO₄. After solvent evaporation, the crude product was purified by crystallization (ethyl acetate/hexanes) to yield Compound **4** as a white solid (64.05 g, 81%). Mp 103.5–104.5°C (Refs. [19–21], 102.5–103.5°C).

¹H NMR (200 MHz, DMSO-*d*₆) [major rotamer]: δ 5.33 (ABq, 2H, *J*=12.0 Hz), 5.20, 4.32–4.35 (m, 1H), 3.35–3.55 (m, 2H), 2.25–2.40 (m, 2H), 1.90–2.10 (m, 2H), 1.94 (s, 3H); [minor rotamer]: δ 5.20–5.33 (ABq, 2H, *J*=24 Hz), 4.52–4.55 (m, 1H), 3.60–3.65 (m, 2H), 2.25–2.40 (m, 2H), 1.90–2.10 (m, 2H) 1.88 (s, 3H).

¹³C NMR (50 MHz, DMSO-*d*₆) [major rotamer]: δ 173.27, 169.13, 140.83, 116.44, 58.25, 48.77, 28.91, 24.72, 19.53; [minor rotamer]: δ 174.04, 170.01, 141.60, 115.28, 60.25, 40.67, 30.97, 22.35, 19.66. MS (CI): *m/z* 184.1 (MH⁺, 100). IR (KBr): 3509, 2960, 1734, 1587, 1458, 1174/cm.

2.3.5. (3*R*, 8- α *R*)-3-(Bromomethyl)-3-methyl-tetrahydro-3*H*-pyrrolo [2,1-*c*] [1,4]oxazine-1,4-dione (**5**) [18]

A solution of NBS (142.20 g, 800 mmol) in dry DMF (500 ml) was added dropwise in light-protected environment at 0°C to a solution of Compound **4** (112.59 g, 615 mmol) in dry CCl₄ (320 ml) and dry DMF (400 ml). After the end of addition, the reaction mixture was stirred at 0°C for 2 h, allowed to warm up to room temperature and stirred for additional 48 h. Following the evaporation of CCl₄, saturated NaCl aqueous solution (700 ml) was added, and the mixture was extracted with ethyl acetate (3×400 ml). The combined organic extracts were washed with water (3×200 ml) and dried over MgSO₄. After ethyl acetate evaporation, the crude product was purified by crystallization (CH₂Cl₂/hexanes) to yield Compound **5** as a white solid (154.64 g, 96%). Mp 158.1–159.9°C (Refs. [19–21], 152–154°C).

¹H NMR (200 MHz, DMSO-*d*₆): δ 4.81 (dd, 1H, *J*₁=7.5, *J*₂=9.5 Hz), 3.96, 4.13 (ABq, 2H, *J*=12.0 Hz), 3.48–3.69 (m, 2H), 2.31–2.43 (m, 2H), 1.85–2.10 (m, 2H), 1.67 (s, 3H). ¹³C NMR (50 MHz, DMSO-*d*₆): δ 167.33, 163.11, 83.92, 57.265, 45.47, 37.86, 29.01, 22.95, 21.63. MS (CI): *m/z* 262.0 (MH⁺, 100). IR (KBr): 3861, 1744, 1686, 1449, 1061, 649/cm.

2.3.6. (*R*)-3-Bromo-2-hydroxy-2-methylpropanoic acid (**6**) [18]

A solution of Compound **5** (10.0 g, 38.15 mmol) in 24% aqueous HBr (170 ml) was heated to 105°C for 90 min. After cooling to room temperature, solid NaCl was added until saturation, and the mixture was extracted with ethyl acetate (4×200 ml). The combined ethyl acetate fractions were extracted with saturated NaHCO₃ aqueous solution (4×200 ml); the resulting aqueous solution was acidified to pH 1 and was then extracted with ethyl acetate (4×200 ml). The combined ethyl acetate extracts were dried over MgSO₄ and evaporated to yield Compound **6** as a white solid (5.86 g, 84%). Mp 111.4–113.1°C (Refs. [19–21], 106.5–109.0°C).

¹H NMR (200 MHz, DMSO-*d*₆): δ 3.62, 3.75 (ABq, 2H, *J*=10.0 Hz), 1.47 (s, 3H).

¹³C NMR (50 MHz, DMSO-*d*₆): δ 174.60, 73.19, 41.02, 24.55. MS (CI): *m/z* 182.9 (M⁺, 25), 103.0 (M⁺-Br, 100).

2.3.7. (*R*)-3-Bromo-2-hydroxy-2-methylpropanoyl chloride (**7**) [18]

A round-bottomed flask with a nitrogen flow connector was charged with Compound **6** (40 mg, 0.22 mmol) in 2.0 ml of dry dichloromethane. Oxalyl chloride (38.5 μl, 0.44 mmol) in dry DMF (100 μl) was added to this solution under nitrogen at -5°C to -10°C. The resulting mixture was stirred for 3 h under the same conditions. The excess of oxalyl chloride and the dichloromethane were evaporated under vacuum. Compound **7** was used for the synthesis without further purification.

2.3.8. (*R*)-3-Bromo-2-hydroxy-2-methyl-*N*-(4-nitro-3-(trifluoromethyl)phenyl) propanamide (**8a**) [18]

Thionyl chloride (2.5 ml, 31.90 mmol) was added dropwise at -12°C under argon to a solution of Compound **6** (5.0 g, 27.32 mmol) in dry dimethylacetamide (32 ml). Under the same temperature, the resulting mixture was stirred for 3 h and then a solution of 4-nitro-3-(trifluoromethyl)benzenamine (5.63 g, 27.32 mmol) in dry dimethylacetamide (37 ml) was added dropwise. The reaction mixture was allowed to warm up to room temperature and stirred for additional 6 h. After dimethylacetamide evaporation, the crude orange oil was purified by flash chromatography (SiO₂; CH₂Cl₂; R_f=0.3) to give a yellow wax that was further purified by selective precipitation from diethyl ether/hexanes solution to yield Compound **8a** as a light yellow solid (4.56 g, 45%). Mp 103.0–103.8°C (Refs. [19–21], 100.0–101.5°C).

¹H NMR (200 MHz, DMSO-*d*₆): δ 10.60 (s, 1H), 8.57 (d, 1H, *J*=2.0 Hz), 8.37 (dd, 1H, *J*₁=2.0, *J*₂=9.0 Hz), 8.21 (d, 1H, *J*=9.0 Hz), 6.43 (s, 1H), 3.60, 3.85 (ABq, 2H, *J*=10.5 Hz), 1.50 (s, 3H).

¹³C NMR (100 MHz, DMSO-*d*₆): δ 173.55, 142.89, 141.60, 127.15, 123.02, 122.98 (q, *J*=33 Hz), 122.37, 118.21 (q, *J*=6 Hz), 74.35, 41.28, 24.79. MS (CI): *m/z* 373.0 (MH⁺, 7), 291.1 (MH⁺ HBr, 100).

2.3.9. (*R*)-3-Bromo-2-hydroxy-2-methyl-*N*-(4-cyano-3-(trifluoromethyl)phenyl) propanamide (**8b**) [19]

The synthesis of Compound **8b** was done as described for Compound **8a** with the following change: 4-cyano-3-(trifluoromethyl)benzenamine was added dropwise to a solution of Compound **6** in dimethylacetamide at -12°C to give 1.8 g (23% yield) of Compound **8b** as a white solid.

Mp $106\text{--}108^{\circ}\text{C}$. (Ref. [19], $106.0\text{--}107^{\circ}\text{C}$). ^1H NMR (200 MHz, DMSO-*d*6): δ 10.60 (s, 1H), 8.63 (d, 1H, $J = 2.0$ Hz), 8.39 (dd, 1H, $J = 8.6$ Hz), 8.18 (d, 1H, $J = 8.6$ Hz), 6.49 (s, 1H), 3.91 (d, $J = 10.36$ Hz, 1H, CHH_b), 3.67 (d, $J = 10.38$ Hz, 1H, CHH_a), 1.58 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 169.42, 169, 141.60, 130.7, 128.54, 122.37, 82.49, 70.68, 22.45, 19.99, 14.78. MS (m/z) 351 (MH^+).

2.3.10. (*R*)-3-Bromo-2-hydroxy-2-methyl-*N*-(4-fluoro-3-(trifluoromethyl)phenyl) propanamide (**8c**) [18]

The synthesis of Compound **8c** was done as described for Compound **8b** with the following change: 4-fluoro-3-(trifluoromethyl)benzenamine was added dropwise to a solution of Compound **6** in dimethylacetamide at -12°C to give 1.4 g (56% yield) of Compound **8c** as a white solid.

Mp $103.0\text{--}104.5^{\circ}\text{C}$. ^1H NMR (400 MHz, CDCl_3): δ 8.85 (s, 1H), 7.92 (dd, 1H, $J = 6.0, 4.0$ Hz), 7.77 (dt, 1H, $J = 9.0, 4.0$ Hz), 7.21 (t, 1H, $J = 9.0$ Hz), 4.04 (ABq, 2H, $J = 10.5$ Hz), 3.63, 3.39 (s, 1H), 1.66 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3): δ 171.48, 156.40 (d, $J = 253.0$ Hz), 133.28, 125.27 (d, $J = 8.0$ Hz), 122.35 (q, $J = 122.0$ Hz), 118.84 (q, $J = 5.0$ Hz), 117.65 (d, $J = 22$ Hz), 117.49, 75.44, 41.41, 24.91. ^{19}F NMR (376 MHz, CDCl_3): δ 119.34, 61.93. MS (CI): m/z 344.1 (MH^+ , 100), 343.1 (M^+ , 20). HRMS (MALDI-TOF): m/z calculated for $\text{C}_{11}\text{H}_{10}\text{NO}_2\text{F}_4\text{NaBr}$ (MNa^+): 365.9723. Found: 365.9692. IR (KBr): 3333, 2928, 1674, 1513, 1133, 609/cm. UV-Vis (MeCN): λ_{max} 283, 241 nm.

2.3.11. (*R*)-3-Monomethylamine-2-hydroxy-2-methyl-*N*-(4-nitro-3-(trifluoromethyl)phenyl) propanamide (**9a**)

Methylamine (2 M in THF, 1 ml) was added to a solution of Compound **8a** (8–10 mg) in dry DMSO (70 μl), at 0°C . After a 15-min reaction, 0.013 M NaOH (10 ml) was added. The solution was passed through 2 \times C-18 cartridges (Waters Sep-Pak Plus, Massachusetts, USA, preactivated with 10 ml EtOH and 20 ml of sterile water), and the cartridges were dried under nitrogen stream. The product was eluted with 4 ml of THF and dried with Na_2SO_4 . Filtration with a 0.45- μm filter and THF evaporation under reduced pressure gave the crude products **9a**, which were used for the radiolabeling without any further purification. MS (m/z) 322 (MH^+).

2.3.12. (*R*)-3-Monomethylamine-2-hydroxy-2-methyl-*N*-(4-cyano-3-(trifluoromethyl)phenyl) propanamide (**9b**)

The synthesis of Compound **9b** proceeded in the same manner as Compound **9a**. Methylamine (2 M in THF, 1 ml)

was added into a solution of Compound **8b** in dry DMSO (70 μl), at 0°C . MS (m/z) 302 (MH^+).

2.3.13. (*R*)-3-Monomethylamine-2-hydroxy-2-methyl-*N*-(4-fluoro-3-(trifluoromethyl)phenyl) propanamide (**9c**)

The synthesis of Compound **9c** proceeded in the same manner as Compound **9b**. Methylamine (2 M in THF, 1 ml) was added into a solution of Compound **8c** in dry DMSO (70 μl), at 0°C . MS (m/z) 295 (MH^+).

2.4. Biological assay

2.4.1. Androgen receptor binding affinity assays

Relative binding affinities were determined by a competitive radiometric binding assay as previously described [22–24], using 10 nM [^3H] R1881 as tracer (methyltrienolone; Perkin Elmer, Boston, MA) and purified ligand binding domain of the AR from Pan Vera/Invitrogen (Carlsbad, CA). Incubations were for 18–24 h at 0°C . Hydroxylapatite (BioRad, Hercules, CA) was used to absorb the receptor–ligand complexes, and free ligand was washed away. The binding affinities are expressed as relative binding affinity (RBA) values with the RBA of R1881 set to 100%. The values given are reproducible to within 30% relative error.

2.5. Radiochemistry

2.5.1. Labeling with carbon-11

2.5.1.1. (*R*)-3-Dimethyl-2-hydroxy-2-methyl-*N*-(4-nitro-3-(trifluoromethyl)phenyl)-propionamide [^{11}C -1]. Carbon-11 MeI was prepared according to well-documented procedures [25]. Briefly, [^{11}C]CO₂ (37 GBq, 1000 mCi) was trapped at -160°C . The temperature of the cooling trap was increased to -50°C , and the activity was transferred by a stream of argon (40 ml/min) into reactor-1 containing 300 μl of 0.25 N LiAlH_4 in THF at -50°C [26]. After 90 s, the solvent was removed under reduced pressure. In this manner, more than 80% of the activity was recovered. The reactor temperature was increased to 160°C , HI was added and [^{11}C]MeI was distilled (argon flow of 15 ml/min) through a NaOH column to the second reactor, containing the precursor, Compound **9a**, in 600 μl of dry dimethylacetamide at -20°C . After 1 min of distillation, an average of 550 ± 30 mCi ($n = 15$) was trapped in the second reactor. The reactor was sealed and heated to 80°C for 5 min. At the end of the 5-min reaction, the solvent was removed under flow of argon at 80°C for 1 min. The mixture was cooled to 40°C ; 0.6 ml of $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (1:1) was added, and the crude product (average of 250 ± 20 mCi ($n = 15$)) was automatically injected to the HPLC [Bischoff Nucleosil 100-7-C18 reverse-phase preparative column (7 μm , 250×16 mm), flow rate of 8.5 ml/min]. The labeled product was collected in a flask containing 450 μl of 1 M NaOH in 20 ml of water. The solution was passed through a 2 \times C-18 cartridge (Waters Sep-Pak Plus, preactivated with 10 ml EtOH and 20 ml of

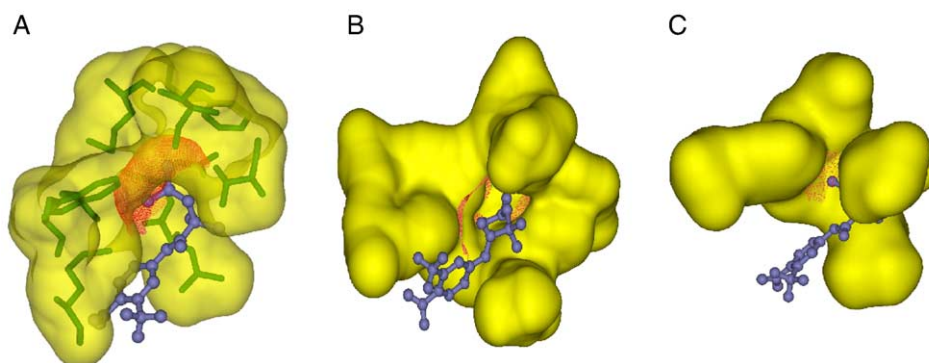


Fig. 3. The binding pocket in the AR of the dimethylamine group in Compound **1** and the bromide atom in 3-bromo-hydroxyflutamide: molecular surface of the residues within 4.5 Å around the dimethylamine group (A and B) and the bromide atom (C). These residues around the dimethylamine group are depicted in green sticks model (A). The molecular surface of the binding pocket is shown in yellow, and that of the dimethylamine group (A and B) and of the bromide atom (C) is shown in red. Compound **1** in Panel A is rotated approximately 115° around the *x*-axis compared with Panel B (the benzyl carbon that is connected to the trifluoromethyl is the center of rotation).

sterile water). The product was eluted with 0.75 ml of EtOH, followed by 4.25 ml of saline and collected into the product vial after a total radiosynthesis time of 50 min, with total activity of 48 ± 6 mCi and radiochemical yield of $15 \pm 3\%$ decay-corrected EOB ($n=5$). Identification of the products and determination of chemical and radiochemical purities were obtained by reverse-phase HPLC C-18 analytical column in comparison to the standard and by coinjection.

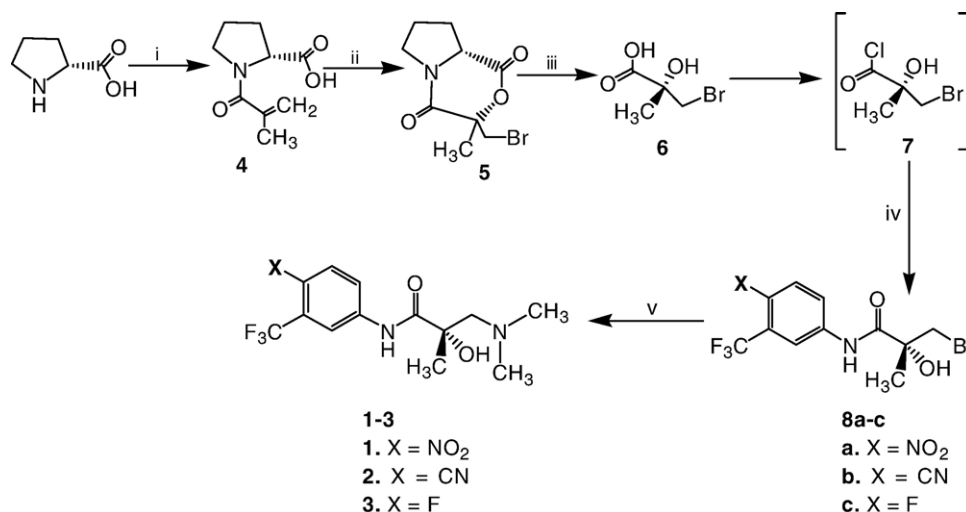
2.5.1.2. (R)-3-Dimethyl-2-hydroxy-2-methyl-N-(4-cyano-3-trifluoromethyl-phenyl)-propionamide [¹¹C-2]. The synthesis of Compound [¹¹C]-**2** proceeded in the same manner as Compound [¹¹C]-**1**. Precursor **9b** reacted with [¹¹C]CH₃I. The average activity obtained was 27 ± 4 mCi with radiochemical yield of $10 \pm 2\%$ decay-corrected EOB ($n=6$).

2.5.1.3. (R)-3-Dimethyl-2-hydroxy-2-methyl-N-(4-fluoro-3-trifluoromethyl-phenyl)-propionamide [¹¹C-3]. The synthesis of Compound [¹¹C]-**3** proceeded in the same manner as Compound [¹¹C]-**2**. Precursor **9c** reacted with [¹¹C]CH₃I. The average activity obtained was 40 ± 4 mCi with radiochemical yield of $12 \pm 3\%$ decay-corrected EOB ($n=4$).

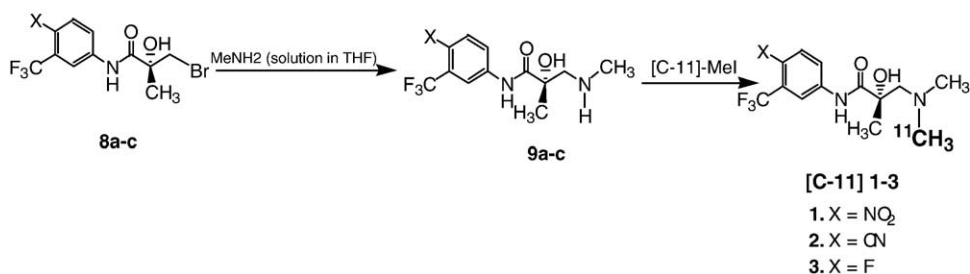
3. Results

3.1. Molecular modeling

The interactions between the 3-bromohydroxyflutamide with the AR binding site pocket were described [27]. The models of the AR complexes with Compounds **1**, **2** and **3** showed that all of the described interactions between AR binding site and 3-bromohydroxyflutamide have been



Scheme 1. Synthesis of Compounds **1**, **2** and **3**. Reagents and conditions: (i) methacryloyl chloride, NaOH_(aq), acetone, 0°C; (ii) *N*-bromosuccinimide, CCl₄, DMF, 0°C; (iii) 24% HBr_(aq), 105°C; (iv) thionyl chloride, dimethylacetamide, (1) 4-nitro-3-(trifluoro-methyl)benzenamine, (2) 4-cyano-3-(trifluoro-methyl)benzenamine, (3) 4-fluoro-3-(trifluoro-methyl)benzenamine, -12°C; (v) dimethylamine 2 mol/L (solution in THF), dimethylacetamide, 40°C.



Scheme 2. Synthesis of novel AR ligands, Compounds **1**, **2** and **3**, labeled with carbon-11.

preserved [27]. However, our model suggests some additional interactions formed by the dimethylamine group that substituted the bromide atom.

A 3.5-Å cutoff distance between the donor and acceptor atoms was taken for putative hydrogen bonds, and a cutoff distance of 4.5 Å was taken for hydrophobic interactions.

The tertiary nitrogen atom of the dimethylamine group is an acceptor in the hydrogen bond formed with the NH₂ group of Asn 705. In addition to the hydrogen bond, the dimethylamine group makes 33 van der Waals (VDW) contacts with residues Thr 877, Phe 891, Ile 899, Met 895, Trp 741, Asn 705, Met 745, Gln 708 and Leu 704. These residues generate a hydrophobic pocket that embedded the dimethylamine group and stabilized it (Fig. 3).

3.2. Chemistry

The synthesis of Compounds **1**, **2** and **3** is outlined in Scheme 1. Initial steps of the synthesis were on the basis of the published procedures [19], which utilize nonnatural D-proline as a chiral auxiliary to generate in three synthetic steps enantiomerically pure 3-bromo-2-hydroxy-2-methylpropionic acid (Compound **6**). Coupling of Compound **6** to different aniline derivatives such as 4-nitro-3-(trifluoromethyl)benzenamine, 4-cyano-3-(trifluoromethyl)benzenamine and 4-fluoro-3-(trifluoromethyl)benzenamine yielded pure *R*-enantiomers of Compounds **8a**, **8b** and **8c**, respectively. Reaction of Compounds **8a**, **8b** and **8c** with dimethylamine (2 M in THF solution) in dimethylacetamide at 40°C for 2 h furnished Compound **1** with a chemical yield of 59% as yellow oil, Compound **2** with a chemical yield of 45% as a white solid and Compound **3** with a chemical yield of 52% as a white oil.

The precursors for the radiolabeling, Compounds **9a**, **9b** and **9c**, were obtained by a reaction of Compounds **8a**, **8b** and **8c** with monomethylamine 2 M in THF at 0°C for 15 min (Scheme 2).

It should be noted that the crude mixture of this reaction could be purified neither by silica gel nor by alumina gel column due to the formation of side products. When kept as a solution at room temperature or when loaded on column for purification, decomposition was observed by MS, HPLC and NMR. The main side product observed was the epoxide (Fig. 4) [18]. At the end of the workup, the solvent was quickly removed under reduced pressure without heating, and the crude product was kept at -20°C and used as is for the radiosynthesis.

3.3. AR binding

In order to test the affinity of the compounds to the AR, the novel AR ligands were tested in RBA assay, which is a competitive binding assay with ³H R1881, a high-affinity AR binding ligand [22–24]. As can be seen in Table 1, Compounds **1**, **2** and **3** had RBAs of 0.03%, 0.036% and 0.029%, respectively, which are similar to those of hydroxyflutamide and bicalutamide and superior to flutamide.

3.4. Radiochemistry

The radiolabeling approach was based on a C-11 methylation reaction of the monomethylamine group. This carbon-11 radiolabeling reaction used a commercial module (GE). Carbon-11 MeI was prepared according to well-documented procedures [25] and reacted with 8–10 mg of the precursors (Compounds **9a**, **9b** and **9c**; Scheme 2). The C-11 methyl-iodide was distilled out to the second reactor, which contained the precursors (Compounds **9a**, **9b** and **9c**) in dry dimethylacetamide at -20°C. Because of the instability of the precursor, the C-11 methylation occurred at low temperature. The optimal temperature for this reaction was found to be 80°C. After a 5-min reaction and evaporation, HPLC solvent was added and the crude mixture was purified by a built-in HPLC to yield the final products.

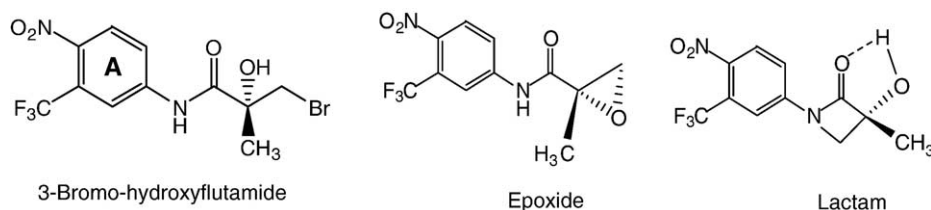


Fig. 4. Nonsteroidal androgen, 3-bromo-hydroxyflutamide and possible side products [18].

Table 1
RBA assays for AR ligands

Compound	RBA (%)
R1881	100
<i>R</i> -Bicalutamide	0.044
<i>R,S</i> -Bicalutamide	0.037
Flutamide	0.001
Hydroxyflutamide	0.027
3-Bromohydroxyflutamide	1.9
(<i>R</i>)-F-1	0.057
(<i>R</i>)-F-2	0.027
(<i>R</i>)-1	0.030
(<i>R</i>)-2	0.036
(<i>R</i>)-3	0.029

The desired fraction was collected into a flask. In order to dilute the acetonitrile in the mobile phase, the flask was preloaded with 450 μ l of 1 mol/L NaOH in 20 ml of water [26]. Basic conditions were provided to obtain Compounds [11 C]-1, [11 C]-2 and [11 C]-3 as a free base. Finally, the products were obtained by two C-18 cartridges and were eluted with ethanol and saline. The described automated procedure enabled us to reproducibly ($n=15$) obtain [11 C]-1, [11 C]-2 and [11 C]-3 in good yields. For Compound [11 C]-1, the highest final product activity dose achieved was 48 mCi; for Compound [11 C]-2, it was 27 mCi, and for Compound [11 C]-3, it was 40 mCi. HPLC analysis of the product solutions showed high radiochemical (>99%) and chemical purities. The high specific radioactivity achieved in the automated radiosynthesis (4 Ci/ μ mol EOB) is an important factor for the development of PET biomarkers that are targeted to low-capacity systems.

4. Discussion

At its initiation, prostate cancer is primarily dependent on the AR. However, aggressive forms of prostate cancer may lose the expression of the AR and are insensitive to anti-AR treatment. As a result, efficient hormonal therapy requires an individualized approach based on tumor biochemical analysis showing AR presence and activity prior to, during and after treatment. While noninvasive PET has the potential to determine AR expression on tumor cells in vivo, appropriate selective imaging agents are lacking.

Anilide analogues such as flutamide and its active metabolite, 2-hydroxyflutamide, were the first nonsteroidal antiandrogens used in the clinical setting (Fig. 1). He et al. [3] have suggested that electron-withdrawing groups located at the aromatic ring A of these compounds, coupled to a branched alkyl group α to the amide, were required for binding and functional activity of androgen ligands (Fig. 1). It is also known that the hydroxyl group of hydroxyflutamide plays an important role in the interaction to the AR [28]. Based on these requirements, a novel group of AR agonists was developed by Dalton et al. [4] (Fig. 1). While these ligands showed high affinity toward the AR, the electron-withdrawing groups induced shifts in electron density

toward the aromatic ring A. This shift of electrons led to the rapid deprotonation of the hydroxyl group, the amide group or both, resulting in instability of the molecules. Indeed, decomposition via intranucleophilic attack to form lactam or epoxide has been previously observed with these bromine or iodine hydroxyflutamide derivatives (Fig. 4) [18,21]. This structural instability decreased the biological half-life of the compounds and led to rapid metabolic rate. When these compounds were labeled with bromine-76 and injected into tumor-bearing mice, low accumulation of activity in tumors has been observed by a micro-PET study [29].

In an attempt to increase biological stability and improve in vivo tumor uptake, we designed, synthesized and radiolabeled with C-11 additional novel nonsteroidal AR ligands. Similar to the previously reported compounds [18], these novel AR ligands (Compounds 1, 2 and 3) are enantiomeric analogues of flutamide and contain the functional electron-withdrawing groups at the aromatic moiety essential for AR binding [3]. In order to stabilize the electron distribution directed toward the aromatic ring A (Fig. 4), an electron-rich group, dimethylamine, was added at the opposite side of the pole, that is, methyl group, replacing one of the hydrogen and forming a chiral center. In addition, dimethylamine is a very ineffective leaving group and unlikely to form side products such as epoxide via intranucleophilic attack like the bromine derivative does. The dimethylamine group can also serve as an “anchor” for future carbon-11 labeling. This enables an easier labeling procedure than for fluorine-18 labeling [18].

In order to estimate the potential new interactions of these compounds with the AR, molecular modeling was carried out for Compounds 1, 2 and 3 (Fig. 3). We also compared the contribution of each electron-withdrawing group (NO₂, CN and F) to these interactions. From the crystal structure of 3-bromo-hydroxyflutamide [27], it was shown that while the NO₂ group on the aromatic ring A could form two hydrogen bonds with the receptor, one of the hydrogen bonds was via one molecule of water [27]. From the modeling results that we have performed, it appears that Compounds 2 and 3 with the cyano and fluorine groups on the aromatic ring A could form one hydrogen bond with the receptor. The dimethylamine group interacted with nine amino acids of the AR, while the bromide group interacted only with four. The dimethylamine group was stabilized by additional 33 VDW bonds and 1 hydrogen bond, creating a hydrophobic core that may increase the interaction time with the receptor (Fig. 3). In contrast, the bromide group had fewer interactions with the AR and was not stabilized by the hydrophobic effect. Overall, the stabilization of Compound 1-AR, Compound 2-AR and Compound 3-AR complexes over the 3-bromo-hydroxyflutamide and hydroxyflutamide was higher.

Since it is well known that the *R*-enantiomers of flutamide derivatives are more potent than the *S*-enantiomers, we designed the synthesis of the target ligands as a

regioselective chemical transformation using D-proline to yield the *R*-enantiomers.

Compounds **8a**, **8b** and **8c** were key intermediates in the synthesis of the *R*-hydroxyflutamide derivatives (Compounds **1**, **2** and **3**; Scheme 1). Reaction of Compounds **8a**, **8b** and **8c** with dimethylamine yielded Compounds **1**, **2** and **3** with a chemical yield of 59%, 45% and 52%, respectively.

In an in vitro RBA assay, Compounds **1**, **2** and **3** (Fig. 2) had higher affinity to the AR than flutamide and similar affinities to hydroxyflutamide and bicalutamide (Table 1). Hence, if these compounds possess an antagonist function to the AR, they may also be better therapeutic agents in AR-dependent prostate cancer.

Diagnosis of pelvic prostatic malignancy with the most commonly used PET biomarker, ^{18}F -FDG, is not practical due to high accumulation of activity in the urine bladder, which masks the prostatic bed. Currently, PET imaging for prostate cancer is done almost exclusively with ^{11}C -choline and ^{11}C -acetate, nonspecific markers that measure metabolism and which are not selective to the AR and therefore cannot monitor and guide AR targeted treatment. Therefore, labeling of Compounds **1**, **2** and **3** with ^{11}C on one of the methyl groups of the dimethylamine group or with ^{18}F on the aromatic ring (if defluorination would prove to be minor) may have a better potential not only for the diagnosis of AR-dependent prostate cancer but also for monitoring and guiding further treatment.

The approach for labeling Compounds **1**, **2** and **3** with carbon-11 is via the reaction of label methyl-iodide with the monomethylamine precursors (Compounds **9a**, **9b** and **9c**; Scheme 2). One major drawback in this approach is the instability of the starting material, the monomethylamine, which decomposed upon purification to give the epoxide that was identified by NMR and MS. The moderate radiochemical yields of the desired products (10–15%) are due to the fact that monomethylamine was used as crude mixture. An automated radiosynthesis for the preparation of the radiolabeled bioprobes (Compounds [^{11}C]-**1**, [^{11}C]-**2** and [^{11}C]-**3**) was developed, leading to the production of these labeled compounds with high specific radioactivity (4 Ci/ μmol EOB), which is an important factor for the development of PET biomarkers in general, as well as for Compounds **1**, **2** and **3**, designed to target in vivo low-capacity receptor systems, in particular.

5. Conclusions

Novel AR ligands have been designed based on molecular modeling, indicating their higher interaction with the receptor over 3-bromo-hydroxyflutamide and hydroxyflutamide. These compounds were synthesized and have demonstrated higher or similar affinities to the AR than the currently used commercial drugs. Unlike other reported nonsteroidal radiolabeled AR ligands, these compounds have an electron-rich group (dimethylamine) located on the methyl moiety, which may confer a better stability to the

molecule and, in addition, serve as an anchor for carbon-11 labeling in a more straightforward approach than labeling with fluorine-18 or bromine-76. An automated radiosynthetic route for the preparation of these novel ^{11}C -containing hydroxyflutamide derivatives was developed. If these compounds demonstrate an antagonist effect on the AR, they may also be superior therapeutic endocrine agents for prostate cancer than the currently used drugs.

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