Synthesis of Androprostamine A and Resormycin

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Syntheses of androprostamine A (1), and resormycin (3), anti-prostate cancer peptidyl natural products produced by microorganisms, were completed. The characteristic enamide structures of these compounds were installed using the Horner–Wadsworth–Emmons reaction from the corresponding phosphonates in reasonable Z-selectivity.

Key words antitumor agent; prostate cancer; natural product; synthesis

Secondary metabolites of microorganisms are rich sources of biologically active natural products from which extremely important medicines for public health can be developed.1) Fascinated by the structural variation and diverse biological activities demonstrated by natural products, we have been involved in exploratory research for anticancer seeds of microbial origin with novel modes of action.

Prostate cancer is one of the leading causes of male mortality, especially in developed countries.2) In the early stage of prostate cancer before metastasis occurs, surgical removal of the prostate or radiation therapy are effective. Even after metastasis is observed, androgen ablation therapy is applicable as long as the cancer remains at an androgen-dependent stage. In many cases, however, successful androgen ablation therapy is followed by relapse of the cancer with exacerbated malignancy at the androgen-independent stage. The cancer in this state is called castration-resistant prostate cancer (CRPC),3) and the choice of treatment for curation is extremely limited. The molecular background of CRPC development has been actively studied, and it is now widely known that the androgen receptor (AR) retains its function even at this advanced level of the disease.4) In fact, AR knockdown inhibits CRPC growth.5) Although conventional antiandrogens such as bicalutamide and flutamide are not effective for the progressed prostate cancer,6) a novel type of inhibitor could potentially affect the AR signaling pathway of CRPC.

Androprostamines A (1) and B (2) produced by Streptomyces sp. MK932-CF8 were discovered as inhibitors of AR functions;5) these natural products inhibit the androgen-dependent proliferation of human prostate cancer LNCaP and VCaP cells (Fig. 1). Interestingly, androprostamines inhibit growth without cytotoxicity and reduce the androgen-induced expression of AR-regulated genes.6) To explore the potential of these natural products as leads for chemotherapeutics against prostate cancer, we aimed to establish synthetic routes to androprostamines and related compounds with which extensive structure–activity relationship (SAR) studies could be performed.

Androprostamines are peptide compounds sharing a part of their structure with that of resormycin8,9) (3) containing the three non-proteinogenic amino acids: a characteristic dehydroamino acid at C-terminus, hydroxyvaline, and β-homolysine. Herein we disclose the first synthesis of natural products of this class, androprostamine A (1) and resormycin (3). Key to the success of the synthetic study was the preparation of the dehydroamino acid moiety by taking advantage of the Horner–Wadsworth–Emmons (HWE) reaction.

![Fig. 1. Structure of Androprostamines and Resormycin](image-url)

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Results and Discussion

Synthesis of Resormycin (3) As resormycin is a core structure of androprostamines, we first undertook the synthesis of resormycin to establish a method of installing the enamide portion of these natural products at the C-terminus. The amino phosphonate is a well-known precursor of a wide range of dehydroamino acid derivatives that can be formed by the HWE reaction. The synthetic procedure of the counterpart of this reaction, aldehyde 8, is depicted in Chart 1. The protocol is rather straightforward: a commercially available resorcinol derivative 4 was esterified to give 5, which was followed by tert-butyldimethylsilyl (TBS)-protection, and conversion of an ester moiety to a formyl group by a reduction–oxidation sequence to afford 8.

Then, a literature-known hydroxyvaline derivative protected with an 9-fluorenylmethyloxycarbonyl (Fmoc) group was subjected to condensation with 10 using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) as the coupling reagent to give a dipeptide intermediate 11 (Chart 2). Further elongation of the peptide was achieved using an Fmoc-protocol: removal of the Fmoc group from the N-terminus by a secondary amine (piperidine), followed by the formation of an amide bond with the protected homolysine 12. In this case also, DMT-MM was used as the coupling reagent. The resultant tripeptide compound 13 was used in the subsequent HWE reaction with resorcinol-derived aldehyde 8 using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as the base. The desired Wittig-type reaction proceeded smoothly, but partial removal of the TBS group was observed, and purification at this stage was troublesome. The crude mixture was therefore treated with tetra-n-butylammonium fluoride (TBAF) to afford 26% (2 steps) of the desilylated product of the desired Z-isomer 14 as the main product. Concomitant formation of a small amount of the E-isomer (7% in 2 steps) was also observed. The final total deprotection was carried out under acidic conditions to complete the synthesis of resormycin (3) in the form of trifluoroacetic acid (TFA) salt. The 1H- and 13C-NMR data of 3 showed good agreement to those of the HCl salt of resormycin reported previously.9

Synthesis of Androprostamine A (1) Androprostamine A was synthesized from a β-homolysine derivative 15 in the direction of the C-terminus. Then, the γ-glutamyl portions were attached, and the HWE reaction was performed (Chart 3). At the outset, the benzylxycarbonyl (Cbz) group of the N-terminal amino functionality of 15 was exchanged with an Fmoc group to afford 16, which, coupled with dipeptide 17 that was easily obtained from 11, afforded the tripeptide intermediate with a phosphonate moiety (18). The N-terminal primary amino group of 18 was unveiled, and was followed by amide bond formation with the commercially available glutamic acid derivative to result in 19. Next, each phosphonate was separately subjected to the HWE reaction with 8, and silyl protecting groups were detached by TBAF-treatment in the same manner as that for resormycin to afford 20. Again, the desired Z-isomer predominated in this reaction sequence (ca. 2:1 as the isolated yield). The final acidic deprotection accomplished the synthesis of androprostamine A (1) in 25% yield after HPLC purification. The 1H- and 13C-NMR data of the synthetic 1 was identical to that of the natural one reported previously.7

Conclusion

Syntheses of androprostamines A (1), and resormycin (3), peptidyl anti-androgen natural products, were completed. The

Reagents and conditions: (a) H2SO4, MeOH, r.t., 3.5 h, 89%; (b) TBSCl, imidazole, CH2Cl2, r.t., 4 h, 95%; (c) DIBAL-H, CH2Cl2, −78°C, 1 h, quant; (d) PCC, silica gel, CH2Cl2, r.t., 1 h, 83%.

Chart 1. Synthesis of Aldehyde 8

Reagents and conditions: (a) 10, DMT-MM, THF, r.t., 5 h, 57%; (b) piperidine, DMF, r.t., 30 min; 12, DMT-MM, MeOH, r.t., 3 h, 40%; (c) 8, DBU, CH2Cl2, r.t., 3 h; TBAF, THF, CH2Cl2, r.t., 40 min, 26% (with 7% of E-isomer); (d) TFA, CH2Cl2, r.t., 30 min, 52%.

Chart 2. Synthesis of Resormycin (3)
characteristic enamide structure at the C-terminus of these compounds was installed using the HWE reaction with the corresponding phosphonates in reasonable Z-selectivity. SAR studies using the synthetic protocol described here aimed at developing leads for clinical anti-prostate cancer agents are ongoing.

**Experimental**

**General Remarks** The reactions were performed in an oven-dried test tube or round bottom flask with a Teflon-coated magnetic stirring bar unless otherwise noted. All work-up and purification procedures were carried out with reagent-grade solvents under ambient atmosphere. Infrared (IR) spectra were recorded on a JASCO FT/IR 4100 Fourier transform infrared spectrophotometer. NMR was recorded on JEOL ECS-400 (1H-NMR: 400 MHz, 13C-NMR: 100 MHz) or on Bruker AVANCE 500 (1H-NMR: 500 MHz, 13C-NMR: 125 MHz) spectrometers. Chemical shifts for proton are reported in parts per million downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent (CDCl₃: δ 7.26 ppm, CD₃OD: δ 3.30 ppm, DMSO-d₆: 2.49 ppm). For 13C-NMR, chemical shifts were reported in the scale relative to NMR solvent (CDCl₃: δ 77.0 ppm, CD₃OD: δ 49.0 ppm, DMSO-d₆: 39.7 ppm) as an internal reference, or calibrated based on independently recorded peak of 3-trimethylsilylpropanoic acid (TSP) at 0 ppm in D₂O. NMR data are reported as follows: chemical shifts, multiplicity (s: singlet, d: doublet, dd: doublet of doublets, t: triplet, m: multiplet, br: broad signal), coupling constant (Hz), and integration. Opti- cal rotation was measured using a 2 mL cell with a 1.0 dm path length on a JASCO polarimeter P-1030. High-resolution (HR)-mass spectra (ESI-Orbitrap) were measured on Thermo Fisher Scientific LTQ Orbitrap XL. Unless otherwise noted, materials were purchased from commercial suppliers and were used without purification. For reaction, tetrahydrofuran (THF), N,N-dimethylformamide (DMF), CH₃CN, and CH₂Cl₂ were purified by passing through a solvent purification system (Glass Contour). Dry MeOH was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and used as received.

**Methyl 4-Chloro-3,5-dihydroxybenzoate (5)** To a solution of 4 (3.00 g, 15.9 mmol) in 15 mL of MeOH was added dropwise 0.3 mL of H₂SO₄ at 0°C. The mixture was stirred at room temperature (r.t.) for 3.5 h, and half of MeOH was removed under reduced pressure and followed by addition of saturated NaHCO₃ until the pH value of the mixture reached to around 7. The resultant white solid was collected on funnel and washed thoroughly with H₂O, then n-hexane to give 5 as a white powder (2.86 g, 14.1 mmol) in 89% yield; mp 145–146°C; IR (MeOH) ν 3419, 3338, 2360, 1705, 1429, 1376, 1041, 764 cm⁻¹; HR-MS electrospray ionization (ESI) Anal. Calcd for C₈H₆ClO₄ m/z 200.9955 [M−H]⁻; Found 200.9964; 1H-NMR (500 MHz, CD₃OD) δ: 7.06 (2H, s), 3.85 (3H, s); 13C-NMR (125 MHz, CD₃OD) δ: 168.1, 155.7, 130.2, 114.6, 109.1, 52.7

**Methyl 3,5-Bis((tert-butyldimethylsilyl)oxy)-4-chlorobenzoate (6)** To a solution of 5 (2.86 g, 14.1 mmol) in 70 mL of CH₂Cl₂ was added imidazole (4.23 g, 62.1 mmol), and TBSCl (4.68 g, 31.1 mmol), successively at 0°C. The mixture was stirred at r.t. for 4 h. The reaction mixture was diluted with CHCl₃, and washed with saturated NH₄Cl, and brine successively. The combined organic layers were dried over Na₂SO₄, and was concentrated to dryness. The residue was purified with silica gel column chromatography (n-hexane/AcOEt=6/1) to give 6 as a white powder (5.80 g, 13.5 mmol) in 89% yield; mp 95–96°C; IR (CHCl₃) ν 3419, 3230, 3089, 2930, 2859, 1724, 1434, 1361, 1253, 841, 783 cm⁻¹; HR-MS (ESI) Anal. Calcd for C₂₀H₃₆Cl₂O₄Si₂
m/z 431.8411 [M+H]+; Found 431.8222; 1H-NMR (500 MHz, CD3OD) δ: 7.21 (2H, s), 3.89 (3H, s), 1.06 (18H, s), 0.25 (12H, s); 13C-NMR (125 MHz, CD3OD) δ: 167.5, 154.3, 130.0, 124.3, 115.3, 53.0, 26.2, 19.3, −4.3.

(3,5-Bis(tert-butyldimethylsilyl)oxy)-4-chlorophenylmethanol (7) To a solution of 6 (5.80 g, 13.5 mmol) in 90 mL of CH2Cl2 was added Dibal-H (disobutylaluminium hydride, 1.0 M in n-hexane, 26.9 mL, 26.9 mmol) at −78°C, and the mixture was stirred for 1 h at the same temperature. Then, 90 mL of aqueous solution of potassium sodium tartrate was added, and extracted with CHCl3. The organic layer was dried over Na2SO4 and concentrated in vacuo to afford 7, as a colorless oil (5.40 g, 13.4 mmol) in quantitative yield; IR (KBr) ν 3301, 2931, 2860, 1731, 1666, 1525, 1248, 1154, 1107 (overlapped with solvent peaks), 28.3, 28.2, 27.7, 26.2.

1H-NMR (500 MHz, CD3OD) δ: 6.90 (2H, s), 4.48 (2H, s), 1.05 (18H, s), 0.23 (12H, s); 13C-NMR (125 MHz, CD3OD) δ: 154.1, 142.4, 117.2, 113.0, 64.4, 26.2, 19.3, −4.2.

3,5-Bis(tert-butyldimethylsilyl)oxy)-4-chlorobenzaldehyde (8) To a solution of 7 (1.00 g, 2.48 mmol) in 12.4 mL of CH2Cl2, 26.9 mL of n-hexane, 26.9 mmol) at −78°C, and the mixture was stirred for 1 h at r.t. Then, 294 mg, 1.36 mmol) was added and stirred for additional 1 h. The reaction mixture was poured onto the pad of Celite, washed with CHCl3 thoroughly, and the filtrate was concentrated in vacuo. The organic layer was dried over Na2SO4, and was concentrated to dryness. The residue was purified with silica gel column chromatography (n-hexane/AcOEt=8/1) to give 8 as a colorless oil (830 mg, 2.07 mmol) in 83% yield; IR (KBr) ν 3272, 2931, 2860, 1737, 1542, 1098, 829 cm−1; HR-MS (ESI) Anal. Calcd for C34H46Cl2O2Si3 m/z 403.1892 [M+H]+; Found 403.1882. 1H-NMR (500 MHz, CD3OD) δ: 6.60 (2H, s), 4.48 (2H, s), 1.05 (18H, s), 0.23 (12H, s); 13C-NMR (125 MHz, CD3OD) δ: 154.1, 142.4, 117.2, 113.0, 64.4, 26.2, 19.3, −4.2.

tert-Butyl 2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-hydroxy-3-methylbutanamido)-2-(dimethoxyphosphoryl)acetae (11) To a mixture of 9 (1.09 g, 3.06 mmol), and 10 (1.10 g, 4.60 mmol) in 10 mL of THF was added DMT-MM (1.27 g, 4.60 mmol), and the solution was stirred at r.t. for 5 h. The reaction was quenched with H2O, AcOEt was added to the mixture for extraction, and the combined organic layers were concentrated to dryness. The residue was suspended in toluene and concentrated, which was repeated 5 times. The residue was purified with silica gel column chromatography (CHCl3/MeOH=1/1) to give 11 as a colorless oil (1.01 g, 1.75 mmol) in 57% yield as a colorless oil; IR (KBr) ν 3301, 2979, 1731, 1666, 1524, 1248, 1154, 1041, 758 cm−1; HR-MS (ESI) Anal. Calcd for C34H44N2O4P2Si3 m/z 599.2134 [M+Na]+; Found 599.2123. 1H-NMR (500 MHz, CD3OD) δ: 7.79 (2H, d, J=6.7 Hz), 7.67 (2H, m), 7.38 (2H, m), 7.30 (2H, m), 4.43–4.36 (2H, m), 4.24–4.22 (2H, m), 3.85–3.74 (6H, m), 1.49 (4.5 H, s), 1.48 (4.5 H, s), 1.27 (1.5H, s), 1.27 (1.5H, s), 1.22 (1.5H, s), 1.22 (1.5H, s); 13C-NMR (125 MHz, CD3OD) δ: 172.6 (d, J=6.3 Hz), 172.4 (d, J=6.3 Hz), 166.3, 166.2, 158.7, 145.4, 145.2, 142.3, 128.8, 128.2, 126.3, 121.0, 84.9, 79.5, 72.8, 68.1, 63.72, 63.67, 55.0–54.7 (m, overlap), 48.6 (overlapped with solvent peaks), 28.2, 28.1, 27.7, 27.6, 26.1.

Resorcylin (3) To a solution of 14 (22.3 mg, 0.03 mmol) in 0.5 mL of CH2Cl2 was added TFA (trifluoroacetic acid, 0.3 mL) at 0°C. The mixture was stirred at r.t. for 30 min. Then the mixture was concentrated to dryness to give 3 as a
white powder (11.3 mg, 15.7 µmol, 52%); \([\alpha]_D^{24} +127.7 (c 0.44, MeOH), \text{for HCl salt, } [\alpha]_D^{23} +146.8 (c 1, MeOH)\); IR (KBr) \(\nu 3026, 1648, 1584, 1428, 1391, 1205, 1004 \text{ cm}^{-1}\); HR-MS (ESI) Anal. Calcd for \(C_{37}H_{37}N_2NaO_6\) m/z 548.1960 [M+H]'; Found 548.1941; \(^1\)H-NMR (500 MHz, DMSO-\(d_6\)) \(\delta\): 8.11 (1H, s), 7.64 (1H, d, \(J=7.5 \text{ Hz}\)), 7.36 (2H, t, \(J=7.5 \text{ Hz}\)), 7.30 (1H, d, \(J=7.5 \text{ Hz}\)), 6.54 (1H, d, \(J=7.5 \text{ Hz}\)), 5.84 (1H, t, \(J=6.6 \text{ Hz}\)), 4.95 (1H, br), 3.85 (1H, m), 3.61–3.39 (6H, m), 2.85 (2H, d, \(J=7.5 \text{ Hz}\)), 1.43 (9H, s), 1.23 (9H, s), 1.21 (9H, s), 1.08 (9H, s), 0.97 (9H, s), 0.94 (9H, m), 0.91 (9H, s), 0.88 (9H, s), 0.76 (9H, s), 0.71 (9H, s), 0.69 (9H, s); \(^13\)C-NMR (125 MHz, DMSO-\(d_6\)) \(\delta\): 173.6, 172.2 (d, \(J=6.3 \text{ Hz}\)), 128.2, 127.0, 121.0, 84.9, 80.1, 79.5, 72.9, 72.8, 76.6, 61.6, 55.0 (d, \(J=7.5 \text{ Hz}\)), 54.8 (d, \(J=6.3 \text{ Hz}\)), 54.7 (d, \(J=7.5 \text{ Hz}\)), 49.6 (overlapped with solvent peaks), 42.6, 42.5, 41.6, 35.5, 30.7, 28.9, 28.2, 27.8, 27.6, 26.2, 26.1, 24.2.

tert-Butyl \(N^5-\{\[(55\text{-S})-7-\{\{(2S)\text{-}1-\{\text{di}-\text{methoxyphosphoryl}\}\text{-}2\text{-oxoethylamino}\}\text{-3-hydroxy-3-methoxyphosphoryl}\}\text{-2-oxoethylamino}\}\text{-7-oxoheptyl}\}-N^5\text{-tert-butyloxycarbonyl}\text{-l-glutamate} (19)

To a solution of (18) (700 mg, 0.855 mmol) in 7 mL of DMF was added 93 µL of piperidine (0.940 mmol), and the mixture was stirred at r.t. for 30 min. Then, the solution was concentrated to dryness. The resultant residue was dissolved in THF, to which were added Boc-L-Glu-O'Bu (130 mg, 0.427 mmol), DMT-MM (237 mg, 0.855 mmol), and the resulting mixture was stirred at r.t. for 18 h. The solution was diluted with AcOEt, washed with 1 M hydrochloric acid, and the organic layer was dried over Na₂SO₄ and concentrated in vacuo. The residue was purified with silica gel column chromatography (CHCl₃/MeOH=1/1) to give 19 (307 mg, 81%) as a colorless oil (mixture of diastereomers); IR (KBr) ν 3309, 2978, 1691, 1654, 1525, 1367, 1156, 1037 cm⁻¹; HR-MS (ESI) Anal. Calcd for \(C_{44}H_{67}ClN_5O_{14}\) m/z 904.4660 [M+Na]⁺; Found 904.4662; \(^1\)H-NMR (500 MHz, CDCl₃) \(\delta\): 4.53 (1H, s), 3.95 (1H, m), 3.88 (1H, m), 3.84–3.81 (6H, m), 3.75 (1H, m), 3.69 (1H, m), 3.61 (1H, m), 3.52 (1H, m), 3.48–3.45 (6H, m), 2.43 (2H, b, \(J=7.5 \text{ Hz}\)), 2.06 (1H, m), 1.85 (1H, m), 1.54–1.34 (4H, m), 1.49 (9H, s), 1.46 (9H, s), 1.44 (9H, s), 1.42 (9H, s), 1.34 (9H, s), 1.22 (9H, s), 1.21 (9H, s), 1.13 (9H, s), 1.04 (9H, s), 0.99 (9H, s), 0.91 (9H, s), 0.87 (9H, s), 0.82 (9H, s), 0.79 (9H, s), 0.70 (9H, s), 0.58 (9H, s), 0.52 (9H, s), 0.43 (9H, s), 0.38 (9H, s), 0.34 (9H, s), 0.30 (9H, s), 0.27 (9H, s), 0.25 (9H, s), 0.22 (9H, s), 0.20 (9H, s), 0.18 (9H, s), 0.16 (9H, s), 0.15 (9H, s), 0.13 (9H, s), 0.11 (9H, s), 0.09 (9H, s), 0.07 (9H, s), 0.05 (9H, s), 0.03 (9H, s), 0.01 (9H, s).
Found 928.4664; 1H-NMR (400 MHz, CD3OD) δ: 7.11 (1H, s), 6.74 (2H, s), 4.49 (1H, s), 3.97–3.81 (2H, m), 3.11 (2H, m), 2.48 (2H, d, J=7.6 Hz), 2.25 (2H, d, J=9.2 Hz), 2.20–2.01 (1H, m), 1.90–1.80 (1H, m), 1.52 (9H, s), 1.50–1.25 (6H, m), 1.46 (9H, s), 1.44 (9H, s), 1.40 (9H, s), 1.38 (3H, s), 1.33 (3H, s); 13C-NMR (125 MHz, CD3OD) δ: 174.8, 174.3, 173.3, 172.4, 165.6, 158.1, 158.0, 155.5, 133.6, 133.5, 127.9, 110.8, 110.1, 83.2, 82.8, 80.6, 80.2, 72.4, 62.6, 55.6, 42.8, 40.3, 35.4, 33.4, 30.0, 28.9, 28.8, 28.4, 28.3, 27.5, 27.0, 24.3.

Androprostamine A (1) To a solution of 20 (11.6 mg, 12.5 µmol) in 0.25 mL of CH2Cl2 was added TFA (125 µL) at 0°C. The mixture was stirred at r.t. for 30 min. Then the mixture was concentrated to dryness to give a crude material containing 1, which was purified with LH-20 (MeOH) and quenched with saturated NaHCO3. The 1H-NMR of the resultant sample was identical to that of natural one. The sample was further purified with HPLC (Capcell Pak C18 UG, Shiseido, Tokyo, Japan, 20×250 mm; eluent, 10% CH3CN in H2O containing 0.1% of TFA; detection, UV at 300 nm; flow rate, 10 mL/min) to give androprostamine A TFA salt as a colorless amorphous. The material was dissolved in H2O, to which 1 M NH4OH was added until the pH reached to around 9. The solution was concentrated and the resulting crude material was purified by LH-20 (MeOH) to give 1 (1.9 mg, 2.05 µmol, 25%); [α]D27 +42.4 (c 0.085, MeOH), (natural sample: [α]D27 +43.2 (c 0.085, MeOH)); IR (KBr) ν3100, 1671, 1397, 1202, 1136 cm−1; HR-MS (ESI) Anal. Calcd for C26H39ClN5O10 m/z 616.2385 [M+H]+; Found 616.2377; 1H-NMR (500 MHz, D2O) δ: 7.20 (1H, s), 6.75 (2H, s), 4.49 (1H, s), 3.75 (1H, t, J=6.5 Hz), 3.60 (1H, m), 3.06 (2H, m), 2.78 (1H, dd, J=14.4, 5.0 Hz), 2.74 (1H, dd, J=14.4, 6.0 Hz), 2.39 (1H, m), 2.12 (1H, m), 1.60 (1H, m), 1.53 (1H, m), 1.36–1.32 (4H, m), 1.33 (1H, s); 13C-NMR (125 MHz, D2O) δ: 177.2, 176.7, 174.9, 174.1, 173.2, 155.4, 136.7, 133.4, 132.5, 111.7, 111.3, 74.5, 64.3, 56.9, 51.6, 41.7, 38.7, 34.3, 33.9, 30.6, 29.3, 28.5, 27.9, 24.7.

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Conflict of Interest The authors declare no conflict of interest.

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