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Synthesis of Cross-linkable 2,5-Diketopiperazine Derivatives.

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Abstract: Synthesis of cross-linkable diketopiperazine derivatives is described. Cross-linkable α -amino acid methyl esters were subjected to peptide synthesis with Boc-protected glycine or L-tyrosine. No protections of cross-linkable functional groups (catechol and phenylazide) are necessary for constructions of diketopiperazine skeleton.

Keywords: cross-link, cyclic dipeptide, diketopiperazine, DOPA, phenylazide, photoaffinity label

1. INTRODUCTION

2,5-Diketopiperazine (2,5-DKP) scaffolds are core templates for numerous bioactive compounds [1]. Stereo-controlled intramolecular cyclization of dipeptide is one of the effective ways to construct 2,5-DKP skeletons [2-5]. Cross-link between bioactive compounds and their target biomolecules are attractive methods to elucidate molecular mechanism of bioactivity. DOPA, which has catechol (*o*-hydroxyphenol) moiety, can be utilized as a cross-linkable α -amino acid in the presence of periodate [6-8]. Two examples for DOPA containing 2,5-DKP derivatives have been reported previously. First is enzymatic hydroxylation of cyclo(Tyr-Tyr) in cultured cell to afford cyclo(DOPA-DOPA) [9]. The another one is condensation of all hydroxyl groups protected Tyr and DOPA followed by deprotection to afford cyclo(Tyr-DOPA) [10]. The protections of phenol and catechol moieties are conducted to prevent the side reactions. To date, diketopiperazine formations without unprotected catechol moiety have not been reported yet. Photoaffinity labeling is the most attractive method for creating a cross-linking method between ligand and biomolecule interactions [11, 12]. Photoreactive aromatic α -amino acid derivatives have been used to investigate bioactive peptides interactions [13-17]. Although arylazides are one of the common photophores for the photoaffinity labeling but no description of 2,5-DKP derivatives has been reported. In this report, we aim to describe the novel synthesis of cross-linkable 2,5-DKP derivatives, based on glycine or tyrosine with catechol and phenylazide, which can be utilized to elucidate their biological activity.

2. RESULTS AND DISCUSSIONS

L-DOPA **1** is converted to corresponding methyl ester treated with thionyl chloride in methanol at room temperature [18]. L-DOPA-OMe hydrochloride **2** was

condensed with Boc-Gly **3** in the presence of DCC in acetonitrile and DMF at 0 °C. The dipeptide was purified with silica column chromatography. Boc-Gly-L-DOPA-OMe **4** was subjected to deprotection of Boc group followed by intramolecular cyclization in the presence of AcOH and *N*-methylmorpholine in 2-BuOH under reflux for several hours [2]. The reaction mixture became suspension and the precipitates was washed with methanol for several times to purify cyclo(Gly-L-DOPA) **5** with moderate yield. During the course of the synthesis, protection of catechol hydroxyl groups were not necessary to form 2,5-DKP skeleton. Boc-L-Tyr **6** was treated with L-DOPA-OMe hydrochloride **2**, followed by treatment of dipeptide **7** with identical manner described above, afforded cyclo(L-Tyr-L-DOPA) **8** with moderate yield.

Figure 1. Synthesis of 2,5-DKP containing L-DOPA

Different protecting groups for L-Tyr and L-DOPA have been utilized in the previous synthesis of compound **8** [10] to inhibit byproduct formations via hydroxyl groups. But our results indicated that no protection of phenolic and catechol

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hydroxyl group was essential to construct 2,5-DKP derivatives (Figure 1).

Photophore containing derivatives, L-Phe(4-N₃) **9** [19], was converted corresponding methyl ester hydrochloride **10** with thionyl chloride in methanol at room temperature. Compound **10** was subjected to peptide coupling with Boc-Gly **3** or Boc-L-Tyr **6** in the presence DCC in acetonitrile and DMF. The dipeptides **11** and **13** were deprotected under acidic condition and intramolecular cyclization in aforementioned conditions to 2,5-DKP derivatives **12** and **14**, respectively. IR measurements of compound **12** and **14** indicated that azide moiety was not influenced by synthetic conditions.

Figure 2. Synthesis of 2,5-DKP containing L-Phe(4-N₃)

Many diketopiperazines are dissolved in acidic media. The cyclo(Gly-Phe(4-N₃)) was easily soluble in trifluoroacetic acid-d to measure NMR spectrum [3]. But the compound **12** was decomposed during NMR measurement and this decomposition was still continued even though the sample was stored at -20 °C for several days. The ¹H-NMR analysis

Table 1. Stability of cyclo(Gly-L-Phe(4-N₃)) **12 in trifluoroacetic acid-d at -20 °C^a**

Time (h)	Residual 12 (%) ^b
0	100
2	58
4	42
10	39
26	24
50	14
70	0

^aCompound **12** (5 mg) was dissolved in trifluoroacetic acid-d (0.5 mL). ^bThe proportion of residual **12** was calculated from ¹H-NMR.

indicated that the compound **12** was decomposed completely after 70 hours at -20 °C (Table 1). The acidic solution of **12** was concentrated and subjected to IR spectrometry. The azide moiety was decomposed under the treatment. In spite of this result, DOPA containing diketopiperazine **8** was stable in trifluoroacetic acid at room temperature over 24 hours. The result consistent with our previous result, that the arylazide moiety was unstable under strong acid conditions [20]. Finally, compound **12** in 1% DCl-D₂O is subjectable to ¹H- and ¹³C- NMR measurement without decomposition of azide moiety.

4. EXPERIMENTAL

General FTIR spectra were recorded on a JASCO FT-IR 4100 spectrometer. ¹H (270 MHz) and ¹³C NMR (67.5 MHz) spectra were recorded on a JEOL EX-270 spectrometer. Optical rotations were measured on a JASCO DIP-370 polarimeter.

(S)-Methyl 2-amino-3-(3,4-dihydroxyphenyl)propanoate hydrochloride (L-DOPA-OMe, 2) Thionyl chloride (13.5 mL, 184 mmol) was added dropwise to a solution of L-DOPA **1** (4.02 g, 20.4 mmol) in methanol (100 mL) at 0 °C. After 21 hours at room temperature, the solvent was evaporated, and the residue was concentrated to afford pale yellow mass (5.01 g, 99%). ¹H NMR (CD₃OD): δ 3.00 (dd, *J* = 14.5, 7.3 Hz, 1H), 3.11 (dd, *J* = 14.5, 5.9 Hz, 1H), 3.82 (s, 3H), 4.21 (dd, *J* = 7.3, 5.9 Hz, 1H), 6.55 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.66 (d, *J* = 2.0 Hz, 1H), 6.75 (d, *J* = 8.2 Hz, 1H); ¹³C NMR (CD₃OD): 36.7, 53.6, 55.4, 116.9, 117.3, 121.9, 126.3, 146.1, 146.7, 170.4; HRMS (ESI): calcd. for C₁₀H₁₄NO₄ (M⁺+H) 212.0923, found 212.0928; [α]_D +9.8 (c 2.0, CH₃OH).

(S)-Methyl 2-(2-((tert-butoxycarbonyl)amino)acetamido)-3-(3,4-dihydroxyphenyl)propanoate (Boc-Gly-L-DOPA-OMe, 4) A solution of L-DOPA methyl ester hydrochloride **2** (1.02 g, 4.11 mmol) and *N*-(tert-butoxycarbonyl)-glycine **3** (0.72 g, 4.11 mmol) in DMF (15 mL) and acetonitrile (60 mL) was cooled in ice. With stirring, triethylamine (0.5 mL, 3.6 mmol) was added followed by dicyclohexylcarbodiimide (0.89 g, 4.33 mmol). After 5 h of continued stirring at 0 °C, the reaction mixture was placed in the freezer for overnight. The insoluble material was filtered off and washed with ethyl acetate. The combined filtrate was evaporated in vacuo leaving a gummy residue, which was taken up in ethyl acetate (100 mL) and water (40 mL). The organic layer was washed successively with 40 mL portions of 0.5 N HCl, H₂O, 0.5 N NaHCO₃ and brine. The organic layer was dried over MgSO₄ and the filtrate was evaporated. The residue was subjected to silica column chromatography (ethyl acetate/hexane = 1:1, 3:2 then 2:1) to afford a colorless amorphous mass (0.91 g, 60%). ¹H NMR (CDCl₃): δ 1.44 (s, 9H), 2.93 (dd, *J* = 14.0, 5.4 Hz, 1H), 3.05 (dd, *J* = 14.0, 4.1 Hz, 1H), 3.72 (s, 3H), 3.77 (d, *J* = 5.6 Hz, 2H), 4.78 (m, 1H), 5.53 (m, 1H), 6.45 (dd, *J* = 7.9 Hz, 1.6 Hz, 1H), 6.59 (d, *J* = 1.6 Hz, 1H), 6.73 (d, *J* = 7.9 Hz, 1H), 6.86 (br d, *J* = 8.2 Hz, 1H); ¹³C NMR (CDCl₃): δ 28.3, 36.7, 44.0, 52.5, 53.4, 81.0, 115.3, 116.7, 121.2, 127.3, 143.7, 143.8, 156.5, 169.8, 172.0; HRMS (ESI): calcd. for C₁₇H₂₅N₂O₇ (M⁺+H) 369.1662, found 369.1667; [α]_D +40.6 (c 5.0, CH₃OH).

(S)-3-(3,4-Dihydroxybenzyl)piperazine-2,5-dione (cyclo(Gly-L-DOPA), 5) Boc-Gly-L-DOPA-OMe **4** (0.36 g, 0.97 mmol) was suspended in 4M HCl-dioxane (5 mL) and the reaction mixture was stirred at room temperature for 2 hours, then concentrated. The residue was co-evaporated with dioxane three times and was dissolved in 0.1 M AcOH-2-butanol (12.5 mL), then *N*-methylmorpholine (0.09 mL) was added. The reaction mixture was refluxed for 3 hours. After cooling at -20 °C, insoluble material was centrifuged and washed with methanol to afford colorless mass (0.12 g, 51%). ¹H NMR (DMSO-d₆): δ 2.68 (dd, *J* = 13.2, 7.6 Hz, 1H), 2.92 (dd, *J* = 13.2, 3.5 Hz, 1H), 3.38 (d, *J* = 16.8 Hz, 1H), 3.50 (d, *J* = 16.8 Hz, 1H), 4.20 (m, 1H), 6.41 (d, *J* = 8.2 Hz, 1H), 6.58 (d, *J* = 8.2 Hz, 1H), 6.61 (s, 1H); ¹³C NMR (DMSO-d₆): 37.0, 40.7, 55.6, 115.4, 117.0, 120.0, 129.2, 143.7, 145.0, 166.2, 174.2; HRMS (ESI): calcd. for C₁₁H₁₃N₂O₄ (M⁺+H) 237.0875, found 237.0876; [α]_D +38.7 (c 2.0, DMSO).

(S)-Methyl 2-((S)-2-((tert-butoxycarbonyl)amino)-3-(4-hydroxyphenyl)propanamido)-3-(3,4-dihydroxyphenyl)propanoate (Boc-L-Tyr-L-DOPA-OMe, 7) A solution of L-DOPA methyl ester hydrochloride **2** (0.27 g, 1.11 mmol) and *N*-(tert-butoxycarbonyl)-L-tyrosine (0.33 g, 1.16 mmol) in DMF (2.2 mL) and acetonitrile (9 mL) was cooled in ice. With stirring, triethylamine (0.16 mL, 1.11 mmol) was added followed by dicyclohexylcarbodiimide (0.25 g, 1.11 mmol). After 7 h of continued stirring at 0 °C, the reaction mixture was cooled in the freezer overnight. The insoluble material was filtered off and washed with ethyl acetate. The combined filtrate was evaporated and the residue was dissolved in ethyl acetate (40 mL) and water (25 mL). The organic layer was washed successively with 25 mL portions of 0.5 N HCl, H₂O, 0.5 N NaHCO₃ and brine and dried over MgSO₄. After filtration and concentration, the crude yellow solid was subjected to silica chromatography (ethyl acetate/hexane = 1:1, then 3:1) to afford a colorless amorphous mass (0.34 g, 64%) ¹H NMR (CD₃OD): δ 1.34 (s, 3H), 2.67 (m, 1H), 2.90 (m, 3H), 3.35 (s, 3H), 3.66 (s, 3H), 4.23 (t, *J* = 6.9 Hz, 1H), 4.59 (t, *J* = 6.8 Hz, 1H), 6.48 (d, *J* = 7.9 Hz, 1H), 6.62 (s, 1H), 6.68 (d, *J* = 7.9 Hz, 1H), 6.69 (d, *J* = 8.6 Hz, 2H), 7.01 (d, *J* = 8.6 Hz, 2H); ¹³C NMR (CD₃OD): δ 28.6, 37.9, 38.4, 52.6, 55.2, 57.5, 80.7, 116.1, 116.3, 117.2, 121.7, 129.0, 129.1, 131.3, 145.3, 146.2, 157.1, 157.5, 173.2, 174.2; HRMS (ESI): calcd. for C₂₄H₃₁N₂O₈ (M⁺+H) 475.2080, found 475.2055; [α]_D -2.1 (c 2.0, CH₃OH).

(3S,6S)-3-(3,4-Dihydroxybenzyl)-6-(4-hydroxybenzyl)piperazine-2,5-dione (cyclo(L-Tyr-L-DOPA), 8) Boc-L-Tyr-L-DOPA-OMe **7** (0.47 g, 1.02 mmol) was treated with 4M HCl-dioxane (3 mL) and the solution was stirred at room temperature for 2 hours, then concentrated. The residue was co-evaporated with dioxane three times. The residue was dissolved in 0.1 M AcOH-2-butanol (15 mL). *N*-Methylmorpholine (0.11 mL) was added. The reaction mixture was refluxed for three hours. After cooling to rt, the reaction mixture was cooled at -20 °C overnight. The precipitates were centrifuged and washed with methanol to afford colorless amorphous mass. (0.20 g, 58%). ¹H NMR (CF₃COOD): δ 2.19 (dd, *J* = 12.9, 7.6 Hz, 1H), 2.47 (dd, *J* = 12.9, 6.8 Hz, 1H), 2.97 (d, *J* = 13.8 Hz, 1H), 3.06 (d, *J* = 13.8 Hz, 1H), 4.48 (m, 1H), 4.55 (m, 1H), 6.66 (d, *J* = 7.9 Hz,

1H), 6.80 (s, 1H), 6.98 (d, *J* = 7.9 Hz, 1H), 6.99 (d, *J* = 8.6 Hz, 2H), 7.07 (d, *J* = 8.6 Hz, 2H); ¹³C NMR (CF₃COOD): δ 41.2, 41.3, 58.8, 58.9, 118.7, 119.5, 120.2, 125.9, 129.7, 130.4, 133.8, 145.0, 145.8, 156.1, 172.8, 172.9; HRMS (ESI): calcd. for C₁₈H₁₉N₂O₅ (M⁺+H) 343.1294, found 343.1281; [α]_D -111 (c 1.0, CF₃COOH).

(S)-Methyl 2-amino-3-(4-azidophenyl)propanoate hydrochloride (L-Phe(4-N₃)-OMe, 10) L-Phe (4-N₃) **9** (0.51 g, 2.47 mmol) was dissolved in methanol (25 mL). Thionyl chloride (1.5 mL, 20.8 mmol) was added to the solution dropwisely at 0 °C and the reaction mixture was warmed to room temperature, then concentrated to afford colorless amorphous mass (0.45 g, 71%). ¹H NMR (CD₃OD): δ 3.19 (dd, *J* = 13.5, 5.9 Hz, 1H), 3.27 (dd, *J* = 13.5, 5.8 Hz, 1H), 3.80 (s, 3H), 4.33 (t, *J* = 6.8 Hz, 1H), 7.06 (d, *J* = 8.6 Hz, 2H), 7.31 (d, *J* = 8.6 Hz, 2H); ¹³C NMR (CD₃OD): δ 36.6, 53.6, 55.1, 120.6, 132.0, 132.1, 141.2, 170.3; HRMS (ESI): calcd. for C₁₀H₁₃N₄O₂ (M⁺+H) 221.1039, found 221.1042; [α]_D +15.8 (c 1.0, CH₃OH); IR (neat): ν 2136 cm⁻¹.

(S)-Methyl 3-(4-azidophenyl)-2-((tert-butoxycarbonyl)amino)acetamido)propanoate (Boc-Gly-L-Phe(4-N₃)-OMe, 11) A solution of Phe(4-N₃) methyl ester hydrochloride **10** (0.45 g, 1.77 mmol) and *N*-(tert-butoxycarbonyl)glycine **3** (0.32 g, 1.84 mmol) in DMF (3.5 mL) and acetonitrile (14 mL) was cooled in ice. With stirring, triethylamine (0.25 mL, 1.79 mmol) was added followed by dicyclohexylcarbodiimide (0.38 g, 1.84 mmol). After 5 h of continued stirring at 0 °C, the reaction mixture was cooled -20 °C overnight. The insoluble material was filtered off and washed with ethyl acetate. The combined filtrate was evaporated and the residue was dissolved in ethyl acetate (30 mL) and water (20 mL). The organic layer was washed successively with 20 mL portions of 0.5 N HCl, H₂O, 0.5 N NaHCO₃ and brine, and dried over MgSO₄. After filtration and evaporation, the residue was subjected to silica chromatography (ethyl acetate/hexane = 1:1, then 3:1) to afford colorless amorphous mass (0.38 g, 58%). ¹H NMR (CDCl₃): δ 1.44 (s, 9H), 3.04 (dd, *J* = 13.8, 6.3 Hz, 1H), 3.13 (dd, *J* = 13.8, 5.6 Hz, 1H), 3.71 (s, 3H), 3.80 (m, 2H), 4.85 (m, 1H), 5.34 (m, 1H), 6.82 (m, 1H), 6.94 (d, *J* = 8.6 Hz, 2H), 7.10 (d, *J* = 8.6 Hz, 2H); ¹³C NMR (CDCl₃): δ 28.2, 37.2, 44.2, 52.3, 53.0, 80.1, 119.0, 130.5, 132.4, 138.8, 155.9, 169.2, 171.5; HRMS (ESI): calcd. for C₁₇H₂₄N₅O₅ (M⁺+H) 378.1777, found 378.1771; [α]_D +55.8 (c 1.0, CHCl₃); IR (solid): ν 2120 cm⁻¹.

(S)-3-(4-Azidobenzyl)piperazine-2,5-dione (cyclo(Gly-L-Phe(4-N₃)), 12) Boc-Gly-L-Phe(4-N₃)-OMe **11** (0.12 g, 0.39 mmol) was suspended in 4M HCl-dioxane (2.5 mL) and the suspension turned to solution with stirring at room temperature for 2 hours, then concentrated. The residue was dissolved in 0.1 M AcOH-2-butanol (5 mL). *N*-Methylmorpholine (0.07 mL) was added and the reaction mixture was refluxed for 3 hours. After cooling, the insoluble material was collected with centrifuge and washed with methanol three times to afford pale brown amorphous mass (0.044 g, 0.18 mmol, 45%). ¹H NMR (1% DCl-D₂O): δ 2.60 (dd, *J* = 13.8, 8.6 Hz, 1H), 2.80 (dd, *J* = 13.8, 5.3 Hz, 1H), 3.30 (d, *J* = 16.2 Hz, 1H), 3.40 (d, *J* = 16.2 Hz, 1H), 4.30 (t, *J* = 7.1 Hz, 1H), 6.63 (d, *J* = 7.9 Hz, 2H), 6.85 (d, *J* = 7.9 Hz, 2H); ¹³C NMR (1% DCl-D₂O): δ 36.7, 41.0, 54.8,

119.8, 131.4, 133.8, 139.4, 167.4, 175.0; HRMS (ESI): calcd. for $C_{11}H_{12}N_5O_2$ ($M^+ + H$) 246.0991, found 246.0988; $[\alpha]_D^{25} +58.4$ (c 1.0, 1% HCl); IR (solid): ν 2125 cm^{-1} .

(S)-Methyl 3-(4-azidophenyl)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-(4-hydroxyphenyl)propanamido)-propanoate (Boc-L-Tyr-L-Phe(4-N₃)-OMe, 13) A solution of Phe(4-N₃) methyl ester hydrochloride **10** (0.45 g, 1.75 mmol) and *N*-(tert-butoxycarbonyl)-L-tyrosine **6** (0.51 g, 1.80 mmol) in DMF (3.5 mL) and acetonitrile (14 mL) was cooled in ice. With stirring, triethylamine (0.25 mL, 1.79 mmol) was added followed by dicyclohexylcarbodiimide (0.37 g, 1.81 mmol). After 2 h stirring at 0 °C, the reaction mixture was cooled at -20 °C overnight. The insoluble material was filtered off and washed with ethyl acetate. The combined filtrate was evaporated and the residue was dissolved in ethyl acetate (30 mL) and water (20 mL). The organic layer was washed successively with 20 mL portions of 0.5 N HCl, H₂O, 0.5 N NaHCO₃ and brine, and dried over MgSO₄. After filtration and evaporation, the residue was subjected to silica chromatography (ethyl acetate/hexane = 1:1, then 3:2) gave a colorless amorphous mass (0.64 g, 75%). ¹H NMR (CDCl₃): δ 1.42 (s, 9H), 2.93 (d, J = 6.3 Hz, 2H), 3.02 (t, J = 5.9 Hz, 2H), 3.66 (s, 3H), 4.24 (br s, 1H), 4.74 (d, J = 6.6 Hz, 1H), 5.02 (br s, 1H), 6.34 (d, J = 6.9 Hz, 1H), 6.72 (d, J = 8.6 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H), 6.99 (d, J = 7.9 Hz, 2H), 7.00 (d, J = 7.9 Hz, 2H); ¹³C NMR (CDCl₃): δ 28.2, 37.3, 37.4, 52.4, 53.3, 56.1, 80.6, 115.6, 119.1, 127.7, 130.4, 130.6, 132.3, 138.9, 155.3, 155.5, 171.1, 171.2; HRMS (ESI): calcd. for $C_{24}H_{30}N_5O_6$ ($M^+ + H$) 484.2196, found 484.2195; $[\alpha]_D^{25} +28.9$ (c 3.0, CHCl₃); IR (solid): ν 2130 cm^{-1} .

(3S,6S)-3-(4-Azidobenzyl)-6-(4-hydroxybenzyl)-piperazine-2,5-dione (cyclo(L-Tyr-L-Phe(4-N₃)), 14) Boc-L-Tyr-L-Phe(4-N₃)-OMe **13** (0.32 g, 0.66 mmol) was suspended in 4M HCl-dioxane (5 mL) and the suspension turned to solution with stirring at room temperature for 2 hours, then concentrated. The residue was dissolved in 0.1 M AcOH- 2-butanol (10 mL). *N*-Methylmorpholine (0.14 mL) was added and the reaction mixture was refluxed for three hours. After cooling, the precipitates were collected with centrifuge and washed with methanol to afford colorless amorphous mass (0.069 g, 30%). ¹H NMR (DMSO-d₆): δ 2.12 (dd, J = 13.7, 5.8 Hz, 1H), 2.38 (dd, J = 13.7, 5.8 Hz, 1H), 2.52 (dd, J = 13.2, 5.1 Hz, 1H), 2.58 (dd, J = 13.2, 5.1 Hz, 1H), 3.87 (m, 1H), 3.97 (m, 1H), 6.67 (d, J = 8.2 Hz, 2H), 6.86 (d, J = 8.2 Hz, 2H), 7.01 (d, J = 8.4 Hz, 2H), 7.03 (d, J = 8.4 Hz, 2H), 7.89 (d, J = 15.8 Hz, 2H); ¹³C NMR (DMSO-d₆): 38.3, 38.5, 55.3, 55.6, 115.0, 118.8, 126.3, 130.9, 131.2, 133.8, 137.5, 156.1, 166.1, 166.3; HRMS (ESI): calcd. for $C_{18}H_{18}N_5O_3$ ($M^+ + H$) 352.1410, found 352.1407; $[\alpha]_D^{25} +134$ (c 0.67, DMSO); IR (solid): ν 2105 cm^{-1} .

CONCLUSION

It is well known that 2,5-DKP skeletons were pivotal roles for many bioactivities, but the derivatives for tools to elucidate their bioactivities have been reported yet. The cross-linkable 2,5-DKP derivatives described in this report will be utilized for this purpose.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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