Supporting Information:

Discover 4,6-O-thenylidene-β-D-glucopyranoside-(2″-acetamido, 3″-acetyl-di-S-5-fluorobenzothizole/5-fluorobenzoxazole)-4'-demethylepipodophyllotoxin as potential less toxic antitumor candidate drugs by reducing DNA damage and less inhibition of PI3K

Jie Cheng ¹, b, Wei Zhao ¹, a, Hui Yao ¹, b, Yuemao Shen a, Youming Zhang a, Yue-zhong Li a,
Qingsheng Qi a, Kanokpan Wongprasert c and Ya-Jie Tang * a

a State Key Laboratory of Microbial Technology, Shandong University, Qingdao 266237, China

b Hubei Key Laboratory of Industrial Microbiology, Hubei Provincial Cooperative Innovation Center of Industrial Fermentation, Key Laboratory of Fermentation Engineering (Ministry of Education), Hubei University of Technology, Wuhan, 430068, China

c Department of Anatomy, Faculty of Science, Mahidol University, Rama 6th Road, Bangkok 10400, Thailand

*Corresponding author. Tel. & Fax: +86-532-5863.2365 Email: yajietang@sdu.edu.cn

¹ with equal contribution to this work

Conflict of Interest: No potential conflicts of interest were disclosed
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**Figure 1S.** Virtual library of teniposide derivatives.

<table>
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Teniposide R¹ = R² = OH
**Figure S2.** Docking of Compounds with Topo II.
Table S1. Calculated docking of the complex of compounds with Topoisomerase II.

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<tr>
<th>Compound</th>
<th>ΔG (kcal/mol)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ki (μM)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Hydrogen bonds</th>
<th>π-π bonds</th>
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<td>DG13, GLN778</td>
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</table>

<sup>a</sup>Estimated free energy of binding (ΔG) in kcal/mol. <sup>b</sup>Estimated inhibition constant (Ki).
Synthetic procedures for 2″-NH₂-Teniposide (10a).

Preparation of 1″,3″,4″,6″-Tetra-O-acetyl-2″-azido-2″-deoxy-D-galactopyranoside (4a).

The synthesis was performed according to previously published methods.¹

A solution of NaN₃ (8.94 g, 137 mmol) in H₂O (22 mL) was cooled to 0 °C and stirred for 5 min, then DCM (40 mL) was added. The mixture was stirred vigorously and Tf₂O (4.68 mL, 27.90 mmol) added over a period of 5 min, and the reaction stirred at 0 °C for 2 h. The organic phase was then separated and the aqueous phase washed twice with DCM. The combined organic phases were washed once with saturated sodium carbonate solution. The total volume of TfN₃ in DCM was 75 mL, and this reagent solution was used without further purification. D-Glucosamine Hydrochloride 3a (3.0 g, 13.95 mmol) was dissolved in H₂O (45 mL) and treated with K₂CO₃ (2.88 g, 20.93 mmol) and CuSO₄ (21 mg, 132 mmol). Methanol (90 mL) was added followed by careful addition of the freshly prepared TfN₃ solution. More methanol was added to achieve a homogeneous solution and the reaction was then stirred for 6 h at room temperature. The solvent was then removed under vacuum and the residue redissolved in pyridine (75 mL). After cooling to 0 °C, Ac₂O (45 mL) was added and the mixture stirred at room temperature overnight. Excess reagents were removed under vacuum and the residue dissolved in EtOAc (300 mL) before washing with saturated CuSO₄ (aq.) (200 mL×3) and saturated sodium bicarbonate solution (200 mL). After drying over anhydrous sodium sulfate, the solvent was removed under vacuum to give a residue which was purified by column chromatography (EtOAc: n-hexanes=3:7) to give the title compound as a colorless syrup (yield: 5.20 g, 97%). Spectral analysis in agreement with ref 1. MS (ESI): 396.31(C₁₄H₁₉N₃O₉, [M+Na]+).
Preparation of 3″,4″,6″-Tri-O-acetyl-2″-azido-2″-deoxy-α-D-glucopyranose (6a).

The synthesis was performed according to previously published methods.²

A solution of 4a (373 mg, 1 mmol) in dry DCM (50 mL) stirred for 5 min, a 33% solution of HBr in AcOH (1 mL, 1.7 mmol) was added. The mixture was stirred under nitrogen for 2-4 h at rt. The reaction mixture was then diluted with DCM (40 mL) and washed with cold saturated sodium bicarbonate solution (15 mL×2) and cold water (15 mL×2). The organic phase was separated, dried with MgSO₄, and concentrated in vacuo. The glycosyl bromide 5a was dissolved with acetone (100 mL) and 0.1 mL H₂O was added, then Ag₂CO₃ (275 mg, 1 mmol) was added, the mixture was stirred at 0 °C for 5 h, the solids were then filtered off through a pad of celite and rinsed successively with DCM. The organic phase was dried with anhydrous anhydrous sodium sulfate and concentrated in vacuo to give a residue which was purified by column chromatography (EA:PE=4:6) to give the title compound as a colorless syrup (yield: 275 mg, 83%).


Preparation of 3″,4″,6″-tri-O-acetyl-2″-azido-2″-deoxy-4-O-DMEP-D-glucopyranoside (7a).

A solution of DMEP (400 mg, 1 mmol) in dry DCM (50 mL) stirred under nitrogen for 5 min at 0 °C, BF₃·Et₂O (475 μL, 3 mmol) was added, the mixture was stirred for 10 min at the same temperature. Then cooled to -20 °C, 6a (496.5 mg, 1.5 mmol) was added slowly by syringe, and the
mixture was stirred for 2 h. After being quenched with ice and was neutralized with saturated sodium bicarbonate solution. The organic phase was separated and washed with saturated brine, extracted with DCM, dried over anhydrous sodium sulfate and concentrated in vacuo. And then purified by column chromatography (DCM:MeOH=50:1) to give the desired compound 7 as white solid (yield: 606 mg, 85%).

^1H NMR (800 MHz, CDCl₃) δ 6.87 (s, 1H), 6.59 (s, 1H), 6.25 (s, 2H), 6.01 (d, J = 14.3 Hz, 2H), 5.08 (d, J = 3.0 Hz, 1H), 4.64 (d, J = 5.4 Hz, 1H), 4.56 (d, J = 8.0 Hz, 1H), 4.41 (dd, J = 10.6, 8.8 Hz, 1H), 4.34-4.23 (m, 2H), 4.20 (dd, J = 12.3, 5.8 Hz, 1H), 3.77 (s, 6H), 3.72-3.63 (m, 1H), 3.55 (dd, J = 9.4, 8.3 Hz, 1H), 3.37 (dd, J = 14.0, 5.4 Hz, 1H), 3.00-2.91 (m, 1H), 2.16 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H).

^13C NMR (201 MHz, CDCl₃) δ 174.64, 170.57, 169.94, 169.63, 149.04, 147.06, 146.45, 134.18, 133.72, 130.66, 126.24, 111.32, 109.08, 107.96, 101.71, 98.78, 77.26, 77.08, 76.93, 72.94, 72.10, 68.39, 67.40, 63.75, 62.13, 56.52, 43.76, 40.94, 37.54, 20.79, 20.62. MS (ESI): 712.47(C₃₃H₃₅N₃O₁₅, [M-H]).

**Preparation of 4′-Demethyl-(epi)-podophyllotoxin 4-O-2″-deoxy-2″-azido-D-glucopyranoside (8a).**

![Chemical Structure](image)

A solution of 7a (713 mg, 1 mmol) in dry MeOH (50 mL), Zinc power (325 mg, 5 mmol) was added, the mixture was stirred under a reflux condenser, after two days, the Zinc power was then filtered off through a pad of celite and rinsed successively with MeOH and concentrated in vacuo.
MS (ESI): 588.17(C_{27}H_{29}N_{3}O_{12}, [M+H]^+).

4′-Demethyl-(epi)-podophyllotoxin-4-O-(2′-deoxy-2′-azido-4′,6′-di-O-thiophenylidene)-β-D-glucopyranoside (9a).

The synthesis was performed according to previously published methods.

To a suspension of 8a (587 mg, 1 mmol) in dry 2-thiophenaldehyde (4 mL) was added anhydrous ZnCl_2 (340 mg, 2.5 mmol) under the atmosphere of nitrogen at 30 °C. After stirring 12 h at the same temperature, during which time a clear solution was gradually formed, DCM was added to dilute the reaction. The resultant solution was washed successively with water, saturated sodium bicarbonate solution and brine, and then dried with anhydrous sodium sulfate. Filtration and concentration generate the crude product which was further purified by column chromatography (ethyl acetate: petroleum ether=55:45) to give 9a.

^1H NMR (500 MHz, DMSO-d_6) δ 8.28 (s, 1H), 7.55 (dd, J = 5.0, 1.2 Hz, 1H), 7.20 (s, 1H), 7.10 (s, 1H), 7.36-6.65 (m, 4H), 7.03 (dd, J = 5.0, 3.6 Hz, 1H), 6.56 (s, 1H), 6.21 (s, 2H), 6.05 (d, J = 4.5 Hz, 2H), 5.93 (s, 1H), 5.90 (d, J = 5.4 Hz, 1H), 5.90 (d, J = 5.4 Hz, 1H), 4.99 (d, J = 3.3 Hz, 1H), 4.99 (d, J = 3.3 Hz, 1H), 4.96 (d, J = 8.1 Hz, 1H), 4.55 (d, J = 5.3 Hz, 1H), 4.36-4.18 (m, 4H), 4.04 (q, J = 7.1 Hz, 1H), 4.04 (q, J = 7.1 Hz, 1H), 3.79 (t, J = 9.7 Hz, 2H), 3.63 (s, 9H), 3.53 (s, 4H), 3.33 (t, J = 8.4 Hz, 1H), 3.21 (dd, J = 14.2, 5.4 Hz, 1H), 3.04-2.86 (m, 1H).^13C NMR (126 MHz, DMSO-d_6) δ 174.89, 148.29, 147.59, 146.78, 140.49, 135.13, 133.12, 130.51, 129.13, 126.87,
126.57, 110.42, 109.77, 108.78, 101.81, 100.28, 98.01, 80.85, 73.24, 71.23, 68.06, 67.49, 66.03, 60.24, 56.41, 43.37, 40.99, 40.44, 40.36, 39.98, 39.94, 39.77, 39.61, 39.44, 37.49.

MS (ESI): 704.32(C_{32}H_{33}NO_{12}S, [M+H]^+).

4′-Demethyl-(epi)-podophyllotoxin-4-O-(2″-deoxy-2″-amino-4″,6″-di-O-thiophenylidene)-β-D-glucopyranoside (10a).

To a solution of 9a (681 mg, 1 mmol) in a mixture of MeOH and ethyl acetate (v/v = 1:1, 50 mL) was added 10% Pd-C. Under an H_2 atmosphere, the resulting suspension was stirred at room temperature for 4 h, at which time TLC shown that the starting material disappeared and a new polar compound appeared. Filtration and concentration generate the crude product which was further purified by column chromatography (DCM/MeOH= 10: 1) to afford 10a (589 mg, 90%) as a white solid.

MS (ESI): 656.41(C_{32}H_{33}NO_{12}S, [M+H]^+).
H NMR spectrum of Compound 2a.

\( ^{13} \text{C} \) NMR spectrum of Compound 2a.
$^1$H NMR spectrum of Compound 2b.

$^{13}$C NMR spectrum of Compound 2b.
$^{1}H$ NMR spectrum of Compound 7a.

$^{13}C$ NMR spectrum of Compound 7a.
$^1$H NMR spectrum of Compound 9a.

$^{13}$C NMR spectrum of Compound 9a.
MS spectrum of Compound 9a.
$^1$H NMR spectrum of Compound 1.

$^{13}$C NMR spectrum of Compound 1.
\textbf{S17}

$^1\text{H}$ NMR spectrum of Compound 2.

$^{13}\text{C}$ NMR spectrum of Compound 2.

S17
\(^1\)H NMR spectrum of Compound 3.

\(^{13}\)C NMR spectrum of Compound 3.
$^1$H NMR spectrum of Compound 4.

$^{13}$C NMR spectrum of Compound 4.
$^1$H NMR spectrum of Compound 5.

$^{13}$C NMR spectrum of Compound 5.
$^1$H NMR spectrum of Compound 6.

$^{13}$C NMR spectrum of Compound 6.
H NMR spectrum of Compound 9.

$^{13}$C NMR spectrum of Compound 9.
$^1$H NMR spectrum of Compound 10.

$^{13}$C NMR spectrum of Compound 10.
$^{1}H$ NMR spectrum of Compound 15.

$^{13}C$ NMR spectrum of Compound 15.
$^1$H NMR spectrum of Compound 16.

$^{13}$C NMR spectrum of Compound 16.
MS spectrum of Compound 15/16.
Figure S2. Effect of Compounds 15 and 16 on cell cycle arrest against A549 (A), MRC-5 (B) cell lines, MCF-7 (C) and HMEC (D) cell lines. Cell cycle arrest was detected in cells by using propidium iodide (PI) staining after treatment with the test Compounds. Each value represents the mean ± SE of three independent experiments.
**Figure S3.** Effect of Compounds 15 and 16 on cellular apoptosis in A549 (A), MRC-5 (B) cell lines, MCF-7 (C) and HMEC (D) cell lines. Apoptosis was detected in cells by using annexin V and propidium iodide (PI) double staining after treatment with the test Compounds. Each value represents the mean ± SE of three independent experiments.
Effect of Teniposide on cell cycle arrest of HepG2 cell lines.
Effect of Compound 15 on cell cycle arrest of HepG2 cell lines.
Effect of Compound 16 on cell cycle arrest of HepG2 cell lines.
Effect of Teniposide on cell cycle arrest of HL-7702 cell lines.
Effect of Compound 15 on cell cycle arrest of HL-7702 cell lines.
Effect of Compound 16 on cell cycle arrest of HL-7702 cell lines.
Effect of Teniposide on cell cycle arrest of A549 cell lines.
Effect of Compound 15 on cell cycle arrest of A549 cell lines.
Effect of Compound 16 on cell cycle arrest of A549 cell lines.
Effect of Teniposide on cell cycle arrest of MRC-5 cell lines.
Effect of Compound 15 on cell cycle arrest of MRC-5 cell lines.
Effect of Compound 16 on cell cycle arrest of MRC-5 cell lines.
Effect of Teniposide on cell cycle arrest of MCF-7 cell lines.
Effect of Compound 15 on cell cycle arrest of MCF-7 cell lines.
Effect of Compound 16 on cell cycle arrest of MCF-7 cell lines.
Effect of Teniposide on cell cycle arrest of HMEC cell lines.
Effect of Compound 15 on cell cycle arrest of HMEC cell lines.
Effect of Compound 16 on cell cycle arrest of HMEC cell lines.
Effect of Teniposide on apoptosis of HepG2 cell lines.
Effect of Compound 15 on apoptosis of HepG2 cell lines.
Effect of Compound 16 on apoptosis of HepG2 cell lines.
Effect of Teniposide on apoptosis of HL-7702 cell lines.
Effect of Compound 15 on apoptosis of HL-7702 cell lines.
Effect of Compound 16 on apoptosis of HL-7702 cell lines.
Effect of Teniposide on apoptosis of A549 cell lines.
Effect of Compound 15 on apoptosis of A549 cell lines.
Effect of Compound 16 on apoptosis of A549 cell lines.
Effect of Teniposide on apoptosis of MRC-5 cell lines.
Effect of Compound 15 on apoptosis of MRC-5 cell lines.
Effect of Compound 16 on apoptosis of MRC-5 cell lines.
Effect of Teniposide on apoptosis of MCF-7 cell lines.
Effect of Compound 15 on apoptosis of MCF-7 cell lines.
Effect of Compound 16 on apoptosis of MCF-7 cell lines.
Effect of Teniposide on apoptosis of HMEC cell lines.
Effect of Compound 15 on apoptosis of HMEC cell lines.
Effect of Compound 16 on apoptosis of HMEC cell lines.
Table S2. qRT-PCR primer sequences.

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<th>Reverse (5’-3’)</th>
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Table S3. Primary antibodies.

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<td>AKT1/2/3-Phospho-S472+S473+S474 Rabbit polyclonal Ab</td>
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<tr>
<td>PI3K/P85 Alpha Antibody Mouse monoclonal Ab</td>
<td>Proteintech, #60225-1-Ig</td>
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<td>Phospho-Pi3 Kinase p85(Tyr458)/p55(Tyr199)</td>
<td>Cell Signaling Technology, #4228T</td>
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<tr>
<td>BAX Rabbit polyclonal Ab</td>
<td>Proteintech, #50599-2-Ig</td>
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<tr>
<td>PARP1 Rabbit polyclonal Ab</td>
<td>Proteintech, #13371-1-AP</td>
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</table>
**Figure S4.** Fold-changes in expression of genes ATM, RPS6, RPS6KA3, PIK3C2B, PIK3R2, PDK1, AKT2, AKT3 in response to 50 µM Compounds 15 and 16 versus Teniposide-treated group, respectively.
Figure S5. The mRNA expression levels of PDK1, ATM, AKT2, AKT3, PIK3R2, PIK3C2B, RPS6KA3 and RPS6 in response to 50 µM Teniposide, Compounds 15 and 16 by using RT-qPCR. Bars that do not share the same letter are significantly different ($p < 0.05$). *$p < 0.05$ versus Teniposide group.
REFERENCES

