Supporting Information

Discovery of 29-O-acyl-toosendanin-based derivatives as potent anti-cancer agents

Ming-Feng Zou[‡] ^a, Run-Zhu Fan[‡] ^a, Ai-Ping Yin ^b, Rong Hu ^a, Dong Huang ^a, Wei Li ^a, Sheng Yin ^a,

Rong Pu^{b,*} and Gui-Hua Tang^{a,*}

^a School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China

^b Department of Clinical Laboratory, the Third People's Hospital of Dongguan, Dongguan 523326, China

* Corresponding Authors

E-mail address: tanggh5@mail.sysu.edu.cn (G.-H. Tang), purong328073@163.com (R. Pu)

[‡] M.-F. Zou and R.-Z. Fan contributed equally to this paper.

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1. Figures S1-S6. ¹H NMR, ¹³C NMR, 2D NMR, and HRESIMS spectra of 1.

Figure S1. ¹H NMR (400 MHz) spectrum of 1 in CDCl₃.



Figure S2. ¹³C NMR (100 MHz) spectrum of 1 in CDCl₃.



Figure S3. HSQC spectrum of 1 in CDCl₃.



Figure S4. ¹H–¹H COSY spectrum of 1 in CDCl₃.



Figure S5. HMBC spectrum of 1 in CDCl₃.



Figure S6. HRESIMS spectrum of 1



2. Figures S7–S8. ¹H NMR and ¹³C NMR spectra of 2.

Figure S7. ¹H NMR (400 MHz) spectrum of 2 in CDCl₃&CD₃OD.



Figure S8. ¹³C NMR (100 MHz) spectrum of 2 in CDCl₃&CD₃OD.



3. Figures S9–S14. ¹H NMR, ¹³C NMR, 2D NMR, and HRESIMS spectra of 3.

Figure S9. ¹H NMR (400 MHz) spectrum of 3 in CDCl₃.



Figure S11. HSQC spectrum of 3 in CDCl₃.



Figure S12. ¹H–¹H COSY spectrum of 3 in CDCl₃.



Figure S13. HMBC spectrum of 3 in CDCl₃.



Figure S14. HRESIMS spectrum of 3.



4. Figures S15–S20. ¹H NMR, ¹³C NMR, 2D NMR, and HRESIMS spectra of 4.

Figure S15. ¹H NMR (400 MHz) spectrum of 4 in pyridine- d_5 .



Figure S16. ¹³C NMR (100 MHz) spectrum of 4 in pyridine- d_5 .









Figure S18. $^{1}H^{-1}H$ COSY spectrum of **4** in pyridine- d_{5} .



Figure S19. HMBC spectrum of 4 in pyridine-*d*₅.



Figure S20. HRESIMS spectrum of 4.



5. Figures S21–S26. ¹H NMR, ¹³C NMR, 2D NMR, and HRESIMS spectra of 5.

Figure S21. ¹H NMR (400 MHz) spectrum of 5 in CDCl₃.



Figure S22. ¹³C NMR (100 MHz) spectrum of 5 in CDCl₃.



Figure S23. HSQC spectrum of 5 in CDCl₃.



Figure S24. ¹H–¹H COSY spectrum of 5 in CDCl₃.



Figure S25. HMBC spectrum of 5 in CDCl₃.



Figure S26. HRESIMS spectrum of 5.



6. Figures S27–S28. ¹H NMR and ¹³C NMR spectra of 6.

Figure S27. ¹H NMR (500 MHz) spectrum of 6 in CDCl₃.





Figure S28. ¹³C NMR (125 MHz) spectrum of 6 in CDCl₃.



7. Figures S29–S30. ¹H NMR and ¹³C NMR spectra of 7.

Figure S29. ¹H NMR (400 MHz) spectrum of **7** in pyridine- d_5 .



Figure S30. ¹³C NMR (100 MHz) spectrum of 7 in pyridine- d_5 .



8. Figures S31–S32. ¹H NMR and ¹³C NMR spectra of 8.

Figure S31. ¹H NMR (400 MHz) spectrum of 8 in CDCl₃.



Figure S32. ¹³C NMR spectra of 8 in CDCl₃.





9. Figures S33–S38. ¹H NMR, ¹³C NMR, 2D NMR, and HRESIMS spectra of 9.

Figure S33. ¹H NMR (400 MHz) spectrum of 9 in CDCl₃.





Figure S35. HSQC spectrum of 9 in CDCl₃.



Figure S36. ¹H–¹H COSY spectrum of 9 in CDCl₃.



Figure S37. HMBC spectrum of 9 in CDCl₃.



Figure S38. HRESIMS spectrum of 9.



10. Figures S39–S44. ¹H NMR, ¹³C NMR, 2D NMR, and HRESIMS spectra of 10.

Figure S39. ¹H NMR (400 MHz) spectrum of 10 in CDCl₃.



Figure S40. ¹³C NMR (100 MHz) spectrum of 10 in CDCl₃.



Figure S41. HSQC spectrum of 10 in CDCl₃.



Figure S42. ¹H–¹H COSY spectrum of 10 in CDCl₃.



Figure S43. HMBC spectrum of 10 in CDCl₃.



Figure S44. HRESIMS spectrum of 10.



11. Figures S45–S46. ¹H NMR and ¹³C NMR spectra of 11.

Figure S45. ¹H NMR (400 MHz) spectrum of 11 in pyrdine- d_5 .



Figure S46. ¹³C NMR (100 MHz) spectrum of 11 in pyrdine- d_5 .



12. Figures S47–S48. ¹H NMR and ¹³C NMR spectra of 12.







13. Figures S49–S50. ¹H NMR and ¹³C NMR spectra of 13.

Figure S49. ¹H NMR (400 MHz) spectrum of 13 in CDCl₃.

7.3292 7.1275 7.1275 7.1275 6.8163 7.1275 6.8163 7.25863 7.25863 7.25863 7.25863 7.25863 7.25863 7.25863 7.25863 7.256



Figure S50. ¹³C NMR (100 MHz) spectrum of 13 in CDCl₃.



14. Figures S51–S52. ¹H NMR and ¹³C NMR spectra of 14.

Figure S51. ¹H NMR (500 MHz) spectrum of 14 in CDCl₃.





Figure S52. ¹³C NMR (125 MHz) spectrum of 14 in CDCl₃.



15. Figures S53–S54. ¹H NMR and ¹³C NMR spectra of 15.

Figure S53. ¹H NMR (500 MHz) spectrum of 15 in CDCl₃.





Figure S54. ¹³C NMR (125 MHz) spectrum of 15 in CDCl₃.



16. Figures S55–S60. ¹H NMR, ¹³C NMR, 2D NMR, and HRESIMS spectra of 16.

Figure S55. ¹H NMR (400 MHz) spectrum of 16 in CDCl₃.



Figure S56. ¹³C NMR (100 MHz) spectrum of 16 in CDCl₃.

| 206.86 | $<^{170.58}_{170.09}$ | -157.31 | $\int 147.40$ $\int 143.81$ ~ 142.58 $\sqrt{140.81}$ | -122.60 -119.53 -119.53 $\int 112.24$ $\int 112.03$ | -95.39 77.16 77.48 77.16 77.16 77.684 73.65 73.65 70.11 70.11 64.91 58.56 | -48.58 -42.69 -42.69 -41.55 -27.99 -21.64 -20.86 -15.91 |
|--------|-----------------------|---------|--|---|--|--|
|--------|-----------------------|---------|--|---|--|--|



Figure S57. HSQC spectrum of 16 in CDCl₃.



Figure S58. ¹H–¹H COSY spectrum of 16 in CDCl₃.



Figure S59. HMBC spectrum of 16 in CDCl₃.



Figure S60. HRESIMS spectrum of 16.



17. Figures S61–S63. ¹H NMR, ¹³C NMR, and HRESIMS spectra of 17.

Figure S61. ¹H NMR (400 MHz) spectrum of 17 in CDCl₃.



Figure S62. ¹³C NMR (100 MHz) spectrum of **17** in CDCl₃.



Figure S63. HRESIMS spectrum of 17.



18. Figures S64–S65. ¹H NMR and ¹³C NMR spectra of 18.

Figure S64. ¹H NMR (400 MHz) spectrum of 18 in CDCl₃.

8.0565 8.0540 8.0540 8.05308 8.0328 8.0328 8.0328 7.55934 7.7574 7.7574 7.7574 7.7574 7.7574 7.7574 7.7574 7.7574 7.7574 7.7574 7.7574 7.75592 7.73365 6.1524 6.1524 6.1524 6.1524 7.73565 6.1524 7.73565 6.1524 7.73565 6.1524 7.73565 7.73565 7.73565 7.73565 7.73565 7.73565 7.73565 7.73565 7.73565 7.73565 7.73565 7.73565 7.735656 7.735656 7.73565 7.73565 7.73565 7.735656 7.735656 7.735656 7.735656 7.735656 7.735656 7.73566 7.735656 7.735656 7.735656 7.735656 7.73566 7.755677 7.755677 7.75567 7.75567 7.7556777 7.7556777 7.7556777777



Figure S65. ¹³C NMR (100 MHz) spectrum of 18 in CDCl₃.



19. Figures S66–S71. ¹H NMR, ¹³C NMR, 2D NMR, and HRESIMS spectra of 19.

Figure S66. ¹H NMR (400 MHz) spectrum of 19 in CDCl₃.

 8.0676

 8.0541

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 8.1384

 8.1384

 9.1428

 7.





Figure S68. HSQC spectrum of 19 in CDCl₃.







Figure S70. HMBC spectrum of 19 in CDCl₃.



Figure S71. HRESIMS spectrum of 19.



20. Figures S72–S73. ¹H NMR and ¹³C NMR spectra of 20.

Figure S72. ¹H NMR (400 MHz) spectrum of 20 in CDCl₃.



Figure S73. ¹³C NMR (100 MHz) spectrum of 20 in CDCl₃.



21. Figures S74–S79. ¹H NMR, ¹³C NMR, 2D NMR, and HRESIMS spectra of 21.

Figure S74. ¹H NMR (500 MHz) spectrum of 21 in CDCl₃.



Figure S75. ¹³C NMR (125 MHz) spectrum of 21 in CDCl₃.



Figure S76. HSQC spectrum of 21 in CDCl₃.



Figure S77. ¹H–¹H COSY spectrum of 21 in CDCl₃.



Figure S78. HMBC spectrum of 21 in CDCl₃.







22. Figures S80–S82. ¹H NMR, ¹³C NMR, and HRESIMS spectra of 22. Figure S80. ¹H NMR (500 MHz) spectrum of 22 in CDCl₃.

| $\begin{cases} 8.05 \\ 8.04 \\ 8.03 \\ 8.02 \\ 8.02 \\ -7.27 \\ -7.27 \\ -7.01 \\ -6.67 \\ 6.67 \\ -6.05 \end{cases}$ | C 235 C 253 C 253 | $\overbrace{\begin{array}{c}3.79\\3.78\\3.78\\2.92\\2.92\\2.92\\2.92\\2.97\\2.89\\2.77\\2.67\end{array}}$ | 2.12 1.99 1.90 1.90 1.80 1.82 2.12 2.12 | -0.79 |
|---|---|---|--|-------|
|---|---|---|--|-------|







Figure S82. HRESIMS spectrum of 22.



23. Figures S83–S84. ¹H NMR and ¹³C NMR spectra of 23.

Figure S83. ¹H NMR (400 MHz) spectrum of 23 in CDCl₃.

8.3377 8.3375 8.3355 8.3355 8.3355 8.3355 8.3339 7.73318 7.73318 7.73318 7.73318 7.73318 7.73318 7.73318 7.73318 7.73318 7.73318 7.73318 7.5459 7.5459 7.5459 7.5459 7.5459 7.5459 7.5459 7.5459 7.5459 7.54599 7.545999 7.545999 7.545999 7.545999 7.241567 7.241567 7.241567 7.241567 7.241567 7.241567767 7.241567767767776777777777777777777777777



Figure S84. ¹³C NMR (125 MHz) spectrum of 23 in CDCl₃.



24. Figures S85–S86. ¹H NMR and ¹³C NMR spectra of 24.

Figure S85. ¹H NMR (400 MHz) spectrum of 24 in CDCl₃.

 8.0099

 7.7.3305

 7.7.33305

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 7.7.33305

 7.6.9270

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 7.6.9270

 7.6.9270

 7.6.9270

 7.6.9209

 7.7.0295

 7.1.3355

 7.1.7357

 7.1.7357

 7.1.7353

 7.1.7793

 7.1.7793

 7.1.7793

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 7.1.7793

 7.1.7793

 7.1.7793



Figure S86. ¹³C NMR (100 MHz) spectrum of 24 in CDCl₃.



25. Figures S87–S89. ¹H NMR, ¹³C NMR, and HRESIMS spectra of 25.

Figure S87. ¹H NMR (400 MHz) spectrum of 25 in CDCl₃.



Figure S88. ¹³C NMR (100 MHz) spectrum of 25 in CDCl₃.



Figure S89. HRESIMS spectrum of 25.



26. Table S1. The IC₅₀ and folds values of compounds 14, 17–19, 21, and 25 on HepG2 cell line.

| Commit | HepG2 | HepG2 | | HepG2 | |
|--------|-----------------------|---------------------------|--------|-----------------------|---------------------------|
| Compa. | IC ₅₀ (µM) | Folds ^{<i>a</i>} | Compa. | IC ₅₀ (µM) | Folds ^{<i>a</i>} |
| 14 | 0.0455 ± 0.00590 | $1.04\downarrow$ | 21 | 0.241 ± 0.0287 | 5.51↓ |
| 17 | 0.136 ± 0.00871 | 3.11↓ | 25 | 0.0352 ± 0.00264 | 1.24 ↑ |
| 18 | 0.0751 ± 0.00967 | $1.72 \downarrow$ | TSN | 0.0437 ± 0.00443 | - |
| 19 | 0.0946 ± 0.0141 | $2.16\downarrow$ | | | |

^{*a*} The increase (\uparrow) or decrease (\downarrow) fold values of the compounds are calculated by comparison their IC₅₀ values with that of TSN in the same cancer cell model.

| 27. Table S2. The cel | ll viability of compound | ls 14, 17–19, 21, | and 25 on normal | cells (MCF10A) at the |
|------------------------------|--------------------------|-------------------|------------------|-----------------------|
| concentration of 0.3 µ | μΜ. | | | |

| Comnd | MCF10A | Comnd | MCF10A |
|--------|--------------------|----------|--------------------|
| Compa. | Cell viability (%) | - Compa. | Cell viability (%) |
| 14 | 0.314 | 21 | 10.7 |
| 17 | 0.658 | 25 | 0.310 |
| 18 | 4.57 | TSN | 0.384 |
| 19 | 0.155 | | |