Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

This journal is © The Royal Society of Chemistry 2023

Design, Synthesis and Structure–Activity Relationship Studies on Erianin Analogues as Pyruvate Carboxylase (PC) Inhibitors Against Hepatocellular Carcinoma Cells

Hailong Shi,<sup>†a,b,c</sup> Jinlian Yang,<sup>†a,b,c</sup> Zeen Qiao, <sup>† a,b</sup> Lingyu Li, <sup>a,b</sup> Gang, Liu, <sup>a,b</sup> Qi Dai,<sup>c</sup> Li Xu,<sup>c</sup> Wei Jiao,<sup>a</sup> Guolin Zhang,<sup>a</sup> Fei Wang,<sup>a</sup> Xiaoxia Lu,<sup>\* a,c</sup>, Xiaofeng Ma. <sup>\* a</sup>

<sup>a.</sup> Natural Product Research Center, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China;

<sup>b.</sup> University of Chinese Academy of Sciences, Beijing 100049, China.

<sup>c.</sup> NMPA Key Laboratory for Quality Monitoring and Evaluation of Traditional Chinese Medicine (Chinese Materia Medica)

\*Correspondence Authors:

Xiaofeng Ma, Natural Product Research Center, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China, E-mail: (maxf@cib.ac.cn)

Xiaoxia Lu, Natural Product Research Center, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China, E-mail: (<u>luxx@cib.ac.cn</u>)

## content

SYNTHESIS OF 2-METHOXY-5-(3-(3,4,5-TRIMETHOXYPHENYL)PROPYL)PHENOL 44
SYNTHESIS OF COMPOUND 5 AND 14
SYNTHESIS OF COMPOUND 6 AND 76
FIGURE S1. IC <sub>50</sub> VALUES OF THE ERIANIN ANALOGUES 1, 2, 3, 4 AND 6 ON CELL GROWTH INHIBITION IN HCCLM37
FIGURE S2 COMPOUND 34 AND HUMAN PC (PDB ID:3BG3) BINDING MODES8
FIGURE S3 COMPOUND 35 AND HUMAN PC (PDB ID:3BG3) BINDING MODES9
FIGURE S4 ERIANIN AND HUMAN PC (PDB ID:3BG3) BINDING MODES10
FIGURE S5. <sup>1</sup> H NMR OF COMPOUND 411
FIGURE S6. <sup>13</sup> C NMR OF COMPOUND 4
FIGURE S7. <sup>1</sup> H NMR OF COMPOUND 14
FIGURE S8. <sup>13</sup> C NMR OF COMPOUND 14
FIGURE S9. <sup>13</sup> C NMR OF COMPOUND 5
FIGURE S10. <sup>13</sup> C NMR OF COMPOUND 5
FIGURE S11. <sup>1</sup> H NMR OF COMPOUND 617
FIGURE S12. <sup>13</sup> C NMR OF COMPOUND 6
FIGURE S13. <sup>1</sup> H NMR OF COMPOUND 7
FIGURE S14. <sup>13</sup> C NMR OF COMPOUND 7
FIGURE S15. <sup>1</sup> H NMR OF COMPOUND 8
FIGURE S16. <sup>13</sup> C-NMR OF COMPOUND 8
FIGURE S17. <sup>1</sup> H NMR OF COMPOUND 9
FIGURE S18. <sup>13</sup> C-NMR OF COMPOUND 9
FIGURE S19. <sup>1</sup> H NMR OF COMPOUND 11
FIGURE S20. <sup>13</sup> C-NMR OF COMPOUND 11
FIGURE S21. <sup>1</sup> H NMR OF COMPOUND 12
FIGURE S22. <sup>13</sup> C-NMR OF COMPOUND 12

FIGURE S23. <sup>1</sup> H NMR OF COMPOUND 13	29
FIGURE S24. <sup>13</sup> C-NMR OF COMPOUND 13	30
FIGURE S25. <sup>1</sup> H NMR OF COMPOUND 15	31
FIGURE S26. <sup>13</sup> C-NMR OF COMPOUND 15	32
FIGURE S27. <sup>1</sup> H NMR OF COMPOUND 16	33
FIGURE S28. <sup>13</sup> C-NMR OF COMPOUND 16	34
FIGURE S29. <sup>1</sup> H NMR OF COMPOUND 17	35
FIGURE S30. <sup>13</sup> C-NMR OF COMPOUND 17	36
FIGURE S31. <sup>1</sup> H NMR OF COMPOUND 18	
FIGURE S32. <sup>13</sup> C-NMR OF COMPOUND 18	
FIGURE S33. <sup>1</sup> H NMR OF COMPOUND 19	
FIGURE S34. <sup>13</sup> C-NMR OF COMPOUND 19	40
FIGURE S35. <sup>1</sup> H NMR OF COMPOUND 20	41
FIGURE S36. <sup>13</sup> C-NMR OF COMPOUND 20	42
FIGURE S37. <sup>1</sup> H NMR OF COMPOUND 22	43
FIGURE S38. <sup>13</sup> C-NMR OF COMPOUND 22	44
FIGURE S39. <sup>1</sup> H NMR OF COMPOUND 23	45
FIGURE S40. <sup>13</sup> C-NMR OF COMPOUND 23	46
FIGURE S41. <sup>1</sup> H NMR OF COMPOUND 24	47
FIGURE S42. <sup>13</sup> C-NMR OF COMPOUND 24	48
FIGURE S43. <sup>1</sup> H NMR OF COMPOUND 25	49
FIGURE S44. <sup>13</sup> C-NMR OF COMPOUND 25	50
FIGURE S45 <sup>1</sup> H NMR OF COMPOUND 26	51
FIGURE S46. <sup>13</sup> C-NMR OF COMPOUND 26	52
FIGURE S47. <sup>1</sup> H NMR OF COMPOUND 27	53
FIGURE S48. <sup>13</sup> C-NMR OF COMPOUND 27	54
FIGURE S49. <sup>1</sup> H NMR OF COMPOUND 28	55
FIGURE S50 <sup>13</sup> C-NMR OF COMPOUND 28	56

FIGURE S51. <sup>1</sup> H NMR OF COMPOUND 29	
FIGURE S52. <sup>13</sup> C-NMR OF COMPOUND 29	
FIGURE S53. <sup>1</sup> H NMR OF COMPOUND 30	
FIGURE S54. <sup>13</sup> C-NMR OF COMPOUND 30	60
FIGURE S55. <sup>1</sup> H NMR OF COMPOUND 31	61
FIGURE S56. <sup>13</sup> C-NMR OF COMPOUND 31	
FIGURE S57. <sup>1</sup> H NMR OF COMPOUND 32	
FIGURE S58. <sup>13</sup> C-NMR OF COMPOUND 32	64
FIGURE S59. <sup>1</sup> H NMR OF COMPOUND 33	
FIGURE S60. <sup>13</sup> C-NMR OF COMPOUND 33	66
FIGURE S61. <sup>1</sup> H NMR OF COMPOUND 34	67
FIGURE S62. <sup>13</sup> C-NMR OF COMPOUND 34	
FIGURE S63. <sup>1</sup> H NMR OF COMPOUND 35	70
FIGURE S64. <sup>13</sup> C-NMR OF COMPOUND 35	71
FIGURE S65. <sup>1</sup> H NMR OF COMPOUND 36	71
FIGURE S66. <sup>13</sup> C-NMR OF COMPOUND 36	
FIGURE S67. <sup>1</sup> H NMR OF COMPOUND 37	
FIGURE S68. <sup>13</sup> C-NMR OF COMPOUND 37	74
FIGURE S70. <sup>13</sup> C-NMR OF COMPOUND 38	76
FIGURE S71. <sup>1</sup> H NMR OF COMPOUND 39	77
FIGURE S72. <sup>13</sup> C-NMR OF COMPOUND 39	
FIGURE S73. 1H NMR OF COMPOUND 41	
FIGURE S74. <sup>13</sup> C-NMR OF COMPOUND 41	80
FIGURE S75. <sup>1</sup> H NMR OF COMPOUND 42	
FIGURE S76. <sup>13</sup> C-NMR OF COMPOUND 42	
FIGURE S77. <sup>1</sup> H NMR OF COMPOUND 43	
FIGURE S78. <sup>13</sup> C-NMR OF COMPOUND 43	
FIGURE S79. <sup>1</sup> H NMR OF COMPOUND 44	85

FIGURE S80. <sup>13</sup> C-NMR OF COMPOUND 44	86
FIGURE S81. <sup>1</sup> H NMR OF COMPOUND 45	87
FIGURE S82. <sup>13</sup> C-NMR OF COMPOUND 45	88
FIGURE S83. <sup>1</sup> H NMR OF COMPOUND 46	89
FIGURE S84. <sup>13</sup> C-NMR OF COMPOUND 46	90
FIGURE S85. <sup>1</sup> H NMR OF COMPOUND 47	91
FIGURE S86. <sup>13</sup> C-NMR OF COMPOUND 47	92
REFERENCES	93

Synthesis of 2-methoxy-5-(3-(3,4,5-trimethoxyphenyl)propyl)phenol 41

The obtained ketone **3** (150.0 mg, 0.43 mmol) was dissolver in acetic acid (2 mL), 10% palladium on carbon (25.2 mg) was added to the solution. The reaction vessel was evacuated and backfilled with hydrogenation (H<sub>2</sub>) for 3 times. The mixture was stirred at 25 °C for 12 h and then filtration through celite gave a filtrate. This filtrate was evaporated to oil, which was purified by chromatography on silica gel (ethyl acetate: hexane = 1: 10) to give 15 (130.6 mg, 91.4%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.77 (d, *J* = 2.24 Hz, 1H), 6.76 (d, *J* = 8.36 Hz, 1H), 6.67 (dd, *J* = 2.00, 8.20 Hz, 1H), 6.39 (s, 2 H), 3.87 (s, 3H), 3.85 (s, 3 H), 3.83 (s, 3H), 2.61 (t, *J* = 7.76 Hz, 4H), 1.89 - 1.95 (m, 2 H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  153.07, 145.50, 144.78, 138.17, 136.05, 135.54, 119.75, 114.71, 110.63, 105.33, 60.86, 56.07, 35.76, 34.82, 33.02.

Synthesis of compound 5<sup>2</sup> and 14<sup>3</sup>

The synthesis of compound **5** and **7** followed the synthesis procedure of compound **15** in scheme 3.

2-(3-hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethan-1-one (5) <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.26 (s, 2H), 6.84 (d, J = 2.00 Hz, 1H), 6.81 (d, J= 8.20 Hz, 1H), 6.75 (dd, J = 2.00 Hz, 8.20 Hz, 1H), 5.64 (s, 1H), 4.14 (s, 2H), 3.40 (s, 3H), 3.88 (s, 6H), 3.85 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  196.61, 153.01, 145.82, 145.62, 142.57, 131.70, 127.97, 120.73, 115.54, 110.94, 106.32, 60.91, 56.26, 55.95, 45.03.

1-(4-hydroxy-3-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethan-1-one (**14**). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.63 (dd, *J* = 1.92 Hz, 8.28 Hz, 1H), 7.57 (d, *J* = 1.92 Hz, 1H), 6.96 (d, *J* = 8.28 Hz, 1H), 6.48 (s, 2H), 6.13 (br s, 1H), 4.17 (s, 2H), 3.94 (s, 3H), 3.83 (s, 3 H), (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 196.19, 153.32, 150.60, 146.77, 136.91, 130.57, 129.48, 124.07, 113.86, 110.33, 106.45, 60.82, 56.11, 56.03, 45.26, 29.69.

Synthesis of compound 6 and 7<sup>4</sup>

The synthesis of compound **6** and **7** followed the synthesis procedure of compound **8** and **9** in scheme 4. 2-methoxy-5-((methyl(3,4,5- trimethoxyphenyl)amino)methyl)-

phenol (6). <sup>1</sup>H NMR (400 MHZ, CDCl<sub>3</sub>) δ 6.84 (s, 1H), 6.81 (d, *J* = 10.52, 1H), 6.76 (d, *J* = 8.04, 1H), 6.00 (s, 2H), 5.64 (s, 1H) + 4.38 (s, 2H), 3.88 (s, 3H) 3.80 (s, 6H) 3.77 (s, 3H), 2.97 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 153.68, 145.79, 145.64, 118.41, 113.25, 110.70, 90.90, 61.09, 57.04, 56.01, 55.99, 38.97.

2-methoxy-5-(methyl(3,4,5-trimethoxybenzyl)amino)phenol (7). <sup>1</sup>H NMR (400 MHZ, CDCl<sub>3</sub>), δ 6.77 (d, *J* = 8.8Hz, 1H), 6.48 (d, *J* = 2.92Hz, 1H), 6.47 (s, 2H), 6.26 (dd, *J* = 2.92Hz, 8.8Hz, 1H), 5.63 (s, 1H) 4.34 (s, 2H), 3.83 (s, 3H), 3.82 (s, 3H), 3.80 (s, 6H), 2.90 (s, 3H), <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 153.40, 146.42, 145.92, 138.94, 136.76, 135.06, 112.27, 104.45, 103.70, 101.25, 60.85, 58.11, 56.78, 56.09, 38.96.

Figure S1.  $IC_{50}$  values of the erianin analogues 1, 2, 3, 4 and 6 on cell growth inhibition in HCCLM3





Figure S2 Compound 34 and human PC (PDB ID:3BG3) binding modes

**Fig.** S2 Binding conformations of **34** with human pyruvate carboxylase (PDB ID: 3BG3). Panel A: binding site of compound **34** in the 3BG3; panel B: binding 3D modes of compound **34** in the active site of 3BG; panel C, binding 2D modes of compound **34** the active site of 3BG3.



**Fig.** S3 Binding conformations of **35** with human pyruvate carboxylase (PDB ID: 3BG3). Panel A: binding site of compound **35** in the 3BG3; panel B: binding 3D modes of compound **35** in the active site of 3BG; panel C, binding 2D modes of compound **35** the active site of 3BG3.

Figure S4 Erianin and human PC (PDB ID:3BG3) binding modes



**Fig.** S4 Binding conformations of erianin with human pyruvate carboxylase (PDB ID: 3BG3). Panel A: binding site of compound erianin in the 3BG3; panel B: binding 3D modes of compound erianin in the active site of 3BG; panel C, binding 2D modes of compound erianin the active site of 3BG3.





S11

Figure S6. <sup>13</sup>C NMR of Compound 4





Figure S7. <sup>1</sup>H NMR of Compound 14

0.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5

5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 f1 (ppm)

Figure S8. <sup>13</sup>C NMR of Compound **14** 





Figure S9. <sup>13</sup>C NMR of Compound 5

Figure S10. <sup>13</sup>C NMR of Compound 5





Figure S11. <sup>1</sup>H NMR of Compound 6

Figure S12. <sup>13</sup>C NMR of Compound 6





Figure S13. <sup>1</sup>H NMR of Compound 7

 ✓ 60.85
58.12
56.78
56.09 ₹ 77.38 ₹ 77.06 76.74 ---- 38.97 -4200 -4000 - 3800 - 3600 - 3400 - 3200 - 3000 -2800 -2600 -2400 -2200 - 2000 -1800 -1600 -1400 -1200 -1000 - 800 -600 - 400 -200 - 0 210 200 190 180 170 160 150 140 130 120 110 100 90 f1 (ppm) 80 70 60 50 40 30 20 10 0 -10

Figure S14. <sup>13</sup>C NMR of Compound 7















Figure S18. <sup>13</sup>C-NMR of Compound 9



Figure S19. <sup>1</sup>H NMR of Compound 11



Figure S20. <sup>13</sup>C-NMR of Compound 11



Figure S21. <sup>1</sup>H NMR of Compound 12



Figure S22. <sup>13</sup>C-NMR of Compound 12



Figure S23. <sup>1</sup>H NMR of Compound 13



Figure S24. <sup>13</sup>C-NMR of Compound 13

Figure S25. <sup>1</sup>H NMR of Compound 15





Figure S26. <sup>13</sup>C-NMR of Compound 15



Figure S27. <sup>1</sup>H NMR of Compound 16



Figure S28. <sup>13</sup>C-NMR of Compound 16



Figure S29. <sup>1</sup>H NMR of Compound 17



Figure S30. <sup>13</sup>C-NMR of Compound 17


Figure S31. <sup>1</sup>H NMR of Compound 18



Figure S32. <sup>13</sup>C-NMR of Compound 18



Figure S33. <sup>1</sup>H NMR of Compound 19



Figure S34. <sup>13</sup>C-NMR of Compound 19



Figure S35. <sup>1</sup>H NMR of Compound 20

Figure S36. <sup>13</sup>C-NMR of Compound 20





Figure S37. <sup>1</sup>H NMR of Compound 22

Figure S38. <sup>13</sup>C-NMR of Compound 22





Figure S39. <sup>1</sup>H NMR of Compound 23







Figure S41. <sup>1</sup>H NMR of Compound 24







Figure S43. <sup>1</sup>H NMR of Compound 25







Figure S45 <sup>1</sup>H NMR of Compound 26









Figure S48. <sup>13</sup>C-NMR of Compound 27







- 153.02 - 147.75 - 147.75 - 137.50 - 136.14 - 136.14 - 134.13 - 133.41-2200 — 77.03 — 69.89 — 60.89 — 56.05 38.4837.46 -2100 -2000 - 1900 -1800 -1700 -1600 -1500 -1400 -1300 -1200 -1100 -1000 - 900 - 800 - 700 - 600 - 500 - 400 - 300 -200 -100 ultupiendystandysta<sup>n</sup>utionspholekologian fungslanderspholekolutukishieldeskelephysionelekologian (hadraksishieldeskelephysionelekologian) n her and a second provided by the full of the full 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 f1 (ppm) 10 0 -10

Figure S50 <sup>13</sup>C-NMR of Compound 28

Figure S51. <sup>1</sup>H NMR of Compound 29



Figure S52. <sup>13</sup>C-NMR of Compound 29



Figure S53. <sup>1</sup>H NMR of Compound 30



 137.39
136.17
136.17
135.49 ∠ 108.96 2 108.13 - 105.36 - 100.79 --- 60.89 --- 56.06 — 77.03 38.62
37.70
37.70 -2100 -2000 - 1900 - 1800 -1700 -1600 -1500 -1400 -1300 - 1200 -1100 -1000 - 900 - 800 - 700 -600 - 500 - 400 - 300 -200 -100 lahy jama wa ing bahawany ni palang bahang miyani wa yani wa ban ari aga ku nu wandi biya ya ku baha ing palang bana na baha ing palang bana ku baha ing palang HAVONT HE HAVE AN A VIEW AND A 80 f1 (ppm) 150 140 130 120 110 100 90 70 . 60 50 40 20 10 0 160 30

Figure S54. <sup>13</sup>C-NMR of Compound 30



Figure S55. <sup>1</sup>H NMR of Compound 31



Figure S56. <sup>13</sup>C-NMR of Compound 31

Figure S57. <sup>1</sup>H NMR of Compound 32



Figure S58. <sup>13</sup>C-NMR of Compound 32



Figure S59. <sup>1</sup>H NMR of Compound 33



Figure S60. <sup>13</sup>C-NMR of Compound 33



Figure S61. <sup>1</sup>H NMR of Compound 34











S69

Figure S64. <sup>13</sup>C-NMR of Compound 35







Figure S66. <sup>13</sup>C-NMR of Compound 36


Figure S67. <sup>1</sup>H NMR of Compound 37



Figure S68. <sup>13</sup>C-NMR of Compound 37





Figure S69. <sup>1</sup>H NMR of Compound 38







Figure S71. <sup>1</sup>H NMR of Compound 39

Figure S72. <sup>13</sup>C-NMR of Compound 39



Figure S73. <sup>1</sup>H NMR of Compound 41











Figure S76. <sup>13</sup>C-NMR of Compound 42





Figure S77. <sup>1</sup>H NMR of Compound 43



Figure S78. <sup>13</sup>C-NMR of Compound 43

Figure S79. <sup>1</sup>H NMR of Compound 44



— 153 04 ✓ 137.66
✓ 136.12
✓ 135.57
─ 127.31 ---- 114 49 -2600 <sup>77, 35</sup>
 <sup>77, 35</sup>
 <sup>77, 03</sup>
 <sup>76, 72</sup> --- 60. 89 --- 56. 06 38 75 37.50 -2500 -2400 -2300 -2200 -2100 - 2000 - 1900 -1800 -1700 - 1600 A DECEMBER OF STREET, S -1500 -1400 -1300 -1200 -1100 -1000 - 900 - 800 - 700 -600 - 500 - 400 - 300 - 200 -100 - 0 90 f1 (ppm) 180 170 160 150 140 130 120 110 100 80 70 60 50 40 30 20 10 0

Figure S80. <sup>13</sup>C-NMR of Compound 44



Figure S81. <sup>1</sup>H NMR of Compound 45





Figure S83. <sup>1</sup>H NMR of Compound 46



Figure S84. <sup>13</sup>C-NMR of Compound 46



Figure S85. <sup>1</sup>H NMR of Compound 47



Figure S86. <sup>13</sup>C-NMR of Compound 47



References

1. Z. Getahun, L. Jurd, P.S. Chu, C.M. Lin, E. Hamel, J. Med. Chem., 35 (1992) 1058-1067.

2. M.-Y. Song, Q.-R. He, Y.-L. Wang, et al. Int. J. Mol. Sci., 2020, 21 (5),1817.

3. M. Medarde, R. Peláez-Lamamiéde Clairac, A. C. Ramos, E. Caballero, J. L. López, D. G. Grávalos and A. S. Feliciano, *Bioorg. Med. Chem. Lett.*, 1995, 5, 229-232.

4. R. Shirai, K. Tokuda, Y. Koiso and S. Iwasaki, Bioorg. Med. Chem. Lett., 1994, 4, 699-704.