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SUPPLEMENTARY MATERIAL TO

Screening the binding affinity of bile acid derivatives for the glucocorticoid receptor ligand-binding domain

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(3EZ, 7Z, 12Z)-3,7,12-Trioximino-5β-cholan-24-oic acid (1)

^{1}H NMR (400 MHz, DMSO-d_6, δ) 10.30 and 10.28 (s, 1H, NOH on C-7), 10.19 and 10.18 (s, 1H, NOH on C-3), 10.14 (s, 1H, NOH on C-12), 1.13-1.12 (overlapping singlets, 3H, H-19), 0.89-0.88 (overlapping doublet (H-21) and singlet (H-18), 6H). ^{13}C NMR (101 MHz, DMSO-d_6, δ) 175.38 (C-24), 162.61(C-12), 157.41 (C-7), 156.87 (C-3), 156.67 (C-3), 53.57, 53.54, 49.23, 46.42, 45.21, 44.07, 43.94, 43.84, 41.52, 36.71, 36.67, 36.29, 35.72, 35.15, 32.76, 31.95, 30.96, 27.72, 27.44, 27.33, 26.39, 25.57, 25.42, 22.50 (C-19), 22.39 (C-19), 20.14, 19.86 (C-21), 19.32, 12.59 (C-18). (+)ESI-HRMS (*m*/*z*): calculated for $C_{24}H_{37}N_3O_5$ $[M+Na]^+$ 470.26309, found 470.26093.

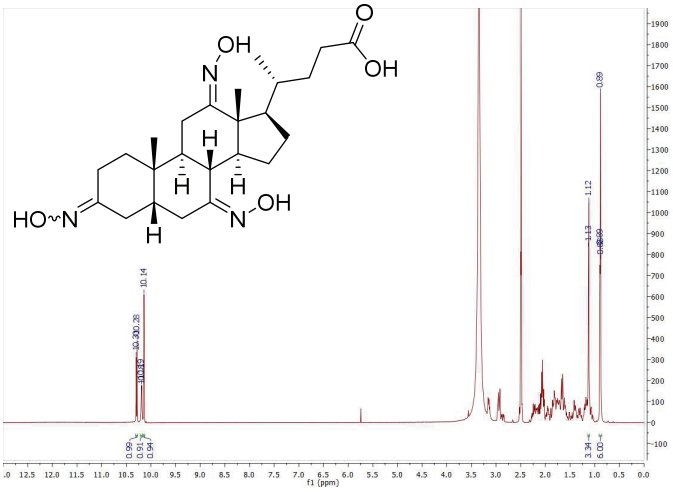

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Fig. S-1. Compound **1** ^{1}H NMR (400 MHz, DMSO-d_6) spectra.

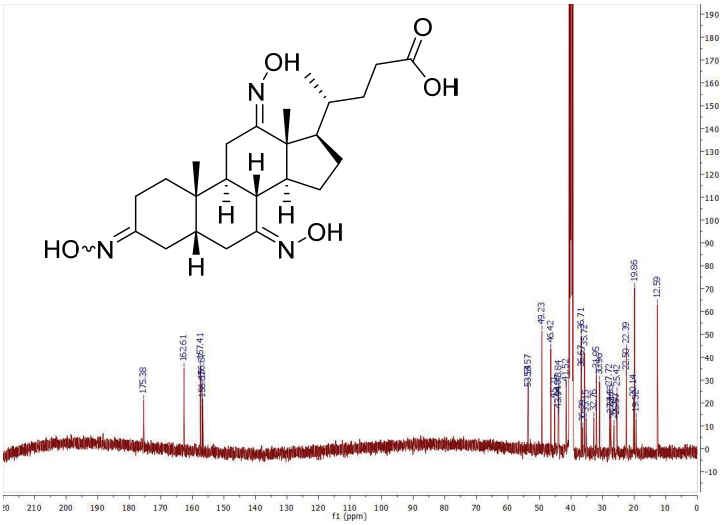

Fig. S-2. Compound **1** ^{13}C NMR (101 MHz, DMSO-d_6) spectra.

Methyl (7Z,12Z)-3,3-dimethoxy-7,12-dioximino-5β-cholan-24-oate (2)

^{1}H NMR (400 MHz, DMSO-d_6, δ) 10.21 (s, 1H, NOH on C-7), 10.11 (s, 1H, NOH on C-12), 3.56 (s, 3H, CH_3 ester), 3.01 (s, 3H, OCH_3), 3.00 (s, 3H, OCH_3), 1.09 (s, 3H, H-19), 0.87 (d, J = 6.8 Hz, 3H, H-21), 0.86 (s, 3H, H-18).^{13}C NMR (101 MHz, DMSO-d_6, δ) 174.17 (C-24), 162.71 (C-12), 157.75 (C-7), 100.07 (C-3), 53.68, 51.67 (CH_3 ester), 49.18, 47.32 (overlapping OCH_3 signals), 46.30, 44.00, 42.06, 41.56, 39.42, 36.16, 35.76, 33.97, 32.60, 31.50, 30.88, 27.80, 27.29, 25.40, 22.69 (C-19), 20.15, 19.75 (C-21), 12.50 (C-18). (+)ESI-HRMS (m/z): calculated for $C_{27}H_{44}N_2O_6$ $[M+Na]^+$ 515.30970, found 515.30725.

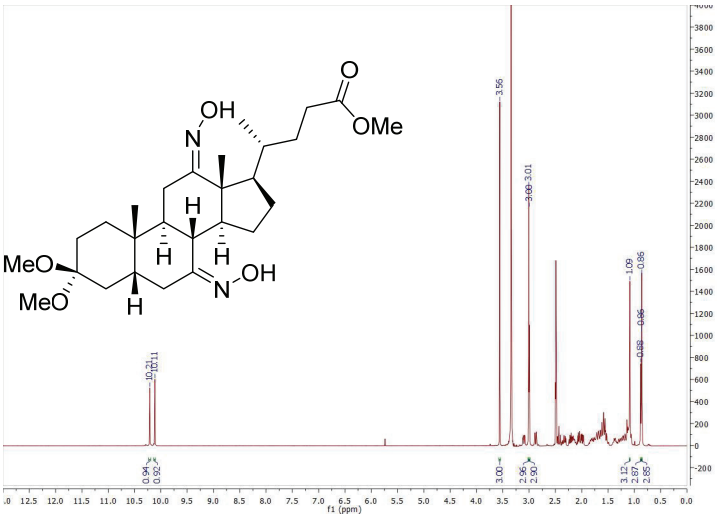

Fig. S-3. Compound **2** 1H NMR (400 MHz, DMSO-d_6) spectra.

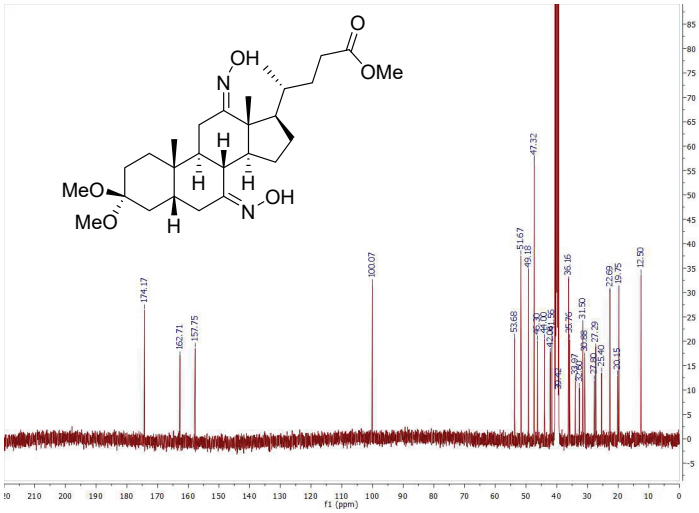

Fig. S-4. Compound **2** ^{13}C NMR (101 MHz, DMSO-d_6) spectra.

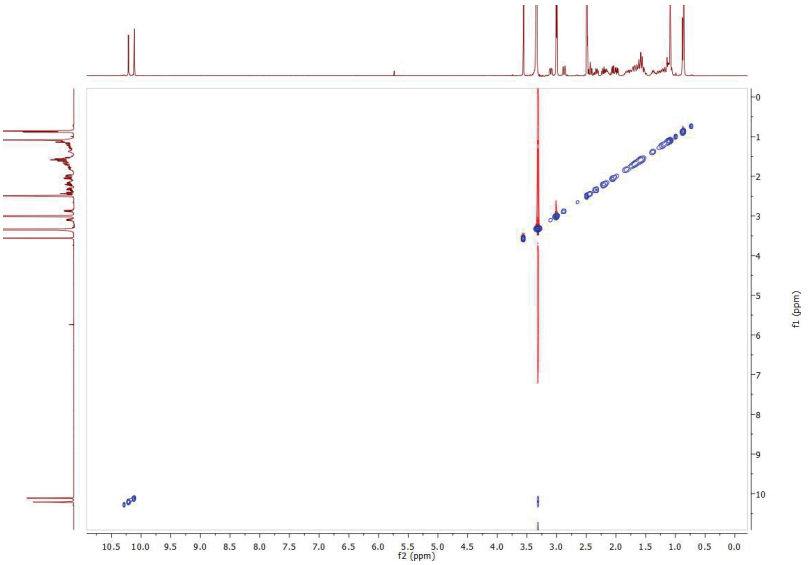

Fig. S-5. Compound **2** NOESY NMR spectra.

Methyl 3,3-dimethoxy-7,12-dioxo-5β-cholan-24-oate (3)

^{1}H NMR (400 MHz, $CDCl_3$, δ) δ 3.65 (s, 3H, CH_3 ester), 3.13 (s, 3H, OCH_3), 3.10 (s, 3H, OCH_3), 1.29 (s, 3H, H-19), 1.02 (s, 3H, H-18), 0.82 (d, J = 6.6 Hz, 3H, H-21).^{13}C NMR (101 MHz, $CDCl_3$, δ) 212.53 (C-12), 209.71 (C-7), 174.56 (C-24), 99.75 (C-3), 56.81, 51.80, 51.47 (CH_3 ester), 48.94, 47.64 (OCH_3), 47.41 (OCH_3), 45.55, 45.21, 45.09, 43.40, 38.55, 36.03, 35.53, 34.65, 31.89, 31.29, 30.46, 27.66, 26.60, 25.17, 22.35 (C-19), 18.61 (C-21), 11.79 (C-18). (+)ESI-HRMS (*m*/*z*): calculated for $C_{27}H_{42}O_6$ $[M+Na]^+$ 485.28791, found 485.28558.

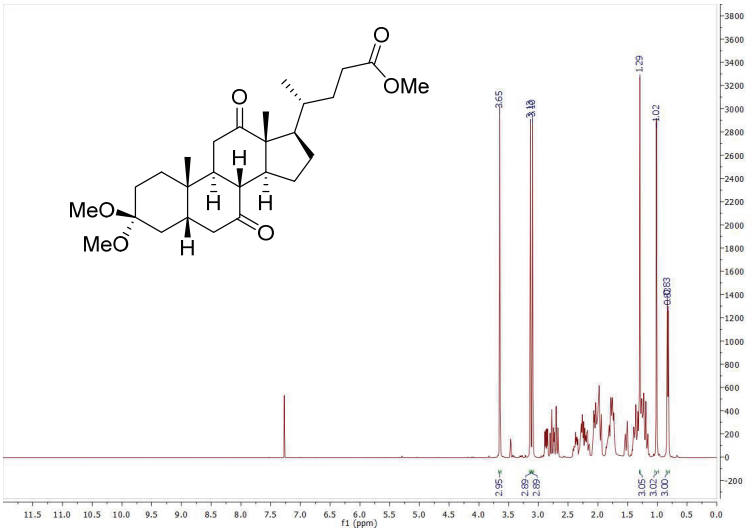

Fig. S-6. Compound **3** ^{1}H NMR (400 MHz, $CDCl_3$) spectra.

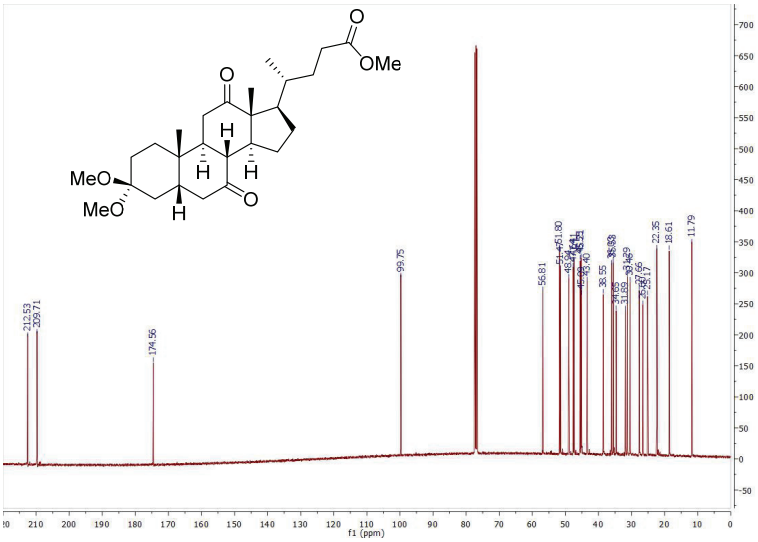

Fig. S-7. Compound **3** ^{13}C NMR (101 MHz, $CDCl_3$) spectra.

12α-Hydroxy-3-oxo-5β-chola-4,6-dien-24-oic acid (25)

^{1}H NMR (400 MHz, DMSO-d_6, δ) 11.95 (bs, 1H, H-24), 6.16 (s, 2H, H-4 and H-6), 5.61 (s, 1H, H-7), 4.31 (d, J = 3.6 Hz, 1H, OH), 3.87 (s, 1H, H-12), 1.04 (s, 3H, H-19), 0.94 (d, J = 6.5 Hz, 1H, H-21), 0.71 (s, 3H, H-18). ^{13}C NMR (101 MHz, DMSO-d_6, δ) δ 198.63 (C-3), 175.42 (C-24), 163.96 (C-5), 142.02 (C-4), 127.90 (C-6), 123.28 (C-7), 70.97 (C-12), 47.28, 46.54, 45.25, 43.71, 37.82, 35.55, 35.38, 34.02, 33.74, 31.31, 31.17, 28.67, 27.43, 23.31, 17.34 (C-21), 16.29 (C-19), 12.53 (C-18). IR (film, cm^{-1}): 3436, 2923, 1733, 1379, 1261. (+)ESI-HRMS (*m/z*): calculated for $C_{24}H_{34}O_4$ $[M-H]^-$ 387.25353, found 387.25246.

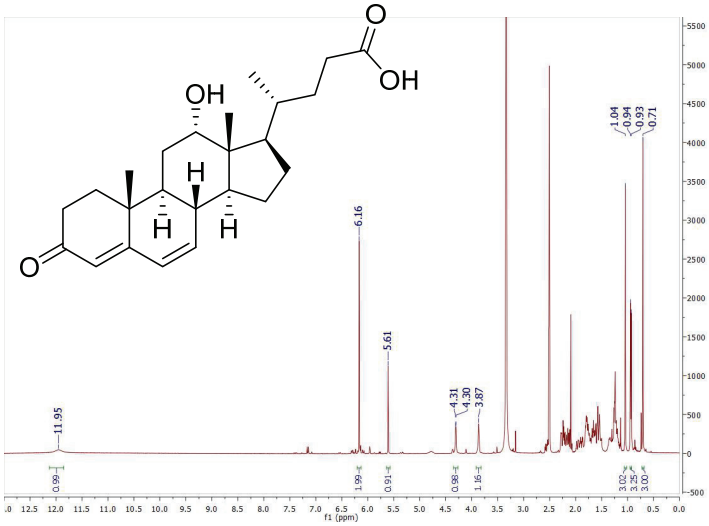

Fig. S-8. Compound **25** ^{1}H NMR (400 MHz, DMSO-d_6) spectra.

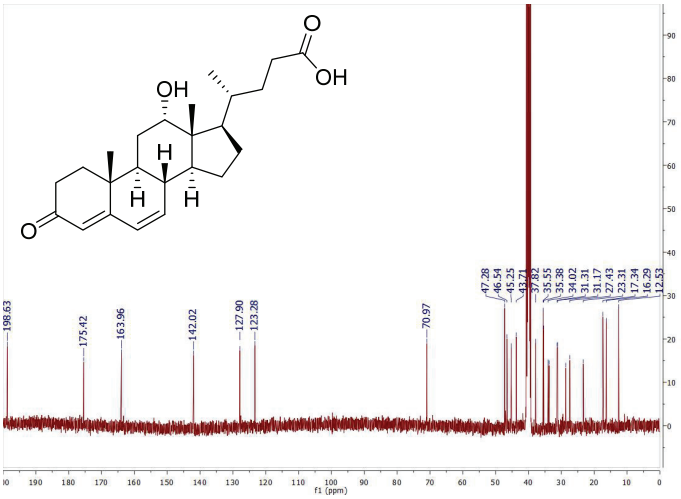

Fig. S-9. Compound **25** ^{13}C NMR (101 MHz, DMSO-d_6) spectra.

Ethyl 12α-hydroxy-3-oxo-5β-chola-4,6-dien-24-oate (26)

^{1}H NMR (400 MHz, DMSO-d_6, δ) 6.15 (s, 2H, H-4 and H-6), 5.59 (s, 1H, H-7), 4.29 (d, J = 4.0 Hz, 1H, OH), 4.03 (q, J = 6.9 Hz, 2H, CH_2 from Et), 3.85 (d, J = 3.1 Hz, 1H, H-12), 1.16 (t, J = 7.1 Hz, 3H, CH_3 from Et), 1.03 (s, 3H, H-19), 0.92 (d, J = 6.5 Hz, 3H, H-21), 0.69 (s, 3H, H-18). ^{13}C NMR (101 MHz, DMSO-d_6, δ) 198.59 (C-3), 173.74 (C-24), 163.92 (C-5), 141.97 (C-4), 127.91 (C-6), 123.29 (C-7), 70.95 (C-12), 60.09 (CH_2 from Et), 47.28, 46.49, 45.25, 43.69, 37.81, 35.54, 35.33, 34.02, 33.73, 31.17, 31.10, 28.66, 27.43, 23.30, 17.29 (C-21), 16.29 (C-19), 14.61 (CH_3 from Et), 12.49 (C-18). IR (film, cm^{-1}): 3457, 2943, 2871, 1734, 1649, 1615, 1447, 1268, 1180, 1034. (+)ESI-HRMS (*m/z*): calculated for $C_{26}H_{38}O_4$ [M-H]$^-$ 415.28483, found 415.28355.

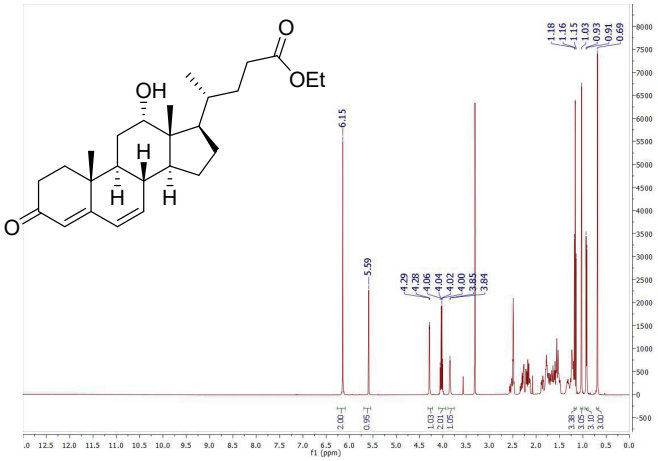

Fig. S-10. Compound **26** ^{1}H NMR (400 MHz, DMSO-d_6) spectra.

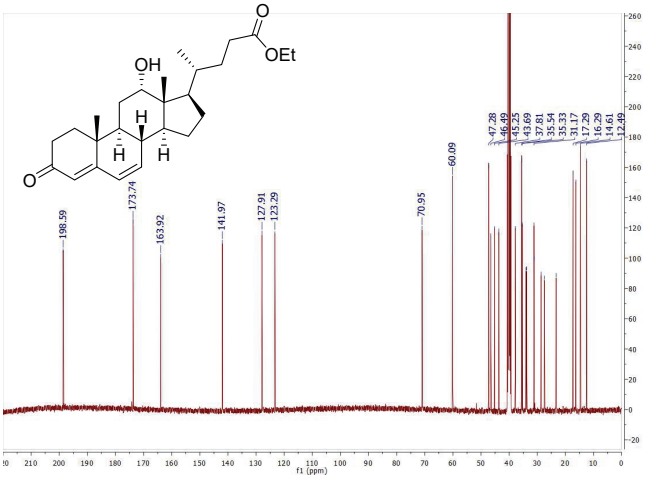

Fig. S-11. Compound **26** ^{13}C NMR (101 MHz, DMSO-d_6) spectra.

2-(5β-chol-3-ene-7α,12α,24-triol)-N-(1-hydroxy-2-methylpropan-2-yl)acetamide (30)

^{1}H NMR (400 MHz, DMSO-d_6, δ) 6.74 (s, 1H, NH), 5.41 (s, 1H, H-4), 4.05 (s, 1H, OH on C-12), 3.85 (s, 1H, OH on C-7), 3.79 (s, 1H, H-12), 3.60 (s, 1H, H-7), 3.46 and 3.37 (both are d, J_2 = 10.8 Hz, 1H, CH_2 from 1-hydroxy-2-methylpropan-2-yl), 3.35 (m, 2H, H-24), 2.79 and 2.54 (both are d, J_2 = 15.1 Hz, 1H, CH_2 from acetamide), 1.20 and 1.18 (s, 3H, CH_3 from 1-hydroxy-2-methylpropan-2-yl), 0.92 (d, J = 6.5 Hz, 3H, H-21), 0.89 (s, 3H, H-19), 0.61 (s, 3H, H-18). ^{13}C NMR (101 MHz, DMSO-d_6, δ) 170.57 (carbonyl from acetamide), 133.02 (C-4), 127.75 (C-3), 71.02 (C-12), 67.81 (CH_2 from 1-hydroxy-2-methylpropan-2-yl), 66.62 (C-7), 61.40 (C-24), 54.45, 46.33, 45.78, 41.64, 41.46, 39.21, 35.45, 34.82, 33.84, 32.84, 31.94, 29.41, 29.13, 27.46, 26.19, 24.28, 23.53, 23.29, 22.86, 21.97 (C-19), 21.17, 17.38 (C-21), 12.30 (C-18). (+)ESI-HRMS (*m/z*): calculated for $C_{30}H_{51}O_5$ $[M+Na]^+$ 528.36649, found 528.36477.

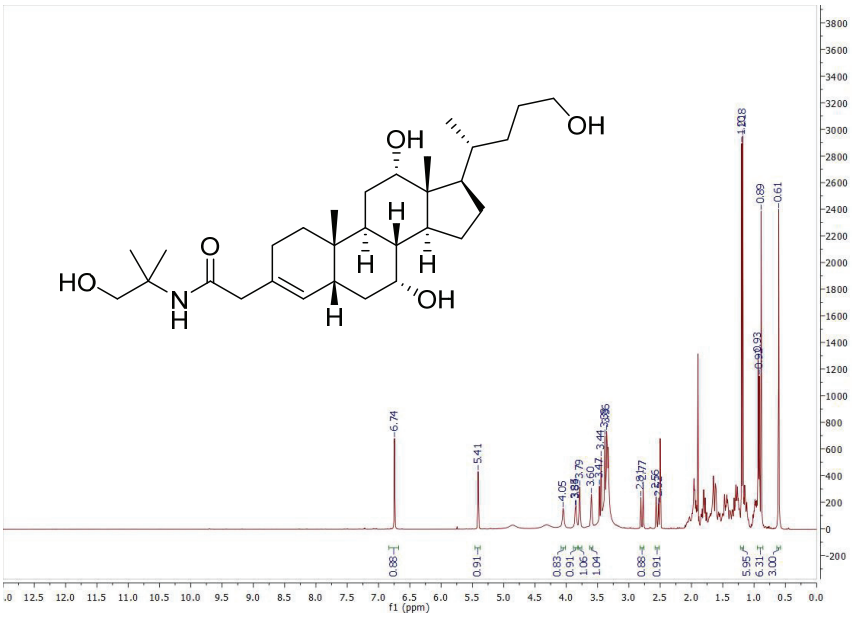

Fig. S-12. Compound **30** ^{1}H NMR (400 MHz, DMSO-d_6) spectra.

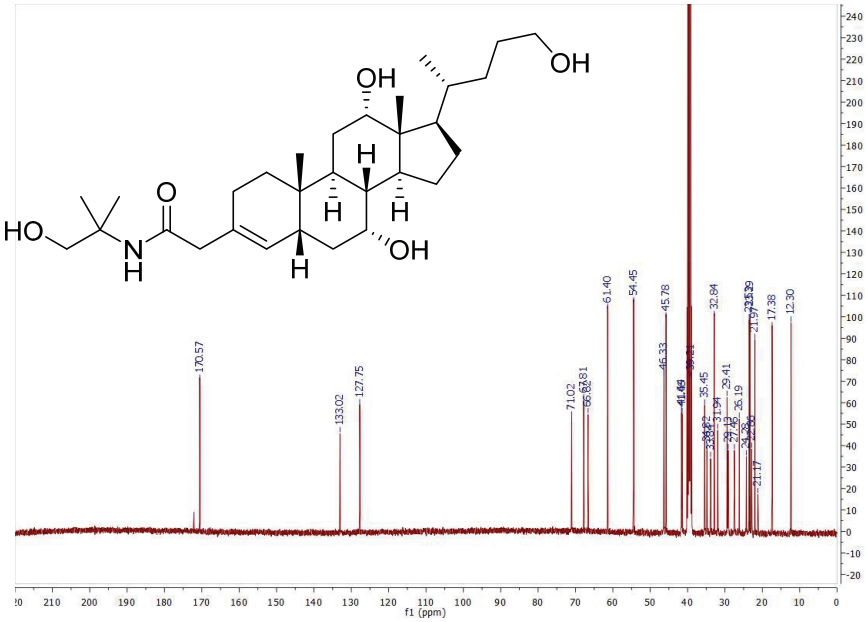

Fig. S-13. Compound **30** ^{13}C NMR (101 MHz, DMSO-d_6) spectra.

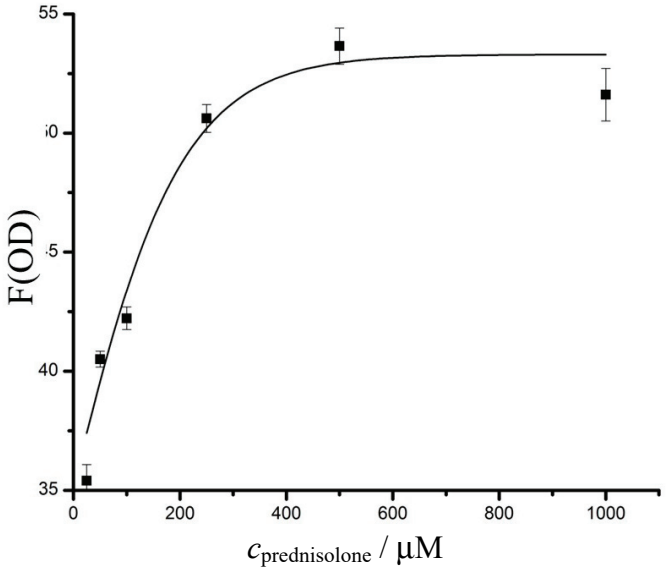

Fig. S-14. Dose-dependent changes in the fluorescence intensity normalized by optical density (F/OD) of yeast cells expressing GR LBD-YFP upon addition of the glucocorticoid receptor ligand, prednisolone, at six different concentrations (25, 50, 100, 250, 500 and 1000 μM) following 15 h exposure.