OxoaporphineMetal Complexes (Co^{II}, Ni^{II}, Zn^{II}) with High Antitumor Activity by InducingMitochondria-Mediated Apoptosis and S-phase Arrest in HepG2

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Table S1 Crystallographic data and refinements of complexes **1−3** and **OD.**

	OD	**1**	**2**	**3**
Formula	$C_{21}H_{19}NO_5$	$C_{42}H_{42}Cl_2CoN_2O_{21}$	$C_{44}H_{46}C_{l2}NiN_2O_{20}$	$C_{42}H_{46}Cl_2ZnN_2O_{22}$
M_r	365.38	1040.61	1052.44	1067.08
Crystal system	*Monoclinic*	*Triclinic*	*monoclinic*	*Triclinic*
Space group	*P2(1)/c*	*P-1*	*P2(1)/n*	*P–1*
a/Å	14.7970(13)	11.9385(3)	7.9782(7)	11.9525(7)
b/Å	8.9351(8)	12.6488(3)	15.9908(17)	12.6917(4)
c/Å	15.8677(16)	15.9365(4)	35.913(5)	16.0413(9)
α/°	90.00(3)	74.630(2)	90.00	74.647(4)
β/°	107.0190(10)	79.728(2)	89.742(10)	79.932(5)
γ/°	90.00(2)	87.418(2)	90.00	87.379(4)
V/Å^3	2006.0(3)	2283.28 (10)	4581.6(9)	2310.5(2)
T/K	293(2)	293(2)	293(2)	293(2)
Z	4	2	4	2
D_c/g cm^{-3}	1.263	1.514	1.526	1.534
θ/°	2.65 to 25.02	2.82 to 26.37	2.83 to 28.63	2.93 to 26.37
F(000)	804	1074	2184	1104
μ(Mo Kα)/mm^{-1}	0.091	0.578	0.624	0.734
Total no. reflns	10136	9319	4140	9408
No. indep. reflns	3525	8004	2839	6074
R1 [$I > 2\sigma(I)$]	0.0766	0.0585	0.0758	0.0686
*wR*2(all data)	0.2175	0.1736	0.2037	0.1084
Gof(F^2)	1.084	1.085	1.078	1.034

Table S2 Selected bond lengths[Å] and angles [°]of **OD** and **1−3**

OD					
N(1)–C(1)	1.342(4)	O(4)–C(14)	1.370(4)	O(1)–C(2)–C(3)	121.9(3)
N(1)–C(13)	1.345(5)	O(5)–C(15)	1.352(4)	O(1)–C(2)–C(1)	120.5(3)
O(1)–C(2)	1.240(3)	O(5)–C(19)	1.444(4)	O(4)–C(14)–C(9)	120.2(3)
O(2)–C(5)	1.355(4)	O(6)–C(22)	1.43(8)	O(2)–C(5)–C(6)	116.9(3)
O(2)–H(2)	0.8200	C(1)–N(1)–C(13)	118.1(3)	O(3)–C(6)–C(7)	124.8(3)
O(3)–C(6)	1.363(4)	N(1)–C(1)–C(10)	123.6(3)	N(1)–C(13)–C(12)	123.7(4)
O(3)–C(17)	1.429(4)	C(6)–O(3)–C(17)	117.3(3)	N(1)–C(1)–C(2)	116.3(3)
O(4)–C(18)	1.310(5)	C(18)–O(4)–C(14)	127.4(4)	O(2)–C(5)–C(4)	124.6(3)
Complex 1					
Co(1)–O(1)	2.138(2)	O(6)–Co(1)–O(1)	175.90(9)	C(6)–O(1)–Co(1)	114.09(18)
Co(1)–O(6)	2.091(2)	O(6)–Co(1)–O(11)	87.73(11)	C(30)–O(6)–Co(1)	114.33(18)
Co(1)–O(11)	2.110(3)	O(6)–Co(1)–N(2)	78.17(9)	C(1)–N(1)–Co(1)	126.7(2)

Co(1)–O(12)	2.052(3)	O(11)–Co(1)–O(1)	88.61(10)	C(5)–N(1)–Co(1)	114.82(18)
Co(1)–N(1)	2.081(2)	O(12)–Co(1)–O(1)	92.27(12)	N(2)–Co(1)–O(11)	89.98(11)
Co(1)–N(2)	2.100(3)	O(12)–Co(1)–O(6)	91.36(12)	C(22)–N(2)–Co(1)	113.06(19)
N(1)–C(1)	1.349(4)	O(12)–Co(1)–O(11)	178.80(13)	N(1)–C(1)–C(2)	121.9(3)
N(1)–C(5)	1.341(4)	O(12)–Co(1)–N(1)	92.50(11)	C(23)–N(2)–Co(1)	129.2(2)
N(2)–C(22)	1.337(4)	O(12)–Co(1)–N(2)	90.61(11)	N(1)–Co(1)–O(11)	86.89(10)
N(2)–C(23)	1.338(4)	N(1)–Co(1)–O(1)	77.33(8)	N(1)–Co(1)–N(2)	176.70(10)
O(6)–C(30)	1.248(3)	N(1)–Co(1)–O(6)	100.62(9)	N(2)–Co(1)–O(1)	103.68(9)
Complex 2					
Ni(1)–O(1)	2.047(3)	O(1)–Ni(1)–O(1A)	180.00(11)	C(1)–N(1)–Ni(1)	129.8(4)
Ni(1)–O(10)	2.059(4)	O(1)–Ni(1)–O(10)	89.50(18)	C(14)–N(1)–Ni(1)	112.2(3)
O(1)–C(15)	1.253(6)	O(1)–Ni(1)–O(10A)	90.50(18)	C(15)–O(1)–Ni(1)	113.0(3)
N(1)–C(1)	1.353(6)	O(10)–Ni(1)–O(10A)	180.0(3)	N(1)–C(1)–C(2)	122.3(5)
N(1)–C(14)	1.341(6)	N(1)–Ni(1)–O(1A)	99.18(15)	N(1)–Ni(1)–O(10A)	89.51(17)
C(1)–C(2)	1.362(7)	N(1)–Ni(1)–O(1)	80.82(15)	N(1)–Ni(1)–O(10)	90.49(17)
O(10)–C(23)	1.391(8)	N(1A)–Ni(1)–N(1A)	180.00(18)	C(23)–O(10)–Ni(1)	127.2(5)
Complex 3					
Zn(1)–O(1)	2.168(3)	O(6)–Zn(1)–O(1)	175.06(13)	N(2)–Zn(1)–O(12)	88.58(14)
Zn(1)–O6	2.116(3)	O(6)–Zn(1)–O(12)	87.89(14)	C(1)–O(1)–Zn(1)	112.9(2)
Zn(1)–O(11)	2.100(4)	O(11)–Zn(1)–O(1)	93.49(15)	C(2)–O(1)–Zn(1)	114.9(2)
Zn(1)–O(12)	2.160(3)	O(11)–Zn(1)–O(6)	91.19(16)	C(3)–O(1)–Zn(1)	126.3(3)
Zn(1)–N(1)	2.069(3)	O(12)–Zn(1)–O(1)	87.42(14)	C(3)–N(1)–C(2)	118.8(3)
Zn(1)–N(2)	2.080(3)	N(1)–Zn(1)–O(1)	78.00(11)	C(39)–N(2)–Zn(1)	128.6(3)
O(1)–C(1)	1.252(5)	N(1)–Zn(1)–O(6)	100.24(12)	C(40)–N(2)–Zn(1)	113.2(3)
N(1)–C(2)	1.342(5)	N(1)–Zn(1)–O(11)	92.45(14)	N(2)–Zn(1)–O(1)	102.66(12)
N(1)–C(3)	1.337(5)	N(1)–Zn(1)–O(12)	87.27(13)	N(2)–Zn(1)–O(6)	78.76(12)
N(2)–C(39)	1.352(5)	N(1)–Zn(1)–N(2)	175.77(14)	N(2)–Zn(1)–O(11)	91.69(15)

Table S3. The inhibitive ratios of OD,**1−3** and corresponding metal salt for the selected cells and one normal liver cell line HL-7702 for 48 h.

	HepG2	T-24	BEL-7404	MGC80-3	SKOV-3/DDP	HL-7702
OD	33.35±0.32	46.91±0.17	36.28±0.18	42.28±0.24	45.57±1.94	46.55±0.67
1	88.34± 1.03	85.35±0.73	63.96±0.78	61.54±0.33	78.17±1.69	56.73±0.14
2	87.68 ±0.92	82.31±0.60	69.38±0.65	71.86±0.28	84.44±0.65	72.34±0.22
3	89.58±1.22	84.16±0.76	61.00±0.19	67.30±0.21	70.80±1.69	59.26±0.15
$Co(ClO_4)_2$	32.87±0.13	36.23±0.09	23.29±0.09	24.74±0.03	21.74±0.03	45.91±0187
$Ni(ClO_4)_2$	32.57±0.29	37.86±0.19	32.31±0.07	16.35±0.09	11.25±0.12	50.14±0.06
$Zn(ClO_4)_2$	30.94±0.39	28.19±0.13	24.07±0.15.	17.63±0.07	12.33±0.05	43.18±0.19
cisplatin[b]	71.93±0.83	75.26±1.00	76.07±2.68	65.58±0.09	15.13±0.07	68.96±1.34

inhibitive ratios are represent mean ± SD of at least five independent experiments(SD represents the standard deviation). The concentration of **OD**, **1−3** and corresponding salts are 20μmol/L[b] cisplatin was dissolved at a concentration of 1mM in 0.154 M NaCl.

Table S4. Primer sequences used in this work.

target protein	Upstream primer sequences	Downstream primer sequences
β-Actin	AAAAGCCACCCCACTTCTCT	GACCAAAAGCCTTCATACATCTCA
caspase-8	CCAGTGCCAGACACACAGTC	CCAGCAGGTTCATGTCATC
cytochrome c	GTTCGTTGTGCCAGCGACTA	GCTTGCCTCCCTTTTCAACG
Apaf-1	AGAGGTAGCGAGTGGACGTG	CGCTGCGGCACCTCAAGTCT
p21	AGGGGACAGCAGAGGAAG	CGTTTTCGACCCTGAGAG
CDK2	AGTTACTTCTATGCCTGATTACA	TCCGTCCATCTTCATCCA
PCNA	TGAAGCACCAAACCAGGA	GCATCTCCAATATGGCTG

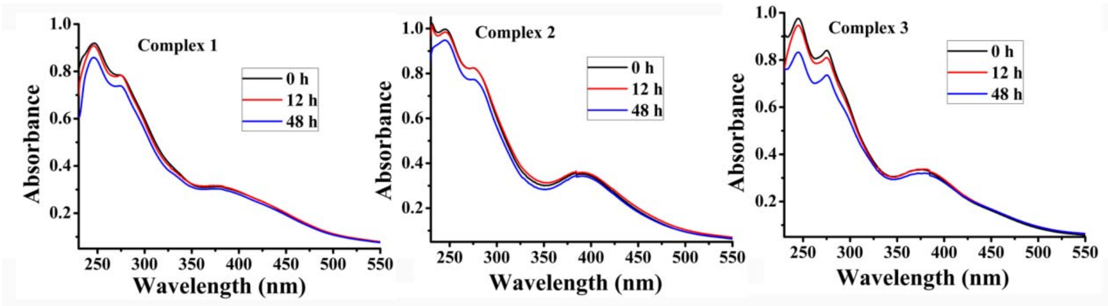


Figure S1. Solution stability of complexes **1–3** in tris buffer solution examined by UV-vis spectra.

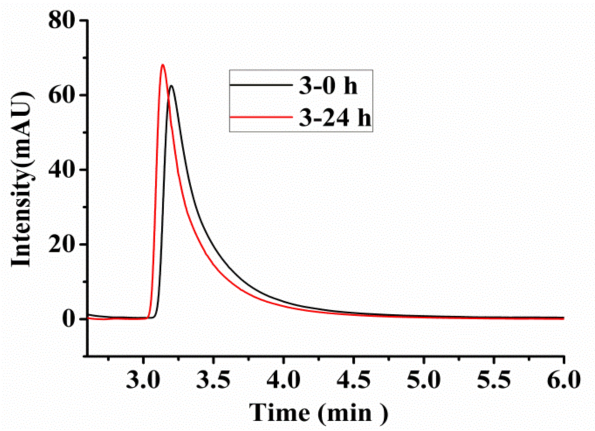


Figure S2. HPLC spectra of complex **3** in aqueous solution (1 mg/mL) with time 0 h and 24 h, respectively. Column: reversed-phase C18 column (YMC HPLC COLUMN, 250×4.6mm I. D.). Column temperature: 35ºC. Mobile phase: Methanol/H_2O (80:20). Flow rate: 1.0 ml/min. Injection volume: 10 μL.

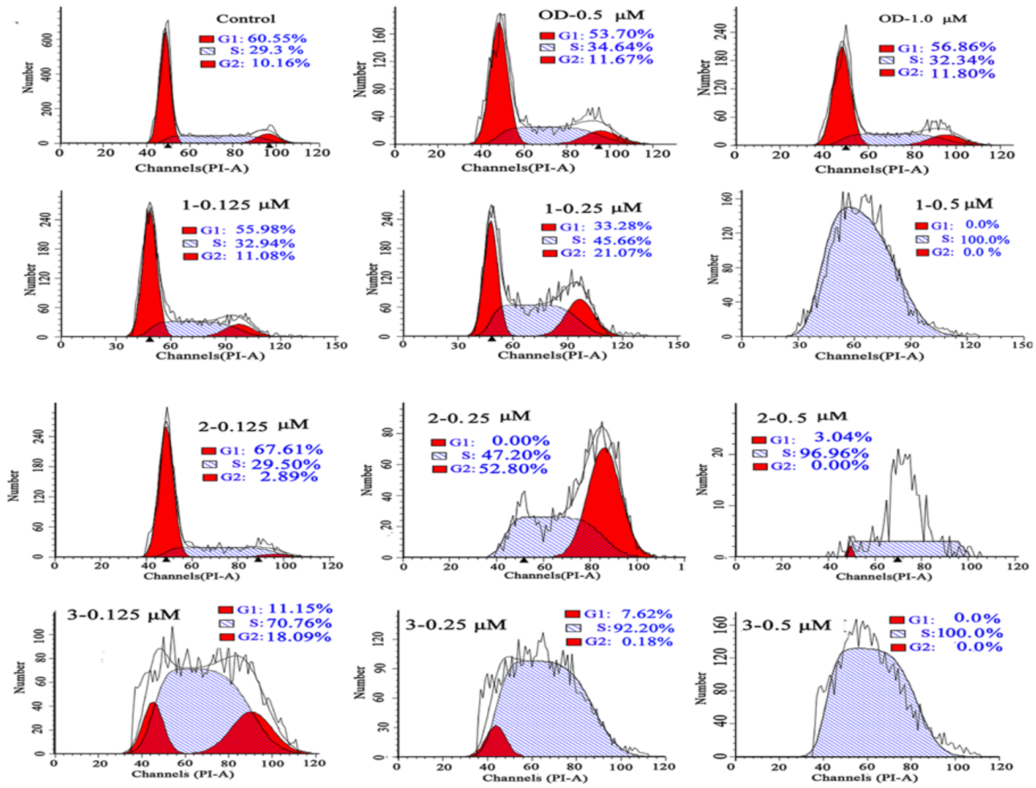


Figure S3. Cell cycle analysis of **1–3** (0.125, 0.25 and 0.5 μM) and **OD** (0.5 and 1.0 μM) for 24 h in HepG2 cells, graph bar show the distributions of cells in the different phases of cell cycle.

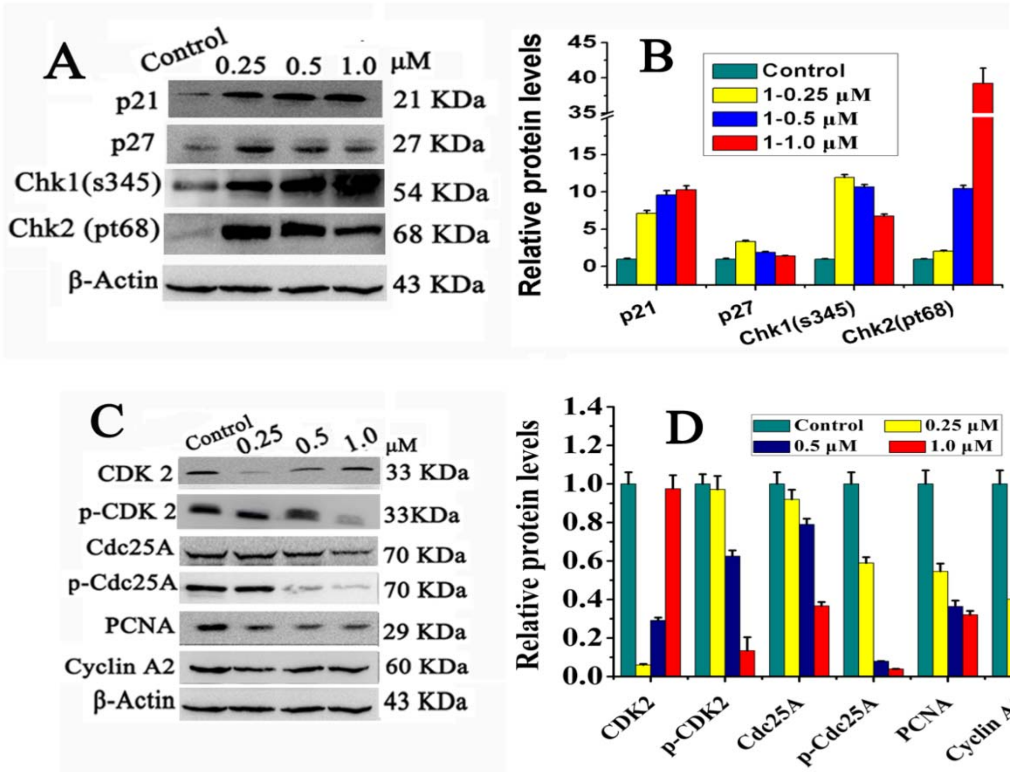


Figure S4. (A,C) Effects of complex **1** treatment in HepG2 cells on cell cycle regulatory proteins at 0.25 μM, 0.5 μM and 1.0 μM for 24 h, respectively. (B,D) The relative protein expression of each band = (density of each band/density of actin band). Mean ± SD was from three independent measurements.

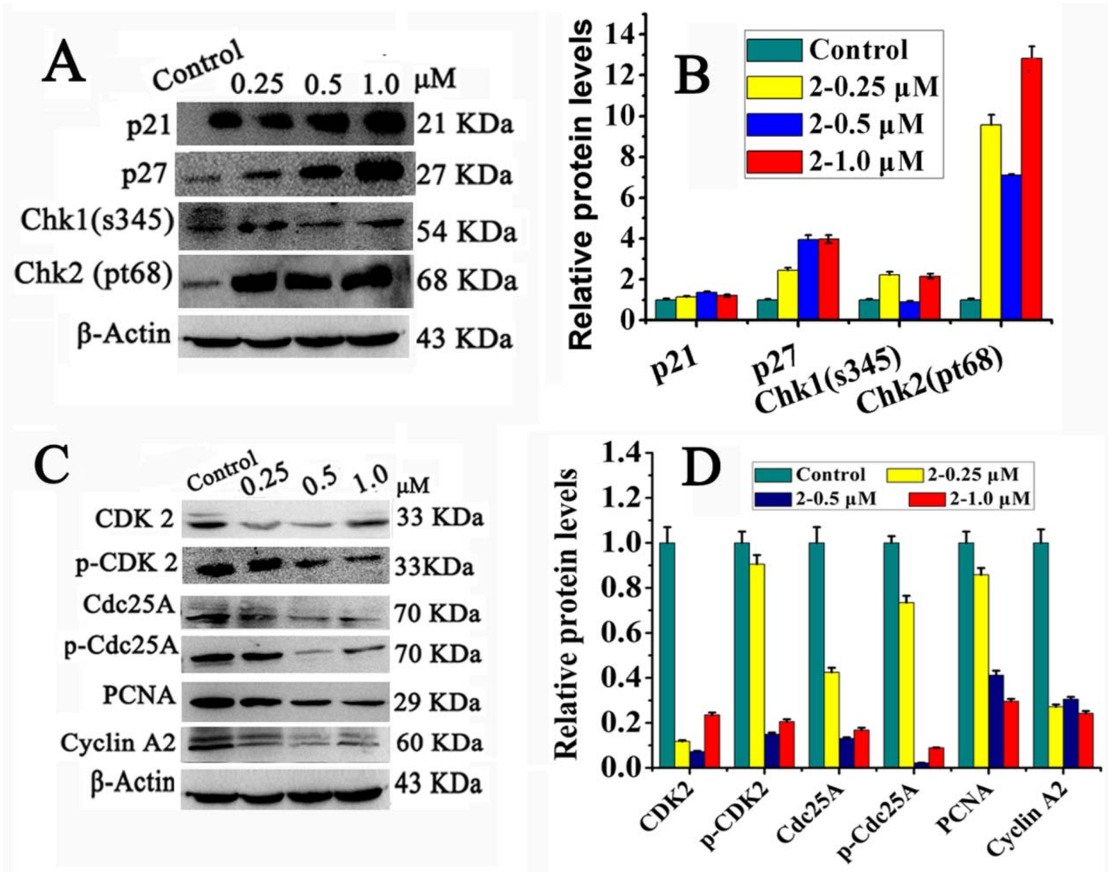


Figure S5. (a) Effects of complex **2** treatment in HepG2 cells on cell cycle regulatory proteins at 0.25 μM, 0.5 μM and 1.0 μM for 24 h, respectively.(b) The relative protein expression of each band = (density of each band/density of actin band). Mean ± SD was from three independent measurements.

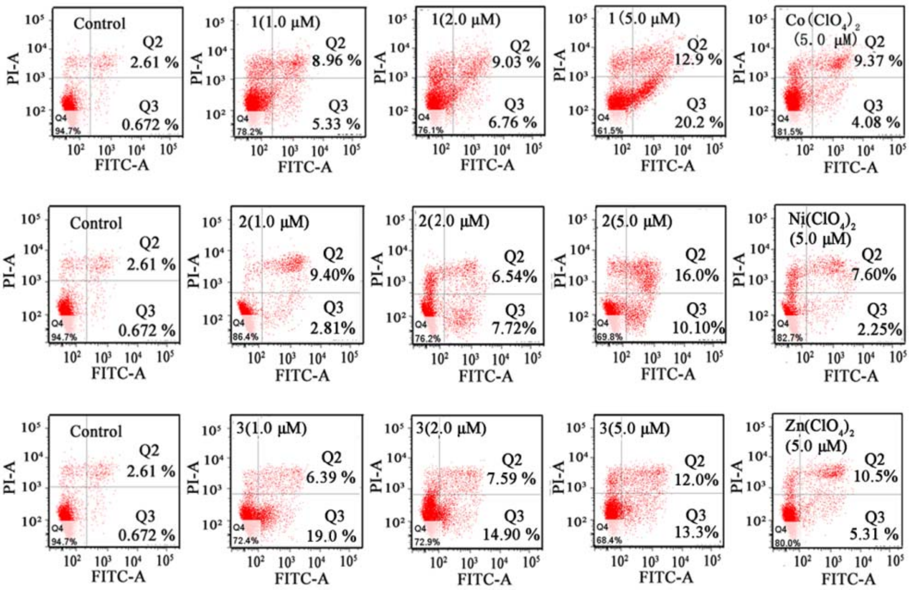


Figure S6. Effect of cell apoptosis of HepG2 treated with **1–3**, **OD** and corresponding metal salts for 24 h compared with the control cells.

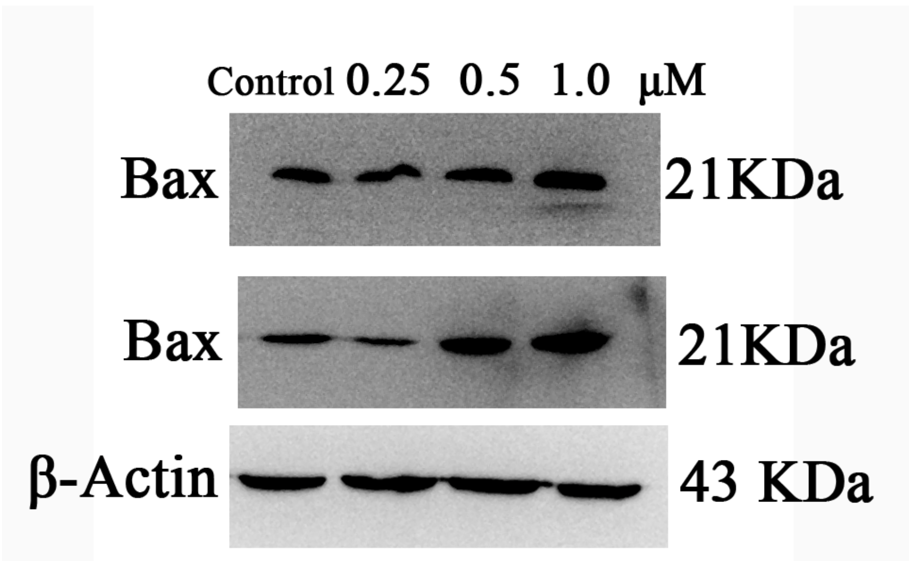
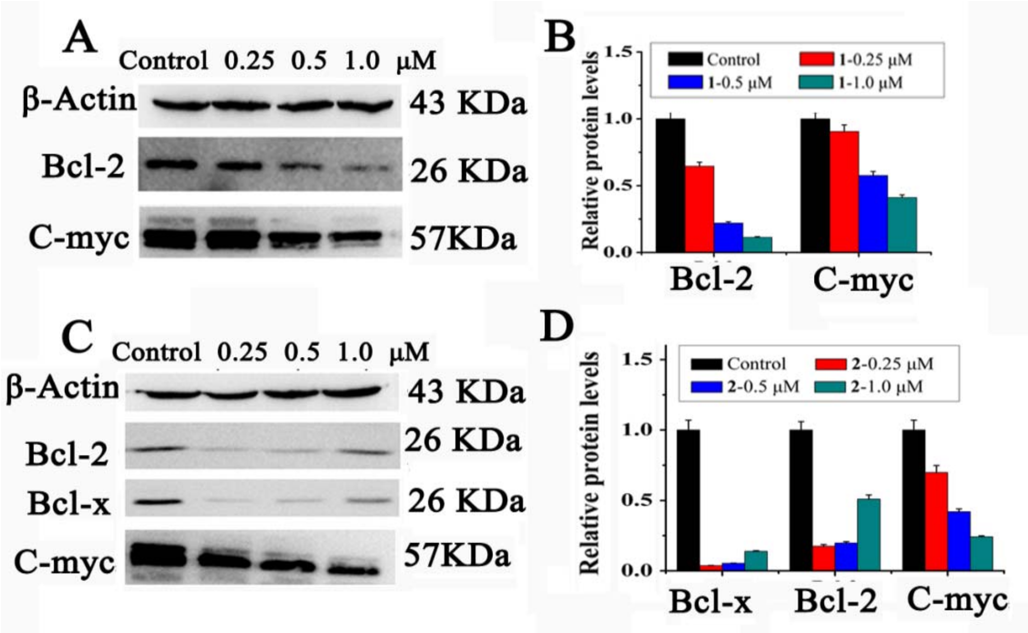


Figure S7 Multiple Western blot analysis of Bax protein after treatment of HepG2 cells with **3** at 0.25, 0.5, 1.0 μM for 24 h, respectively.



FigureS8 (a) Western blot analysis of Bcl-2, and C-myc after treatment of HepG2 cells with complex **1** at 0.25, 0.5, 1.0 μM for 24 h, respectively. (b) Densitometric analysis of Bcl-2 and C-myc band from part A. (c) Western blot analysis of Bcl-2, Bcl-xl, and C-myc after treatment of HepG2 cells with complex **2** at 0.25, 0.5, 1.0 μM for 24 h, respectively. (d) Densitometric analysis of Bcl-2, Bcl-xl and C-myc band from part C. The relative expression of each band = (density of each band/ density of actin band). Mean and SD values were from three independent measurements.

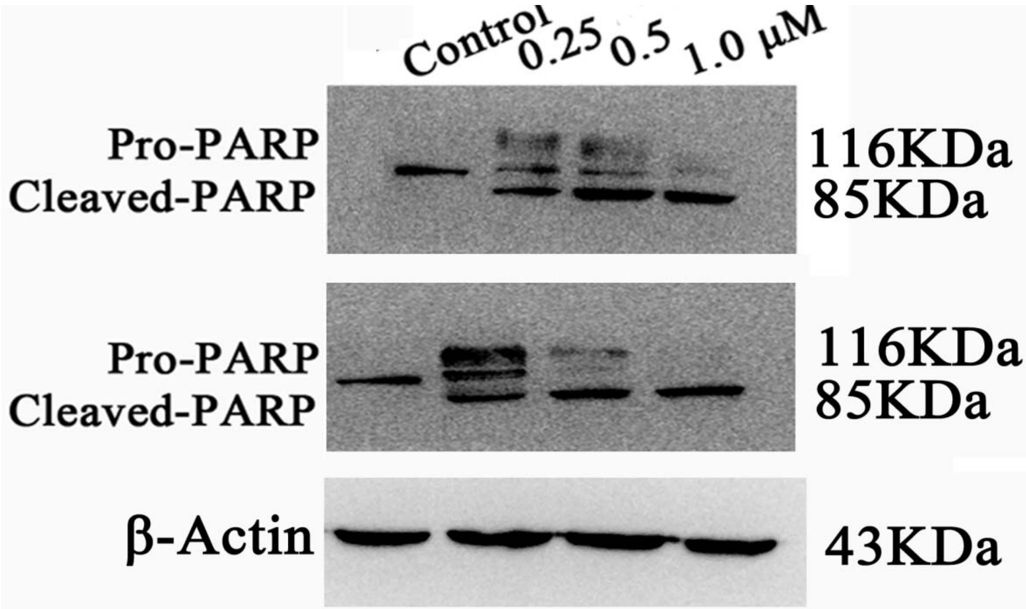


Figure S9. Multiple Western blot analysis of Pro-PARP and Cleaved-PARP proteins after treatment of HepG2 cells with **3** at 0.25, 0.5, 1.0 μM for 24 h, respectively.

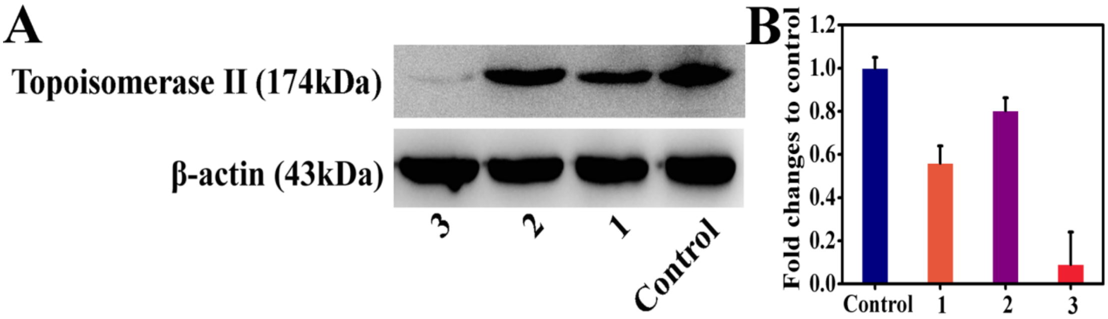


Figure S10. The protein levels of Topoisomerase II in HepG2 cells after treatment with complexes **1–3** (0.5 μM), respectively. (A) Topoisomerase II protein regulators protein levels in HepG2 cells were analyzed by western blot. (B) The whole-cell extracts were prepared and analyzed by Western blot analysis using antibodies against cell cycle protein regulators proteins. The same blots were stripped and reprobed with β-actin antibody to show equal protein loading. Western blotting bands from three independent measurements were quantified with Image J. in (B).

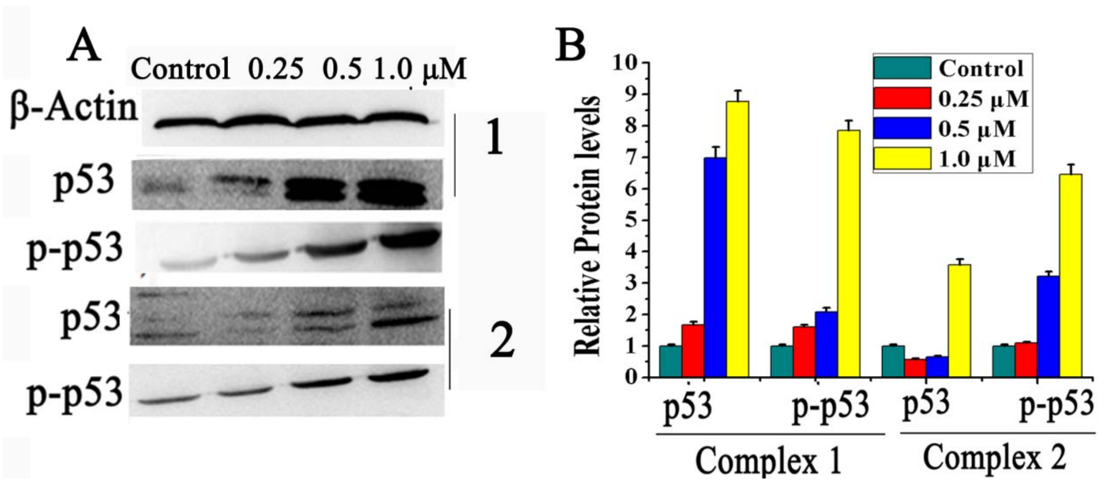


Figure S11.(a) Western blot analysis of p53 and p-p53 proteins in HepG2 cells with complex **1** and **2** at 0.25,0.5 and 1.0 μM for 24 h. (b) The relative expression of proteins from A, each band = (density of each band/density of β-actin band). Mean ± SD was from three independent measurements.

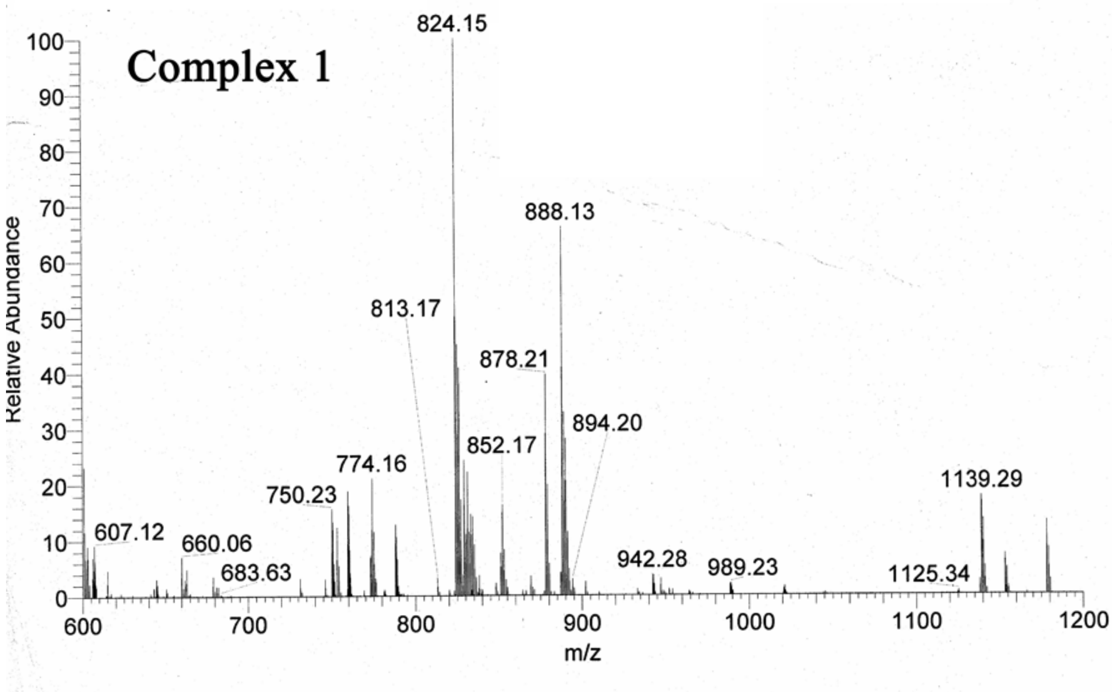


Figure S12 ESI-MS spectrum of complex **1** in MeOH / H_2O (1:500)

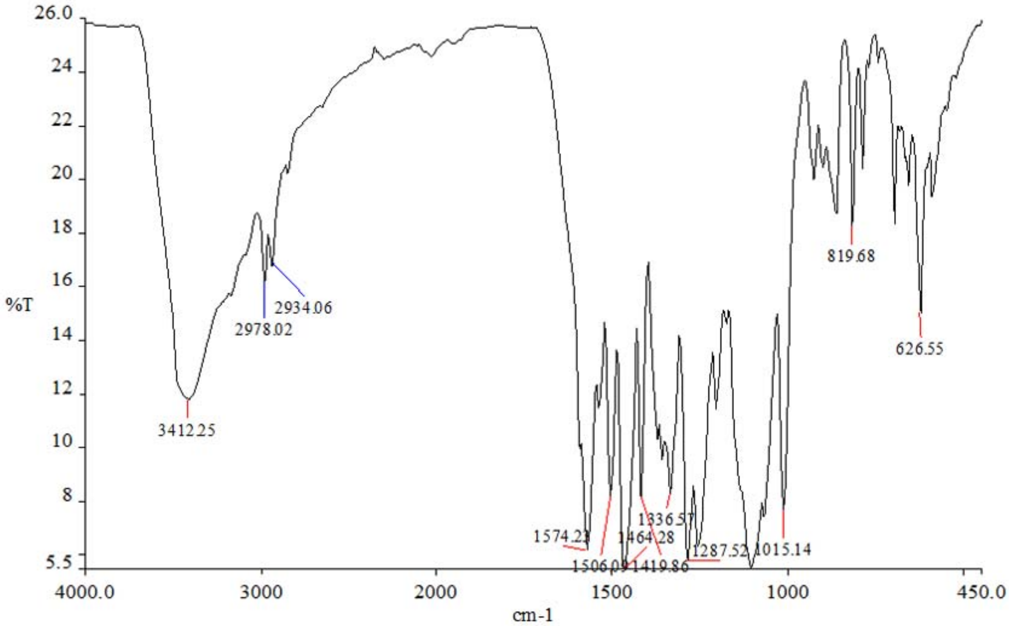


Figure S13. IR spectrum of complex **1.**

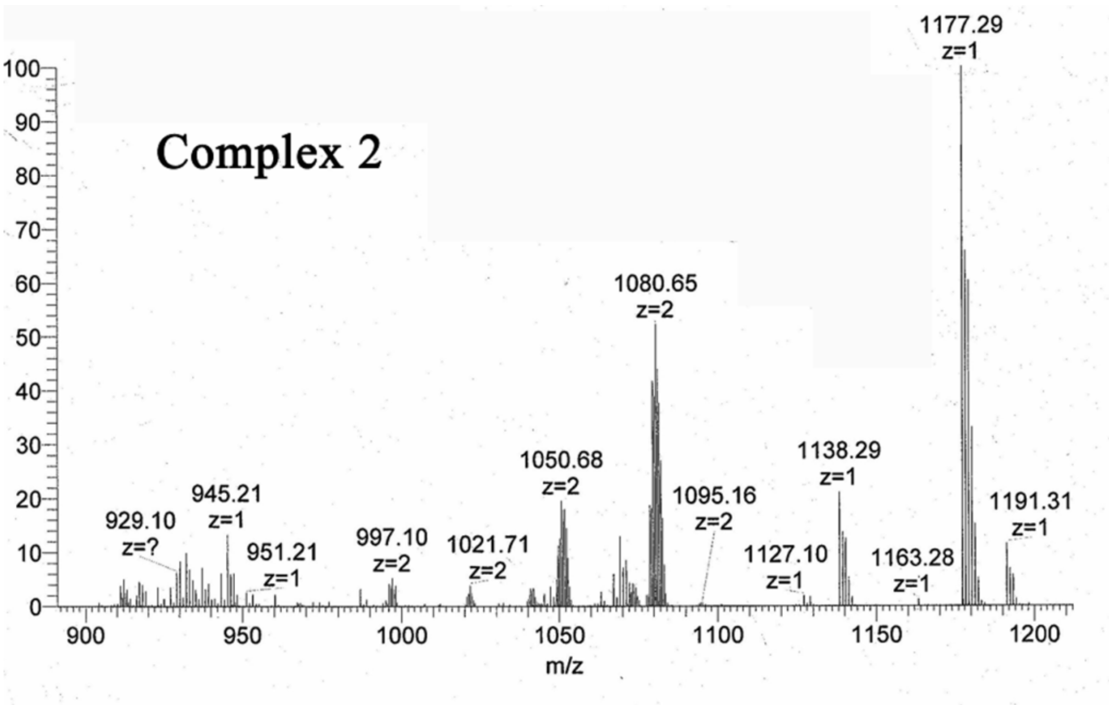


Figure S14. ESI-MS spectrum of complex **2** in MeOH / H_2O (1:500)

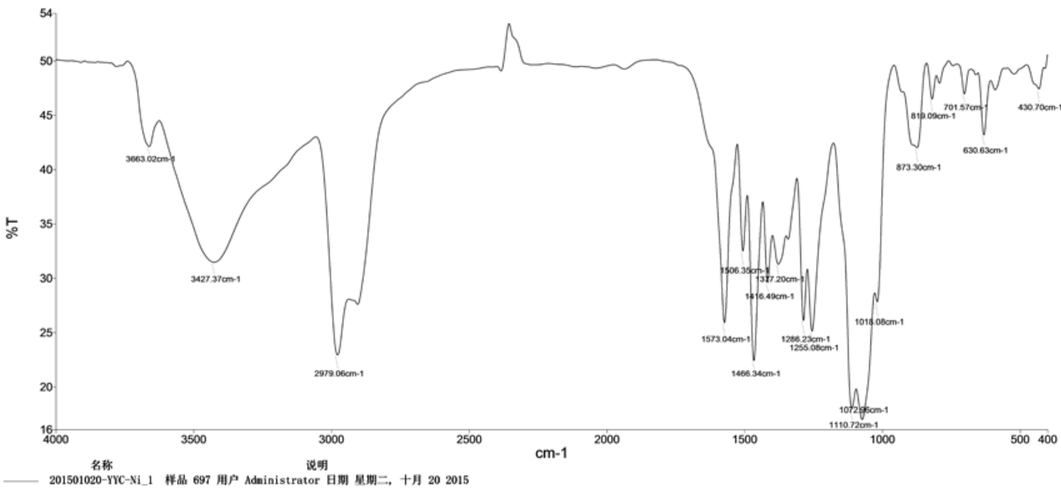


Figure S15.IR spectrum of complex **2.**

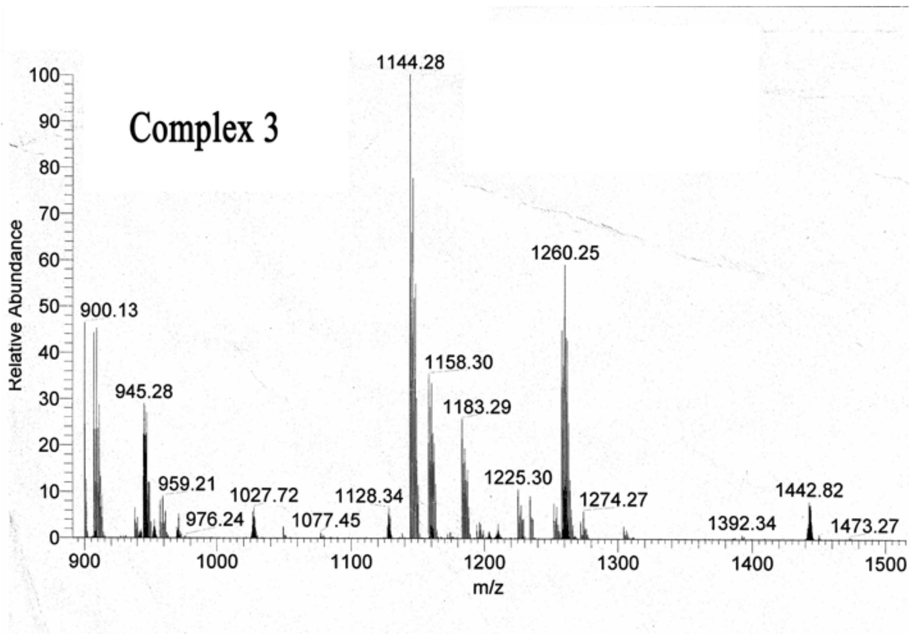


Figure S16. ESI-MS spectrum of complex **3** in MeOH / H_2O (1:500)

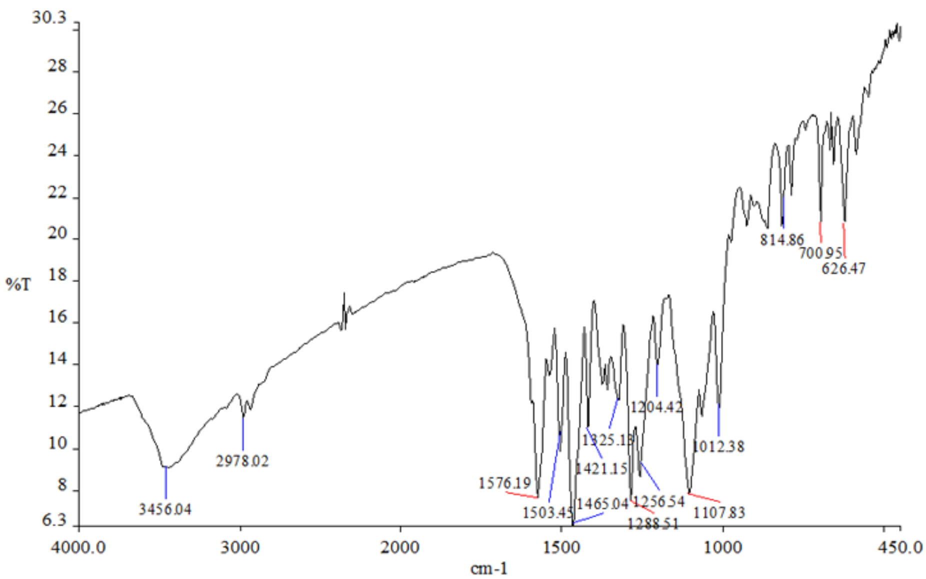


Figure S17.IR spectrum of complex **3.**