

Cobalt(II) Diphenylazodioxide Complexes Induce Apoptosis in SK-HEP-1 Cells

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Supporting Information

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1. Effect of cobalt(II) diphenylazodioxide complexes on PARP cleavage of additional cell lines

HEK 293, HT-29, MCF-7, and PC-3 cells were subjected to treatment for 12 h with each of compounds **1** and **2** at 2 μ M and 10 μ M concentrations, and western blots were used to assess the cleavage of PARP, as a marker of apoptosis. Figures S1-S4 below show that compounds **1** and **2** did not induce extensive PARP cleavage compared with the DMSO vehicle control in these cell lines.

HEK 293 cells (12 h treatment)

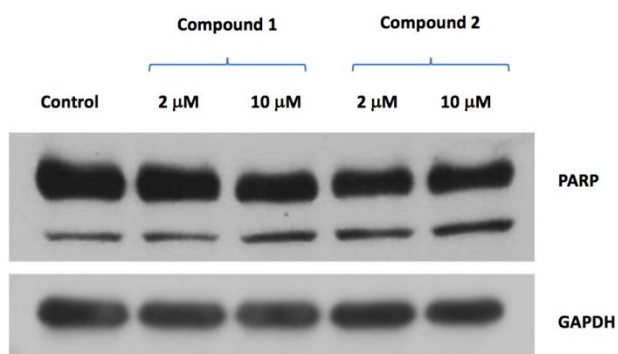


Figure S1. Effect of compounds **1** and **2** on PARP cleavage in HEK 293 cells.

HT-29 cells (12 h treatment)

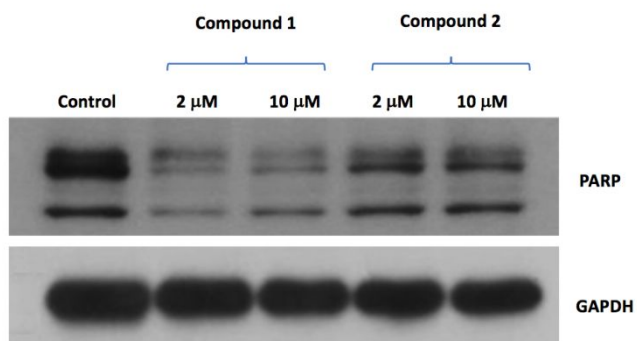


Figure S2. Effect of compounds 1 and 2 on PARP cleavage in HT-29 cells.

MCF-7 cells (12 h treatment)

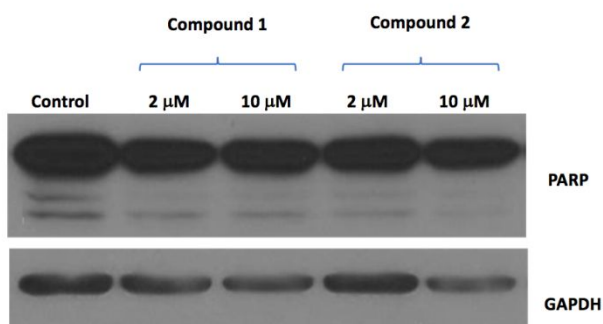


Figure S3. Effect of compounds 1 and 2 on PARP cleavage in MCF-7 cells.

PC-3 cells (12 h treatment)

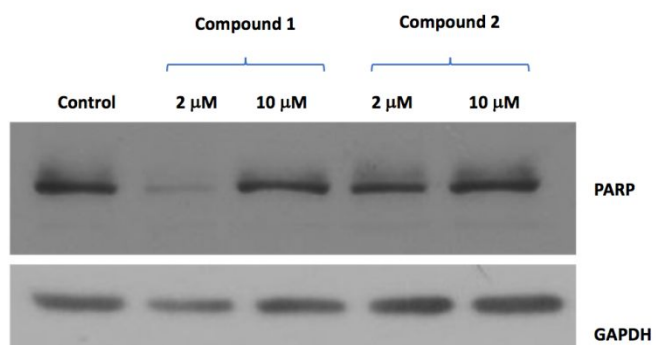
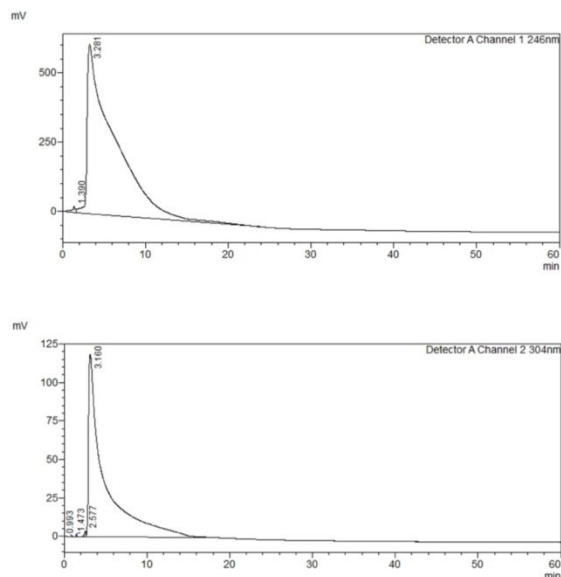


Figure S4. Effect of compounds 1 and 2 on PARP cleavage in PC-3 cells.

2. HPLC chromatograms of cobalt(II) diphenylazodioxide complexes

HPLC analysis of compounds **1** and **2** was performed, supplementing the characterization provided in the initial report of these compounds.¹ The analyses were performed on a Shimadzu LC20 HPLC with a 190-320 nm UV detector. The compounds were eluted on a reverse-phase C₁₈ column (Waters Corporation), with an isocratic mobile phase of pure acetonitrile. Figure S5 below shows chromatograms of compound **1** with detection wavelengths of 246 and 304 nm, and Figure S6 shows the chromatogram of compound **2** with detection wavelengths of 282 and 305 nm. The product peak area is seen to be >98% of the total in all cases.



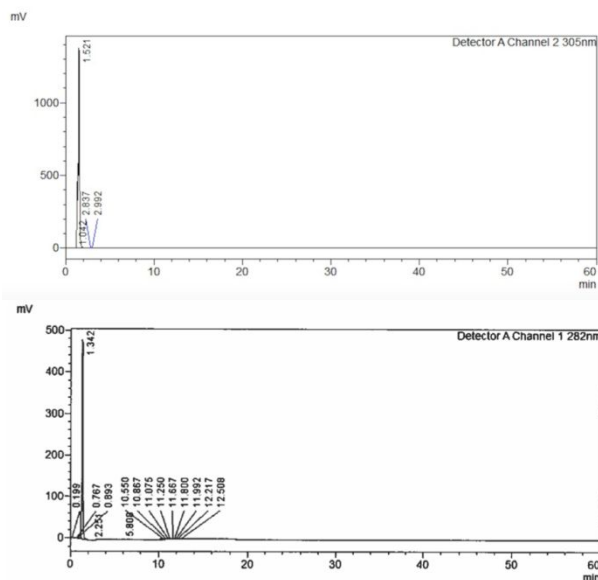
Peaks from wavelength 246 nm

| Peak# | Ret. Time | Area | Height | Area% |
|-------|-----------|-----------|--------|---------|
| 1 | 1.390 | 679276 | 22502 | 0.494 |
| 2 | 3.281 | 136777955 | 609136 | 99.506 |
| Total | | 137457231 | 631638 | 100.000 |

Peaks from wavelength 304 nm

| Peak# | Ret. Time | Area | Height | Area% |
|-------|-----------|----------|--------|---------|
| 1 | 0.993 | 1080 | 164 | 0.007 |
| 2 | 1.473 | 13887 | 1790 | 0.090 |
| 3 | 2.577 | 55736 | 4035 | 0.361 |
| 4 | 3.160 | 15370264 | 118298 | 99.542 |
| Total | | 15440967 | 124287 | 100.000 |

Figure S5. Chromatograms of compound **1** at 246 and 304 nm detection wavelengths.



Peaks from wavelength 305 nm

| Peak# | Ret. Time | Area | Height | Area% |
|-------|-----------|----------|---------|---------|
| 1 | 1.042 | 4806 | 540 | 0.030 |
| 2 | 1.521 | 15816686 | 1378967 | 99.933 |
| 3 | 2.837 | 4179 | 202 | 0.026 |
| 4 | 2.992 | 1617 | 150 | 0.010 |
| Total | | 15827288 | 1379659 | 100.000 |

Peaks from wavelength 282 nm

| Peak# | Ret. Time | Area | Height | Area% |
|-------|-----------|---------|--------|---------|
| 1 | 0.199 | 1484 | 135 | 0.043 |
| 2 | 0.767 | 6739 | 804 | 0.195 |
| 3 | 0.893 | 16359 | 1534 | 0.473 |
| 4 | 1.342 | 3417463 | 479303 | 98.721 |
| 5 | 2.251 | 2288 | 233 | 0.066 |
| 6 | 5.808 | 1683 | 79 | 0.049 |
| 7 | 10.550 | 4029 | 127 | 0.116 |
| 8 | 10.867 | 1690 | 128 | 0.049 |
| 9 | 11.075 | 1280 | 127 | 0.037 |
| 10 | 11.250 | 1953 | 124 | 0.058 |
| 11 | 11.667 | 1773 | 123 | 0.051 |
| 12 | 11.800 | 1268 | 128 | 0.037 |
| 13 | 11.992 | 1275 | 125 | 0.037 |
| 14 | 12.217 | 1117 | 110 | 0.032 |
| 15 | 12.508 | 1299 | 94 | 0.038 |
| Total | | 3461739 | 483172 | 100.000 |

Figure S6. Chromatograms of compound **2** at 282 and 305 nm detection wavelengths.

3. References

1. Emhoff, K. A.; Balaraman, L.; Simpson, S. R.; Stromyer, M. L.; Kalil, H. F.; Beemiller, J. R.; Sikatzki, P.; Eshelman, T. S.; Salem, A. M. H.; DeBord, M. A.; Panzner, M. J.; Youngs, W. J.; Boyd, W. C. Synthesis and Characterization of Cobalt(II) *N,N'*-Diphenylazodioxide Complexes. *ACS Omega* **2018**, *3*, 16021-16027.