

Supplementary Materials for

**A small-molecule inhibitor of human DNA polymerase η potentiates the effects
of cisplatin in tumor cells**

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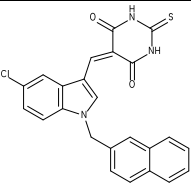
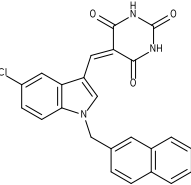
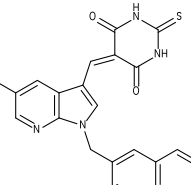
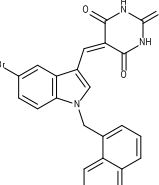
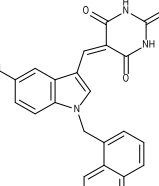
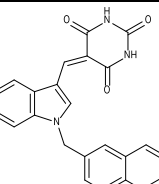
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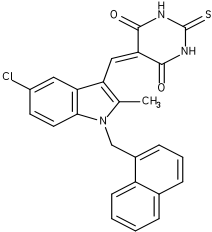
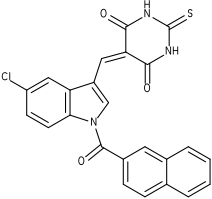
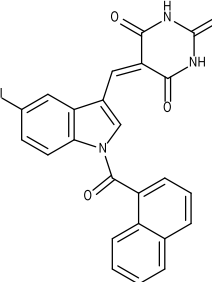
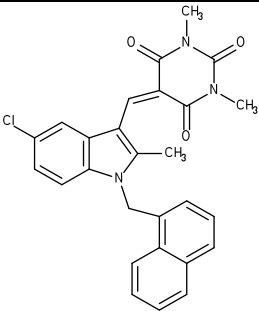
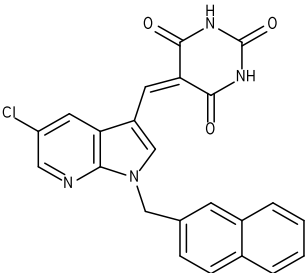
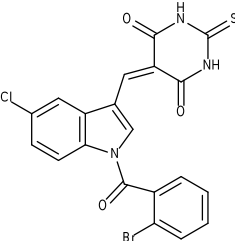
E-mail: RLEOFF@UAMS.EDU

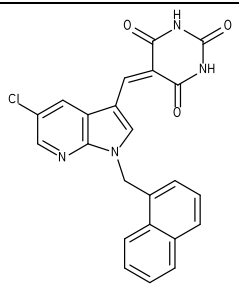
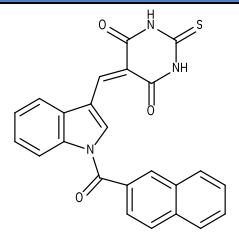
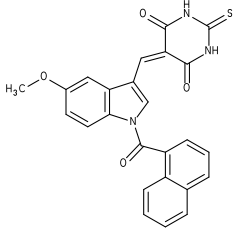
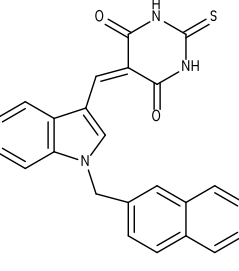
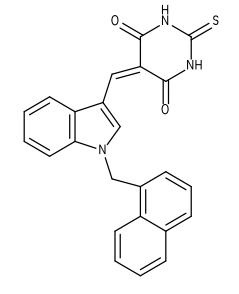
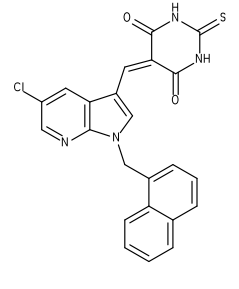
Running Title: Inhibitors of hpol η to improve genotoxic drugs

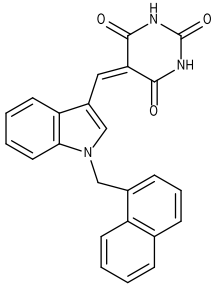
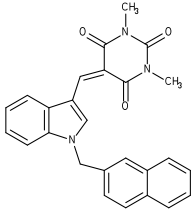
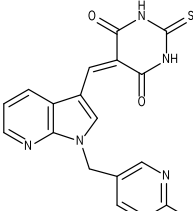
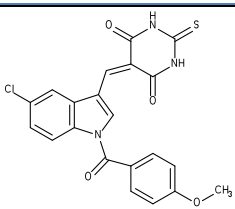
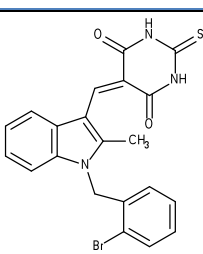
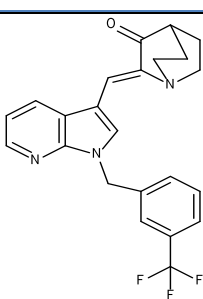
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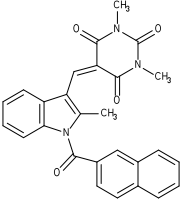
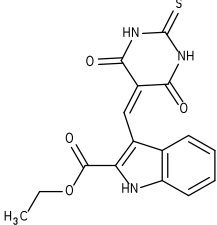
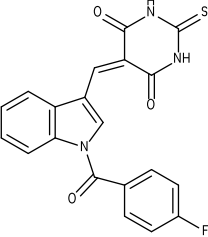
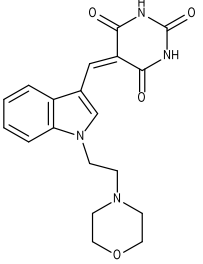
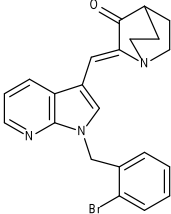
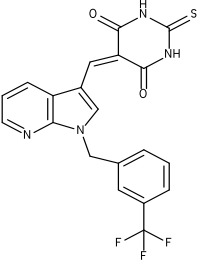
Table S1. Summary of IBA/ITBA chemical structures and the inhibition of DNA polymerase eta

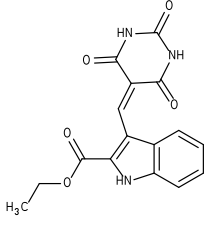
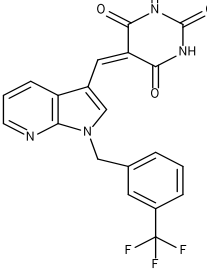
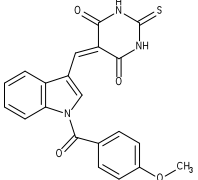
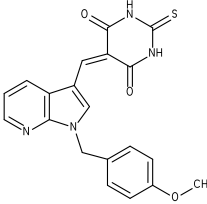
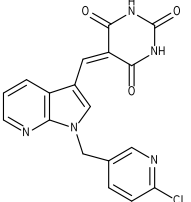
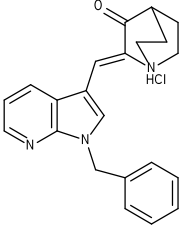
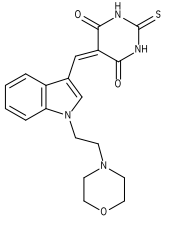
Number	Compound I.D.	Structure	Mean % activity of hpol η	S.D. in mean % activity
1.	PNR-7-02		No pol activity detected	No pol activity detected
2.	PNR-7-01		No pol activity detected	No pol activity detected
3.	PNR-9-66B		No pol activity detected	No pol activity detected
4.	PNR-6-92		No pol activity detected	No pol activity detected
5.	PNR-6-89		No pol activity detected	No pol activity detected
6.	PNR-6-97		No pol activity detected	No pol activity detected

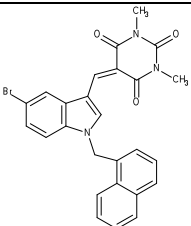
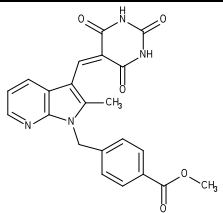
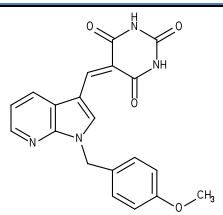
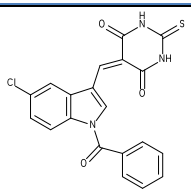
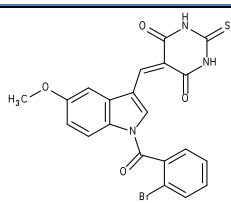
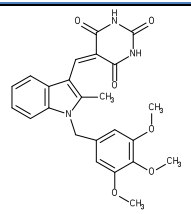
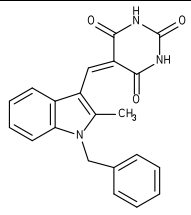
7.	<u>PNR-7-39</u>		<u>16.2</u>	<u>12.8</u>
8.	<u>PNR-3-80</u>		<u>18.4</u>	<u>1.6</u>
9.	<u>PNR-3-84</u>		<u>33.6</u>	<u>4.4</u>
10.	<u>PNR-7-40</u>		<u>44.9</u>	<u>38.8</u>
11.	<u>PNR-9-66A</u>		<u>45.1</u>	<u>16.8</u>
12.	<u>PNR-3-51</u>		<u>45.5</u>	<u>5.8</u>

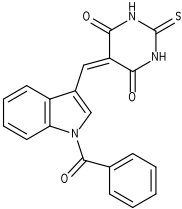
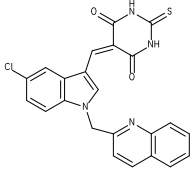
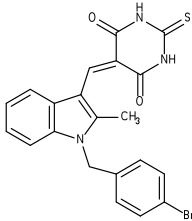
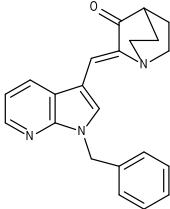
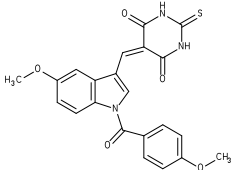
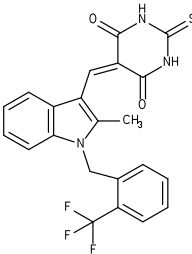
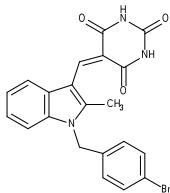
13.	<u>PNR-9-65A</u>		<u>48.1</u>	<u>34.6</u>
14.	<u>PNR-3-79</u>		<u>56.6</u>	<u>20.0</u>
15.	<u>PNR-3-85</u>		<u>57.8</u>	<u>27.2</u>
16.	<u>PNR-6-98</u>		<u>58.7</u>	<u>33.4</u>
17.	<u>PNR-6-83</u>		<u>62.3</u>	<u>22.8</u>
18.	<u>PNR-9-65B</u>		<u>62.2</u>	<u>39.2</u>

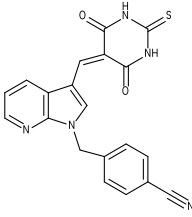
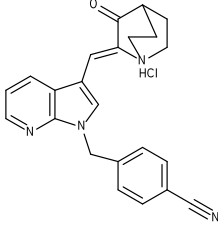
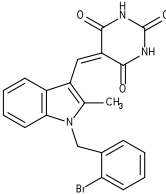
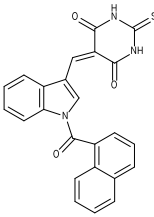
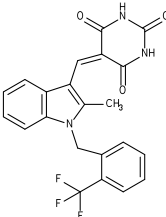
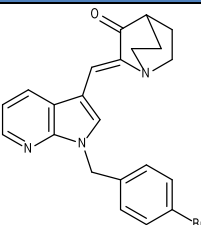
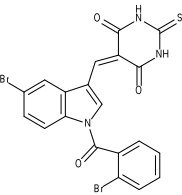
19.	<u>PNR-6-82</u>		<u>64.3</u>	<u>23.5</u>
20.	<u>PNR-6-99</u>		<u>71.2</u>	<u>16.6</u>
21.	<u>PNR-9-14C</u>		<u>76.1</u>	<u>11.9</u>
22.	<u>PNR-3-62</u>		<u>77.1</u>	<u>15.5</u>
23.	<u>PNR-6-02</u>		<u>78.6</u>	<u>5.9</u>
24.	<u>PNR-9-41</u>		<u>78.9</u>	<u>7.7</u>

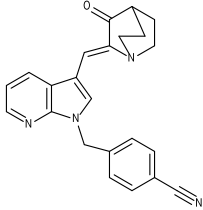
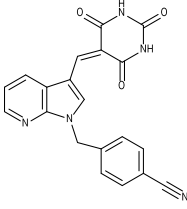
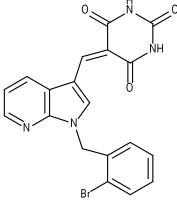
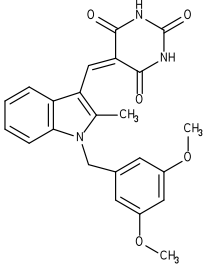
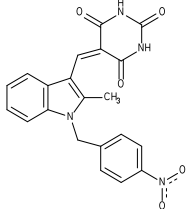
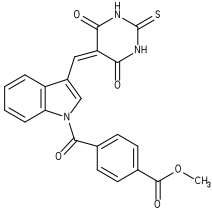
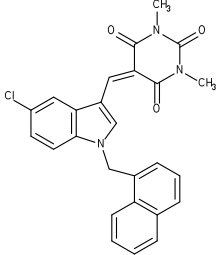
25.	PNR-6-70		81.6	38.1
26.	PNR-6-57		82.4	9.2
27.	PNR-3-68		82.5	8.3
28.	PNR-9-63B		84.1	16.5
29.	PNR-9-47		84.2	7.7
30.	PNR-9-37B		87.1	18.6

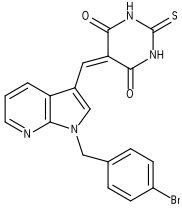
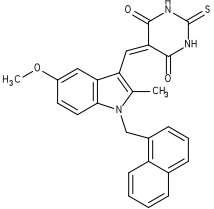
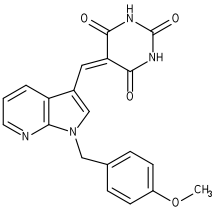
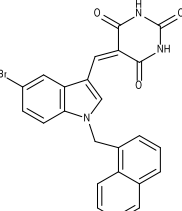
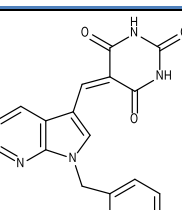
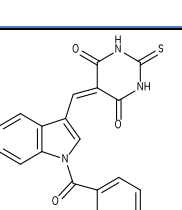
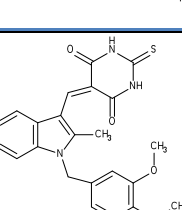
31.	PNR-6-56		89.1	18.9
32.	PNR-9-37A		89.3	10.6
33.	PNR-3-60		90.9	15.3
34.	PNR-9-36B		91.0	6.5
35.	PNR-9-14B		91.3	9.4
36.	PNR-9-33 salt		92.9	0.9
37.	PNR-9-63C		92.4	17.7

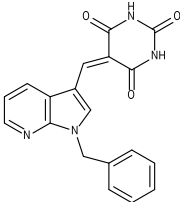
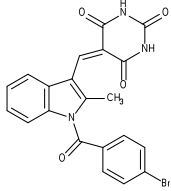
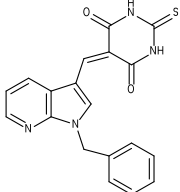
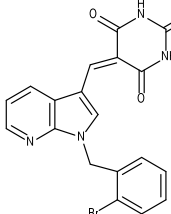
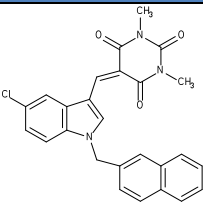
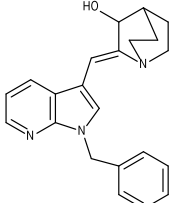
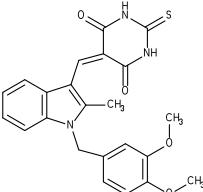
38.	PNR-6-93		93.6	33.8
39.	PNR-5-87		93.9	4.7
40.	PNR-9-39		94.1	10.8
41.	PNR-3-55		94.6	8.4
42.	PNR-3-53		94.8	10.6
43.	PNR-5-90		94.8	5.8
44.	PNR-5-81		96.5	9.6

45.	<u>PNR-3-40</u>		<u>97.8</u>	<u>23.4</u>
46.	<u>PNR-7-82</u>		<u>97.8</u>	<u>7.3</u>
47.	<u>PNR-6-05</u>		<u>98.1</u>	<u>11.0</u>
48.	<u>PNR-9-33 base</u>		<u>98.1</u>	<u>9.1</u>
49.	<u>PNR-3-66</u>		<u>98.3</u>	<u>5.9</u>
50.	<u>PNR-6-11</u>		<u>99.4</u>	<u>9.5</u>
51.	<u>PNR-6-04</u>		<u>98.8</u>	<u>18.7</u>

52.	<u>PNR-9-32B</u>		<u>99.6</u>	<u>11.0</u>
53.	<u>PNR-9-38 salt</u>		<u>99.9</u>	<u>11.5</u>
54.	<u>PNR-6-01</u>		<u>99.4</u>	<u>7.8</u>
55.	<u>PNR-3-83</u>		<u>100.1</u>	<u>17.7</u>
56.	<u>PNR-6-10</u>		<u>100.3</u>	<u>14.9</u>
57.	<u>PNR-9-40</u>		<u>101.3</u>	<u>13.5</u>
58.	<u>PNR-3-57</u>		<u>101.8</u>	<u>2.7</u>

59.	<u>PNR-9-38 base</u>		<u>102.4</u>	<u>9.1</u>
60.	<u>PNR-9-32A</u>		<u>102.6</u>	<u>11.3</u>
61.	<u>PNR-9-42A</u>		<u>102.7</u>	<u>14.2</u>
62.	<u>PNR-5-95</u>		<u>103.3</u>	<u>3.7</u>
63.	<u>PNR-6-16</u>		<u>103.6</u>	<u>2.2</u>
64.	<u>PNR-3-89</u>		<u>104.6</u>	<u>2.2</u>
65.	<u>PNR-6-90</u>		<u>104.3</u>	<u>2.9</u>

66.	<u>PNR-9-30B</u>		<u>105.0</u>	<u>16.0</u>
67.	<u>PNR-7-27</u>		<u>105.4</u>	<u>13.3</u>
68.	<u>PNR-9-36A</u>		<u>106.3</u>	<u>1.7</u>
69.	<u>PNR-6-91</u>		<u>106.3</u>	<u>15.8</u>
70.	<u>PNR-9-30A</u>		<u>106.9</u>	<u>7.4</u>
71.	<u>PNR-3-88</u>		<u>107.2</u>	<u>1.7</u>
72.	<u>PNR-5-91</u>		<u>107.9</u>	<u>17.2</u>

73.	<u>PNR-9-28A</u>		<u>108.1</u>	<u>5.1</u>
74.	<u>PNR-5-82</u>		<u>109.3</u>	<u>6.4</u>
75.	<u>PNR-9-28B</u>		<u>111.7</u>	<u>2.8</u>
76.	<u>PNR-9-42B</u>		<u>111.7</u>	<u>3.6</u>
77.	<u>PNR-7-03</u>		<u>111.7</u>	<u>15.6</u>
78.	<u>PNR-9-44</u>		<u>113.0</u>	<u>10.5</u>
79.	<u>PNR-6-08</u>		<u>114.2</u>	<u>6.3</u>

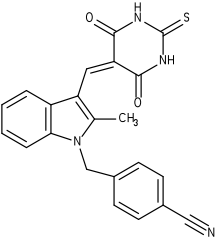
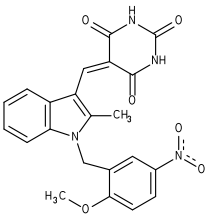
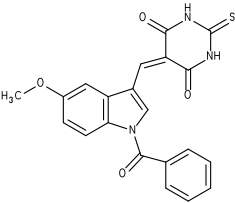
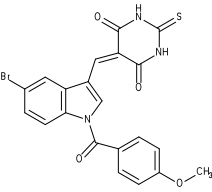
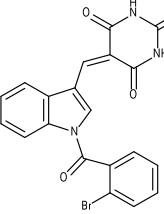
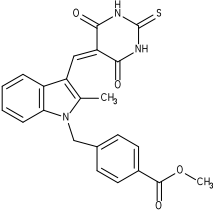
80.	<u>PNR-5-85</u>		<u>118.4</u>	<u>12.2</u>
81.	<u>PNR-6-14</u>		<u>122.4</u>	<u>10.9</u>
82.	<u>PNR-3-56</u>		<u>123.4</u>	<u>11.1</u>
83.	<u>PNR-3-64</u>		<u>124.7</u>	<u>1.1</u>
84.	<u>PNR-3-50</u>		<u>129.6</u>	<u>7.4</u>
85.	<u>PNR-5-88</u>		<u>130.8</u>	<u>17.8</u>

Table S2. Hpol η -derived peptides identified by mass spectrometry.

Sequence (NH ₂ -COOH)	<i>m/z</i>	Charge state	M.W.	Residue : Start-End
(R)VVALVDmDcFFVQVEQR(Q) ^a	691.0	+3	2,069.9	8-24
(R)VVALVDMdCFFVQVEQR(Q)	685.6	+3	2,054.0	8-24
(R)VVALVDMdCFFVQVEQRQNPHLR(N)	733.8	+4	2,931.4	8-30
(R)QNPHLRNKPCAVVQYK(S)	521.7	+4	2,083.0	25-40
(R)QNPHLRNKPCAVVQYK(S)	695.3	+3	2,083.0	25-40
(R)NKPCAVVQYK(S)	402.8	+3	1,205.6	31-40
(R)NKPCAVVQYKSWK(G)	536.6	+3	1,606.8	31-43
(R)NKPCAVVQYKSWKGGGIIAVSYEARAFGVTR(S)	683.3	+5	3,411.7	31-61
(K)GGGIIAVSYEAR(A)	596.8	+2	1,191.6	44-55
(R)AFGVTRSmWADDAKK(L)	425.4	+4	1,697.8	56-70
(R)AFGVTRSMWADDAK(K)	562.9	+3	1,685.7	56-69
(R)AFGVTRSMWADDAKK(L)	605.6	+3	1,813.8	56-70
(R)SmWADDAK(K)	470.1	+2	938.380	62-69
(R)SMWADDAKK(L)	526.2	+2	1,050.4	62-70
(K)KLcPDLLLAQVR(E)	475.9	+3	1,424.8	70-81
(K)LcPDLLLAQVRESR(G)	601.3	3	1,800.9	71-84
(K)YREASVEVMEIMSR(F)	567.2	+3	1,698.8	92-105
(K)YREASVEVMEImSR(F)	621.9	+3	1,862.8	92-105

(R)EASVEVMEIMSR(F)	690.8	+2	1,379.6	94-105
(R)ASIDEAYVDLTSVQER(L)	622.9	+3	1,865.9	112-128
(K)LQGQPISADLLPSTYIEGLPQGPTTAEETVQK(E)	846.1	+4	3,380.7	132-163
(R)KQGLFQWLDSLQIDNLTSPDLQLTVGAVIVEEMR(A)	965.0	+4	3,856.0	168-201
(R)KQGLFQWLDSLQIDNLTSPDLQLTVGAVIVEEMRAAIER(E)	1,100.0	+4	4,396.3	168-206
(K)QGLFQWLDSLQIDNLTSPDLQLTVGAVIVEEMR(A)	1,243.6	+3	3,727.9	169-201
(R)AAIERETGFQcSAGISHNK(V)	692.66	+3	2,074.9	202-220
(R)AAIERETGFQcSAGISHNK(V)	736.67	+3	2,207.0	202-220
(R)AAIERETGFQcSAGISHNKVLAK(L)	829.76	+3	2,486.2	202-224
(R)ETGFQcSAGISHNK(V)	768.34	+2	1,534.6	207-220
(R)ETGFQcSAGISHNKVLAK(L)	649.66	+3	1,945.9	207-224
(K)VLAKLAcGLNKPNR(Q)	389.22	+4	1,552.8	221-234
(K)LAcGLNKPNR(Q)	571.80	+2	1,141.6	225-234
(R)QTLVSHGSPQLFSQMPPIR(K)	709.04	+3	2,124.1	235-253
(R)QTLVSHGSPQLFSQMPPIRK(I)	597.06	+4	2,384.2	235-254
(R)KIRSLGGK(L)	495.79	+2	989.565	254-261
(K)IrSLGGK(L)	431.74	+2	861.471	255-261
(R)SLGGKLGASVIEILGIEYMGELTQFTESQLQSHFGEK(N)	1,000.0	+4	3,996.0	257-

				293
(R)SLGGKLGASVIEILGIEYMGELTQFTESQLQSHFGEKNGSW LYAMcR(G)	1,309.6	+4	5,234.5	257- 303
(K)LGASVIEILGIEYMGELTQFTESQLQSHFGEK(N)	1,185.6	+3	3,553.7	262- 293
(K)NGSWLYAMcR(G)	629.27	+2	1,256.5	294- 303
(R)GIEHDPVKPR(Q)	383.28	+3	1,146.6	304- 313
(R)GIEHDPVKPrQLPK(T)	873.46	+2	1,744.9	304- 317
(R)QLPKTIGcSK(N)	377.8	+3	1,130.6 1	314- 323
(K)TIGcSKNFPKG(T)	403.54	+3	1,207.6 0	318- 328
(K)NFPGKTALATR(E)	392.55	+3	1,174.6	324- 344
(K)NFPGKTALATREQVQWWLLQLAQELEER(L)	839.44	+4	3,353.7	324- 351
(K)TALATREQVQWWLLQLAQELEER(L)	981.83	+3	2,942.4	329- 351
(K)TALATREQVQWWLLQLAQELEER(L)	937.83	+3	2,810.4	329- 351
(K)TALATREQVQWWLLQLAQELEERLTKDRNDNDRVATQL VVSIR(V)	851.79	+6	5,104.7	329- 371
(R)EQVQWWLLQLAQELEER(L)	1,099.5	+2	2,197.1	335- 351
(R)EQVQWWLLQLAQELEERLTK(D)	891.46	+3	2,671.3 6	335- 354
(R)EQVQWWLLQLAQELEERLTKDR(N)	703.62	+4	2,810.4	335- 356
(R)LTKDRNDNDR(V)	416.20	+3	1,245.6	352- 361

(K)DRNDNDR(V)	452.69	+2	903.378	355-361
(K)DRNDNDRVATQLVVSIR(V)	657.68	+3	1,970.0	355-371
(R)VATQLVVSIR(V)	543.33	+2	1,084.6	362-371
(R)VQGDKRLSSLR(R)	420.24	+3	1,257.7	372-382
(R)RccALTR(Y)	468.72	+2	935.442	383-389
(R)RccALTR(Y)	534.73	+2	1,067.4	383-389
(R)ccALTRYDAHK(M)	763.83	+2	1,525.6	384-394
(R)ccALTRYDAHK(M)	465.54	+3	1,393.6	384-394
(R)YDAHKMSHDAFTVIK(N)	588.29	+3	1,761.8	390-404
(K)MSHDAFTVIK(N)	574.79	+2	1,147.5	395-404
(K)MSHDAFTVIKNcNTSGIQTEWSPPLTMLFLcATK(F)	975.47	+4	3,897.8	395-428
(K)NcNTSGIQTEWSPPLTMLFLcATK(F)	1,385.1	+2	2,768.3	405-428

^alowercase 'm' indicates oxidation (+16) and lowercase 'c' indicates carbamidomethyl (+57) modification.

^bResidues highlighted in red text were modified with HPG (+132).

FIGURES

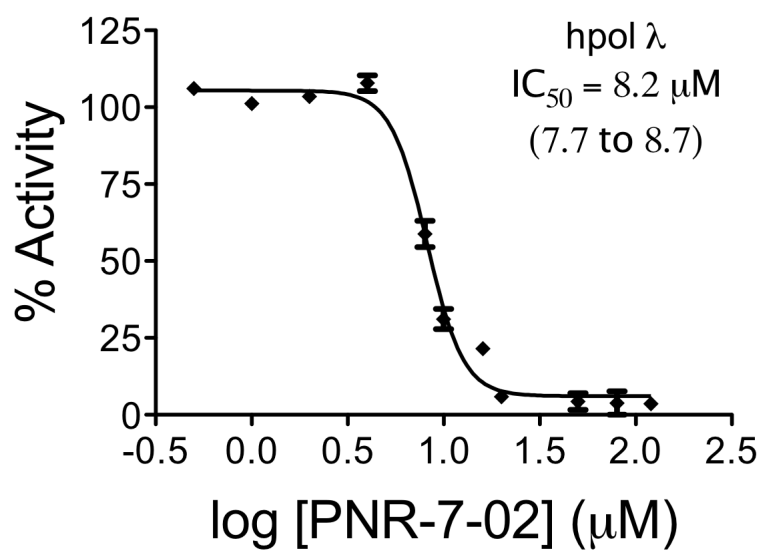


FIGURE S1. Determination of IC_{50} value for PNR-7-02 against hpol λ . The results shown represent the mean (\pm s.d.), $n = 3$. The IC_{50} value shown represents the best-fit value (95% confidence interval) derived from the fit of the data.

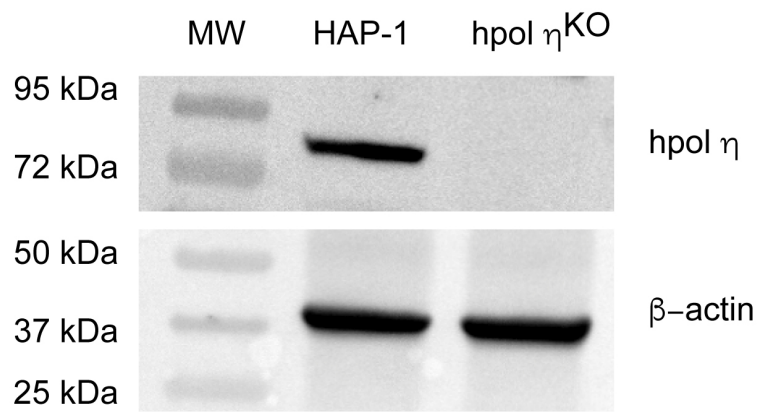


FIGURE S2. Validation of hpol η knock-out in HAP-1 cells. Immunoblotting was used to verify knock-out (KO) of hpol η in the HAP-1 cell line.

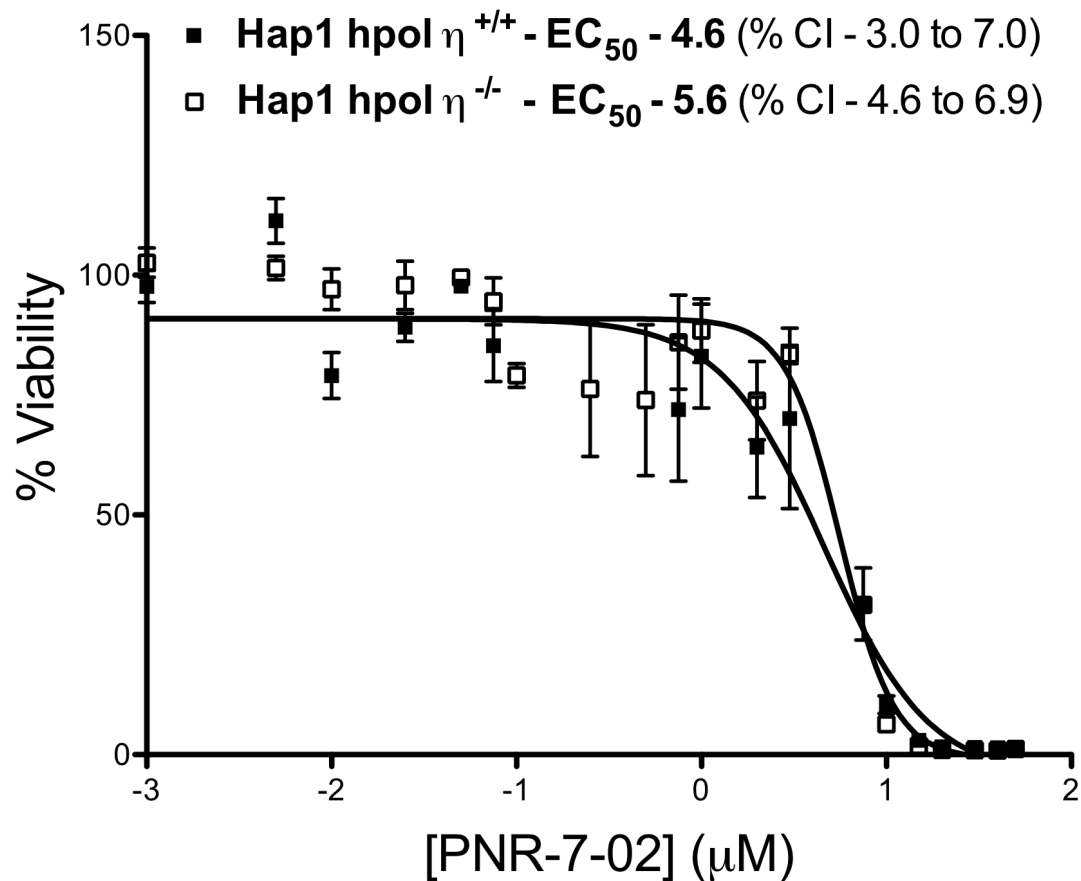


FIGURE S3. Determination of EC_{50} values for PNR-7-02 in hpol η -proficient and hpol η -deficient HAP-1 cells. The viability of hpol η -proficient and hpol $\eta^{-/-}$ knock-out HAP-1 cells was measured as a function of PNR-7-02 concentration. Dose-response results were fit to a four-parameter logistic model using Prism software (GraphPad, San Diego, CA). The experiments were performed in triplicate and the mean (\pm s.e.m.) was plotted. The best-fit EC_{50} values (95% confidence interval) derived from the fit of the data for each cell line are reported above the plot.

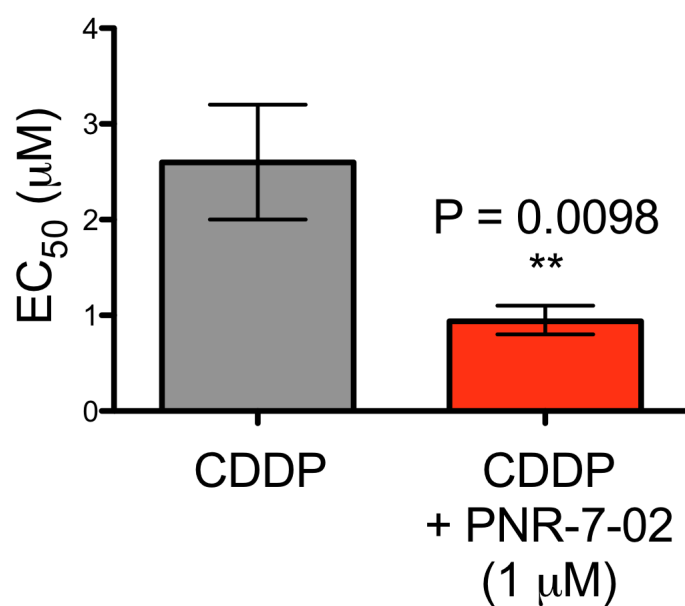


FIGURE S4. PNR-7-02 sensitizes ovarian cancer-derived cells to CDDP. OVCAR3 cells were exposed to varying concentrations of CDDP alone or in the presence of PNR-7-02 (1 μM). Cell viability was measured using the Calcein-AM assay. The EC₅₀ value for each treatment condition is shown (n=3, mean ± 95% confidence interval). An unpaired Student's t-test was used to compare treatment conditions. The Chou-Talalay method was used to calculate a combination index (CI) value of 0.40 using an EC₅₀ value of 24 μM for OVCAR3 cells treated with PNR-7-02 alone (data not shown).