Supplementary Material

# The size of aryl linker between two polyaza-cyclophane moieties controls the binding selectivity to ds-RNA vs ds-DNA

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**Supplementary Material include:**

**Table S1.** Electronic absorption data of **PHENPOD** and **PYPOD** at pH=6, 5 and 7.

**Scheme S1.** Distribution diagrams of PYPOD and PHENPOD.

**Fig. S1.** Fluorimetric titrations of **PHENPOD** ($c$ = 2.5 × $10^{-6}$ mol $dm^{-3}$, $\lambda_{exc}$=369 nm) at pH 5 with ct-DNA at pH 5.0 (left) and pH 6.0 (right) performed in sodium cacodylate buffer, $I$ = 0.05 mol $dm^{-3}$.

**Fig. S2**. Fluorimetric titrations of **PHENPOD** ($c$ = 2.5 × $10^{-6}$ mol $dm^{-3}$, $\lambda_{exc}$=369 nm) at pH 5 with poly A –poly U at pH 5.0 (left) and pH 6.0 (right) performed in sodium cacodylate buffer, $I$ = 0.05 mol $dm^{-3}$.

**Fig. S3.** Fluorimetric titrations of **PHENPOD** ($c$ = 2.5 × $10^{-6}$ mol $dm^{-3}$, $\lambda_{exc}$=369 nm) at pH 5 with poly dA –poly dT at pH 5.0 (left) and pH 6.0 (right) performed in sodium cacodylate buffer, $I$ = 0.05 mol $dm^{-3}$.

**Fig. S4**. Fluorimetric titrations of **PHENPOD** ($c$ = 2.5 × $10^{-6}$ mol $dm^{-3}$, $\lambda_{exc}$=369 nm) at pH 5 with poly dC –poly dG at pH 5.0 (left) and pH 6.0 (right) performed in sodium cacodylate buffer, $I$ = 0.05 mol $dm^{-3}$.

**Fig. S5**. CD titrations of ct-DNA ($c$ = 1.0 × $10^{-5}$ mol $dm^{-3}$) with **PYPOD (left)** and **PHENPOD (right)** at molar ratios $r_{[compound] [polynucleotide]}$ = 0.1; 0.2; 0.3 at pH 5.0 (sodium cacodylate buffer, $I$ = 0.05 mol $dm^{-3}$

**Fig. S6**. CD titrations of ct-DNA ($c$ = 1.0 × $10^{-5}$ mol $dm^{-3}$) with **PYPOD (left)** and **PHENPOD (right)** at molar ratios $r_{[compound] [polynucleotide]}$ = 0.1; 0.2; 0.3 at pH 6.0 (sodium cacodylate buffer, $I$ = 0.05 mol $dm^{-3}$

**Fig. S7**. CD titrations of poly A – poly U (left) and poly dA – poly dT (right) ($c$ = 1.0 × $10^{-5}$ mol $dm^{-3}$) with **PHENPOD** at molar ratios $r_{[compound] [polynucleotide]}$ = 0.1; 0.2; 0.3 at pH 5.0 (sodium cacodylate buffer, $I$ = 0.05 mol $dm^{-3}$.

**Fig. S8**. CD titrations of poly A – poly U (left) and poly dA – poly dT (right) ($c$ = 1.0 × $10^{-5}$ mol $dm^{-3}$) with **PHENPOD** at molar ratios $r_{[compound] [polynucleotide]}$ = 0.1; 0.2; 0.3 at pH 6.0 (sodium cacodylate buffer, $I$ = 0.05 mol $dm^{-3}$.

**Fig. S9**. CD titrations of poly A – poly U (left) and poly dA – poly dT (right) ($c$ = 1.0 × $10^{-5}$ mol $dm^{-3}$) with **PYPOD** at molar ratios $r_{[compound] [polynucleotide]}$ = 0.1; 0.2; 0.3 at pH 5.0 (sodium cacodylate buffer, $I$ = 0.05 mol $dm^{-3}$.

**Fig. S10**. CD titrations of poly A – poly U (left) and poly dA – poly dT (right) ($c$ = 1.0 × $10^{-5}$ mol $dm^{-3}$) with **PYPOD** at molar ratios $r_{[compound] [polynucleotide]}$ = 0.1; 0.2; 0.3 at pH 6.0 (sodium cacodylate buffer, $I$ = 0.05 mol $dm^{-3}$.

**Fig. S11**. CD titrations of poly dC – poly dG ($c$ = 1.0 × $10^{-5}$ mol $dm^{-3}$) with **PYPOD (left)** and **PHENPOD (right)** at molar ratios $r_{[compound] [polynucleotide]}$ = 0.1; 0.2; 0.3 at pH 5.0 (sodium cacodylate buffer, $I$ = 0.05 mol $dm^{-3}$.

**Fig. S12**. CD titrations of poly dC – poly dG ($c$ = 1.0 × $10^{-5}$ mol $dm^{-3}$) with **PYPOD (left)** and **PHENPOD (right)** at molar ratios $r_{[compound] [polynucleotide]}$ = 0.1; 0.2; 0.3 at pH 6.0 (sodium cacodylate buffer, $I$ = 0.05 mol $dm^{-3}$.

**Fig. S13**. CD titrations of poly A – poly U (left) and poly dA – poly dT (right) ($c$ = 1.0 × $10^{-5}$ mol $dm^{-3}$) with **PHENPOD** at molar ratios $r_{[compound] [polynucleotide]}$ = 0.3 and 0.6 at pH 6.0 (sodium cacodylate buffer + NaCl; $I$ = 0.15 mol $dm^{-3}$.

**Fig. S14**. CD titrations of poly A – poly U (left) and poly dA – poly dT (right) ($c$ = 1.0 × $10^{-5}$ mol $dm^{-3}$) with **PYPOD** at molar ratios $r_{[compound] [polynucleotide]}$ = 0.3 and 0.6 at pH 6.0 (sodium cacodylate buffer + NaCl; $I$ = 0.15 mol $dm^{-3}$.

**Fig. S15**. Dose-response profiles for **PHENPOD** tested *in vitro* on a panel of human cell lines.

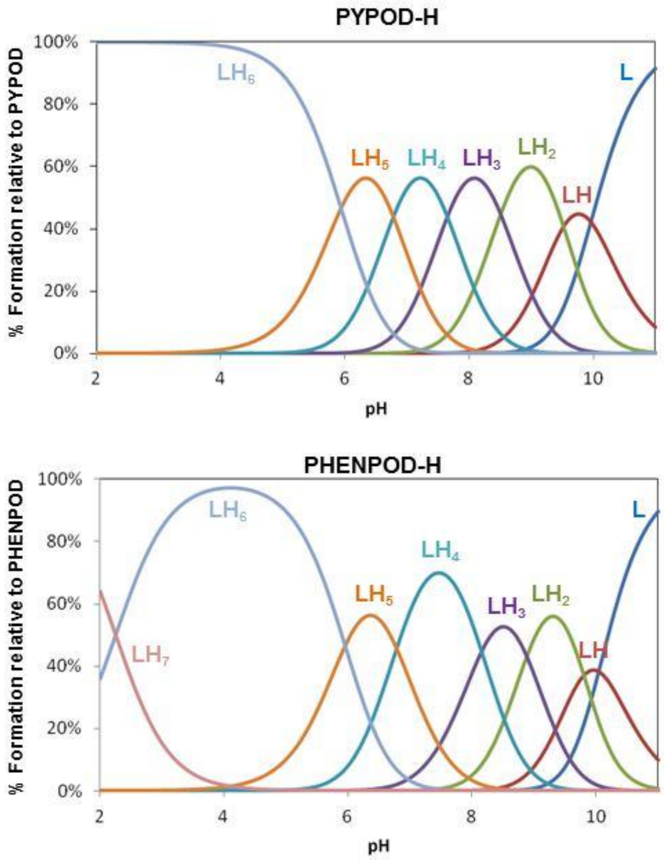
**Additional experimental details about antitumor evaluation assay.**

## Supplementary Material

**Table S1.** Electronic absorption data of **PHENPOD** and **PYPOD** at pH=6, 5 and 7.[a]

| Compound | pH = 5.0 | | pH = 6.0 | | pH = 7.0 | |
|---|---|---|---|---|---|---|
| | $\lambda_{max}$ / nm | $\varepsilon \times 10^3$ / mmol$^{-1}$ cm$^2$ | $\lambda_{max}$ / nm | $\varepsilon \times 10^3$ / mmol$^{-1}$ cm$^2$ | $\lambda_{max}$ / nm | $\varepsilon \times 10^3$ / mmol$^{-1}$ cm$^2$ |
| **PHENPOD** | 269 | 25.72 | 269 | 22.46 | 269 | 19.63 |
| **PYPOD** | 260 | 9.50 | 260 | 9.52 | 262 | 8.88 |

[a] Sodium cacodylate buffer, $I$ = 0.05 mol dm$^{-3}$,




The number of charges in table 1 at each pH value is the % of each species multiplied by the charge of the respective species
Nº Charges = [% H6L*6+% H5L*5+% H4L*4+% H3L*3+% H2L*2+% HL*1]

**Scheme S1.** Distribution diagrams of PYPOD (up) and PHENPOD (down), details see in Ref 22 of the manuscript.

# Supplementary Material

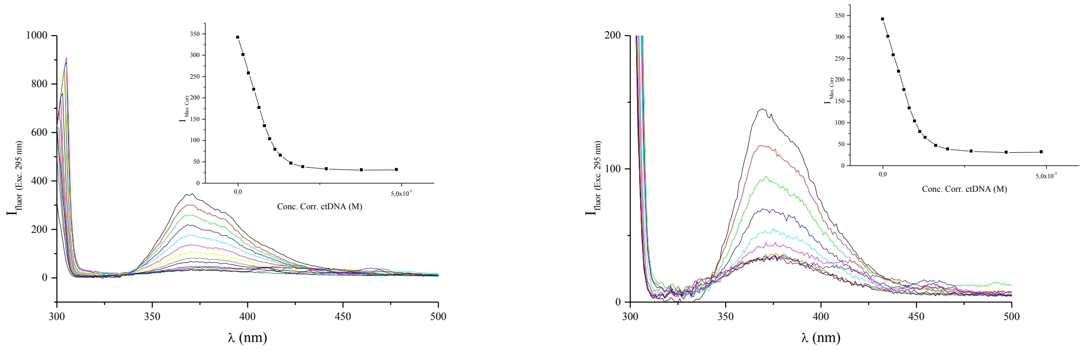



**Fig. S1.** Fluorimetric titrations of **PHENPOD** ($c = 2.5 \times 10^{-6}$ mol dm$^{-3}$, $\lambda_{exc}$=369 nm) at pH 5 with ct-DNA at pH 5.0 (left) and pH 6.0 (right) performed in sodium cacodylate buffer, $I$ = 0.05 mol dm$^{-3}$.

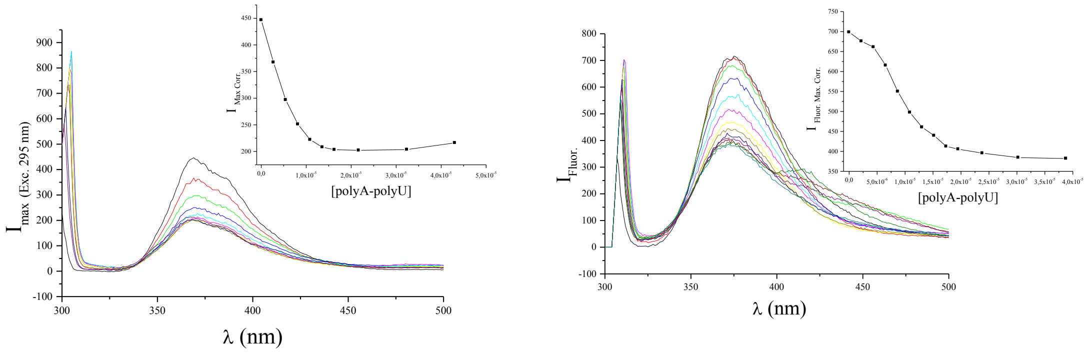
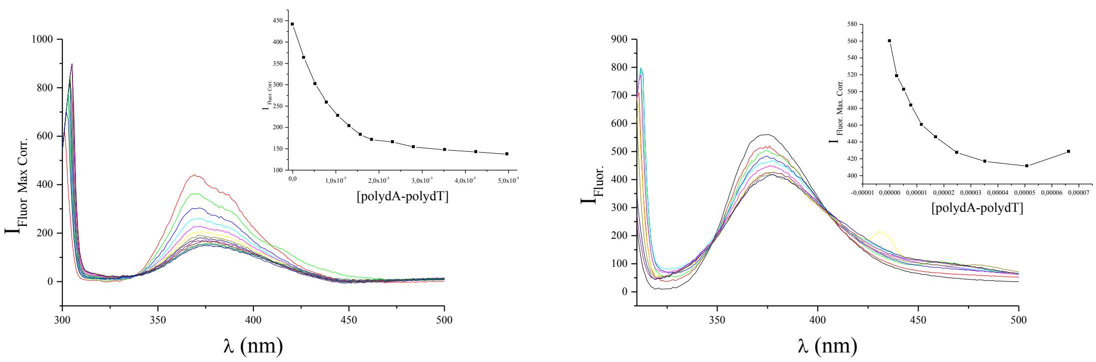



**Fig. S2**. Fluorimetric titrations of **PHENPOD** ($c = 2.5 \times 10^{-6}$ mol dm$^{-3}$, $\lambda_{exc}$=369 nm) at pH 5 with poly A –poly U at pH 5.0 (left) and pH 6.0 (right) performed in sodium cacodylate buffer, $I$ = 0.05 mol dm$^{-3}$.

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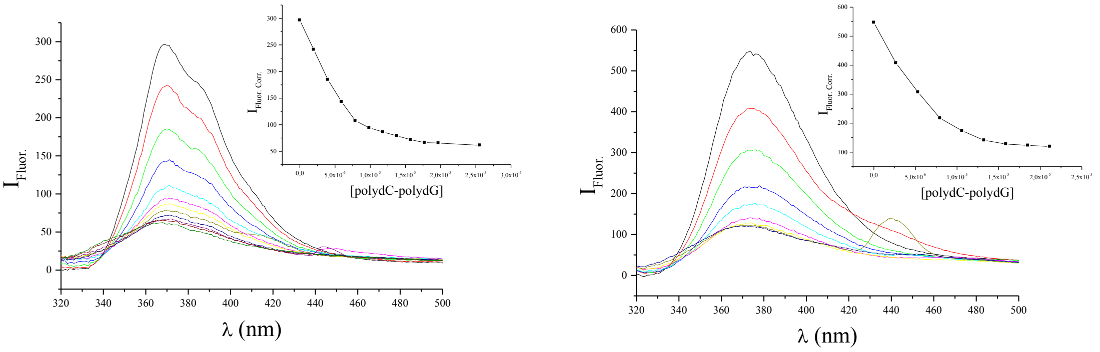


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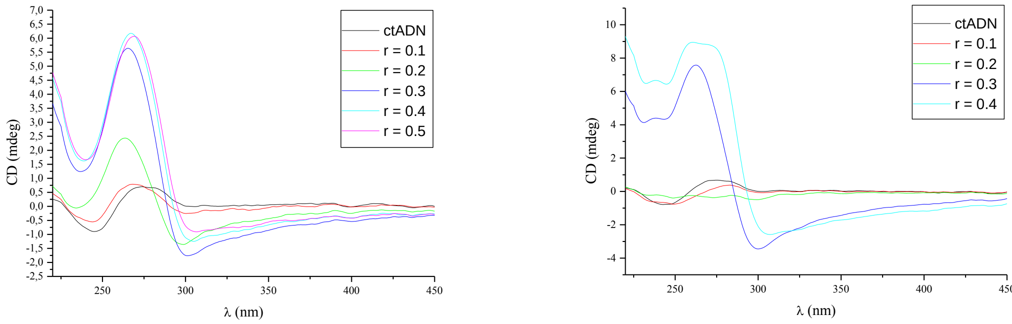
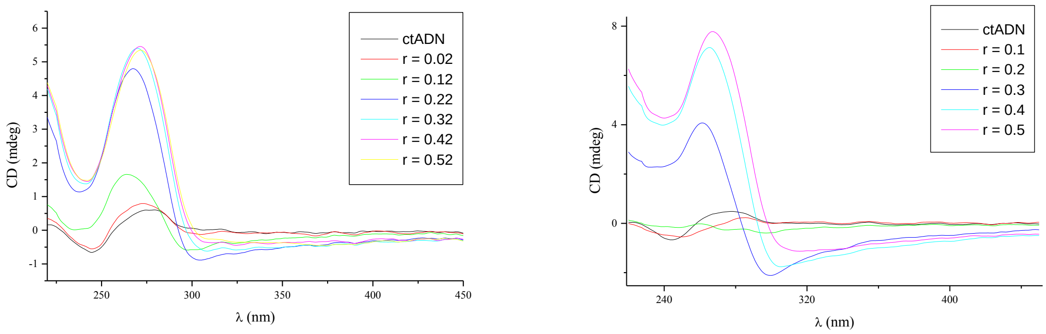



**Fig. S5**. CD titrations of ct-DNA ($c$ = 1.0 × $10^{-5}$ mol $dm^{-3}$) with **PYPOD (left)** and **PHENPOD (right)** at molar ratios $\boldsymbol{r}_{[compound] / [polynucleotide]}$ = 0.1; 0.2; 0.3, 0.4, 0.5 at pH 5.0 (sodium cacodylate buffer, $I$ = 0.05 mol $dm^{-3}$

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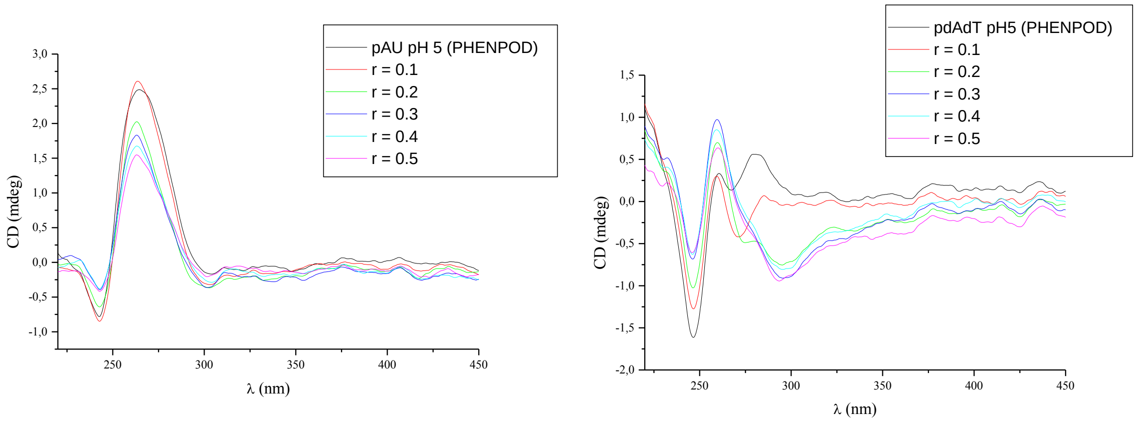


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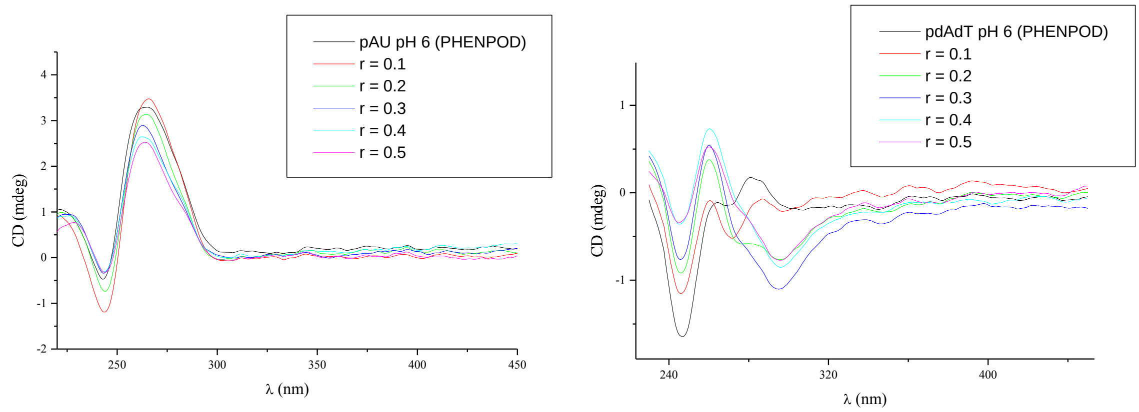


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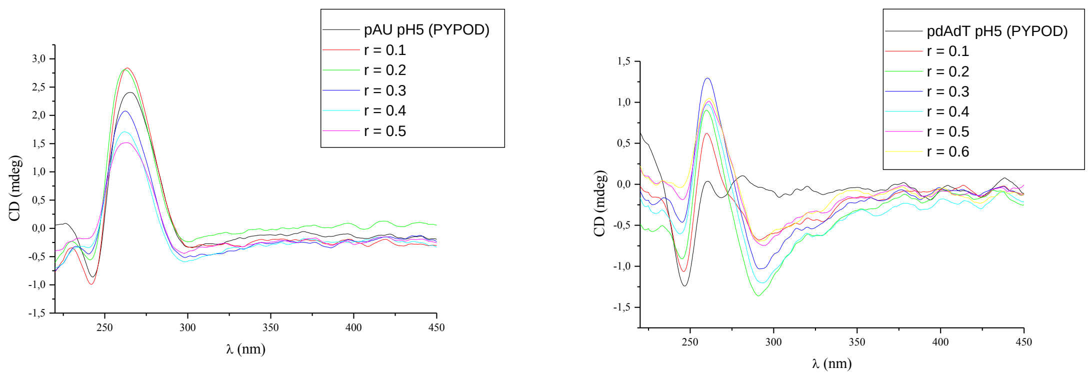


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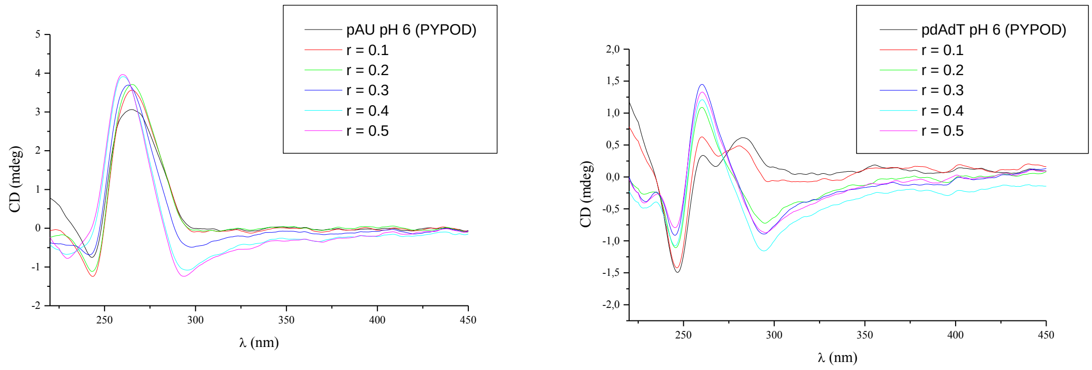
## Supplementary Material

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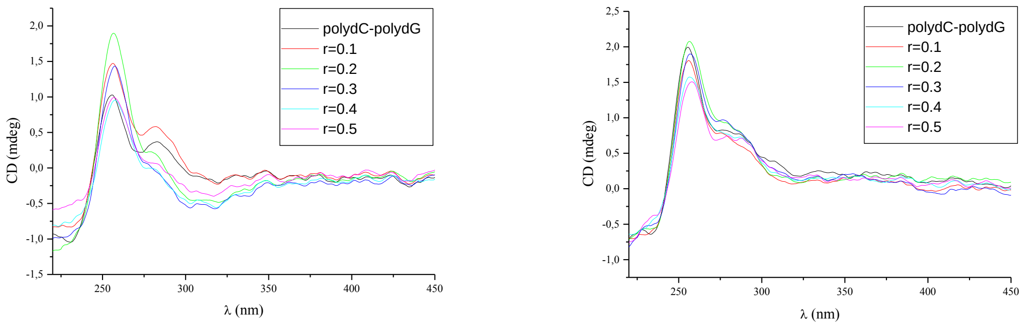


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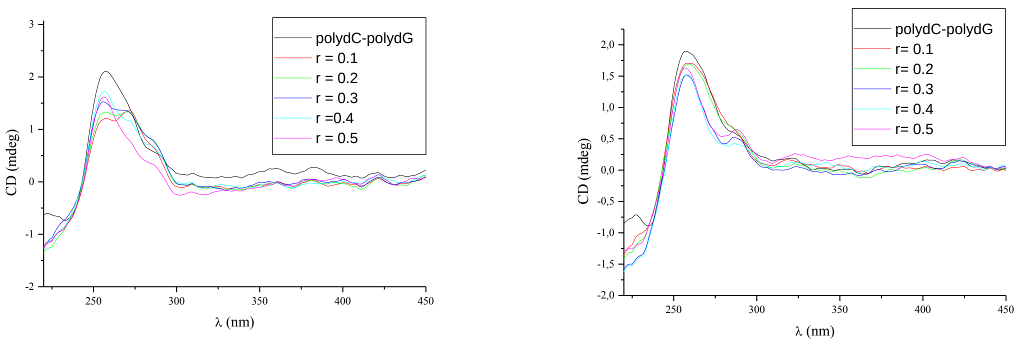
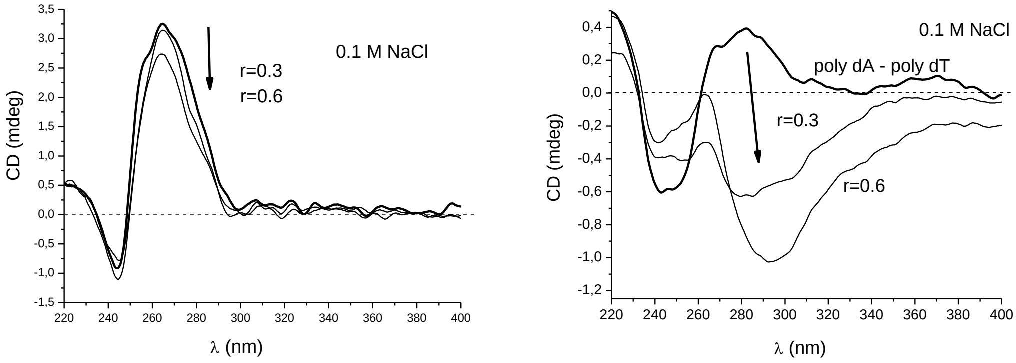



**Fig. S12**. CD titrations of poly dC – poly dG ($c = 1.0 \times 10^{-5}$ mol dm$^{-3}$) with **PYPOD (left)** and **PHENPOD (right)** at molar ratios $\boldsymbol{r}_{\text{[compound] [polynucleotide]}}$ = 0.1; 0.2; 0.3, 0.4, 0.5 at pH 6.0 (sodium cacodylate buffer, $I$ = 0.05 mol dm$^{-3}$.

Supplementary Material

## CD experiments at high ionic strength, pH 6.0 (sodium cacodylate buffer, I = 0.05 M, NaCl = 100 mM concentration, thus in total I=150 mM)

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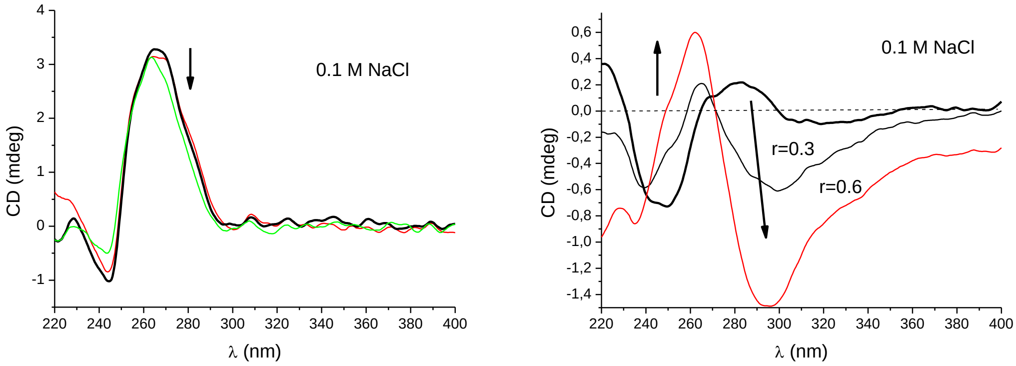


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Supplementary Material

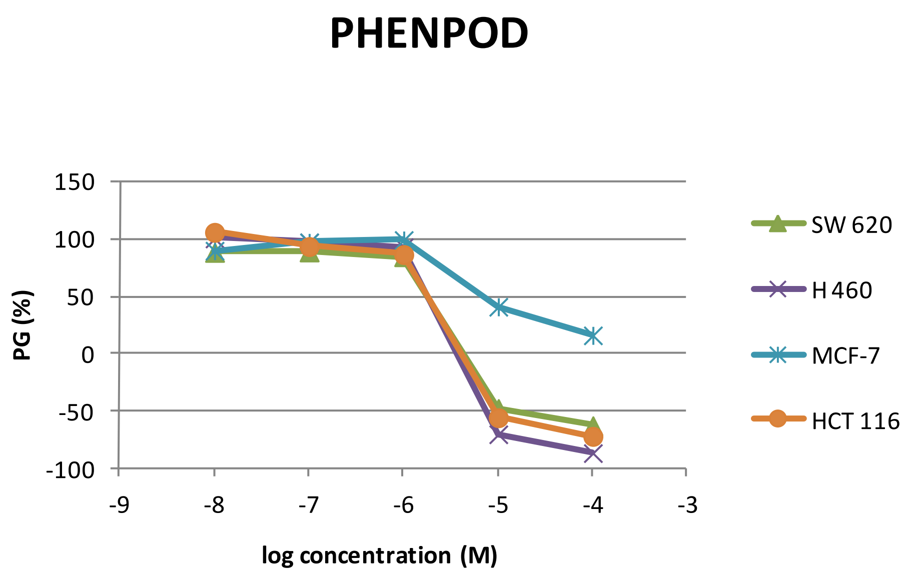


**Figure S15.** Dose-response profiles for PHENPOD tested *in vitro* on a panel of human tumor cell lines. PG – percentage of growth.

## Antitumor evaluation assay

The experiments were carried out on four human cell lines, which are derived from three cancer types. The following cell lines were used: SW 620, HCT 116 (colon carcinoma), H 460 (lung carcinoma) and MCF-7 (breast carcinoma). The cells were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere with 5% $CO_2$ at 37˚C.

The growth inhibition activity was assessed as described previously[1,2]. The cell lines were inoculated onto a series of standard 96-well microtiter plates on day 0, at $3\times10^4$ cells/mL (SW 620, HCT 116, H 460) to $5\times10^4$ cells/mL (MCF-7), depending on the doubling times of a specific cell line. Test agents were then added in ten-fold dilutions ($10^{-8}$ to $10^{-4}$ M) and incubated for further 72 h. Working dilutions were freshly prepared on the day of testing. After 72 h of incubation the cell growth rate was evaluated by performing the MTT assay, which detects dehydrogenise activity in viable cells. The absorbance (A) was measured on a microplate reader at 570 nm. The absorbance is directly proportional to the number of living, metabolically active cells. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions:

If (mean $A_{test}$ – mean $A_{tzero}$) ≥ 0, then PG = 100 × (mean $A_{test}$ – mean $A_{tzero}$) / (mean $A_{ctrl}$ – mean$A_{tzero}$).

If (mean $A_{test}$ – mean $A_{tzero}$) < 0, then: PG = 100 × (mean $A_{test}$ – mean $A_{tzero}$) / $A_{tzero}$, where the mean $A_{tzero}$ is the average of optical density measurements before exposure of cells to the test compound, the mean $A_{test}$ is the average of optical density measurements after the desired period of time and the mean $A_{ctrl}$ is the average of optical density measurements after the desired period of time with no exposure of cells to the test compound.

The results are expressed as $IC_{50}$, which is the concentration necessary for 50% of inhibition. The $IC_{50}$ values for each compound are calculated from concentration-response curves using linear regression analysis by fitting the test concentrations that give PG values above and below the reference value (*i.e.* 50%). If however, for all of the tested concentrations produce PGs exceeding the respective reference level of effect (*e.g.* PG value of 50), then the highest tested concentration is assigned as the default value, which is preceded by a ">" sign. Each test was performed in quadruplicate in at least two individual experiments.

**References**

[1] G. Malojčić, I. Piantanida, M. Marinić, M. Žinić, M. Marjanović, M. Kralj, K. Pavelić and H-J Schneider, *Org. Biomol. Chem.*, 2005, **3**, 4373

[2] M. Marjanović, M. Kralj, F. Supek, L. Frkanec, I. Piantanida, T. Šmuc and L. Tušek-Božić, *J. Med. Chem.*, 2007, **50**, 1007.