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

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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}, λ_em=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}, λ_em=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}, λ_em=369 nm) at pH 5 with poly dA – poly dU 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}, λ_em=369 nm) at pH 5 with poly dG – poly dC 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 – polydT (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 – polydT (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 – polydT (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 – polydT (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 – polydT (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 – polydT (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. S15. Dose-response profiles for PHENPOD tested in vitro on a panel of human cell lines.

Additional experimental details about antitumor evaluation assay.
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**Table S1.** Electronic absorption data of PHENPOD and PYPOD at pH=6, 5 and 7.*

<table>
<thead>
<tr>
<th>Compound</th>
<th>pH = 5.0</th>
<th>pH = 6.0</th>
<th>pH = 7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \lambda_{\text{max}} ) / nm</td>
<td>( \varepsilon \times 10^3 ) / mmol(^{-1}) cm(^2)</td>
<td>( \lambda_{\text{max}} ) / nm</td>
</tr>
<tr>
<td>PHENPOD</td>
<td>269</td>
<td>25.72</td>
<td>269</td>
</tr>
<tr>
<td>PYPOD</td>
<td>260</td>
<td>9.50</td>
<td>260</td>
</tr>
</tbody>
</table>

* Sodium cacodylate buffer, \( I = 0.05 \text{ mol dm}^{-3} \).

The number of charges in table 1 at each pH value is the % of each species multiplied by the charge of the respective species.

\[
\text{N}^\circ \text{ Charges} = [\% H6L^6+ \% H5L^5+ \% H4L^4+ \% H3L^3+ \% H2L^2+ \% HL^1]
\]

**Scheme S1.** Distribution diagrams of PYPOD (up) and PHENPOD (down), details see in Ref 22 of the manuscript.
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**Fig. S1.** Fluorimetric titrations of PHENPOD ($c = 2.5 \times 10^{-6}$ mol dm$^{-3}$, $\lambda_{ex}=369$ 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$^{-3}$, $\lambda_{ex}=369$ 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 ($c = 2.5 \times 10^{-6}$ mol dm$^{-3}$, $\lambda_{ex}=369$ 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$^{-3}$. 
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**Fig. S4.** Fluorimetric titration of PHENPOD \( (c = 2.5 \times 10^{-6} \text{ mol dm}^{-3}, \lambda_{\text{exc}}=369 \text{ nm}) \) at pH 5 with poly dC–poly dG at pH 5.0 (left) and pH 6.0 (right) performed in sodium cacodylate buffer, \( I = 0.05 \text{ mol dm}^{-3} \).

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**Fig. S6.** CD titrations of ct-DNA \( (c = 1.0 \times 10^{-5} \text{ mol dm}^{-3}) \) with PYPOD (left) and PHENPOD (right) at molar ratios \( r_{\text{compound}}/\text{polynucleotide} = 0.1; 0.2; 0.3, 0.4, 0.5 \) at pH 6.0 (sodium cacodylate buffer, \( I = 0.05 \text{ mol dm}^{-3} \)).
**Fig. S7.** CD titrations of poly A – poly U (left) and poly dA – poly dT (right) \((c = 1.0 \times 10^{-5} \text{ mol dm}^{-3})\) with PHENPOD at molar ratios \(r_{[\text{compound}]}/[\text{polynucleotide}] = 0.1; 0.2; 0.3; 0.4, 0.5\) at pH 5.0 (sodium cacodylate buffer, \(I = 0.05 \text{ mol dm}^{-3}\)).

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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)

![Graph](image)

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

![Graph](image)

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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% CO₂ at 37°C.

The growth inhibition activity was assessed as described previously¹,². The cell lines were inoculated onto a series of standard 96-well microtiter plates on day 0, at 3×10⁴ cells/mL (SW 620, HCT 116, H 460) to 5×10⁴ 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
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one or the other of the following two expressions:

If \( (\text{mean } A_{\text{test}} - \text{mean } A_{\text{tzero}}) \geq 0 \), then \( \text{PG} = 100 \times \frac{(\text{mean } A_{\text{test}} - \text{mean } A_{\text{tzero}})}{(\text{mean } A_{\text{ctrl}} - \text{mean } A_{\text{tzero}})} \).

If \( (\text{mean } A_{\text{test}} - \text{mean } A_{\text{tzero}}) < 0 \), then: \( \text{PG} = 100 \times \frac{(\text{mean } A_{\text{test}} - \text{mean } A_{\text{tzero}})}{A_{\text{tzero}}} \),

where the mean \( A_{\text{tzero}} \) is the average of optical density measurements before exposure of cells to the test compound, the mean \( A_{\text{test}} \) is the average of optical density measurements after the desired period of time and the mean \( A_{\text{ctrl}} \) 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 \( \text{IC}_{50} \), which is the concentration necessary for 50% of inhibition. The \( \text{IC}_{50} \) 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
