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Dihydroimidazophenanthridinium (DIP)-Based DNA Binding Agents with Tuneable Structures and Biological Activity

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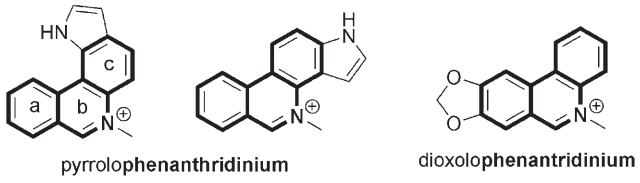
*We have synthesised a library of dihydroimidazophenanthridinium cations (DIPs) with large structural diversity (**1**–**29**) using a "one-pot" approach. The DNA binding constants of DIPs range from 2×10^4 to $1.3\times10^5\,M^{-1}$, and the free energies for binding range from -5.9 to $-6.40\,kcal\,mol^{-1}$. Viscosity measurements demonstrated that the binding of the compounds caused DNA lengthening, thus signifying binding by intercalation. The cytotoxicities of the compounds were determined by tetrazolium dye-based microtitration assays and showed a large range of values (0.09–11.7 μM). Preliminary molecular modelling studies of the DNA–DIP interactions suggested that the DIP moieties can interact with DNA by intercalation, and some R groups might facilitate binding by minor-groove binding. The results provide insight into how to design biologically active DNA binding agents that can interact in these ways.*

Introduction

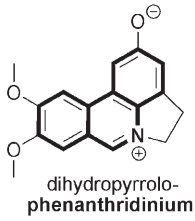
Nitrogen heteroaromatic cations are interesting compounds due to their reactivity and biological properties.[1,2] In particular, research has focussed on the phenanthridinium moiety because of its implication in the scaffold of a number of DNA intercalating agents with antitumour properties,[3,4] DNA drug targeting applications[5,6] and DNA probes,[7] see Scheme 1. Consequently many researchers have investigated the ring expansion of the phenanthridine framework, with the exception of the heteroaromatic middle ring.[8–11]



Scheme 1. Examples of systems based around the phenanthridinium core.[8–11]

We have recently developed a methodology to exploit the reactivity of the phenanthridinium iminium moiety in an annelation reaction.[12] A primary amine reacts with 2-(bromoethyl)-phenanthridinium bromide to yield a new phenanthridinium-ring-extended framework in one pot: 2,3-dihydro-1*H*-imidazo-[1,2-*f*]phenanthridinium (DIP), see Scheme 2. Furthermore, our recent studies have demonstrated that these compounds are stable to reduction and pseudobase formation through delocalisation of the positive charge, an advantage over previously reported phenanthridinium antitumour agents.[13]

This reaction allows the derivatisation of virtually any primary amine, by connecting it to a polyaromatic cationic core. Therefore it was anticipated that the DIPs would bind to DNA by intercalation, and could have potential as anticancer therapeutics. Indeed, we recently gathered preliminary data on our first DIP compounds that indicated DNA interactivity, and the compounds did exhibit some cytotoxicity.[13] Thus, these studies encouraged us to synthesise a large library of DIP compounds from a wide variety of primary amines, including aromatic, aliphatic and chiral monomers. A variety of dimers were also designed and synthesised as potential bis-intercalators. As has been shown by others,[14] with the correct spacer, it is theoretically possible to obtain a binding constant that is the square of that of the monomer. Given the DNA binding and cellular activity of the compounds, coupled with the extremely simple "one-pot" synthetic approach, we have undertaken a study to attempt to understand and work towards a possible connection between the structure, affinity for DNA and cytotoxicity. Although it is attractive



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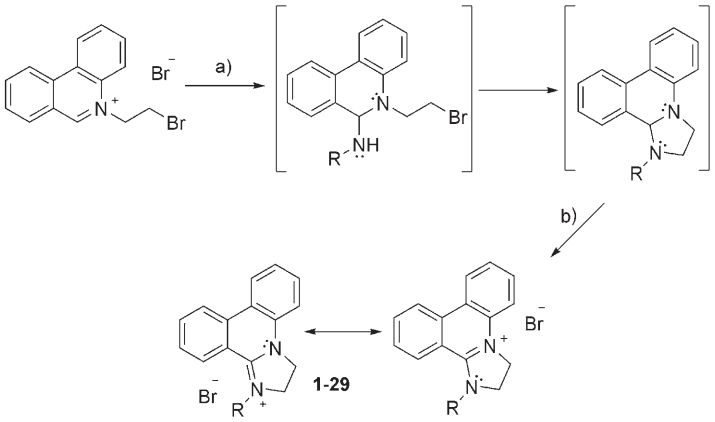


Scheme 2. Annelation reaction forming Dihydroimidazophenanthridinium derivatives.[12] Reagents and conditions: a) R–NH_2 (virtually any primary amine can undergo this reaction),[12] Na_2CO_3, water/ethyl acetate, N_2, RT, 3 h. b) Aqueous wash, NBS, RT, 3 h, in the dark.

to consider DNA as the cellular target, this assumption is not proven. The work presented here allows a *preliminary* correlation between structure and DNA binding; however, at this stage, any correlation with the tetrazolium dye-based microtitration (MTT) assay results will be done only in a circumstantial manner. What is perhaps more relevant is the new understanding of how the DIP moiety interacts with DNA, and how this can be adjusted by selecting new R groups.

The ultimate goal of anticancer therapeutics is to design compounds that can prevent tumour proliferation, reduce tumour size and even prevent the initial development of cancerous cells, whilst having no or only limited toxicity towards normal cells.[15] Understanding the structure–affinity relationships for the design of DNA-interactive molecules is extremely important, and the design of new therapeutics, along with gaining more fundamental understanding regarding DNA–small-molecule interactions, is a big motivation for this work. In order to work effectively towards such ambitions, it is important to use molecular design and assays together to develop a structure–activity relationship.

Here we opted to synthesise a set of structurally diverse DIPs from a range of primary amines and to analyse these compounds for DNA binding and cytotoxicity in in vitro cell lines. By using such an approach, we hope to be able to understand in more detail how our DIP family of molecules interacts with DNA and to appreciate the possible consequences of different design approaches.

Results

DNA binding affinities

To evaluate the DNA binding affinity of the DIP framework,[16] isothermal titration calorimetry (ITC) experiments were undertaken on salmon testes (ST) DNA. All results were compared to the known intercalator ethidium bromide (EtBr), which contains the same aromatic framework as DIP, and are shown in Table 1, with a break down of the thermodynamic components shown in Figure 1. These results show that the standard entropies of binding (ΔS^0) of the DIP ligands are generally favourable (under standard conditions). This is in contrast to many other intercalators, notably ethidium bromide, and serves to reinforce the small favourable enthalpic (ΔH^0) components of the standard free energies of binding (ΔG^0). This observation is interesting since it implies that binding of the DIP-R moieties is dominated by hydrophobic interactions or other interactions involving desolvation.

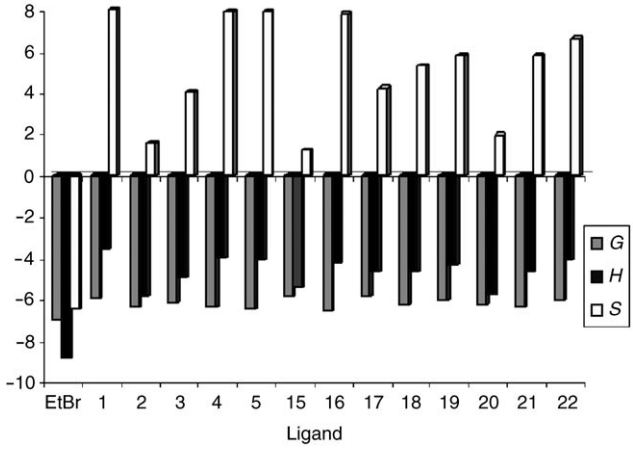
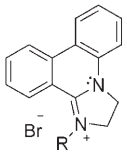


Figure 1. Plot of ΔG^0 and ΔH^0 given in kcal mol^{-1}, ΔS^0 given in cal mol^{-1} K^{-1}.

The basic framework for DIP is denoted by molecule **1**, see Table 1. This molecule itself shows moderate binding to DNA ($\sim 10^4$), compared to that of EtBr, ($\sim 10^5$), a strongly binding intercalator. Examination of the values across the compounds tested (not all the compounds—**6**–**9**, **11**–**14**, **23**–**29**—could be examined due to solubility constraints) show that the affinity of the DIP-based molecules for DNA varies depending on their R groups. In general, addition of a functional group onto the DIP moiety increases its affinity for DNA by up to 60%[16] [for example, the basic DIP moiety with R = H, **1**, with a binding constant of 2.6×10^4 vs. **16** with a binding constant of 7.0×10^4 here R = $(CH_2)_6$–OH], suggesting further interaction between the DIP-R group and the DNA helix, most probably in the minor groove. This general trend indicates that increasing the size or length of the R group increases the molecules' affinity for DNA. For instance the DIP-based molecules **3**–**5**, with long aliphatic chains (C_3–C_6), showed an increase in binding affinity.[16,17] Unfortunately, the binding affinities of the compounds with very long chains (**11**–**14**, C_{13}–C_{18}) could not be evaluated due to solubility constraints.[18] However the trend for molecules **3**–**5** does seem to demonstrate the importance of hydrophobic interactions arising from possible binding in the minor groove of DNA. Also, the chemical nature of the R group has an effect and this can be shown by the relative binding constants of compounds **16** and **17**; the compound with the terminal hydroxy group, **16**, binds slightly better than **17**, which has an ether functionality, although both **16** and **17** have comparable pendant chain lengths (X_{7-8}). This demonstrates the importance of hydrophobic (from the long chain) and hydro-

Table 1. DNA binding results and cytotoxicity results of DIP molecules with varying R groups on DIP core. Compound code with R group structure followed by DNA binding constant (ST) $10^4\,M^{-1}$ and cytotoxicity (A2780) μM.



EtBr	Cisplatin	Carboplatin	**1** R = H
12.9 ± 0.5	[a]	[a]	2.6 ± 0.1
0.30 ± 0.04	0.34 ± 0.05	5.22 ± 0.14	1.56 ± 0.18
2 R = CH_3	**3** R = $(CH_2)_2CH_3$	**4** R = $(CH_2)_5CH_3$	**5** R = $(CH_2)_6CH_3$
4.6 ± 0.2	3.5 ± 0.1	4.9 ± 0.3	5.4 ± 0.4
2.60 ± 0.19	6.71 ± 0.8	1.22 ± 0.16	0.66 ± 0.03
6 R = $(CH_2)_7CH_3$	**7** R = $(CH_2)_8CH_3$	**8** R = $(CH_2)_9CH_3$	**9** R = $(CH_2)_{10}CH_3$
[a]	[a]	[a]	[a]
0.34 ± 0.06	0.27 ± 0.04	0.89 ± 0.01	0.46 ± 0.05
10 R = $(CH_2)_{11}CH_3$	**11** R = $(CH_2)_{12}CH_3$	**12** R = $(CH_2)_{13}CH_3$	**13** R = $(CH_2)_{15}CH_3$
[a]	[a]	[a]	[a]
0.09 ± 0.01	0.81 ± 0.05	0.49 ± 0.04	0.15 ± 0.01
14 R = $(CH_2)_{17}CH_3$	**15** R = $(CH_2)_2OH$	**16** R = $(CH_2)_6OH$	**17** R = $(CH_2)_3O(CH_2)_3CH_3$
[a]	2.0 ± 0.1	7.0 ± 0.8	2.2 ± 0.1
0.20 ± 0.04	11.70 ± 1.20	0.89 ± 0.12	1.38 ± 0.33
18 R = Bn	**19** R = CH_2-4-$C_6H_4OCH_3$	**20** R = 4-$C_6H_4OCH_3$	**21** R = (*R*) $CH(CH_3)$-4-$C_6H_4OCH_3$
4.3 ± 0.2	2.9 ± 0.1	4.2 ± 0.2	5.2 ± 0.2
2.32 ± 0.33	1.53 ± 0.09	1.45 ± 0.15	1.03 ± 0.06
22 R = (*S*) $CH(CH_3)$-4-$C_6H_4OCH_3$	**23** R = Ph	**24** R = 4-C_6H_4Et	**25** R = 4-C_6H_4Bn
2.8 ± 0.1	[a]	[a]	[a]
2.74 ± 0.02	2.40 ± 0.07	1.54 ± 0.12	0.32 ± 0.04
26 R = 3-C_6H_4Bn	**27** R = $(CH_2)_9$DIP	**28** R = $(CH_2)_{10}$DIP	**29** R = $(CH_2)_{12}$DIP
[a]	[a]	[a]	[a]
4.57 ± 0.54	1.54 ± 0.58	1.60 ± 0.01	0.68 ± 0.04

[a] Due to poor solubility, the DNA affinity of every DIP molecule could not be tested.

gen-bonded interactions. The addition of supplementary aromatic moieties offers molecules with stronger affinities for DNA, especially if the aromatic functionality is directly attached to the DIP backbone, for example, **20**. The difference between the binding constants for the enantiomers, compounds **21** and **22**, is very interesting, because it confirms that the R group does have a real effect. Indeed, this observation could be exploited further by the synthesis of chiral DIPs with bulkier R groups, which should magnify the difference between enantiomers. Although the results clearly indicate binding to DNA, ITC alone does not specify the manner in which this binding occurs. The planar, polyaromatic core of the DIP molecules are typical of intercalating moieties, where π–π interactions between the aromatic base pairs of DNA and ligand disrupt and distort the DNA helix. However viscometric studies,[16] which examine the increase in viscosity arising from increased lengthening and stiffening of the DNA chain when planar ligands insert between adjacent base pairs, is a strong indicator of intercalation.[19,20] Indeed, we have shown that the viscosities of ST-DNA solutions are increased in the presence of a classic intercalator (EtBr) or DIP-based ligands for example, **1** and **20**, but not by a known minor-groove binder (netropsin). For the basic DIP ligand, **1**, this increase in viscosity depends on the relative concentrations, and saturates at a ligand/bp molar ratio of around 0.4, similar to the apparent N values obtained by calorimetric titrations.[16]

Cytotoxicity

Drug sensitivity was determined by a tetrazolium dye-based microtitration (MTT) assay that measures the number of viable cells.[21] All compounds with the required solubility were screened against human ovarian cell line A2780, and compared to the clinically used cisplatin and carboplatin, see Table 1. Their IC_{50} values, defined as the concentration of drug required to inhibit 50% of cancer cell growth, are given in μM.

It can be seen from the table that all the synthesised DIP molecules have an IC_{50} value within the range of cisplatin and carboplatin. As with DNA affinity, the sensitivity tests demonstrate a varying degree of cytotoxicity, depending on the functional group attached to the DIP core. The core DIP framework, **1**, shows moderate cytotoxicity towards this cancer cell line, whilst several DIP molecules display higher cytotoxicity than cisplatin. The addition of long chain, aliphatic regions onto the DIP molecule appears to greatly increase its cytotoxicity, for example, compound **10** has an IC_{50} value an order of magnitude less than compound **1**, however the specific length of aliphatic chain appears to be crucial to this value. Notably, molecules **27**–**29** are less toxic than their monomeric counterparts (**8**–**9**, **11**). An interesting result is obtained from isomers **25** and **26**, with one compound an order of magnitude more toxic than the other (ΔIC_{50}). As these isomers have the same molecular weight and hydrophilicity, it seems likely that this toxicity is

due to a third component other than simple intercalation, for example, a protein–DNA interaction,[22] enzyme inhibition,[23] cell membrane distortion,[24] or minor-groove binding. Compound **15** shows the lowest cytotoxcity, and comparison with **3** shows that changing the terminal methyl group to a hydroxy group significantly decrease the effects in vitro.

Molecular modelling

Molecular-modelling studies were performed to evaluate the binding mode of DIP with DNA and in general investigate if the R group can modulate the binding or be utilised in further molecular design. Fourteen DIP-R compounds, see Table 2, were examined due to the nature of the related R groups.

In general, the modelling studies indicated that the binding energy is modulated by the R group to a small extent and increasing the steric bulk can have two effects: i) a small amount of steric bulk appears to help position the DIP moiety into the helix and thus maximises the intercalation and ii) longer-chain R groups can also interact with the minor groove, which decreases the energy of binding,[25] see Figure 2.

Both of these effects are partially corroborated by the ITC studies. The interaction of the hydrophobic tails suspended from the DIP moiety with the minor groove is also shown in Figure 2, and this hydrophobic interaction should entropically aid binding; this is indicated experimentally by the high binding constants for the DIP-based molecules **3**–**5**, with long aliphatic chains (C_3–C_6), Table 1. These studies also offer the intriguing possibility that the introduction of further steric bulk into the R group may be able to modulate DNA intercalation; stronger binding could also be engineered by the addition of hydrogen-bond donor–acceptor groups on these chains.

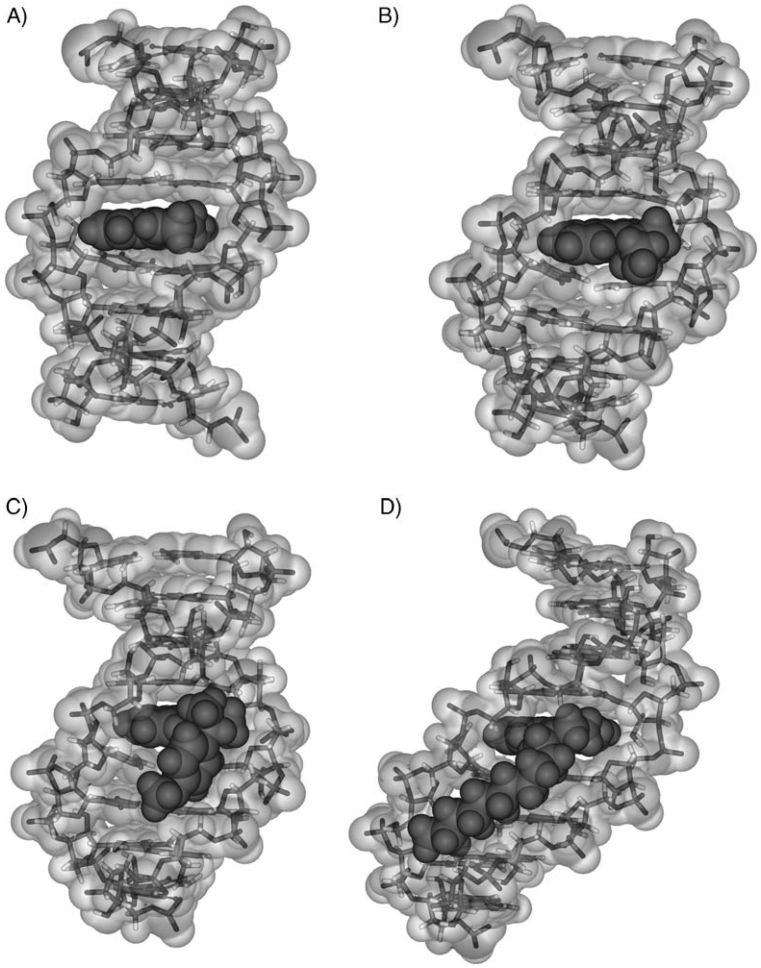


Figure 2. Plots of the energy-minimised models of the DIP compounds **1** (A), **15** (B), **22** (C) and **10** (D) docked into double-stranded B-DNA (5′-CCCCGGGG-3′). The DNA is shown in stick form with a transparent space-filling surface overlaid and the DIP-R is shown in a space-filling representation.

Table 2. Difference in energy between docked and undocked DIP into the DNA strand as a function of the R group.

DIP	DIP R; R =	$-\Delta E_{inter}$ complex [kcal mol^{-1}]
1	H	83
2	CH_3	94
3	$CH_2CH_2CH_3$	90
15	CH_2CH_2OH	101
19	CH_2-4-$C_6H_4OCH_3$	85
20	4-$C_6H_4OCH_3$	84
21	(*R*) $CH(CH_3)$-4-$C_6H_4OCH_3$	82
22	(*S*) $CH(CH_3)$-4-$C_6H_4OCH_3$	79
25	4-C_6H_4Bn	81
26	3-C_6H_4Bn	86
10	$(CH_2)_{11}CH_3$	57
14	$(CH_2)_{17}CH_3$	38
16	$(CH_2)_6OH$	66
17	$(CH_2)_3O(CH_2)_3CH_3$	69

Discussion

Although it would seem reasonable that an increase in DNA binding would directly lead to an increase in cytotoxicity, it is widely known that in general, there is no clear correlation between these two parameters,[3] and there are many examples of weak DNA binders showing good antitumour properties.[26–29] At this point it is important to highlight that although it has been shown here the DIP-based molecules do bind to DNA through intercalation, their actual mode of cytotoxicity has yet to be confirmed. However, it is interesting that the aliphatic chain length does make a difference. The increase in toxicity correlates with increasing chain length from compound **2**, (C_2) to the highest cytotoxicity for compound **10** (C_{12}); further, this toxicity decreases slightly for the increasing chain lengths to C_{18}. ITC and modelling studies clearly suggest that supplementary contributions to DNA binding could account for these observations, and it therefore appears that both intercalation and minor groove interactions are possible. The DIP aromatic framework acts as the intercalator, whilst the pendent R group interacts with the DNA minor groove. Exami-

nation of the lipophilicity against DNA affinity, see Figure 3, and lipophilicity against IC_{50}, see Figure 4, were plotted to aid this discussion.[30] The results presented in Figure 3 suggest that molecules with a very low lipophilicity are poorly correlated with IC_{50}. However, the trend is that more lipophilic molecules do bind more strongly to DNA. Furthermore, Figure 4 indicates that increasing lipophilicity generally increases cytotoxicity. Although all the DIP molecules with aliphatic side chains show generally high cytotoxicity, there is great variation within this subgroup of molecules, as with DNA affinity. In addition, molecules **1** and **10** were examined for potential cell membrane[31] interaction by conducting preliminary ITC measurements on a DMPC lipid bilayer vesicle, which was prepared by extrusion. This showed that molecule **1**, with no side chain, did not bind to the membrane whereas molecule **10**, with a C_{12} side chain did show some form of interaction to the membrane (see the Supporting Information). In fact, the chain length of the R group in compound **10** is ca.1.5 nm; this is the length necessary to insert in between the phospholipids of one layer. This could disrupt the permeability of the membrane, leading to "osmotic bursting" as in the case of many lytic peptides.[32] It should be noted here that this effect is nonspecific and could lead to general, unwanted toxic effects on nontumour cells.

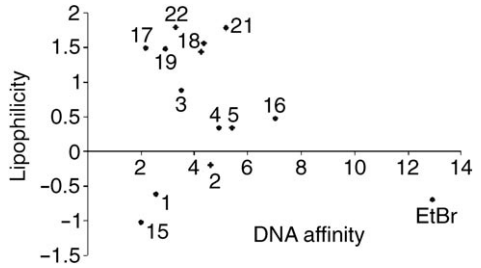


Figure 3. Graph showing effect of lipophilicity (log *P*) on DNA affinity ($K \times 10^4\ M^{-1}$).

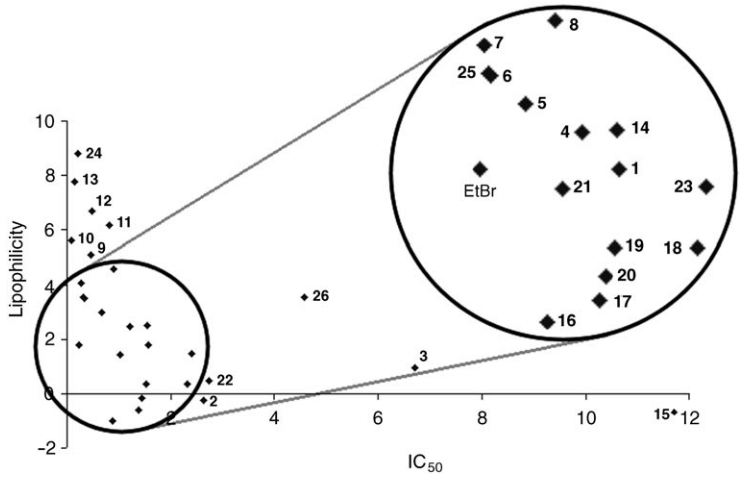


Figure 4. Graph showing effect of lipophilicity (log *P*) on IC_{50} [μM].

The modelling studies demonstrate that one possible reason for good in vitro cytotoxicity arises from the hydrophobic nature of the DIP-R group. Indeed, many potent anticancer drugs, such as daunorubicin, bind to DNA not only through intercalation but also through minor-groove binding.[33] As our results indicate that compound **25** is much more toxic than its isomer, it is possible that the spatial arrangement of this molecule allows for better binding within the minor groove. This result also supports the idea that these molecules are involved in alternative modes of binding besides intercalation. It is unsurprising that the chiral compounds **21** and **22** vary in toxicity due to the chiral nature of DNA, however modelling studies did not convincingly reveal if variations in the DNA binding result from the different absolute configurations.

Conclusion

Applying the "one-pot" methodology to dihydroimidazophenanthridinium bromide from 2-bromoethylphenanthridinium bromide, and a library of primary amines, has allowed us to generate a structurally diverse library of compounds. The range of DIPs studied allows us to postulate that the R groups of the DIP compounds also interact with DNA through minor-groove binding, as shown by ITC and investigated by preliminary modelling studies. These studies show that the DIP-R group can subtly and effectively modulate DNA binding. Further, we have shown that DIPs with high binding constants also have low IC_{50} values in ovarian cancer cell lines. Therefore we will aim, in future work, to use modelling, molecular design and evaluation of binding/IC_{50} values in synergy to design new DIP-based DNA binders. Possible strategies include changing the intrinsic affinity of the DIP framework for DNA (for instance the inclusion of groups capable of hydrogen bonding onto the polyaromatic framework) as well as choosing R groups that also interact with the DNA minor groove more effectively. In further studies we also aim to examine the DNA–DIP interactions in more detail using solution NMR, crystallography and circular dichroism.

Experimental Section

DIP-based ligands **1**–**29** were synthesised, purified and characterised by following our established techniques.[12,13] Other reagents, purchased from Sigma–Aldrich and used without further purification, were as follows: salmon testes DNA ("ST-DNA", D1626), ethidium bromide ("EtBr", E8751), netropsin hydrochloride (N9653). All solutions were prepared in pH 7.0 phosphate buffered saline (12 mM Na_2HPO_4, 4 mM NaH_2PO_4, 1 mM EDTA, 0.2 M NaCl). Concentrations were determined by weight (for DIP ligands) or from UV absorbance (for DNA, expressed per mole of base pairs) by using the following extinction coefficients: $\varepsilon_{260}(DNA) = 12\,824\ Mbp^{-1}\,cm^{-1}$, $\varepsilon_{480}(EtBr) = 5600\ M^{-1}\,cm^{-1}$, $\varepsilon_{296}(netropsin) = 21\,500\ M^{-1}\,cm^{-1}$. The vesicles for the membrane study were prepared as follows: 1 mg mL^{-1} DMPC (dimyristoylphosphatidylcholine) dispersion was prepared in buffer (12 mM Na_2HPO_4, 4 mM

NaH_2PO_4, 1 mM EDTA, 0.1 M NaCl, pH 7) by continuous stirring and vortexing over a period of 4 h. Unilamellar vesicles with a diameter of 100 nm were prepared by extrusion by using an Avanti Polar Lipids Mini-Extruder. A total of 11 passes through a 0.1 μm Nucleopore Polycarbonate membrane were performed.

Isothermal titration calorimetry: DNA–ligand complexation thermodynamics in solution were measured by isothermal titration calorimetry (MicroCal VP-ITC) in the 10–40 °C temperature range following standard instrumental procedures.[35,36] A typical experiment involved an initial 1 μL preinjection followed by 25–30 sequential 10 μL injections of ligand solution (ca. 1 mM) into the ITC cell containing DNA (ca. 0.3 mM base pairs, 1.4 mL working volume, 320 rpm stirring). Control experiments involved identical injections into buffer alone for ligand dilution heats. Titration data were corrected for dilution heats and analysed by using a single-set-of-sites equilibrium binding model (MicroCal Origin™) to give the apparent binding stoichiometry (N), association/dissociation constants ($K_A = 1/K_D$). Other thermodynamic quantities were calculated by using standard expressions: $\Delta G^\circ = -RT\ln K_A = \Delta H^0 - T\Delta S^\circ$; $\Delta C_p = d\Delta H^0/dT$; 1 cal = 4.184 J. Membrane binding studies: 1 mM solutions of the compounds were made in an identical buffer to that of the vesicles (determined by weight), sonicated then incubated at 50 °C for the duration of the experiment.

MTT assays: Drug cytotoxicity was determined by a tetrazolium dye-based microtitration assay.[21] Human ovarian cancer cell line *A2780*,[37] was plated out in 96 well plates at a density of 500–1000 cells per well and allowed to attach and grow for 2 days. Cells were exposed to the drug at a range of concentrations for 24 h and the medium replaced with drug-free medium for a further 3 days. On the final day MTT, (50 μL of a 5 mg mL^{-1} solution), was added to the 200 μL of medium in each well and the plates incubated at 37 °C for 4 h in the dark. Medium and dead cells were removed and the MTT formazan crystals dissolved in 200 μL DMSO. Glycine buffer (25 μL per well, 0.1 M, pH 10.5) was added and the absorbance measured at 570 nm in a multiwell plate reader. A typical dose–response curve consisted of 8 drug concentrations with 2 wells used per drug concentration. Results were obtained from three independent reactions ($n = 3$) and are expressed in terms of the drug concentration required to kill 50% of the cell (IC_{50}), estimated as the absorbance value equal to 50% of that of the control untreated wells.

Computational methodologies: Calculations were preformed on a Pentium Dual Core PC with 2 GB RAM by using Hyperchem 7.52.[38] AMBER force field parameters were used for the nucleic acid interactions.[39] The models were constructed by using the HyperChem programme. The double-stranded B-DNA (5′-CCCCGGGG-3′) was generated by using the nucleic acid feature in the database of HyperChem and the intercalation site initially defined by manual docking of the DIP moiety. The DNA–DIP complex was initially regularised by conjugate-gradient molecular modelling to reduce poor intermolecular steric contacts so as to minimise the energy of the bound ligand alone and for minimisation of the unrestrained complex to an energy gradient of < 0.1 kcal Å^{-1} mol^{-1}. Molecular dynamics (MD) relaxation of the DNA complex was subsequently performed for 2 ps (integration time step = 1 fs) at 300 K. Potential energy analysis during molecular dynamics progress showed that the systems reached equilibrium rapidly within 2 ps and a final molecular mechanics relaxation was done. A distance dependent dielectric constant was used as solvent and counterions were not explicitly included due to computational expense. The models were geometrically minimised by using Polak–Ribiere conjugate-gradient minimisation. Convergence was defined when the gradient of the average root mean square (RMS) shift reached 0.1 kcal Å^{-1} mol^{-1}. Starting geometries for all the DIP molecules before docking were based upon crystallographic coordinates of the main moiety,[12] the various R groups were added manually and the geometry was relaxed by using molecular mechanics geometry optimisations (Polak–Ribiere conjugate-gradient minimisation with convergence criteria as above). The apparent relative interaction energies of the DIPs and DNA were calculated as the energy of the complex minus the energy of the ligand, minus the energy of the DNA, as shown in Equation (1).[40]

$$\Delta E_{inter} = E_{(complex)} - (E_{(L)} + E_{(rDNA)}) \quad (1)$$

Acknowledgements

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Keywords: cytotoxicity · DNA binding agents · drug design · heterocyclic cations · molecular modeling · structure–activity relationships

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