A new phenanthroline–oxazine ligand: synthesis, coordination chemistry and atypical DNA binding interaction†

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1,10-Phenanthroline-5,6-dione and l-tyrosine methyl ester react to form phenanthroline-oxazine (PDT) from which [Cu(PDT)2][ClO4]2 and [Ag(PDT)2]ClO4 2MeOH are obtained. Binding to calf-thymus DNA by Ag(i) and Cu(ii) PDT complexes exceed bis-1,10-phenanthroline analogues and the minor groove binding drugs, pentamidine and netropsin. Furthermore, unlike the artificial metallonuclease, [Cu(phen)2]2+, the [Cu(PDT)2]2+ complex does not cleave DNA in the presence of added reductant indicating unique interaction with DNA.

1,10-Phenanthroline (phen), its organic derivatives and the plethora of metal complexes containing these N,N-chelating heterocycles, find use as optical devices,1,2 catalysts3 and as integral components of supramolecular structures.4 In addition, these compounds have found application in the biological field as antimicrobial and anti-cancer agents,5 DNA intercalators,6 and as nucleoside constituents for incorporation into the DNA backbone.7

The quest to make new and tailored phenanthrolines is ongoing, and methods include synthesising the bases from elementary building blocks8–10 and extending existing, functionalised phenanthrolines.11 In the latter context, the quinone, 1,10-phenanthroline-5,6-dione (phen dio), has been used as the starting material for Schiff base condensation reactions with primary amines.7,9–14 How ever, not all reactions of phendio with primary amines have resulted in the formation of phen dio from which [Cu(PDT)2][ClO4]2 and [Ag(PDT)2]ClO4 2MeOH are obtained. Binding to calf-thymus DNA by Ag(i) and Cu(ii) PDT complexes exceed bis-1,10-phenanthroline analogues and the minor groove binding drugs, pentamidine and netropsin. Furthermore, unlike the artificial metallonuclease, [Cu(phen)2]2+, the [Cu(PDT)2]2+ complex does not cleave DNA in the presence of added reductant indicating unique interaction with DNA.

Generally, phendio and its Ag(i) and Cu(ii) complexes are considerably more biologically active than phen and its corresponding metal complexes.5,6 With this in mind, we sought to improve biological activity, selectivity and compatibility by attempting to prepare a double Schiff base, phen-type ligand by reacting phendio with two equivalents of (S)-methyl 2-amino-3-(4-hydroxyphenyl)-propanoate (l-tyrosine methyl ester).

Phendio was initially treated with l-tyrosine methyl ester (1 : 2 mol ratio) in anticipation of forming a double Schiff base condensation product (Scheme 1). Unexpectedly, this combination produced 1,10-phenanthroline-5,6-diol and a relatively low yield of the new orange-yellow tetracyclic oxazine, PDT. The structure of PDT:MeOH (Fig. 1) showed that chirality within the compound is retained, with C13 being the stereogenic centre (originally it was C20). The molecule crystallises in the centrosymmetric space group, P21/c, and so is a racemic mixture. Interestingly, the phenol ring is almost orthogonal to the oxazine ring (bond angle 89.12(2) ) forming a ‘chair’ profile. The pdt me molecules are linked into zig-zag chains via hydrogen bonding through the methanol solvate; the phenol group makes a H-bond to the oxygen of the methanol (2.6288(18) Å), and the methanol OH makes an unsymmetric, bifurcated H-bond to the phenanthroline nitrogen atoms of a second molecule (3.028(2) and 2.907(2) Å to N1 and N2, respectively, under symmetry operation −x + 2, y − 1/2, −z + 3/2).

What is the mechanism of the cyclization reaction that leads to the formation of the oxazine ring in pdt me? In their paper on the oxidation of l-ami no acids by quinones, Lourak and co-workers17 obtained cyclic derivatives containing oxazine rings. These researchers

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**Scheme 1** Proposed reaction mechanism of phendio with l-tyrosine methyl ester leading to the formation of PDT.
proposed a mechanism for their reaction in which a second quinine molecule acted as a dehydrogenating agent to give the cyclic product. In the present work, the isolation of 1,10-phenanthroline-5,6-diol as a by-product of the reaction suggested that reduction of some phenedio was occurring as part of the reaction mechanism. A likely first step is the formation of a Schiff base (molecule A in Scheme 1). A plausible, concerted mechanism for formation of the oxazine ring is offered in Scheme 1 and involves the participation of a second phendio molecule as a dehydrogenating agent. Due to steric restraints, resulting from both A and phenedio, this reaction is expected to be slow, as was observed.

Interestingly, in the preparation of the phenedio-amino acid ternary complexes, [Cu(phenido)-L-Phe](H2O)2ClO4 and [Ni(phenido)-Gly][H2O]ClO4·H2O, no interaction between the phendio carbonyl functions and the amine group of the amino acid was reported.18 Reaction of the cation in [Cu(phen)2](ClO4)2 ([CuPDT]2+), was obtained in moderate yield. Again, it is believed that [CuPDT]2+ is structurally similar to the known tetrahedral geometry. In comparison to the 1H NMR spectrum of metal-free phen, the spectrum of CuPDT showed shifts in the signals associated with the phen part of the pdtme molecule rather than the ester part of the molecule, indicating that the Ag+ ion is chelated by the two nitrogen atoms. In a similar reaction using Cu(ClO4)2·6H2O, the green solid, [CuPDT](H2O)2ClO4 (CuPhen), was obtained in moderate yield. Again, it is believed that [CuPDT]2+ is structurally similar to the known tetrahedral cation in [Cu(phen)]2+ClO42− (CuPDT).20

In an effort to establish how the metal-free PDT ligand and its Cu(n) and Ag(i) complexes interact with DNA four distinct assays were conducted. To establish an apparent DNA binding constant (Kapp) a sample of high-purity calf thymus (CT) DNA is firstly treated with an excess of EtBr and then this highly fluorescent, EtBr-saturated DNA sample is exposed to a range of concentrations of the competitor test compound. A reduction in fluorescence indicates ejection of the bound EtBr from the DNA backbone and replacement by the test species. Samples are compared based on their Kapp values, which are calculated from the concentration of sample required to accomplish a 50% reduction of the initial fluorescence (Fig. 2 and Table 1). Metal-free PDT, phen and the simple metal salts all showed considerably less DNA binding affinities than AgPDTr and CuPDT, which have almost identical Kapp values. The Ag(i) and Cu(n) PDT complexes also showed a higher binding capacity than the known groove-binding drugs, pentamidine and netropsin.

Table 1  Apparent DNA binding constants (Kapp) of the test compounds and fluorescence quenching (Q) values

The binding of metal-free PDT to DNA is significantly stronger than that of metal-free phen, suggesting that the additional functionalities on the backbone provide secondary binding interactions with the nucleic acid. The contribution of these extra interactions is also apparent when the binding constants of PDT and phen metal complexes are compared (Kapp for AgPDT is ~3 times larger than AgPhen and CuPDT is ~11 times that of CuPhen).

Competitive displacement of DNA-bound Hoechst 33258 (minor groove binder) and ethidium cations (Et+, intercalator) was assessed using fluorescence quenching, which utilises an unsaturated dye-DNA combination in which a limited number of binding spaces on the DNA polymer are occupied at any one time by the interacting dye, which strongly fluoresces once bound with a relatively high number of unoccupied sites remaining for test compound interaction. The introduction of a new compound to the DNA may then displace Hoechst or Et+ either directly or indirectly (i.e. through a conformational change which results in dye ejection) and can give valuable information regarding a binding interaction mode,21 although, caution must be exercised here considering the binding constant and binding stoichiometry of ethidium bromide and Hoechst to CT-DNA. The minor groove binders, pentamidine and netropsin, displayed high quenching affinity (Q) for Hoechst 33258 bound DNA and were, as expected, an order of magnitude less effective in their quenching of ethidium (Table 1). Both AgPhen and CuPDT displayed higher Q values toward Et+ than compared with Hoechst. Interestingly, both AgPDT and CuPDT displayed higher Q values, along with different profiles,17 and were almost identical in their ejection of bound Hoechst and Et+. From these quenching data it is evident that the
The Cu(II)/Cu(I) redox couple was centred around in their efficacy as DNA nuclease agents (Fig. 3 and Fig. S4–S9).\[Cu(phen)\textsubscript{2}\]^2+. This dication renders almost complete degradation e.g. between DNA bases, while surface binding species (dsDNA (1 mM in DNAp) (Fig. 3). Intercalating agents an alternate binding mode compared with their bis-phen analogues. PDT inhibition of pBR322 cleavage (Fig. S3). The redox behaviour of both complexes, indicative of a more facile electron transfer regime. The original Cu(a) reduction peak for CuPDT is observed at \(-0.47\) V but, interestingly, is not evident at scan rates \(\geq 40\) mV s\(^{-1}\), which suggests a transient species. While CuPDT tends toward reversibility in the presence of ascorbate (AE\(_{pK} = 65\) mV), CuPDT remains quasi-reversible (AE\(_{pK} = 119\) mV, Table S1, ESI\(^{†}\)). Thus, the reversibility of the Cu\(^{2+}$$\text{Cu}^{+}\) couple may justify the greater DNA cleavage ability of CuPDT.

For concluding remarks please see ESI.\(^{†}\)

Fig. 3 Relative viscosity changes in salmon testes dsDNA (left) and cyclic voltammograms describing the redox behavior of 1 mM CuPhen (centre) and CuPDT (right), at a scan rate of 100 mV s\(^{-1}\), in the absence (solid black trace) and presence (dashed black trace) of 2 mM ascorbate.\(^{†}\)

Notes and references