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Alkyl-linked diquinolines are monofunctional AT-selective DNA-intercalating agents

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The binding of a homologous series of alkyl-linked 4-aminodiquinolines to circular and linear DNAs was studied using viscometric titrations and equilibrium dialysis. The compounds are monofunctional intercalators with the capacity for intercalative binding reaching a peak for the heptane homologue. They show marked AT-base pair selectivity, which suggests that the non-intercalated quinoline ring may lie in the DNA minor groove. Affinities for calf thymus DNA increase as the alkyl chain is lengthened, falling in the range 6 to $>250 \times 10^4$ M^{-1} in a buffer of I 0.01. The association constant of the heptane diquinoline decreases with increasing ionic strength, 2.1 cations being released per bound dipositive ligand molecule. All the agents are growth inhibitory towards mouse leukemia cells in culture with IC_{50} values in the range 0.06–18 μM.

Alkyl-linked diquinoline; DNA binding; Intercalation; Cell growth inhibition

1. INTRODUCTION

Many compounds which bind to DNA by intercalation are useful chemotherapeutic agents and much effort has gone into defining the structural requirements for intercalative binding. It has generally been accepted that three fused aromatic rings constitute an optimum flat area for intercalation, and aminoacridines, for example, are archetypal DNA-intercalating ligands. In contrast, quinolines like chloroquine are poor intercalators [1]. Among the many studies of acridines are those (review [2]) that have established the necessary structural requirements for bifunctional intercalation of 9-aminoacridine dimers, and the *N,N'*-bis-(4-acridinyl)-α,ω-diaminoalkanes [2] stand as type-specific bisintercalating agents. Interestingly, the bisintercalating members of this series have antitumour activity whereas the monofunctional dimers and 9-aminoacridine do not [2]. The quinoline analogues of these compounds have also been synthesized, and have been evaluated as antibacterial and antileprotic agents [3]. In addition, they are known to be potent and selective ligands for α-adrenoceptors [4]. The structurally related, ring-substituted, bisquinaldines have antimalarial [5] and antitumour activity [6]. Despite the chemotherapeutic properties of binuclear quinolines, and evidence that they interfere with nucleic acid metabolism [2], no detailed study of their binding to DNA has been reported. Here we examine the mode, strength and specificity of DNA binding of the homologous series of *N,N'*bis-(4-quinolyl)-α,ω-diaminoalkanes (fig.1), in order both to evaluate the importance of chromophore surface area as a determinant of the capacity to bisintercalate and to investigate the relationship between biological activity and the mode of binding for diquinolines.

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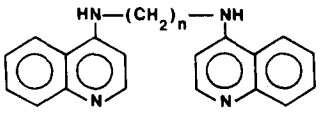

Fig.1. Structure of the *N,N'*-bis(4-quinolyl)-α,ω-diaminoalkanes. Compounds are referred to by the pseudonym C_n where n is the number of carbon atoms in the linker chain.

2. MATERIALS AND METHODS

Experiments were conducted in Hepes/NaOH buffers at pH 7.0 containing 2 mM Hepes, 10 μM EDTA and sufficient NaCl to yield the required ionic strength. This solvent is referred to below as SHE buffer. Calf thymus and bacterial DNAs were purchased from Sigma. A strain of the *E. coli* bacterium bearing the plasmid pNZ116 (miniF inserted at the *Eco*RI site in pBR322, 50% G+C) was provided by Professor H.E.D. Lane, Auckland University, and closed circular DNA prepared from it by standard procedures. DNA concentrations were determined with respect to base pairs using an E_{260} value of 13 200 $M^{-1} \cdot cm^{-1}$, and all binding ratios *(r)* and affinity constants are expressed in base pair units. The bisquinolines were synthesized and purified as the crystalline hydrated dihydrochlorides following the method of Deshpande and Singh [3]. Full synthetic details will be reported elsewhere. Binding affinities and sequence selectivities were measured by equilibrium dialysis using established procedures [7]. When determining binding isotherms, free ligand concentrations were measured spectrophotometrically using the molar absorption coefficients shown in table 1. On the DNA side of the dialysis cell the complex was dissociated by adding SDS to a final concentration of 1% and the total drug concentration determined using the molar absorption coefficients for 1% SDS-buffer mixtures listed in table 1. Dialysis equilibrium was established within 20 h at 20°C. Viscometric titrations were performed according to published methods [7]. Sonicated calf thymus DNA at a concentration of 300 μM was used in the helix extension measurements. When measuring helix unwinding angles pNZ116 DNA was used at the lower concentration of 100 μM. The ability of the compounds to inhibit the growth of murine leukaemia L1210 cells in culture was assessed as described [8].

3. RESULTS

The spectroscopic data reported in table 1 reveal bathochromic shifts in ligand absorption spectra on binding to calf thymus DNA in a buffer of I 0.01. For 4-aminoquinoline and the C6–C10 diquinolines this is accompanied by hypochromicity in each spectral band, whereas for the lower homologues in the series both hypo- and hyper-

Table 1

Spectral properties, association constants and specificity data for the diquinolines

Compound	Free ligand		DNA-bound		SHE/1% SDS		Binding constant $K_{(0)} \times 10^{-4}$ (M^{-1})	Specificity α
	λ (nm)	$\epsilon \times 10^{-4}$ ($M^{-1} \cdot cm^{-1}$)	λ (nm)	$\epsilon \times 10^{-4}$ ($M^{-1} \cdot cm^{-1}$)	λ (nm)	$\epsilon \times 10^{-4}$ ($M^{-1} \cdot cm^{-1}$)		
4-Aminoquinoline								
	335	1.13	336	0.765	337	1.18	0.5	–
	322	1.26	323	0.802				
C2	344	1.91	346	2.19	346	3.86	6.0	0.25
	329	1.79	335	1.87				
C4	342	1.28	343	1.77	345	3.16	6.0	0.20
	327	2.00	330	1.71				
C5	342	1.72	343	2.00	343	3.54	6.0	–
	326	2.56	330	1.92				
C6	342	1.78	343	1.74	343	2.81	10	0.22
	327	2.23	330	1.69				
C7	342	2.89	343	2.18	343	3.91	160 (0.01)	0.55
	328	3.05	331	2.13			2.3 (0.1)	
							0.26 (0.3)	
C8	342	2.49	344	1.80	343	2.91	>250	0.35
	328	2.58	331	1.76				
C9	343	3.22	343	2.58	343	4.24	>250	–
	328	3.24	331	2.19				
C10	343	2.39	344	1.44	343	2.89	>250	0.50
	329	2.65	332	1.35				

Molar absorption coefficients were measured in a 0.01 SHE buffer at a ligand concentration of 5 μM. Spectra of the ligands bound to calf thymus DNA were recorded at $r = 0.025$. Binding measurements were conducted in a 0.01 SHE buffer at 20°C, unless otherwise indicated, and α values determined in a 0.1 SHE buffer at 20°C

chromic effects are found. The C7 diquinoline showed no deviation from Beer's Law up to 1 mM at the ionic strengths used for binding studies. This indicates that intermolecular aggregation, a feature of numerous heteroaromatic chromophores such as acridines [9] and phenanthridines [10], does not occur to any significant extent. Similarly, solutions of 4-aminoquinoline also showed no evidence of aggregation under these conditions.

Scatchard plots for the interaction of C7 diquinoline with calf thymus DNA in SHE buffers of I 0.01, 0.1 and 0.3 at 20°C are shown in fig.2. The $K_{(o)}$ values at I 0.1 and I 0.3 of 2.3×10^4 M^{-1} and 0.26×10^4 M^{-1} respectively, were derived from a linear least-squares fit to eqn 10 of McGhee and Von Hippel [11]. The data at I 0.01 are not amenable to this analysis which corresponds to a simple excluded site model. In this situation the intrinsic association constant to an isolated binding site $K_{(o)}$, can be estimated by extrapolation to the ordinate axis [11] yielding a value for $K_{(o)}$ of 160×10^4 M^{-1} at I 0.01. Affinity is weakened by an

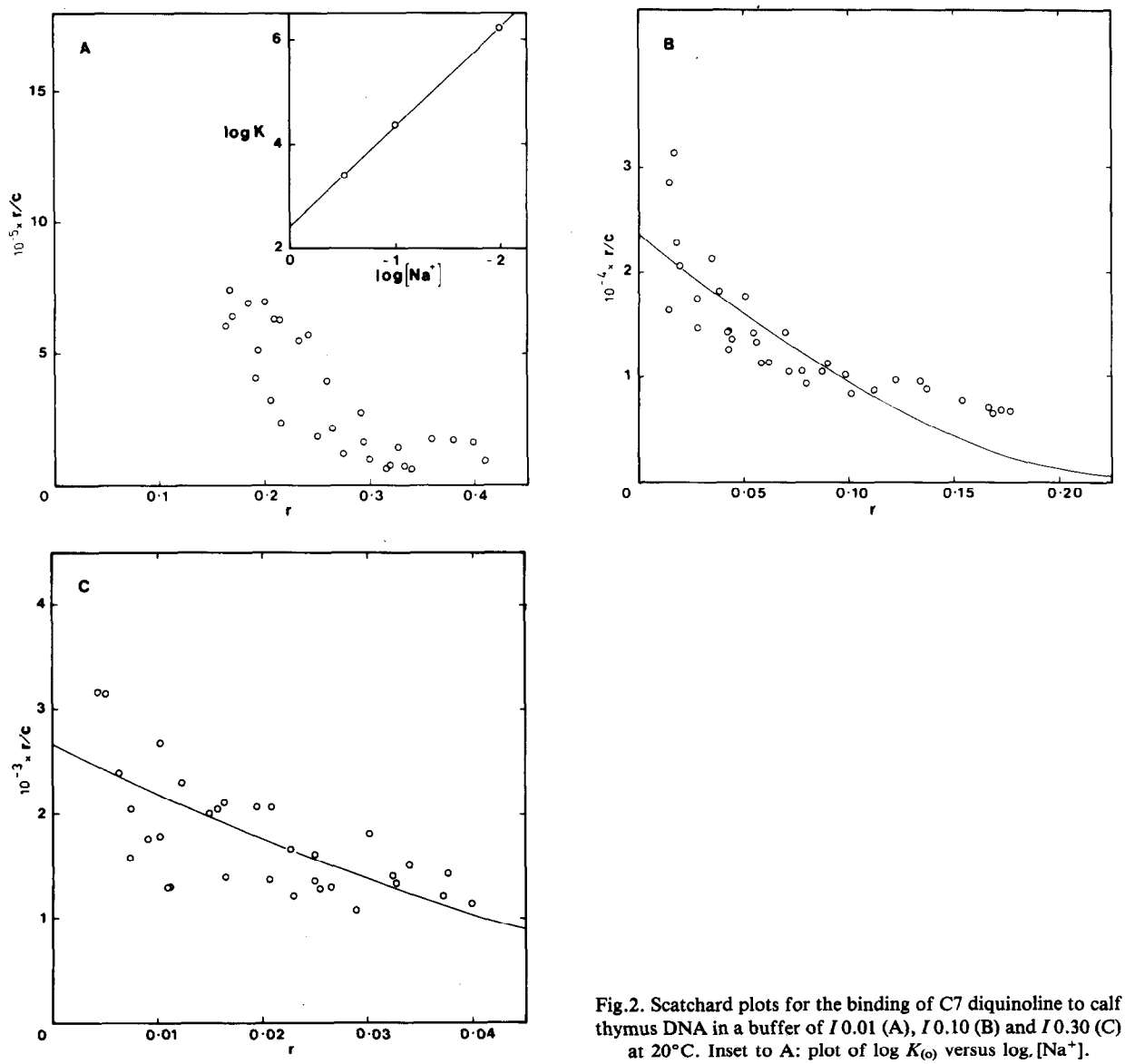

Fig.2. Scatchard plots for the binding of C7 diquinoline to calf thymus DNA in a buffer of I 0.01 (A), I 0.10 (B) and I 0.30 (C) at 20°C. Inset to A: plot of log $K_{(o)}$ versus log. $[Na^+]$.

increase in cation concentration and a linear relationship between log $K_{(o)}$ and log $[Na^+]$ is observed (fig.2A) consistent with polyelectrolyte theory as developed by Friedman and Manning [12] and Record et al. [13]. The latter treatment may be used to estimate whether binding of the dicationic C7 ligand takes place with the concomitant release of 2.1 Na^+ from the condensation sheath of DNA. Binding parameters for 4-aminoquinoline and the other bisquinolines were estimated in the same way (not shown) and the $K_{(o)}$ values so obtained at I 0.01 and 20°C are recorded in table 1. Across the series there is a marked increase in association constant with increasing chain length: notable jumps in affinity occur for C2 diquinoline compared to 4-aminoquinoline and for C7 diquinoline compared to the C6 and lower homologues.

The nucleotide sequence selectivity of the diquinolines was assessed by partition dialysis using DNAs from *Micrococcus lysodeikticus* DNA (72% G+C) and *Clostridium perfringens* (30% G+C) in a buffer of I 0.1. The parameter α' [14], the ratio of the r values for the two DNAs in equilibrium with the same free drug concentration, was determined at three ligand to DNA input ratios for each compound. The plot of α' versus r for *M. lysodeikticus* DNA was extrapolated to r = 0 to yield α, which is the ratio of the affinity for *M. lysodeikticus* DNA to the affinity for *Cl. perfringens* DNA [14]. α' values were obtained at binding ratios up to 0.03 and are found to decrease as r approaches zero, except in the case of C10 diquinoline where α' is independent of the degree of binding. The measured α values are presented in table 1 and indicate that the diquinolines show a marked AT-selectivity. Where optimum binding requires one AT base pair at the binding site α is theoretically equal to the ratio of the A+T content of *M. lysodeikticus* DNA to that of *Cl. perfringens* DNA (0.4). If two AT pairs were required α would be equal to the ratio of the square of the A+T content of the respective DNAs (0.16). The α values found for C2, C4 and C6 diquinoline suggest that at least some binding sites for these ligands contain more than one AT base pair. Binding of 4-aminoquinoline was undetectable in a buffer of I 0.1.

The capacity of 4-aminoquinoline and its dimers to intercalate was determined by their ability to increase the contour length of sonicated calf thymus DNA fragments, and to remove and reverse the supercoiling of covalently closed circular DNA in a buffer of I 0.01. Helix extension parameters and unwinding angles are reported in table 2. Measurements were made in duplicate for each ligand, and input ratios in helix extension plots

Table 2

Intercalation parameters and growth inhibition properties of diquinolines

Compound	Helix extension parameter	Helix unwinding angle (°)	Growth inhibition, IC_{50} (μM)
4AQ	0.60 ± 0.10	8.0 ± 0.5	>25
C2	0.25 ± 0.05	11.5 ± 0.5	18
C3	–	–	15
C4	0.25 ± 0.05	5.2 ± 0.5	6.5
C5	–	–	0.83
C6	0.28 ± 0.05	5.5 ± 0.5	0.65
C7	0.36 ± 0.05	9.4 ± 0.5	0.66
C8	0.35 ± 0.05	4.7 ± 0.5	0.12
C10	0.16 ± 0.05	5.5 ± 0.5	0.063

Helix extension parameter is the slope of the plot of L/L_0 vs r, where L/L_0 is the ratio of the contour length of calf thymus DNA in the presence of the drug to the contour length in the absence of the ligand. Theoretically, an ideal monofunctional intercalator would give a plot with slope equal to 1. Measurements were made in a buffer of I 0.01. Helix unwinding angles are expressed with respect to an equivalence binding ratio of 0.072, and an unwinding angle of 26°, for ethidium. Measurements were made in a buffer of I 0.01. IC_{50} is the micromolar concentration of ligand that when added to cultures of L1210 cells for a period of 70 h reduces cell numbers to 50% of control cultures

were corrected to binding ratios using the measured binding isotherms. With the exception of that for 4-aminoquinoline, helix extension plots are linear up to r values about 0.3 (not shown). The plot for 4-aminoquinoline becomes curvilinear, concave downward, above a binding ratio of 0.1. Representative data for the interaction of the C7 diquinoline with closed circular DNA are shown in fig.3. The rise in reduced viscosity to about 27 dl/g for the fully relaxed circular DNA complex typifies the magnitude of the change caused by the other diquinolines. Binding ratios causing complete relaxation of supercoiling (termed the equivalence binding ratio) were deduced by correcting the appropriate drug to DNA input ratios using the binding isotherm for calf thymus DNA. Unwinding angles were calculated by comparison with ethidium whose unwinding angle is 26° [15], and whose equivalence binding ratio is 0.072 for this DNA under these experimental conditions [15]. The greatest helix extension of 2.0 Å per bound ligand molecule (assuming a slope parameter of 1 is equivalent to 3.4 Å), is found for 4-aminoquinoline; C7 and C8 diquinoline increase contour length by 1.2 Å; the C2, C4 and C6 dimers by 0.9 Å; and the C10 homologue by only 0.5 Å. Similarly, helix unwinding angles fall into definable groups; those of 4-aminoquinoline, C2 and C7 diquinoline lie in the vicinity of 10°,

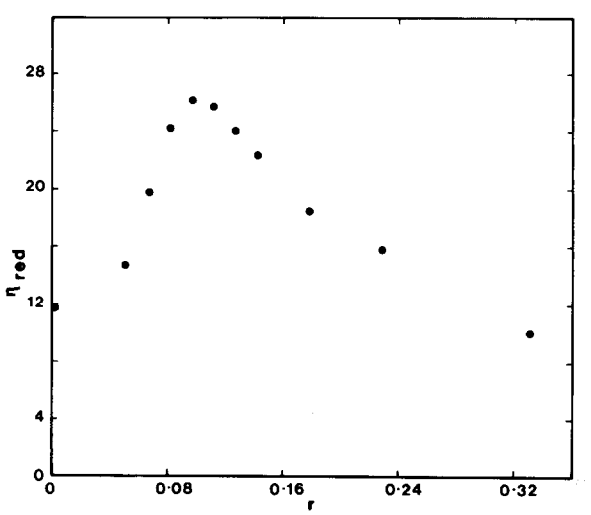

Fig.3. Effects of C7 diquinoline on the reduced viscosity of covalently closed circular pNZ116 DNA. The ordinate shows the reduced viscosity in dl/g and the abscissa the drug to DNA binding ratio expressed in base pairs.

whereas those of the remaining compounds cluster around 5°.

The diquinolines are growth inhibitory towards L1210 murine leukemia cells in culture, and their potency (IC_{50} values in the range of 18–0.06 μM) increases monotonically with increasing DNA affinity and lipophilicity (see table 2).

4. DISCUSSION

The results of the viscometric and dialysis measurements clearly reveal 4-aminoquinoline to be a weakly binding intercalating agent. Its unwinding angle and extension parameter are indistinguishable from those observed for chloroquine [1], whose intercalative mode of binding has been confirmed by NMR spectroscopy [16]. However, compared to its acridine analogue, 9-aminoacridine, in a buffer of I 0.01 its affinity is reduced 60-fold, its unwinding angle is halved and its binding induces only two-thirds of the increase in contour length [17,18]. In addition, given that the helix extension plot for 4-aminoquinoline is strongly bent above binding ratios of 0.1, and the fact that the measured binding isotherm reaches binding levels up to 0.63 at r/c values well above zero, it appears that the quinoline also binds by external attachment to the DNA duplex. If so, the measured unwinding angle of 4-aminoquinoline may depend upon the binding ratio needed to reach equivalence in the circular DNA assay, and could thus appear to be a function of initial superhelix density (see [1] for a related argument concerning the ionic strength dependence of unwinding angles for quinolines). Hence, our value of 8° should be viewed as a lower limit since the equivalence point occurred at an r value well into the bent portion of the extension plot.

Given the observed range of helix unwinding angles and extension parameters, it is clear that no member of this homologous series of diquinolines has the capacity for bifunctional intercalation. This is in stark contrast to the analogous diacridine series in which bisintercalation is found for the C6 and higher homologues [19]. Indeed, the actual values of the unwinding and extension parameters suggest that the C2 and C7 diquinolines intercalate monofunctionally, whereas the other derivatives are poor intercalators and interact with a fair proportion bound wholly externally. Unwinding

angles of a similar magnitude to those of C2 and C7 diquinoline have been reported for a bis-4-anilinoquinoline [20] and for the antibacterial agent dequalinium [21], a dimer comprising 4-aminoquinaldine chromophores linked via ring nitrogens with a decane bridge. The small helix extension caused by monointercalated C2 and C7 diquinoline compared to 4-aminoquinoline is presumably related to DNA bending, kinking or base pair tilting at the binding site [22]. Since AT-base pair selectivity is a prominent feature of minor groove binding ligands [22], it seems probable that the selectivity of the diquinolines is a consequence of the non-intercalated chromophore interacting favourably with the narrow minor groove of AT-rich sequences. In this respect it is noteworthy that the bisintercalating diacridines show no sequence preferences [7]. Although the origins of the enhanced affinity of C2 diquinoline compared to 4-aminoquinoline are self evident (dication compared to monocation, increased van der Waals interactions, etc.), the reason for the jump in affinity for the C7 and higher homologues is less obvious to us. Speculation on this point seems inappropriate in the absence of further structural information about the drug-DNA complexes. The efficacy of the diquinolines in inhibiting the growth of leukaemia cells shows no obvious relationship to ther intercalative capacity. This again contrasts with the behaviour of the diacridines which show a sharp increase in potency for the bisintercalating derivatives [2].

While virtually all cationic compounds containing an acridine chromophore intercalate into DNA, the binding mode of ligands possessing a quinoline ring is much more sensitive to molecular structure: thus, 4-aminoquinoline and chloroquine intercalate [1,16], whereas 4-anilinoquinoline does not [20]. Moreover, when the flexible polymethylene linker chain of the diquinolines is replaced by a rigid cyclic depsipeptide, as is the case in the bisquinoline analogue of echinomycin [23] and luzopeptin [24] bisintercalation occurs. Our findings presented here, along with those of other workers, indicate that the quinoline ring is poised on the edge between intercalative and non-intercalative binding. At present we are investigating the effects of chromophore substituents, as well as the introduction of rigid elements into the linker chain, on diquinoline binding mode.

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