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NUCLEIC ACID BINDING DRUGS—XIV

THE CRYSTAL STRUCTURE OF 1-METHYL AMSACRINE HYDROCHLORIDE; RELATIONSHIPS TO DNA-BINDING ABILITY AND ANTI-TUMOUR ACTIVITY

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Abstract—The crystal structure of the 1-methyl derivative of the anticancer drug amsacrine [4′-(acridin-9-ylamino)-3′-methoxy-methanesulphonanilide] as its hydrochloride salt has been determined. The compound crystallizes in the monoclinic space group $P2_1/n$ with cell dimensions $a = 15.302(3)$, $b = 8.035(2)$, $c = 18.258(4)$ Å and $\beta = 102.68(2)°$, and has been refined to a final R of 0.055. The acridine chromophore is significantly non-planar, with a butterfly conformation about the C(9)—N(11) bond. The bonding geometry about the C(9) atom has been significantly altered compared to non-distorted amsacrine structures, as a result of this non-planarity. Energy calculations have been used to examine the flexibility of the molecule with respect to rotations about the C(9)—N(11) and N(11)—C(12) bonds, and with respect to intercalation into a dinucleoside duplex model for DNA. The latter calculations have been compared with solution DNA-binding and *in vitro* activity data for 1-methyl-amsacrine hydrochloride. The molecular modelling studies find that the energy of interaction between 1-methyl-amsacrine and a DNA intercalation fragment is significantly higher than for amsacrine itself, in accord with the biological data.

The anti-cancer acridine derivative amsacrine [m-AMSA; 4′-(acridin-9-ylamino)-3′-methoxy-methanesulphonanilide] has clinically useful activity against disseminated tumours, especially acute leukaemia. A continuing search for derivatives active against other tumours has led to the study of several hundred compounds and structure–activity relationships have shown that the ability to bind to DNA by intercalation is a necessary condition for biological activity both *in vitro* and *in vivo* [1–4].

In this study, we examine the molecular structure of the hydrochloride salt of 1-methyl-amsacrine. Computerised molecular modelling and energy calculations are used to simulate the intercalation process. The results of this study are compared with measurements of DNA binding ability in solution and biological activity. This study thus provides a test case for the comparative examination of these various approaches. In particular, we wish to examine the proposition that computerised modelling of a drug and its receptor site can relate to biological response parameters.

EXPERIMENTAL

Crystals of 1-methylamsacrine HCl were grown from ethanolic solution. The orange prismatic needle-shaped crystals showed monoclinic symmetry with preliminary oscillation and Weissenberg X-ray photographs. Accurate cell dimensions were obtained by least-squares analysis of 25 θ values measured on a CAD4 diffractometer.

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Crystal data: $a = 15.302(3)$, $b = 8.035(2)$, $c = 18.258(4)$ Å, $\beta = 102.68(2)°$, $C_{22}H_{22}NO_3Cl_1S_1$, $M = 443.96$, $\mu = 26.66$ cm^{-1} space group $P2_1/n$, $Z = 4$, $D_c = 1.345$ g cm^{-3} Cu–K_α radiation ($\lambda = 1.54178$ Å).

Intensity data were collected on the diffractometer using a -2 scan technique, in the range $1.5 < \theta < 55$, for a crystal of dimensions $0.3 \times 0.1 \times 0.02$ mm. The maximum scan time was set at 120 sec with a scan speed chosen so as to have a constant $\sigma(I)/I$ of 0.03. The intensities of three standard reflections were periodically monitored during the course of data collection; no appreciable crystal decay was observed. The intensities of 2777 reflections were measured, which reduced to 2304 unique reflections. Of these, 965 had $I < 2\sigma(I)$ and were used in structure refinement.

The structure was solved by routine use of the MULTAN 82 direct methods program [5]. Full-matrix least-squares methods were used for refinement of non-hydrogen atom positional and individual anisotropic thermal parameters. Hydrogen atom positions were calculated by geometric considerations; these were not included in least-squares refinement, but were taken account of in the calculation of structure factors. An empirical absorption correction was applied to the data. The orientations of the 1-methyl group atoms were optimised by calculation of semi-empirical intramolecular energy using an interactive molecular graphics system; the orientation corresponding to the lowest energy resulting in a lowering of the non-bonded energy term from -18.4 to -32.2 kcal mol^{-1}.

Refinement converged to an R value of 0.055

and a weighted R_w of 0.056, with weights $w = 1/\sigma^2(I) + 0.041^2$ e.s.d. of an observation of unit weight was 1.639, and the maximum shift/error in the final least-squares cycle was 0.01. Calculations were performed with the Enraf–Nonius SDP package, with atomic scattering factors taken from ref. [6]. Positional parameters are given in Table 1.

Molecular modelling of interactions between 1-methylamsacrine salt and DNA, was accomplished by use of interactive computer graphics combined with semi-empirical energy calculations [7]. The intercalated DNA molecule was approximated by crystallographic co-ordinates from the study of an intercalated self-complementary dinucleoside, dCpG [8]. This approach has been described in detail elsewhere [9, 10].

Intermolecular energy was approximated as

$$E_{TOT} = E_{E\text{-}S} + E_{N\text{-}B}$$

$$E_{E\text{-}S} = \frac{q_1 q_2}{\varepsilon r_{12}}; \quad E_{N\text{-}B} = \frac{A}{r^6} - \frac{B}{r^{12}}$$

with parameters A and B chosen so as to control atom–atom contact distances [9, 11]. Partial charges for 1-methylamsacrine were calculated by the CNDO/2 method [12]; those for dCpG were taken from ref. [13]. The dielectric constant was approximated by a distance-dependence formalism: $\varepsilon = 1$ for $r < 3$ Å, $\varepsilon = 4$ for $r < 7$ Å, and $\varepsilon = 0.75r - 1.25$ for 3Å $< r <$ 7Å.

The 1-methylamsacrine salt molecular structure was manipulated in the intercalation site of dCpG by means of interactive computer graphics, and low energy structures for the complex were recorded. Manipulations and associated calculations were performed on a Gresham-Lion Supervisor 214 system linked to PDP11/34A–VAX 11/750 computers.

DNA binding constants were determined in 0.01 SHE buffer to poly(dA-dT), using the fluorometric ethidium displacement technique [26], which has been shown to give reliable determinations of acridine-DNA binding affinity. Unwinding angles were also determined in 0.01 SHE buffer at 25°, using plasmid PNZ 116 DNA [27]; pK_a values [3], cytotoxicity [28] and *in vivo* antileukemic activity [29] were determined using literature procedures.

Table 1. Positional parameters and their estimated standard deviations

Atom	x	y	z	B(Å^2)
Cl	0.4686(2)	0.0668(4)	0.7252(1)	4.04(7)
S19	0.9348(2)	0.1945(5)	0.6119(2)	5.69(9)
O20	0.9179(4)	0.150(1)	0.5363(4)	6.9(2)
O21	0.8653(4)	0.266(1)	0.6422(4)	8.8(2)
O23	1.3133(4)	0.5396(9)	0.6542(3)	4.7(2)
N1O	1.4501(4)	0.1323(9)	0.3962(4)	3.1(2)
N11	1.3628(4)	0.287(1)	0.5767(3)	3.4(2)
N18	1.0157(4)	0.327(1)	0.6310(4)	4.1(2)
C1	1.5554(5)	0.321(1)	0.5771(4)	3.1(2)
C1Me	1.5371(6)	0.428(1)	0.6376(5)	3.9(3)
C2	1.6433(5)	0.287(1)	0.5761(4)	3.2(3)
C3	1.6646(5)	0.200(1)	0.5170(5)	3.8(3)
C4	1.6014(6)	0.143(1)	0.4588(5)	4.0(3)
C4A	1.5117(5)	0.180(1)	0.4576(4)	2.8(3)
C5A	1.3644(6)	0.189(1)	0.3836(4)	3.8(3)
C5	1.3093(6)	0.171(1)	0.3114(5)	4.6(3)
C6	1.2272(6)	0.244(1)	0.2961(5)	4.7(3)
C7	1.1945(6)	0.332(1)	0.3512(5)	4.7(3)
C8	1.2457(5)	0.346(1)	0.4208(5)	3.9(3)
C8A	1.3316(5)	0.272(1)	0.4393(4)	2.9(3)
C9	1.3898(5)	0.274(1)	0.5131(4)	2.8(2)
C9A	1.4858(5)	0.258(1)	0.5182(4)	2.8(3)
C12	1.2724(5)	0.297(1)	0.5876(4)	3.1(3)
C13	1.2482(6)	0.421(1)	0.6290(4)	3.4(3)
C14	1.1623(6)	0.432(1)	0.6436(4)	3.7(3)
C15	1.1012(5)	0.307(1)	0.6133(4)	3.3(3)
C16	1.1241(6)	0.178(1)	0.5720(5)	4.0(3)
C17	1.2114(6)	0.171(1)	0.5611(5)	3.7(3)
C22	0.9721(9)	0.024(2)	0.6613(8)	10.9(5)
C24	1.2933(7)	0.676(1)	0.6939(6)	6.5(3)
H5	1.3286	0.1005	0.2722	
H6	1.1878	0.2364	0.2443	
H7	1.1364	0.3887	0.3366	
H8	1.2205	0.4085	0.4580	
H10	1.4674	0.0549	0.3598	
H11	1.4096	0.2844	0.6237	
H14	1.1462	0.5305	0.6713	
H16	1.0800	0.0909	0.5525	
H17	1.2281	0.0764	0.5325	
H18	1.0050	0.4314	0.6571	
H221	0.9296	−0.0723	0.6565	
H222	1.0272	−0.0306	0.6526	
H223	0.9879	0.0348	0.7186	
H241	1.3416	0.7577	0.7083	
H242	1.2423	0.7455	0.6655	
H243	1.2751	0.6520	0.7408	
HMe1	1.5839	0.5085	0.6551	
H2	1.6914	0.3205	0.6194	
HMe2	1.4819	0.4930	0.6211	
H3	1.7278	0.1819	0.5199	
HMe3	1.5285	0.3695	0.6815	
H4	1.6215	0.0770	0.4201	

RESULTS

Molecular structure

The molecular structure of 1-methylamsacrine is shown in Figs 1 and 2, and calculated bond lengths and angles are given in Table 2.

It is apparent that the acridine ring system in the title compound is significantly non-planar, and adopts a butterfly conformation about the C(9)—

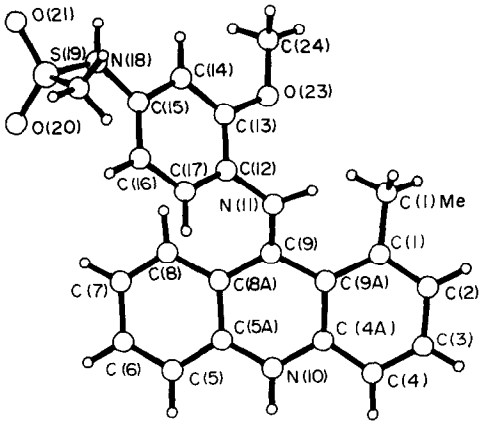


Fig. 1. A computer-drawn view of the molecular structure of 1-methylamsacrine, projected onto the mean plane of the acridine.

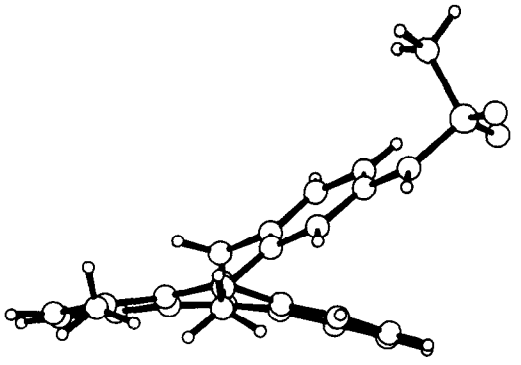


Fig. 2. 1-Methylamsacrine, shown projected approximately along the N(10) and C(9) atoms.

N(10) axis. The angle between the two arms of the butterfly (planes 1 and 2 in Table 3) is 21.5(8)°. This non-planarity contrasts with the closely coplanar acridine rings in amsacrine itself, as salt [14] and free base [15, 16], as the mesyl derivative [15], and as the 2-methoxy derivative [17]. The 1-nitro acridines, by contrast, generally adopt this butterfly conformation. The anti-tumour agent nitracrine [18] [9-(3-dimethylaminopropylimino) - 1 - nitro - 9,10 - dihydroacridine] has a 20° angle between the planes of the two outer acridine rings [19]. The crystal structure of an analogue with slight variation in the side-chain has a 19° inter-plane angle [20], whereas the 2-nitro analogue has a completely coplanar acridine ring system [21]. In the case of the 1-nitroacridines this distortion has been ascribed to interaction between the 1-nitro group (which is thus also distorted out of the acridine plane) and substituents on the 9-position of the acridine when the compound exists as the iminoacridan tautomer [22]. It has been suggested [25] that the electron-withdrawing properties of the nitro group are responsible for nitracrine adopting the non-planar iminoacridan form, but this seems unlikely in view of the planar 2-nitro derivative. In the case of 1-methylamsacrine, the acridine non-planarity must be due to steric rather than electronic factors, suggesting that the latter may also be of lesser importance in the case of the 1-nitro acridines. Table 2 shows that this non-planarity has produced significant changes in the bonding geometry around the N(11) substituent at the 9-position. The C(9)—N(11) bond is considerably shortened in 1-methyl-

Table 2. (a) Selected bond lengths (Å) with estimated standard deviations in parentheses

	Amsacrine HCl [10]	1-Methyl-amsacrine HCl	Amsacrine base [11]	Nitracrine analogue [18]
C(8A)—C(9)	1.424(5)	1.440(10)	1.411(3)	1.472(5)
C(9)—C(9A)	1.441(5)	1.458(9)	1.408(3)	1.482(5)
C(9)—N(11)	1.359(6)	1.318(8)	1.387(2)	1.285(5)
N(11)—C(12)	1.431(6)	1.443(9)	1.409(2)	1.460(5)

(b) Selected bond angles (degrees), with estimated standard deviations in parentheses

	Amsacrine HCl	1-Methyl-amsacrine HCl	Amsacrine base	Nitracrine analogue
C(4A)—C(9A)—C(9)	118.3(5)	116.4(8)	118.6(2)	119.7(1)
C(5A)—C(8A)—C(9)	118.9(5)	116.2(8)	118.3(2)	118.7(1)
C(8A)—C(9)—C(9A)	118.6(5)	117.8(7)	118.7(2)	113.9(1)
C(8A)—C(9)—N(11)	123.9(5)	125.1(7)	120.7(2)	130.8(1)
C(9A)—C(9)—N(11)	117.4(2)	117.1(7)	120.5(2)	114.9(1)
C(9)—N(11)—C(12)	129.6(5)	128.4(7)	125.4(2)	126.1(1)

Table 3. Least-squares planes for 1-methylamsacrine–HCl with deviations from the planes in Å and estimated standard deviations in parentheses. Atoms excluded from a plane calculation are indicated by *

Plane 1

C(1) 0.019(10), C(2) 0.003(10), C(3) − 0.004(10), C(4) − 0.018(10), C(4A) 0.040(10), C(9A) − 0.040(10), C(9)* − 0.106(9), N(10)*0.124(7), C(1Me)*0.172(11), N(11)* − 0.662(8)

Plane 2

C(5) − 0.014(11), C(6) 0.000(11), C(7) 0.005(11), C(8) 0.004(10), C(5A) 0.024(10), C(8A) − 0.019(9), C(9)* − 0.080(9), N(10)*0.158(7), N(11)* − 0.602(8)

Plane 3

C(12) 0.022(9), C(13) − 0.007(9), C(14) − 0.007(9), C(15) 0.007(9), C(16) 0.008(9), C(17) − 0.022(9), N(11)* − 0.027(7), H(11)*0.80(7), C(9)*0.789(8)

Table 4. Selected torsion angles (degrees) with estimated standard deviations in parentheses

	Amsacrine HCl [10]	1-Methyl-amsacrine HCl	Amsacrine base [11]
C(8A)—C(9)—N(11)—C(12)	21(1)	2.2(8)	54.2(3)
C(9)—N(11)—C(12)—C(13)	48(1)	−127.8(8)	−163.5(3)
C(8A)—C(9)—N(11)—H(11)	−149(1)	178.7(8)	3.0(3)
N(11)—C(12)—C(13)—O(23)	−3(1)	4.8(8)	3.0(3)
H(11)—N(11)—C(12)—C(13)	−147(1)	55(1)	34(3)

amsacrine compared to amsacrine itself, although it is not as short as found in the 1-nitro acridines [19–22]. Bonds C(8A)—C(9) and C(9A)—C(9) are shorter in the amsacrine structures, which together with the C(9)—N(11) bond length change, indicate that the steric hindrance produced by 1-substitution results in changes in the electronic distribution of the 9-amino acridines. In the case of the 1-nitro derivatives, the result is a 9-imino,9,10-dihydro acridine since the C(9)—N(11) bond has true double bond character and N(11) does not have a bonded hydrogen atom. This extreme change is not produced in the 1-methylamsacrine reported here, as witnessed by the unequivocal location of a hydrogen atom on N(11).

The conformation of 1-methylamsacrine is primarily defined by the torsion angles around the C(9)—N(11) and N(11)—C(12) bonds (Table 4). It is apparent that these angles have significantly different values from those in amsacrine itself, as free base [15] and hydrochloride salt [14]. These latter two structures themselves adopt distinct conformations, which have been shown to be interconvertible at modest energy cost [15]. Calculation of the intramolecular energy for 1-methylamsacrine with respect to rotations about these two bonds (Fig. 3) shows that the observed conformation is not in the vicinity of the global energy minimum, but is at a point some 6 kcal mol^{-1} higher in energy. This conformational map indicates that the introduction of the 1-methyl group has diminished the flexibility of the anilino group, and that the several low-energy forms are less readily interconvertible than in the amsacrine structures.

DNA interaction

The interaction of 1-methylamsacrine salt with DNA was studied by determining its association constant K for calf thymus DNA, and its unwinding angle using closed circular supercoiled plasmid PNZ 116 DNA. The results are shown in Table 5, together with comparative data for salts of amsacrine, the isomeric 3-methylamsacrine, and 9-aminoacridine. While addition of a methyl group to amsacrine in the unhindered 3-position slightly increases the acridine pK_a as expected, placement at the 1-position

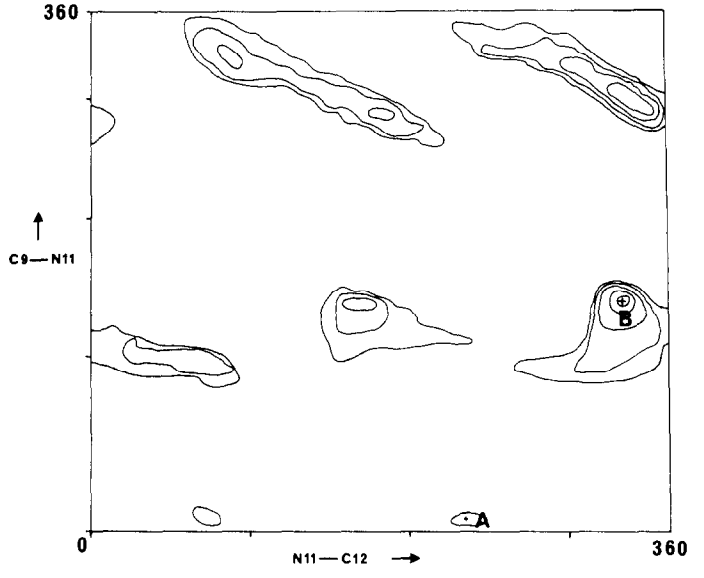
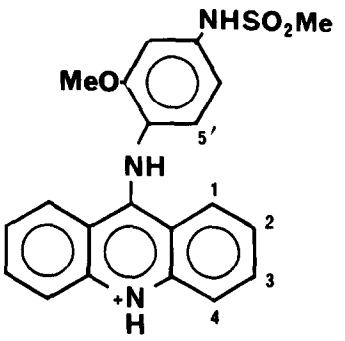


Fig. 3. Energy-contour plot for 1-methylamsacrine. Torsion angles C(8A)—C(9)—N(11)—C(12) and C(9)—N(11)—C(12)—C(13) are varied along the vertical and horizontal axes respectively. Contours have been drawn at intervals of 1 kcal mol^{-1}. Point A marks the crystallographically observed conformation, and B the global minimum.

Table 5. Physicochemical and biological data for amsacrine analogues



Compound	pK_a*	DNA Binding log K†	DNA Binding unwinding‡	Cytotoxicity IC_{50}§	Antileukemic Activity OD‖	Antileukemic Activity ILS¶
9-Aminoacridine	9.9	5.59	17	2600	20	inactive
Amsacrine	7.43	5.57	21	33	13.3	78
1-Methylamsacrine	6.71	5.87	13	1300	200	inactive**
3-Methylamsacrine	7.70	5.95	20	12	10	120
5'-Methoxyamsacrine	7.63	4.69	19	4900	>500	inactive

* pK_a of acridine nitrogen, in 20% DMF [3].
† Association constant, calf thymus, DNA 0.01 SHE buffer.
‡ Unwinding angle, PNZ 116 plasmid DNA [27].
§ Nanmolar concentration of drug needed to reduce L1210 cell numbers by 50% [28].
‖ Optimal dose of drug in mg/kg/day, given on days 1, 5 and 9 after implantation of 10^6 P388 leukemia cells (ref. [29]).
¶ Percentage increase in lifespan of treated animals over controls; values over 25% regarded as significant [29].
** Determined using L1210 leukemia.

decreases it by nearly a whole unit, providing further evidence of the molecular distortion induced. A similar lowering of pK_a has been noted [3] for other 1-substituents. However, the binding of the monocations to DNA at pH 7 is affected very little by the addition of the methyl group, with 1-methylamsacrine rather surprisingly showing the strongest binding. On addition of the side-chain to 9-aminoacridine a small increase in unwinding angle is seen, due presumably to additional interaction of the anilino ring with the DNA, and this is not affected by a 3-methyl group. By contrast, the measured unwinding angle for 1-methylamsacrine is lowered by over 30%, suggesting either a redistribution of drug between "intercalated" and "externally bound" forms, or a "partially-intercalated" complex which induces a smaller unwinding of the DNA. Accordingly the interaction between amsacrine and DNA was explored by molecular modelling techniques using computer graphics and energy calculations on a dinucleoside model for intercalation.

Molecular modelling

Intercalation of 1-methylamsacrine salt from the major groove of DNA was simulated with the acridine perpendicular to the base-pair hydrogen bond directions. This had a total energy of -69 kcal mol^{-1} (-34 kcal mol^{-1} for the non-bonded term and -35 kcal mol^{-1} for the electrostatic one). A 30° diagonal orientation, together with translation so that the acridine is asymmetrically stacked between bases of just one strand, resulted in a lower energy, of -81 kcal mol^{-1} for E_{N-B} and -41 kcal mol^{-1} for E_{N-S}). This is the lowest energy found for the major groove side (Fig. 4) and has the 1-methyl group tucked into a hydrophobic cleft around the ribose groups of a backbone.

Intercalation of 1-methylamsacrine from the DNA minor groove was less easy to achieve due primarily to steric hindrance between the atoms of the sugar rings and the intercalator. Few low-energy positions were found. The best orientation was almost perpendicular to the base-pair hydrogen bonds (Fig. 5), with the only stacking being between guanine bases. The energy of interaction was -87 kcal mol^{-1} (-41 and -46 kcal mol^{-1} for E_{N-B} and E_{E-S} respectively). At this position, the methoxy substituent was able to interact hydrophobically with a 3′ sugar residue.

1-Methylamsacrine was thus found to be capable of only minimal intercalation between base pairs in the DNA model used in this study. For intercalation from either groove, overlap between nucleotide bases and acridine is restricted to the ring of the acridine that is substituted by the methyl group. Further entry is hindered by the proximity of hydrogen atoms on the nitrogen bridge and on the anilino ring, as well as the 1-methyl group. The conformation of 1-methylamsacrine is such as to render sterically inaccessible to intercalation the terminal ring of the acridine that does not carry the methyl group.

Intercalation from either groove is probably not differentiated by the energy calculations. Both are of significantly higher energy than calculated for amsacrine itself with the same dinucleoside duplex

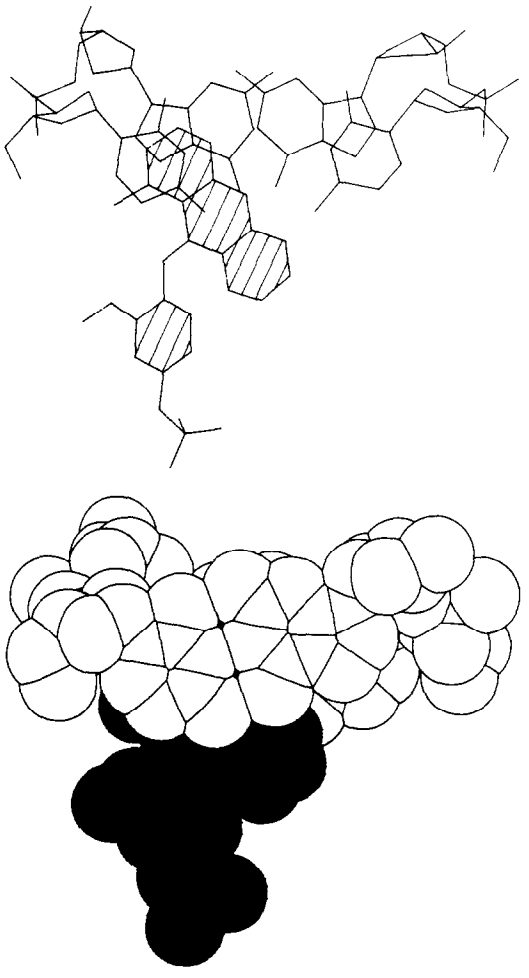


Fig. 4. Computer-drawn views of 1-methylamsacrine intercalated into the major groove of a dinucleoside fragment of double-stranded DNA.

model for DNA, due to the greater extent of intercalation, and hence base pair–acridine chromophore overlap with amsacrine. Intercalation from the minor groove for amsacrine has an associated energy of -113 kcal mol^{-1} at low-energy positions. This in turn is less than for a simple acridine such as proflavine (3,6-diaminoacridine). Thus, 1-methylamsacrine can be considered as a "pseudo intercalator" that, by comparison with amsacrine itself, only weakly stabilises a 6.8 Å base-pair-separated DNA site.

The inability of 1-methylamsacrine to more than partially intercalate is due to two principal factors. The non-planarity of its acridine chromophore together with the steric hindrance resulting from the 1-methyl group render full intercalation in the normal sense, difficult to achieve. In addition, the conformation of the anilino group with respect to the acridine provides in itself constraints to intercalation.

Biological activity

Addition of the 3-methoxymethanesulfonanilide side-chain to the biologically inactive 9-aminoacridine provides the clinically useful antileukemic drug amsacrine [30]. The reasons for this marked change in biological profile are many, and it cannot be explained totally in terms of alterations in the interaction of the compound with DNA. Nevertheless, studies on a large number of amsacrine analogues [1–4] have demonstrated the absolute dependence of biological activity in this series on *efficient* DNA intercalation. Thus the inactive 5′-methoxyamsacrine derivative has a measured unwinding angle of 19° but NMR data suggests only a very small degree of overlap with the base-pairs of the intercalation site [30]. The crystal structure and molecular modelling data presented here suggests a similarly low degree of overlap of the acridine chromophore of 1-methylamsacrine in the binding site, which may account for its complete lack of biological activity and that of other 1-substituted amsacrines [3]. Finally, it should be noted that the nitracrine series of 1-nitro acridines, which have an analogous butterfly-shaped, non-planar acridine chromophore, have also been reported to bind non-covalently to DNA by intercalation [23]. However, the anti-cancer and cytotoxic activities are not due to this intercalative interaction, but to covalent DNA binding via enzymatic activation to reactive species [24].

The data for binding of 1-methylamsacrine to DNA appears to conflict with the molecular modelling results, which indicate that the compound should be a weak intercalator. However, its high DNA association constant also contrasts with the markedly lower unwinding angle compared to amsacrine. We

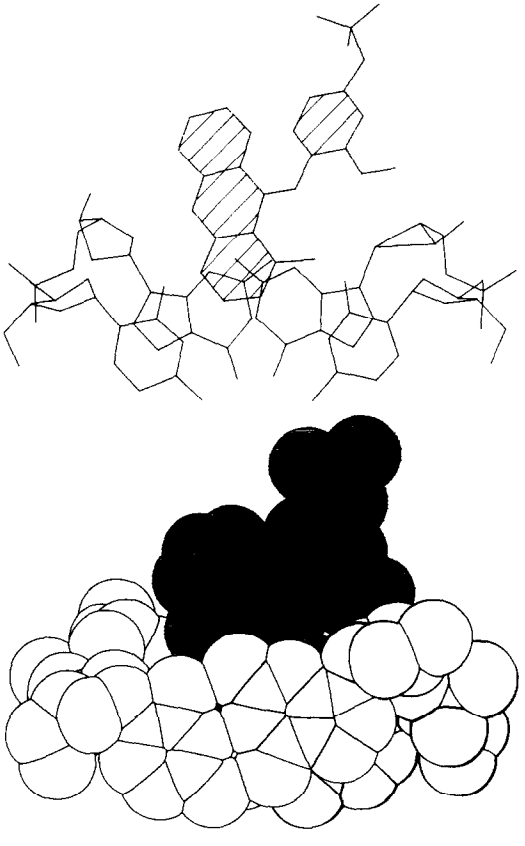


Fig. 5. As in Fig. 4, but for minor-groove intercalation.

tentatively conclude that the association constant is not solely a measure of intercalative binding, as discussed above, and therefore that the calculated relative energies of intercalation may be more relevant parameters in this context, particularly in relation to the prediction of biological activity in the series. However, since the measurement of DNA binding used is an indirect one, further experimental data is required for firm conclusions to be made. The situation is rendered more complex by evidence that intercalating drugs when bound to DNA, form ternary complexes *in vivo* with topoisomerase II, that may at least in part be responsible for cytotoxicity [31].

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