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[1] CYTOTOXICITY OF BIS(PHENYLAMIDINIUM)FURAN ALKYL DERIVATIVES IN HUMAN TUMOUR CELL LINES: RELATION TO DNA MINOR GROOVE BINDING

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Abstract A series of alkyl derivatives of bis(phenylamidinium)furan are shown to have cytotoxic activity in several human tumour cell lines. Xray crystallographic studies have previously shown that these compounds bind in the AT region of the DNA minor groove. It is suggested that activity is linked to this AT groove binding.

The majority of low molecular-weight molecules which bind non-covalently to the minor groove of DNA and show AT sequence preferences[1-3], also have anti-parasitic, anti-bacterial, anti-fungal or anti-viral activity[4]. Well-established members of this class of drugs include pentamidine (**1**), netropsin and berenil. Pentamidine has clinically useful activity[5] against the *Pneumocystis carinii* pathogen, which afflicts the majority of AIDS patients. The mechanism of action of these drugs have not as yet been fully elucidated, although there is evidence that they act by preferentially inhibiting protein-DNA interactions that may be particular to the target rather than the host organism. It has been shown[6] for several minor groove drugs, that interference with the function of DNA topoisomerases I and/or II can play an important role in their selective toxicity. Studies of pentamidine analogues have shown a correlation between DNA affinity and anti-PCP activity[7-10], which can be interpreted as being due to drug binding selectively interfering[11] with the normal processing of DNA by DNA topoisomerases. Anti-tumour activity has not been well studied for this type of minor-groove binding agents,

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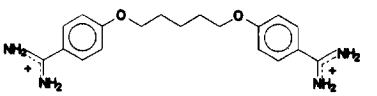
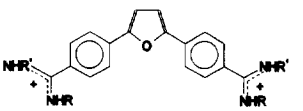
in contrast to the well-established activity of covalent binders such as anthramycin, CC1065 and the distamycin alkylating mustard derivative tallimustine.



(1)



(2)

Significant activity against PCP is also shown by several dicationic diaryl furan derivatives[9], with the parent compound furamidine (2: R=R'=H) binding more strongly to duplex DNA, as well as showing greater activity and reduced toxicity in the rat PCP model compared to pentamidine. We have recently shown[12] by means of a combined X-ray crystallographic, molecular modelling and solution biophysical approach, that the increased DNA affinity shown by furamidine is due to an increase in the non-bonded interactions between the furan group of the drug and the hydrophobic walls of the minor groove, compared to the -$(CH_2)_5$- linker chain in pentamidine or the triazene linker in berenil. Crystallographic analyses have now been performed on oligonucleotide complexes of three aryl derivatives of furamidine, with isopropyl (3), cyclopropyl (6)[13] and cyclobutyl (7)[14] R' substituents. These studies have shown that in all three structures, the drug is in the same position in the A/T region of the minor groove as furamidine[12], but that there are significant differences in minor groove width as a result of the differences in size of the R' groups.

We report here on the cytotoxicities of several compounds in this furamidine series, using cell lines derived from human ovarian tumours. Their syntheses are similar to other previously-described furamidines[9]. The A2780 and CH1 cell lines are sensitive to cis-platinum[15], which binds covalently in the DNA major groove. The A2780R and SKOV-3 lines are resistant to cis-platinum. DNA binding has been assessed by the extent to which the compounds are able to stabilise the helix↔coil transition, using the dodecamer d(CGCGAATTCGCG)$_2$ as a model for genomic DNA. This sequence has also been used in crystallographic studies on several of these complexes[12-14].

Table 1 shows that in broad terms the cytotoxic potency of the furamidine derivatives increases with the size of the alkyl group substituent, in accord with their increases in DNA affinity, as shown by the ΔT_m values. The cyclobutyl and cyclopentyl compounds

Table 1. Cytotoxicity[16] of substituted furamidine derivatives, expressed as IC_{50} values in μM, together with ΔT_m values showing the extent of stabilisation of the dodecanucleotide d(CGCGAATTCGCG)$_2$ produced by derivative binding.

COMP NO.	R	R′	A2780	A2780R	CH1	SKOV-3	ΔT_M
2	H	H	46	15.5	46.5	42.5	11.7
3		H	38	6.4	44.5	20.5	14.4
4		H	22.5	12	9	52	11.2
5		H	36	13	15	56	13.3
6		H	37	15.5	18	48	12.4
7		H	11	3.3	4.2	25	14.8
8		H	12	3.8	3.6	17	15.8
9		H	4.8	2.5	2.1	5.2	15.4
10		H	32.5	7.1	16	31	7.7
Cis-Pt			0.3	3.3	0.1	4.4	-

are approximately equi-active, and the cyclohexyl slightly more active, especially in the A2780 and SKOV-3 resistant lines, where all these three derivatives (as well as several others), show consistent and significant activity. This is especially seen with the A2780 versus A2780R lines; the great majority of DNA damaging agents confer cross-resistance in this pair of lines.

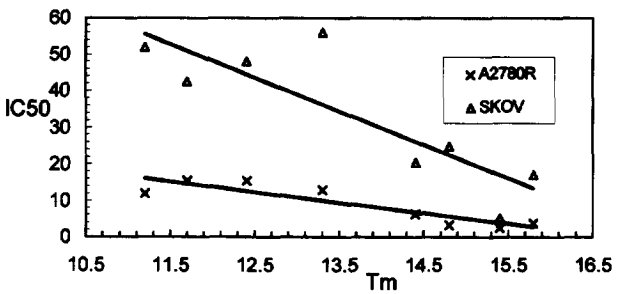


Fig 1. Plots of T_m vs IC_{50} for the SKOV-3 and A2780R cell lines. Data for compound **10** has been omitted in view of its abnormal behaviour in these cell lines.

An approximate link between cytotoxicity and DNA-affinity is apparent from Table 1. It is striking that there is a good correlation between the two for the resistant cell lines

(Fig. 1), with correlation coefficients of 0.89 and 0.85 for the A2780R and SKOV-3 data respectively. The other cell lines do not follow such a consistent trend.

DNA footprinting studies have shown that compounds in this series, in common with related drugs such as pentamidine and berenil[18], preferentially bind at A/T sequences in DNA, with eg 5'-AATT and 5'-AAAA being preferred sites[17]. Binding site sizes are consistent with the crystallographic results[12-14]. Other analogues with amidoalkyl groups replacing the amidiniums have cytotoxicities in the >100μM range, and do not show any pattern of sequence preferences by DNA footprinting[17], although they are still DNA binders (data not shown here). This suggests that DNA-binding to A/T sequences may be implicated in the cytotoxicity of these compounds, possibly by interfering with the binding of a regulatory or processing protein in the minor groove, for example at the TATA box[19,20], thus inhibiting the initiation of transcription. We cannot exclude the possibility that the differences in cytotoxicity are due to differences in cell uptake; however this appears not to be a major factor since compounds with equal numbers of carbon atoms in their alkyl groups show significant differences in cytotoxicity (cf compounds **4** vs **7**, and **8** vs **10**), and that the analogous alkylated amido compounds are inactive in these cell lines.

The crystal structures of **2**, **3**, **6** and **7** complexed to the dodecamer duplex $d(CGCGAATTCGCG)_2$, all have ligand bound in the same region of the minor groove, at the 5'-AATT sequence[12-14]. In all four structures, there are inter-strand hydrogen bonds from the terminal amidinium groups to O2 atoms on the minor-groove edges of the thymine bases in this sequence. The minor-groove widths in these structures are somewhat more variable, with maximal differences of ca 1 Å. The alkyl substituent groups are positioned at the mouth of the groove, and this results in some narrowing of groove width compared to the complex with furamidine itself, as a result of increasing numbers of attractive van der Waals contacts with atoms lining the minor groove walls, as the size and hence surface area of alkyl group increases. This trend is approximately in accord with the differences in DNA binding ability between the derivatives. The importance of groove width has been suggested by others, for example with the binding of the bisquaternary drug SN6999 to an oligonucleotide

duplex[21]. None of the furamidine complex crystal structures show any large-scale structural deformations of the DNA, in accord with data from other minor-groove complexes[3]. The cytotoxicity data presented here is thus broadly consistent with the structural and DNA-binding results, providing further evidence for the suggestion that DNA interaction can be an important (and perhaps key) element of the cellular response to these agents, especially in the resistant lines.

The DNA binding of these compounds could result in them being effective inhibitors of regulatory protein binding in the minor groove, as has been shown for other minor-groove drugs[19,20,22]. It is also possible that the hydrophobic alkyl groups are acting as recognition signals for hydrophobic residues in these proteins, and that their larger surface area in compounds **5**, **8** and **9** results in improved levels of such recognition, and ultimately in the biological responses detailed here. The weaker DNA binding of the 3-pentyl compound **10**, which is paralleled by its lower cytotoxicity compared to, say compound **8**, suggests that entropic as well as steric factors are involved.

Cytotoxic activity (associated with DNA binding) in the platinum-resistant cell lines is a notable feature of these compounds. DNA repair is one of the mechanisms whereby platinum lesions are removed in resistant cells and tumours, with the repair process being triggered by the recognition of major perturbations in DNA structure, such as have been observed in the crystal structure of a cis-platinum oligonucleotide adduct[23]. This shows that the major groove intra-strand cross-linking results in a 26° bend of the duplex, which can readily be recognised by repair proteins[24]. By contrast, the non-covalent minor groove binding of the furamidine series of compounds does not produce significant change in DNA structure[12-14], thereby circumventing this type of major-groove adduct repair. Binding to more extended minor-groove sites can however require structural changes in order for effective base recognition to take place. Local unwinding and disruption of A•T base pairs was observed in the 7½ base pair site required for a tris-benzimidazole ligand[25], suggesting that analogous extended furamidines may be less active in these resistant cell lines.

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