Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2593–2597

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

DNA Minor Groove Interactions and the Biological Activity of 2,5-Bis-[4-(*N*-alkylamidino)phenyl] Furans

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Received 22 June 2000; revised 23 August 2000; accepted 6 September 2000

Abstract—2,5-Bis-[4-(*N*-cyclobutyl-amidino)phenyl] furan and 2,5-bis-[4-(*N*-cyclohexyl-amidino)phenyl] furan have activity against *Pneumocystis carinii* and also show cytotoxicity against several tumour cell lines. These activities are correlated with DNA-binding abilities; the crystal structures of complexes with the DNA sequence d(CGCGAATTCGCG) is reported here. Interactions with, and effects on, the DNA minor groove, are found to be factors in the biological properties of these compounds.

Introduction

The aromatic bis-amidines typified by pentamidine, propamidine, berenil and the furamidines, exhibit a wide range of biological activities, including antiviral, antimicrobial, anticancer and antibiotic effects.[1–3] These compounds bind to the minor groove of duplex DNA in A/T-rich regions,[4] where they are thought to exert their biological activity through the inhibition of DNA-associated enzymes such as DNA topoisomerases I and/or II, or possibly by direct inhibition of transcription.[5–8]

Pentamidine has found use as an effective treatment for *Pneumocystis carinii* pneumonia (PCP) within infants. The advent of acquired immunodeficiency syndrome (AIDS) has increased therapeutic and clinical interest in the applications of pentamidine in view of the widespread prevalence of PCP among AIDS patients.[9] The acute toxicity originally associated with this drug has been only partially overcome through aerosol administration. There is thus a need for new compounds that have greater efficacy and potency combined with diminished toxicity.[10]

A number of bis[4-(*N*-alkylamidino)phenyl] furan compounds (Fig. 1) have been evaluated against PCP in an immunosuppressed rat model.[10–12] There are correlations between their binding to the AT-region in the dodecamer d(CGCGAATTCGCG), as shown by ΔT_m measurements, the inhibition of DNA topoisomerase activity, and anti-PCP activity. The parent compound in the series, furamidine[11] (R = H), displays potent activity at nanomolar concentrations against PCP. Substitution of the amidino nitrogens with alkyl groups (R) increases the drugs' overall binding affinity for DNA, with cycloalkyl groups showing the largest increase in ΔT_m.

These alkyl derived furamidines have also been shown to have selective toxicity in several human ovarian tumour cell lines.[13] Cytotoxic potency is related both to the size of the alkyl group substituent, and to DNA binding affinity (ΔT_m values). The cyclohexyl furamidine derivative was particularly active against the two cell lines A2780 and SKOV-3 (resistant to *cis*-platinum). This suggests that cytotoxic activity may be a consequence of binding to A/T-rich genes in DNA.

The crystal structures have previously been reported for a number of bis-amidino ligands and drugs bound to oligonucleotide sequences.[11,14–16] All show binding in the minor groove of A/T regions, with the majority having hydrogen bonding to base edges hydrogen-bond

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0960-894X/00/$ - see front matter
PII: S0960-894X(00)00511-4

acceptor atoms in the groove. The present paper examines the structural consequences of the size of the terminal alkyl groups, using the drug bound to the dodecamer d(CGCGAATTCGCG)$_2$ as a model system.

Results

The two bis-furamidine (R = cyclobutyl, cyclohexyl)–DNA crystal structures determined[17] in this study are isomorphous (and also isomorphous with the cyclopentyl furamidine complex[16]), and show the ligands bound at the sequences 5′-AATT within the minor groove of a right-handed B-form DNA (Fig. 2). Both ligands cover the 4 bp 5′-AATT site, with the bis-phenyl furamidine moiety located between the two backbones and the terminal cycloalkyl groups orientated away from the minor groove floor. The ligands adopt conformations so that the non-alkylated charged nitrogen atoms of the amidinium groups are all orientated inwards and lie in close proximity to the floor of the minor groove. The binding sites are essentially identical, between the base pairs Ade5·Thy20 and Thy8·Ade17. The cyclobutyl and cyclopentyl[16] furamidines form hydrogen bonds with base-pair donor atoms O2 of Thy20 and Thy8 at each end of the binding site (Fig. 2), identical to the hydrogen-bonding arrangements observed in other bis-furamidine–oligonucleotide structures.[11,14–16] The cyclohexyl complex has only one (weak) hydrogen bond contact with a DNA base, with instead water contacts from the ligand bridging to bases beyond the A/T region.

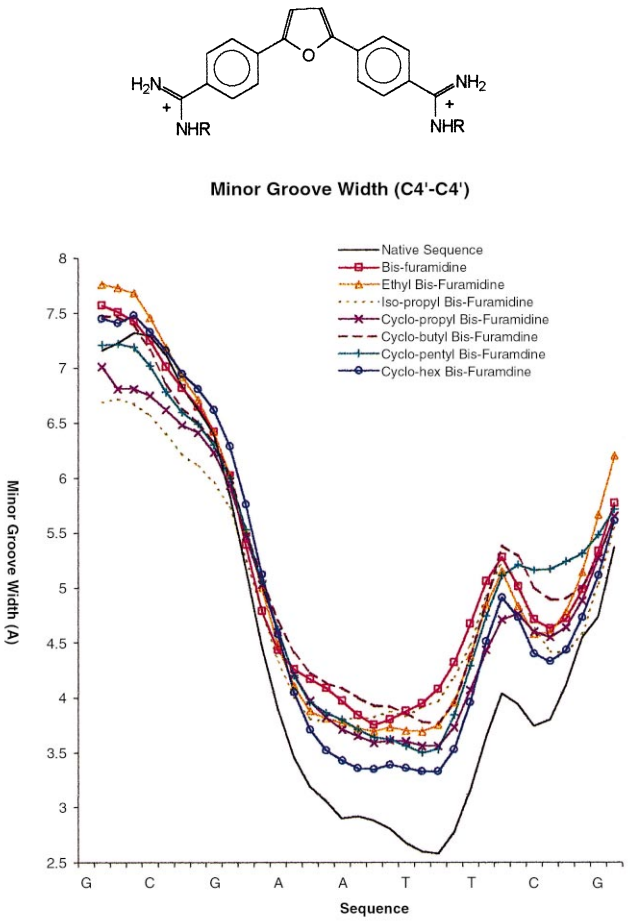

Figure 1. Formulae of the furamidines (top); plot of minor groove widths (bottom).

In accord with previous furamidine complex crystal structures, the hydrophobic alkyl groups sit at the mouth of the groove forming non-bonding interactions with both DNA strands. Hydration of the crystal structures is extensive (Fig. 2). The ligands participate in water networks that solvate them within their binding positions, stabilising them within the minor groove and augmenting their A/T sequence recognition.

The A·T base pairs in all three complexes display high propeller twisting, with a consequent loss of one of the two of the Watson–Crick hydrogen bonds between adenine and thymine (between N6 of Ade to O4 of Thy) in the cyclobutyl and cyclopentyl complexes. By contrast, all four A·T base pairs in the cyclohexyl complex have a full complement of hydrogen bonds, suggesting that the loss of base-pair hydrogen bonding seen in the other two structures is a consequence of the structural changes needed to produce effective direct hydrogen bonding between bases and drug.

Each cycloalkyl bis-furamidine ligand within the minor groove is approximately isohelical with the floor of the groove. This is achieved by means of rotation of the two bis-phenyl-furan dihedral angles. Each phenyl ring is located parallel to the walls of the groove, forming close van der Waals interactions with sugar and phosphate atoms of the DNA backbones. The overall bis-phenyl-furan dihedral angles of the cyclobutyl and cyclopentyl bis-furamidines are almost zero, and are comparable to the cyclopropyl derivative.[14] However, the cyclohexyl analogue requires a more substantial twisting of the drug molecule, by 17°, in order to fit effectively into the minor groove. The furan groups of the cyclobutyl and cyclopentyl analogues make fewer close van der Waals contacts with the walls of the minor groove (C1′ or C4′ backbone atoms of either DNA strand) than the cyclohexyl furan ring, due to its large twist around its bis-phenyl-furan linkage.

Previous structures of cycloalkyl bis-furamidines complexed with the same DNA sequence have indicated that the alkyl group can adopt two distinct orientation at the mouth of the groove through rotation around the N–C dihedral angles of the amidinium group.[14] The alkyl group is either parallel or perpendicular to the walls of the minor groove. The small cyclobutyl group is not in close contact with the groove walls, a factor in the small number of van der Waals contacts which this drug makes with the DNA. At the 3′ end of the binding site, both the cyclobutyl and cyclopentyl groups have a perpendicular orientation, whereas the cyclohexyl group adopts a parallel one.

Minor groove widths are shown in Figure 1. At the central 5′-AATT tract, the ranking order of increasing groove width (based on inter-strand C4′–C4′ distances), is:

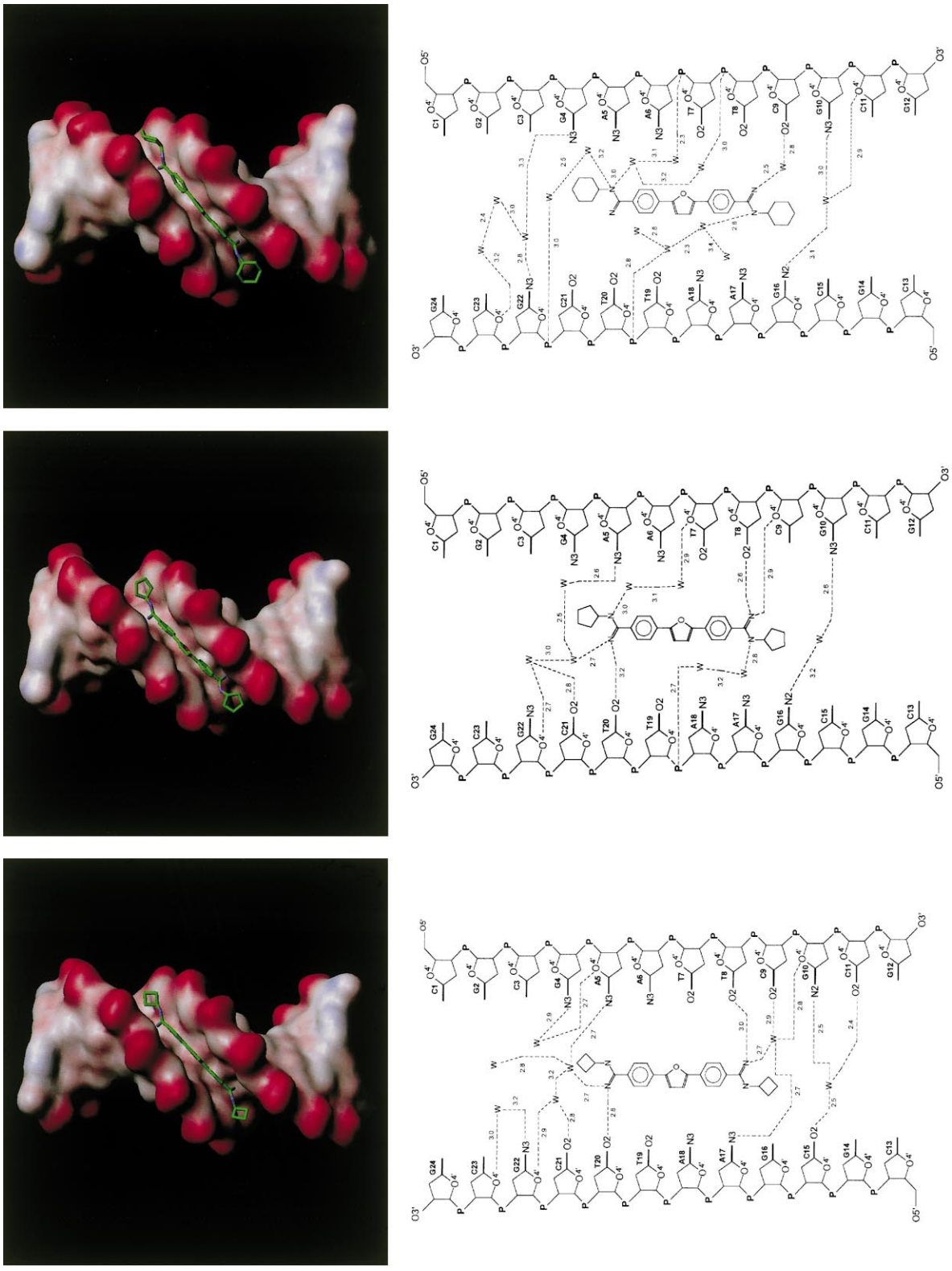

Figure 2. Crystal structures of cyclobutyl, cyclopentyl[16] and cyclohexyl complexes, and respective hydrogen-bonding schemes.

cyclobutyl > cyclopentyl > cyclohexyl

The overall order for all alkyl furamidines is:

isopropyl > cyclobutyl > ethyl ~ cyclopentyl

> cyclopropyl > cyclohexyl > native

Of all the cycloalkyl derivatives examined, the cyclobutyl bis-furamidine complex has the greatest increase in minor groove width compared to the native dodecamer. Comparison of minor groove widths indicates that the smaller the size of the alkyl-substituent, the wider the groove becomes at the central 5′-AATT region. This variation in width is in part a result of the conformation of the diphenyl-furan group situated at the centre of the DNA sequence. Indeed, both cyclobutyl and cyclopentyl substituted analogues are almost co-planar, thereby optimising the orientation of the amidinium groups with respect to the pyrimidine bases to which they hydrogen bond. Both structures indicate that the narrow minor groove can accommodate regions of extended planarity for the moiety linking the two amidinium groups without sacrificing hydrogen bonding ability. Such an increase in groove width results in fewer van der Waals interactions between the diphenyl-furan group and the walls of the minor groove. The diphenyl-furan group in the cyclohexyl structure has high dihedral twist, allowing this drug to adopt a conformation following more closely the curvature of the minor groove compared to that of the almost co-planar cycloalkyl derivatives, which is reflected by a reduction in the perturbation of the minor groove. In doing so, the cyclohexyl analogue establishes significantly more non-bonding contacts, which increase the stability of the drug within the minor groove.

The terminal alkyl groups, as well as affecting the overall planarity of the diphenyl-furan moiety, also dictate hydrogen bond interactions that are fundamental to A/T sequence recognition. Comparison of phenyl-amidinium dihedral twist and direct hydrogen bond interactions shows that near co-planarity of the central three rings induces considerable twisting of the terminal groups establishing O2···N hydrogen bond distances < 3.2 Å. Increasing the size of the cycloalkyl group to a five-membered ring still permits such direct contacts. However, the larger size of the cyclohexyl group, through hydrophobic and steric limitation at the mouth of the groove, orientates the amidinium nitrogen away from the floor of the minor groove. This shift in position in the amidinium groups of cyclo-hexyl bis-furamidine results in two important features: (i) an absence of direct hydrogen bonding to the DNA duplex; and (ii) an increase in dihedral twist of the three-ring chromophore generating additional non-bonding van der Waals interactions.

We have quantitated these non-bonded contacts by means of a simple contact-scoring formalism. N_c, the square of the normalised sum of all non-bonded distances r_j is defined:

Table 1. Contact scores calculated from the bis-furamidine crystal structures, together with in vitro cytotoxicity and in vivo activity against PCP (values from ref 12)

Alkyl group	N_c	ΔT_m	Cytotoxicity[a]	PCP activity[b]	PCP activity[c]
Cyclobutyl	3.46	14.8	11	0.05	0.67
Cyclopentyl[d]	10.15	15.8	12	0.03	0.11
Cyclohexyl	9.00	15.4	4.8	0.02	26.2

[a]Expressed as IC_{50} values in μM, for A2780 ovarian carcinoma cells using the standard sulforhodamine 96 h assay. Values from ref 13.
[b]Expressed as mean cysts/g of lung tissue, for approximately equivalent doses of 9–10 μM/kg/day.
[c]Expressed as mean cysts/g of lung tissue, for approximately equivalent doses of 0.9 μM/kg/day.
[d]See ref 16 for further details of this structure.

$$\sum (r_{max}/r_j)^2$$

where r_{max} is the maximum observed van der Waals distance, taken as 3.5 Å. Table 1 details N_c values calculated for several bis-furamidine structures, together with cytotoxicity and in vivo PCP data. There is an approximate correlation between cytotoxicity and N_c. DNA-binding, at least as estimated by ΔT_m values with the dodecamer $d(CGCGAATTCGCG)_2$, is a less reliable indicator, although the trends are still apparent. The rationale for the design of these and related new DNA minor-groove binding drugs is that DNA-affinity plays an essential role in determining ultimate biological responses, even though by itself it is not the sole factor involved. Thus, correlations between DNA binding and biochemical or biological parameters can be useful in guiding further rational design.

A high level of PCP activity is similarly correlated with a high value for N_c. At higher doses of drug, there is little discrimination between the various derivatives. However at low dosages, the cyclopentyl derivative is outstandingly active. It is currently being further evaluated as a candidate drug for *P. carinii* infections, with the added advantage that it has relatively low acute toxicity. The fact that the cyclopentyl derivative is correctly indicated by the contact-scoring procedure as the most active against PCP is further evidence in favour of a DNA-associated mode of action against the pathogen. This study also demonstrates the usefulness to the drug design process of X-ray crystallographic studies of binary oligonucleotide complexes of bis-(amidinophenyl) furans. Quantitative analysis of the observed minor-groove contact distances between drug and DNA can thus be used as an effective alternative to computational approaches for calculating intermolecular interactions, which are still not consistently able to reliably predict binding energies.

Acknowledgements

Studies at The Institute of Cancer Research have been supported by the Cancer Research Campaign (Grant SP1384), and by a research studentship to IJS from The Institute of Cancer Research. Studies at Georgia State

University are supported by NIH Grant NIAID AI-33363. We are grateful to Dr. L. R. Kelland for the cytotoxicity data.

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17. Both *N*-alkyl bis-furamidine compounds were synthesised by published procedures. Their complexes with d(CGCGA ATTCGCG) were crystallised by hanging-drop methods. Diffraction data for the cyclobutyl complex was collected at room temperature; that for the cyclohexyl complex was obtained from a flash-frozen crystal at 100 K. A Rigaku R-AXISII image plate was used on a RU200 X-ray generator with mirror optics. Cyclobutyl bis-furamidine complex: $a = 24.86$, $b = 40.71$ and $c = 66.44$ Å, 4992 unique reflections to a resolution of 2.0 Å; cyclohexyl bis-furamidine complex: $a = 25.60$, $b = 40.27$ and $c = 67.57$ Å, 5492 unique reflections to a maximum resolution of 1.9 Å. Both structures are in space group $P2_12_12_1$. They were solved by molecular replacement, and refined using the X-PLOR and SHELX-97 programs to R values of 18.8 and 20.8%, respectively, with R_{free} values of 20.9 and 28.0%. Final positional and thermal parameters have been deposited with the Nucleic Acid Database as entry Nos. 1FMQ and 1FMS.