Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Structures of two minor-groove-binding quinolinium quaternary salts complexed with d(CGCGAATTCGCG)$_2$ at 1.6 and 1.8 Å resolution

Adrienne Adams,[a]* Caroline Leong,[a] William A. Denny[b] and J. Mitchell Guss[a]

[a]School of Molecular and Microbial Biosciences, University of Sydney, NSW 2006, Australia, and [b]Auckland Cancer Society Research Centre, Faculty of Medicine and Health Science, University of Auckland, Private Bag 92019, Auckland, New Zealand

Correspondence e-mail: a.adams@mmb.usyd.edu.au

Received 17 May 2005
Accepted 19 July 2005

PDB References: SN8315–d(CGCGAATTCGCG)$_2$, 1zph, r1zphsf; SN8224–d(CGCGAATTCGCG)$_2$, 1zpi, r1zpisf.

The structures of the complexes formed between two anticancer minor-groove-binding quinolinium quaternary salts and the dodecamer d(CGCGAATTCGCG)$_2$ have been refined to resolutions of 1.6 and 1.8 Å. The complexes crystallized in space group $P2_12_12_1$ and the structures are isomorphous with previously solved dodecamer structures. The ligands both bind in the central AT region of the minor groove and although the crystallization conditions and structures of the ligands are very similar, they bind in opposing orientations. The structures are compared with two previously published structures of quinolinium quaternary salts, refined at 2.5 Å resolution, complexed with d(CGCGAATTCGCG)$_2$ and an e^{6}G-modified DNA.

1. Introduction

Quinolinium quaternary salts (QSs; see Fig. 1 for structures of compounds) are a class of DNA minor-groove ligands that bind selectively to AT-rich sequences with a binding-site size of 5–6 base pairs (Baguley, 1982; Kittler *et al.*, 1996). They were originally developed as anticancer drugs, with structure–activity studies showing that activity correlated most strongly with the degree of AT-selective binding (Denny *et al.*, 1979). One example was considered for clinical trial, but was found to be too toxic (Plowman & Adamson, 1978). The mechanism of anticancer action of the compounds has not been defined, but is almost certainly related to their DNA-binding ability. They also show good anti-trichomonal activity, with minimal

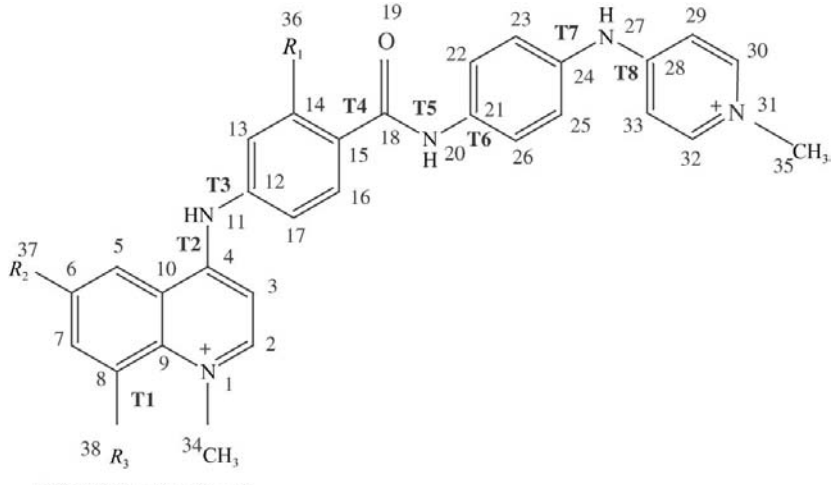

Figure 1
Structure of the compounds.

inhibition concentrations equivalent to that of metronidazole against *Trichomonas vaginalis* (which has 71% AT base pairs in its genome). This is of interest owing to the increasing prevalence of metronidazole-resistant *T. vaginalis* (Chavalitshewinkoon-Petmitr *et al.*, 2003).

NMR studies of the QS SN6999 bound to the oligonucleotide d(GCATTAATGC)$_2$ (Chen *et al.*, 1992; Leupin *et al.*, 1986) showed that the drug binds in the minor groove in the central AT region, equally populating the two possible binding orientations (5′→3′ and 3′→5′) and rapidly exchanging between the two ($k > 100\ s^{-1}$; Leupin *et al.*, 1986). In addition, the crystal structures of SN6999 (Gao *et al.*, 1993) and SN7167 (Squire *et al.*, 1997) complexed with CGC(e^{6}G)-AATTCGCG and CGCGAATTCGCG, respectively, have been solved at a resolution of about 2.5 Å. Although these two compounds both bind in the central region of the minor groove of the duplex DNA and the central amide N atom of each compound donates a hydrogen bond to the DNA, they bind in opposing orientations and SN6999 binds more deeply in the groove. The unusually bent DNA of the SN6999 complex structure was attributed to a combination of the rigid drug and the alkylated DNA; however, other minor-groove-binder/alkylated DNA complex structures do not have such bent DNA (Squire *et al.*, 1997). The SN7167 compound did not penetrate as far into the minor groove of the DNA, making many fewer close contacts than the SN6999 compound in its DNA complex. This may be a consequence of the additional amino groups on SN7167, although neither of these groups is in close contact with the DNA. The DNA and crystallization conditions of these two complexes were significantly different and both structures are at moderate resolution.

We have solved the structure of two new QS compounds, SN8224 and SN8315, complexed with the dodecamer CGC-GAATTCGCG. These two structures are at higher resolution than previous QS–DNA complex structures; both use unmodified DNA and have similar crystallization conditions. We have compared the structures of the four available complexes in detail and reached conclusions for the requirements for minor-groove binding by QS analogues. A detailed structure-based understanding of the binding mode of these compounds should prove useful in the design of derivatives with optimized DNA binding and therefore with possibly optimized biological activity.

2. Materials and methods

2.1. Crystallization and data collection

The HPLC-purified self-complementary deoxyribonucleotide CGCGAATTCGCG was purchased from Oswel DNA Service (University of Southampton, England). SN8224 and SN8315 were synthesized as described previously (Atwell & Cain, 1973). The DNA and the ligand were both dissolved in water and stored frozen at 253 K. Crystals were grown at 285 K by vapour diffusion from sitting drops in Cryschem 24-well crystallization plates. The drop which produced the SN8224 complex crystals initially contained 30 m*M* sodium cacodylate buffer pH 7.0, 0.6 m*M* DNA (duplex concentration), 1.2 m*M* ligand, 0.6 m*M* spermine tetrahydrochloride, 5 m*M* $MgCl_2$ and 8% MPD equilibrated against a 1 ml reservoir of 55% MPD. Colourless rectangular crystals measuring approximately 0.4 × 0.2 × 0.2 mm appeared in six months. For the SN8315 complex crystal, the drop initially contained 20 m*M* sodium cacodylate buffer pH 7.0, 0.4 m*M* DNA (duplex concentration), 0.4 m*M* ligand, 0.6 m*M* spermine tetrahydrochloride, 10 m*M* $MgCl_2$ and 4% MPD equilibrated against a 1 ml reservoir of 50% MPD. Colourless rectangular crystals measuring approximately 0.25 × 0.2 × 0.2 mm appeared in five months. Crystals were removed from the drops, placed in Riedel de Haen perfluoropolyether RS 3000 oil, mounted in a cryoloop and frozen at 100 K in an N_2 cryostream. Diffraction intensities were recorded in two passes on a MAR image-plate system mounted on a Rigaku RU-200 (80 mA, 44 kV) rotating-anode generator with 300 µm focus Osmic blue optics (Cu $K\alpha$, 1.5418 Å) and crystal-to-detector distances of 100 and 200 mm. Diffraction data were integrated and scaled using the *DENZO* and *SCALEPACK* programs from the *HKL* suite (Otwinowski, 1993). Crystals of the SN8224 complex diffracted to 1.6 Å resolution and crystals of the SN8315 complex diffracted to 1.8 Å resolution; see Table 1 for crystal details and data-collection parameters.

Table 1
Crystal details, data-collection and final refinement parameters.

Values in parentheses are for the last shell.

	SN8224 complex	SN8315 complex
Unit-cell parameters (Å)	$a = 25.19$, $b = 39.87$, $c = 65.96$	$a = 24.87$, $b = 39.91$, $c = 65.71$
Space group	$P2_12_12_1$	$P2_12_12_1$
No. of observations	103297	109986
No. of unique reflections	9114	6294
Resolution range (Å)	40–1.6	40–1.8
R_{sym}† (%)	5.4 (38.9)	9.2 (51.1)
Completeness	98.2 (94.3)	97.2 (98.9)
Mean $I/\sigma(I)$	22.5 (2.5)	15.3 (2.6)
R/R_{free} (%)	22.2/25.8	23.4/31.4
R.m.s.d. from ideal		
Bond lengths (Å)	0.017	0.017
Bond angles (°)	1.693	1.672

† $R_{sym}(I) = \sum_{hkl}\sum_i |I_i(hkl) - \overline{I(hkl)}| / \sum_{hkl}\sum_i I_i(hkl)$.

2.2. Solution and refinement

Crystals of the complexes were isomorphous with the previously refined high-resolution structure of d(CGCGAA-TTCGCG)$_2$ (NDB code BD0007; Tereshko *et al.*, 1999). The structure of the SN8224–DNA complex was refined using the program *REFMAC*5 (Collaborative Computational Project, Number 4, 1994), commencing with a model of the high-resolution d(CGCGAATTCGCG)$_2$ structure with all the ions and solvent molecules removed. 8% of the reflections were separated into a reference set to monitor R_{free}. Initially, the model was treated as a single rigid body and data were limited to a resolution of 4 Å. The model was then divided into 24 rigid bodies and the refinement was extended to 2 Å resolu-

tion ($R = 0.35$, $R_{free} = 0.36$). Finally, all data to 1.6 Å resolution were used in rounds of restrained refinement ($R = 0.30$, $R_{free} = 0.34$). An iterative refinement procedure was then conducted interspersed with inspection of electron-density maps and manual model rebuilding with the program *O* (Jones *et al.*, 1991). Inspection of $(2F_o - F_c)$ and $(F_o - F_c)$ electron-density difference maps after the initial rounds of refinement revealed the positions of a magnesium ion and a number of water molecules. These were included in the subsequent refinement. The position of the drug molecule and its orientation were also obvious from the electron-density maps (Fig. 2*a*) and the drug was included in the model in the last rounds of refinement. The topology files for the ligands were generated by *PRODRG* (Schüttelkopf & van Aalten, 2004). Disorder was apparent around several phosphate groups and alternate conformations were modelled for four of these groups. The final refinement parameters are given in Table 1.

The structure of the SN8315 complex was refined using the SN8224 complex structure (with the magnesium ion, waters and ligand removed) as a starting model. The magnesium ion, waters and drug position were apparent in $(F_o - F_c)$ electron-density maps and were modelled into the structure. The electron density for this drug was not as well defined as it was for SN8224. We initially tried to place the drug in the same orientation as SN8224, but the quinolinium ring clashed with G12 and the fit to the density was poor. Moving the drug along the minor groove away from G12 resulted in a worse fit to the electron density. By flipping the drug lengthwise, it was apparent that this new position was the only orientation that would fit the electron density. The R/R_{free} values dropped from 0.264/0.350 to 0.253/0.326 on addition of the drug. Although some of the phosphate groups were apparently disordered, the data were not of sufficient resolution to model more than one conformer.

3. Results

3.1. Global structure and crystal packing

The DNA duplex structure and crystal packing of the CGCGAATTCGCG–SN8224 complex is very similar to that of the high-resolution dodecamer structure (Tereshko *et al.*, 1999) as expected since the crystals were isomorphous. An SN8224 molecule lies in the central portion of the minor groove (Fig. 2*a*). The asymmetric unit of the unit cell contains a duplex of DNA dodecamer, one SN8224 molecule, one magnesium ion and 82 water molecules. The nucleotides are labelled C1–G12 and C13–G24 in the 5′→ 3′ direction and the numbering scheme used for the drug is given in Fig. 1. The SN8224 molecule in the minor groove adopts a very similar position and orientation to the SN6999 molecule in the CGCe^{6}GAATTCGCG–SN6999 complex structure (Gao *et al.*, 1993). The DNA duplex structure and crystal packing of the CGCGAATTCGCG–SN8315 complex is again very similar to that of the high-resolution dodecamer structure (Tereshko *et al.*, 1999). The SN8315 molecule lies in the central portion of the minor groove but in the opposite orientation to the SN8224 molecule of the CGCGAATTCGCG–SN8224 structure (Fig. 2*b*). An asymmetric unit contains a duplex of the DNA dodecamer, one SN8315 molecule, one magnesium ion and 33 water molecules.

3.2. DNA conformation

The DNA main-chain and glycosidic torsion angles, as well as the furanose conformations, for the SN8224 and the SN8315 complex structures are very similar to canonical B-DNA values, the high-resolution structure used as a model and also to the SN7167 complex structure (Squire *et al.*, 1997). The torsion angles for the DNA structure of the SN6999 (Gao *et al.*, 1993) complex are quite different; see, for example, Fig. 3,

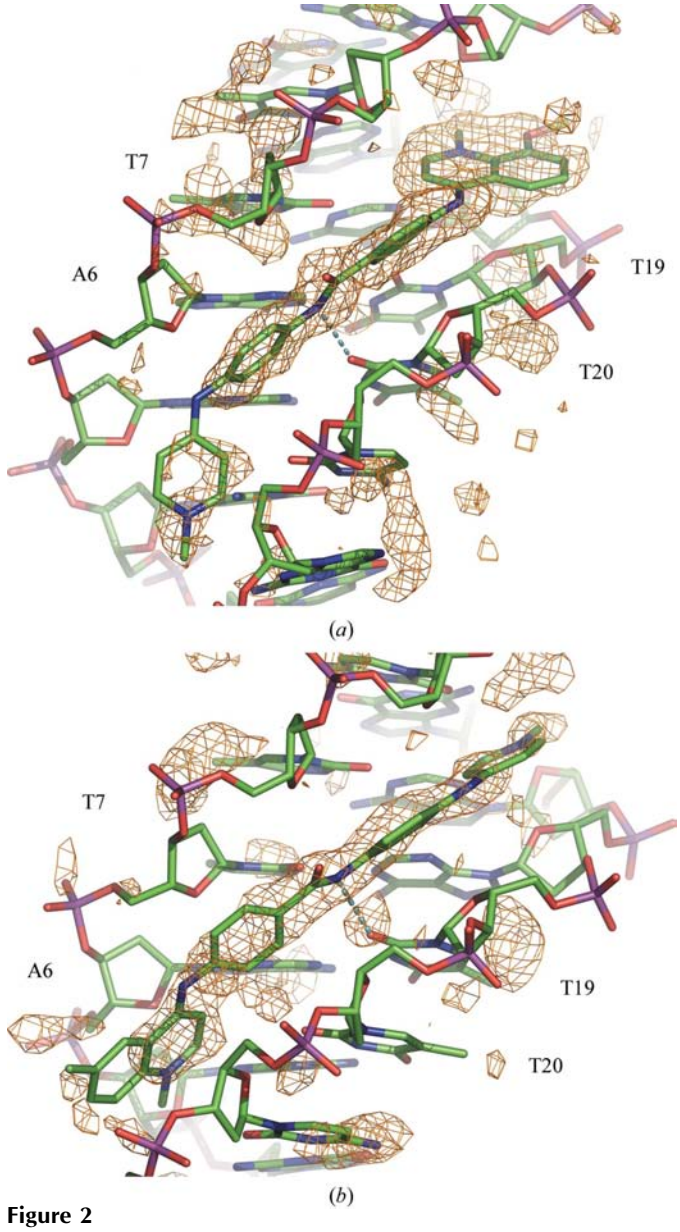

Figure 2
Difference electron density ($F_o - F_c$) maps (gold chicken wire) of the minor groove of (*a*) the SN8224 complex structure and (*b*) the SN8315 complex structure with the drugs omitted from the map calculations. The final refined positions of the drugs are superposed. Hydrogen bonds are shown as dotted cyan lines.

Table 2
Biological data, curvature of DNA and number of close contacts between ligand and DNA for the native DNA and four complex structures.

	Native	SN6999	SN7167	SN8224	SN8315
Antileukaemia log ILS_{max}†		2.23	2.45	2.14	2.17
Antileukaemia dose for 40% increase in lifespan‡ (mg kg^{-1})		2.0	2.9	0.30	1.0
DNA-axis bend UU (°)	11.5	21.5	12.7	11.0	9.9
DNA-axis bend PP (°)	19.6	39.1	12.1	13.2	14.7
No. of close contacts (drug to DNA), 2.0–3.4 Å		46§	15¶	37	31

† Denny *et al.* (1979). A larger number indicates higher activity. ‡ Denny *et al.* (1979). A lower number indicates higher therapeutic potency. § Gao *et al.* (1993). ¶ Squire *et al.* (1997).

where the γ angles for the five structures are plotted. The geometrical properties of base-pair steps and base pairs again are very similar to the values for the high-resolution BD0007 structure. The propeller twist is large and negative for the AATT central region as has previously been observed for this DNA.

3.3. Drug–DNA interactions

Both drugs sit in the central portion of the minor groove, making one hydrogen bond to an O2 atom of a thymine residue, either T19 or T20. There are many other close contacts between the drugs and the DNA (Tables 2 and 3).

The ligands are bent in different ways to fit the minor groove. For SN8315 most of the dihedral angles indicate a planar conformation (Table 4), except for the central amide bond, which has a dihedral angle of 27°. Although SN7167 also has a bent amide bond (17°; Squire *et al.*, 1997), SN8315 is noticeably more bent. For SN8224 it is the T3 (35°) and T7 (−132°) dihedral angles which show a significant deviation from planarity, allowing the outer two rings to twist away from the plane of the central two rings and creating the curvature of the drug (see Fig. 2 and Table 4).

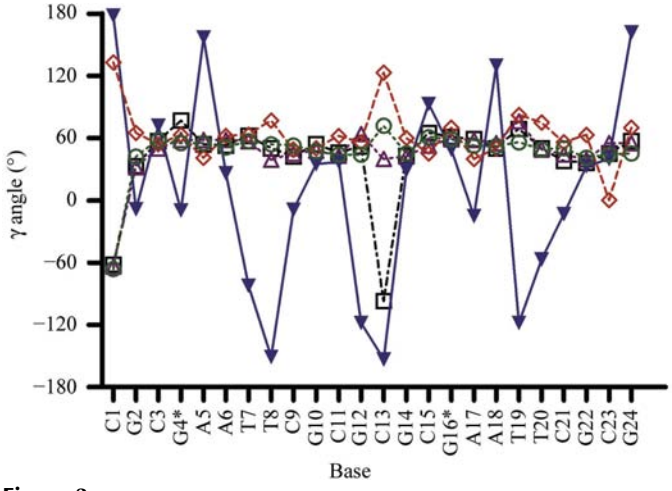

Figure 3
γ angles for each base of the native structure and the four complex structures. Green circles, NDB code BD0007; purple triangles, SN8224; black squares, SN8315; red diamonds, SN7167; blue filled triangles, SN6999. Angles calculated using the program *Curves*5.2 (Lavery & Sklenar, 1989).

Table 3
Close contacts (2–3.5 Å) between SN8224 (or SN8315) and $d(CGCGAATTCGCG)_2$.

SN8224	DNA	Distance (Å)	SN8315	DNA	Distance (Å)
C39	C9 O3′	3.48	C37	G22 O1P	3.49
C8	T19 C4′	3.37	C7	G22 C5′	3.39
C8	T19 C5′	3.48	C34	C21 O2	3.42
C7	T19 C4′	3.40	C2	A5 N3	3.29
C7	T19 C5′	3.30	C2	A5 C2	3.36
C6	T19 C4′	3.49	C2	A6 O4′	3.17
C6	T19 O3′	3.21	C13	T20 O2	3.21
C5	T19 O3′	3.01	C14	T20 O2	3.46
C9	T19 C4′	3.44	C14	T20 O4′	3.48
N1	C9 O4′	3.28†	N20	T7 O2	3.20‡
C34	A18 N3	3.43	N20	T19 O2	2.98‡
C34	T8 O2	3.47	C21	T19 O2	3.38
C34	C9 O4′	3.05	C26	A18 N3	3.35
C2	A18 C2	3.47	C26	A18 C2	3.31
C2	A18 N3	3.47	C26	T19 O2	3.22
C2	T8 O2	2.90	C25	A18 N3	3.16
C3	T7 O2	3.49	C25	T8 O2	3.41
C3	T8 O4′	3.19	C25	T19 O4′	3.28
C12	T20 O4′	3.48	N27	C9 C4′	3.36
C17	T19 O2	3.11	N27	C9 O4′	3.33
C17	T20 O4′	3.03	C28	C9 O4′	3.15
C16	T20 O4′	3.29	C29	C9 O4′	3.13
N20	A6 N3	3.48	C30	A17 N3	3.37
N20	T20 O2	3.05‡	C30	A17 C2	3.36
C26	A5 C2	3.49	C30	A18 O4′	3.29
C26	A6 N3	3.29	C30	C9 O2	2.93
C26	T20 O2	3.22	N31	A18 C4′	3.46
C25	A5 N3	3.47	N31	A18 O4′	3.29†
C25	A6 O4′	3.26	N31	C9 O2	3.30†
N31	G22 O4′	3.41†	C35	A18 O4′	3.49
C35	C23 O4′	3.13	C35	C9 O2	3.39
C32	G4 N2	3.34			
C32	G22 C4′	3.48			
C32	G22 O4′	2.83			
C32	C21 O2	3.20			
C33	G22 O4′	3.47			
C33	C21 O2	2.97			

† Possible charge-induced dipole interaction. ‡ Hydrogen bond.

Table 4
Torsion angles (°) for SN8224 and SN8315 complexes (see Fig. 1 for torsion-angle definitions).

	T1	T2	T3	T4	T5	T6	T7	T8
SN8224	168	−163	35	−167	−179	−180	−132	2
SN8315		165	175	27	−178	−169	−174	−169

3.4. SN8224–DNA interactions

SN8224 sits in the minor groove in the region of the C3–G22 base pair to the C9–G16 base pair, making one hydrogen-bonding interaction between T20 O2 and the amide N atom (N20) of 3.05 Å. There are 36 other close contacts within the minor groove (see Table 3). The two amine linkers of the drug point out of the minor groove and do not interact with the DNA. One (N11) makes a hydrogen bond to a water molecule (W151, 3.11 Å). The only interaction between the drug and a symmetry-related molecule is a contact between C35 of the drug and G12 O3′ (3.24 Å) of a symmetry-related molecule

which enters the minor groove and forms a G–G inter-duplex hydrogen-bonding interaction, typical of dodecamer crystal structures, with G2. G24 of another symmetry-related molecule enters the minor groove at the other end of the duplex, forming another inter-duplex hydrogen-bonding interaction with G14 but making no direct contact with the ligand. There are two possible charge-induced dipolar interactions between the quaternary N atoms of the drug and the O4′ atoms of the DNA in this structure: one between N1 and C9 O4′ (3.28 Å) and the other between N31 and G22 O4′ (3.41 Å).

3.5. SN8315–DNA interactions

SN8315 sits in the minor groove in the region of the G4–C21 base pair to the C9–G16 base pair, with the amide N atom at hydrogen-bonding distance to T19 O2 (2.98 Å) and A7 O2 (3.2 Å). There are 29 other close contacts within the minor groove (see Table 3). There is also one water molecule hydrogen bonded to SN8315, W74, which is 2.75 Å from N27. In this structure, the closest drug contact to the symmetry-related G12 O3′ atom is C34 at a distance of 6.52 Å, indicating that the drug in this structure is displaced towards the G12–C13 end compared with SN8224 in its complex structure. The SN8315 complex is similar to the structure to the SN7167–CGCGAATTCGCG complex (Squire *et al.*, 1997) in terms of drug orientation, but SN8315 makes many more contacts in the minor groove (Tables 2 and 3). There is one possible charge-induced dipolar interaction, between N31 and A18 O4′ (3.29 Å), in the SN8315 complex structure.

4. Discussion

We have studied the DNA-complex structures of two new minor-groove-binding compounds which are potential lead compounds in a search for anticancer agents. Owing to the relatively high resolution of these structures, we are able to examine in detail how these compounds bind to DNA. Both complexes contain the dodecamer CGCGAATTCGCG, the structure of which was first solved by Dickerson and Drew in 1981 (Drew *et al.*, 1981). There is a rich literature of structures of compounds bound in the minor groove of this dodecamer, generally at a resolution of no better than 2.2 Å, from several different groups (for example, Brown *et al.*, 1990; Edwards *et al.*, 1992; Pjura *et al.*, 1987; Sriram *et al.*, 1992; Tabernero *et al.*, 1993). The only other minor-groove DNA-binding structures of a higher resolution than those reported here are those of the dehydrated crystals of *m*-iodo Hoechst bound to CGCGAATTCGCG (Clark *et al.*, 2000). We are able to model disordered phosphates in a similar manner to the high-resolution structures of a guanine tetraplex (Phillips *et al.*, 1997) and GCGAATTCG (Soler-Lopez *et al.*, 2000), neither of which have bound ligands. It has been suggested that alkylation leading to increased bending of DNA, as observed in the SN6999-complex structure (Gao *et al.*, 1993), is necessary for deep penetration of QS into the minor groove. Our structures, with unmodified DNA and an average degree of bending, contradict this hypothesis since they allow QS to bind deeply in the minor groove. The large curvature of the SN6999-complex structure compared with the other three structures (Table 2) may be a consequence of the presence of ammonium acetate in the crystallization buffer rather than the QS in the minor groove as previously proposed (Gao *et al.*, 1993).

SN8224 and SN8315 differ only by the presence of substituents in the R_2 and the R_3 positions (Fig. 1), yet they bind in opposite orientations. If SN8224 is replaced by SN8315 in its complex structure then C37 is 3.2 Å from T20 O1P. Conversely, if SN8315 is replaced by SN8224 in its complex structure then C39 makes a close contact with G22 C5′ (3.01 Å). These contacts may be enough to orient the drugs in the minor groove. We note that the two compounds with a group at R_2, SN7167 and SN8315, bind in the same orientation; the presence of this group may prevent binding in the alternative direction and hence it may be the R_2 group that dictates the binding orientation. There was no evidence in the electron-density maps that a particular drug could bind in both orientations. The crystal structure of the SN6999 complex also has the drug bound in a single orientation. However, NMR studies indicate that SN6999 can bind in two orientations in the minor groove (Chen *et al.*, 1992; Leupin *et al.*, 1986); it may be that the longer stretch of AT in the NMR study allows alternative orientations.

Clearly, it is important for QS binding in the minor groove to have a hydrogen bond between the amide group on the drug and DNA, but this can be with T19 O2 or T20 O2. SN7167 has fewer contacts in the minor groove than the other three compounds. The crystallization buffer for this complex has a lower cacodylate concentration, a lower pH and a higher Mg concentration than the buffers used to crystallize the other three complexes. The crystallization conditions may contribute to the degree to which the drug enters the minor groove, highlighting the necessity to standardize the crystallization conditions when comparing complex structures.

The four complex structures compared here are all of antileukaemic compounds with a similar biological activity but with a tenfold variation in therapeutic potency (Table 2). We are unable to correlate antileukaemic activity with complex structure at this time; however, the solution of additional structures with a broader range of activity may show a relationship.

We wish to thank the National Health and Medical Research Council of Australia for financial support and Stephen Graham for assistance with preparing the figures.

References

Atwell, G. J. & Cain, B. F. (1973). *J. Med. Chem.* **16**, 673–678.
Baguley, B. C. (1982). *Mol. Cell. Biochem.* **43**, 167–181.
Brown, D. G., Sanderson, M. R., Skelly, J. V., Jenkins, T. C., Brown, T., Garman, E., Stuart, D. I. & Neidle, S. (1990). *EMBO J.* **9**, 1329–1334.
Chavalitshewinkoon-Petmitr, P., Ramdja, M., Kajorndechakiat, S., Ralph, R. K., Denny, W. A. & Wilairat, P. (2003). *J. Antimicrob. Chemother.* **52**, 287–289.

Chen, S. M., Leupin, W., Rance, M. & Chazin, W. J. (1992). *Biochemistry*, **31**, 4406–4413.
Clark, G. R., Squire, C. J., Baker, L. J., Martin, R. F. & White, J. (2000). *Nucleic Acids Res.* **28**, 1259–1265.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Denny, W. A., Atwell, G. J., Baguley, B. C. & Cain, B. F. (1979). *J. Med. Chem.* **22**, 134–150.
Drew, H. R., Wing, R. M., Takano, T., Broka, C., Tanaka, S., Itakura, K. & Dickerson, R. E. (1981). *Proc. Natl Acad. Sci. USA*, **78**, 2179–2183.
Edwards, K. J., Jenkins, T. C. & Neidle, S. (1992). *Biochemistry*, **31**, 7104–7109.
Gao, Y. G., Sriram, M., Denny, W. A. & Wang, A. H.-J. (1993). *Biochemistry*, **32**, 9639–9648.
Jones, T. A., Zou, J.-Y., Cowan, S. W. & Kjeldgaard, M. (1991). *Acta Cryst.* A**47**, 110–119.
Kittler, L., Wahnert, U., Baguley, B. C., Bailly, C., Waring, M. J. & Lober, G. (1996). *Anticancer Drug Des.* **11**, 101–115.
Lavery, R. & Sklenar, H. (1989). *J. Biomol. Struct. Dyn.* **6**, 655–667.
Leupin, W., Chazin, W. J., Hyberts, S., Denny, W. A. & Wuethrich, K. (1986). *Biochemistry*, **25**, 5902–5910.
Otwinowski, Z. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 56–62. Warrington: Daresbury Laboratory.
Phillips, K., Dauter, Z., Murchie, A. I., Lilley, D. M. & Luisi, B. (1997). *J. Mol. Biol.* **273**, 171–182.
Pjura, P. E., Grzeskowiak, K. & Dickerson, R. E. (1987). *J. Mol. Biol.* **197**, 257–271.
Plowman, J. & Adamson, R. H. (1978). *Pharmacology*, **17**, 61–68.
Schüttelkopf, A. W. & van Aalten, D. M. F. (2004). *Acta Cryst.* D**60**, 1355–1363.
Soler-Lopez, M., Malinina, L. & Subirana, J. A. (2000). *J. Biol. Chem.* **275**, 23034–23044.
Squire, C. J., Clark, G. R. & Denny, W. A. (1997). *Nucleic Acids Res.* **25**, 4072–4078.
Sriram, M., Van der Marel, G. A., Roelen, H. L. P. F., Van Boom, J. H. & Wang, A. H.-J. (1992). *EMBO J.* **11**, 225–232.
Tabernero, L., Verdaguer, N., Coll, M., Fita, I., van der Marel, G. A., van Boom, J. H., Rich, A. & Aymami, J. (1993). *Biochemistry*, **32**, 8403–8410.
Tereshko, V., Minasov, G. & Egli, M. (1999). *J. Am. Chem. Soc.* **121**, 470–471.