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Mini-Review

Lexitropsins in antiviral drug development

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Antigene; Antisense; Lexitropsin; Sequence selective; Drug development

Introduction

Antiviral chemotherapy continues to be one of the important means of controlling viral diseases (Sidwell and Witkowsky, 1979). Successes to date in terms of clinically used drugs that have received FDA approval include ribavarin, acyclovir, vidarabine, zidovudine, idouridine, trifluridine, and amantidine (Montgomery, 1989). Despite these successes new drugs are needed, not only for infections caused by viruses for which agents are available, but also for those infections that are not adequately controlled. These include influenza, hepatitis, gastroenteritis, respiratory tract infections, as well as, most notably, AIDS, the causative agents of which appear to be HIV viruses.

In principle there are a number of steps in virus infection and reproduction which could permit effective chemical intervention based on exploitable biochemical differences that exist between virus-specific processes and cellular processes. These include adsorption of the virus, cell penetration, uncoating, transcription, translation assembly and release (Montgomery, 1989). In practice most of the antivirals that have been developed to date, and most of the current efforts, both industrial and academical, are devoted to purine-pyrimidine antimetabolites that interfere with viral replication.

One of the most challenging problems in the use of drugs in the treatment of human disease is of specificity. Many antineoplastic and antiviral drugs, as noted above, interfere with replication and transcription of DNA where a lack

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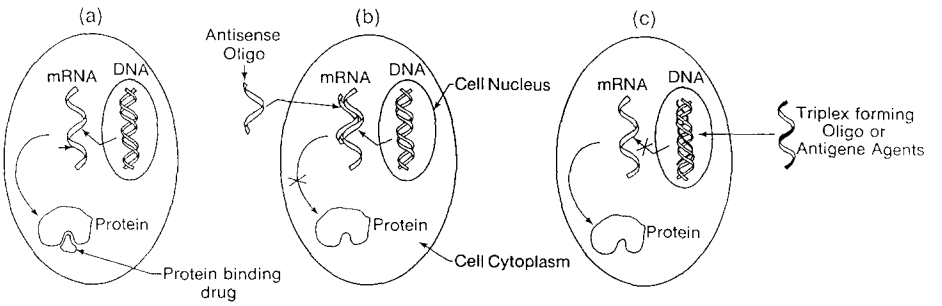


Fig. 1. Schematic representation of alternative strategies of chemical intervention in the cell cycle. (a) Introduction of protein-binding drug, (b) antisense oligomer approach, (c) triplex formation or introduction of a groove binding antigene agent.

of specificity is in evidence. The antisense approach (Cohen, 1989) has special appeal for antiviral agents where problems of selectively inhibiting virus replication have proven almost intractable by conventional medicinal chemistry. The utilization of host biochemical processes by viruses makes discrimination extremely difficult. Although the antisense concept of selective chemical intervention in the life-cycle of a transformed cell or of viral replication (Fig. 1b) (Stephenson and Zamecnik, 1978; Izant and Weintraub, 1984) is elegant in principle, in practice it is plagued with difficulties associated with cellular uptake of antisense oligonucleotides, their intracellular instability and subcellular distribution and diastereomer problems associated with backbone modification (Stein and Cohen, 1988). An additional problem has been that of unexpected cellular responses from random or homopolymer control sequences and the resistance of antisense α-DNA:mRNA hybrids to RNase H degradation (Gagnor et al., 1989; Gmeiner et al., 1990). Nevertheless the antisense strategy for chemotherapeutic agent development is being pursued vigorously on an international scale and, for several reasons, candidate drugs for FDA approval and prospective clinical usage are likely to be antivirals (Stein and Cohen, 1988). Inhibition of splicing by antisense oligonucleotides was suggested early as an antiviral approach (Zamecnik and Stephenson, 1978) and splice sites in viral pre-mRNA have been used as targets for hybridization.

One also has to be aware of non-specific effects on gene expression that can be due to the interactions between antisense agents and key proteins. An example of such unexpected effects was brought about by phosphorothioate analogues that terminate translation in a non-specific way both in eukaryotic cell-free extracts and in injected oocytes in the micromolar concentration range (Cazenave et al., 1989). Phosphorothioate DNAs probably exhibit at least part of their anti-HIV property in their high affinity for the viral reverse transcriptase (Matsukura et al., 1987).

Interest in the therapeutic application of antisense oligonucleotides has been confined almost exclusively to antivirals so far. This is because more is known

about the molecular biology and RNA sequences of viruses than about other systems. There is also a pressing need for new approaches in this area conspicuously lacking in therapeutics, and targeting viral nucleic acids seems particularly suited to addressing the problems of selective toxicity. While the replication of several viruses in tissue culture has been inhibited in this way (Goodchild, 1989), in very few cases has the mechanism of the antiviral effect been established. In such examples the target has been predominantly viral RNA. For viruses that become integrated into host DNA, such as HIV or herpes, this approach may contain the spread of the virus and control its outbreak but is unlikely to solve the underlying problem of latency. Prolonged or repeated treatment would be necessary with the attendant problems of side-effects and development of resistance to the drug (Goodchild, 1989).

A complementary strategy in genetic targetting to antisense agents, which are designed to seek out mRNA, is that of antigene agents which are designed to target individual cellular or proviral gene sequences (Fig. 1c) (Cooney et al., 1988; Durland et al., 1990; Helene et al., 1990: Beal and Dervan, 1991; Riordan and Martin, 1991; Strobel and Dervan, 1991). The design of antigene agents targeted against DNA will not be as straightforward as for RNA, as so far there are no examples of their use in cells. If they can be developed, they will be a major new addition to agents for the control of gene expression. The antigene concept is attractive at first sight from the crucial viewpoint of selectivity. Thus RNA species typically exist at a copy number of 100–100 000 per cell and are being regenerated over time, if the encoding gene is transcriptionally active (Riordan, 1991). However at the DNA level, the copy number of the corresponding sequence is in the range of 1, 2 or only a few per cell and, unlike RNA, DNA is only being regenerated at the modest rate of cell division. These considerations suggest that DNA-directed agents could potentially be effective at lower dose levels than those of the corresponding antisense or conventional antimetabolite agents. However, pharmacological factors may tend to decrease this apparent advantage. A referee has pointed out that even if only one drug molecule is required to attack one target in DNA, this will still require a large number of molecules to be present to provide the concentration gradient for this to occur. In addition, since the antigene molecule must pass through the cytosol to reach the nuclear target, it may still require dose levels comparable with that of an antisense drug.

Efforts within the framework of antigene strategy are currently concentrated on triple helix formation wherein the oligomer probe binds within the major groove of the target duplex DNA sequence (Fig. 1c) (Cooney et al., 1988; Helene et al., 1990; Beal and Dervan, 1991). There are, however, constraints on the types of DNA code that can be recognized by triple helix agents. The purine and pyrimidine motifs both require homopurine tracts within the target DNA. Sequence searches of therapeutically relevant genes reveal that this homopurine requirement greatly limits the number of targets that can be addressed. Circumvention of this code restriction will require, inter alia, devising novel heterocycles that can engage in hydrogen bonding schemes to recognize the

base couplets that currently cannot participate in triple helix formation (Riordan and Martin, 1991).

Recently it has become evident that DNA sequence selectivity is an important component contributing to the cytotoxic potency of several chemotherapeutic agents derived from natural sources. Examples include: the pyrrolo(1,4)-benzodiazepinone antitumor antibiotics (Hurley et al., 1988), saframycins (Rao and Lown, 1990), CC-1065 (Hurley et al., 1988), caleachimycin (Lee et al., 1987), and bleomycin (Stubbe and Kozarich, 1987). Therefore the question arises whether one could tailor the binding preference of DNA-binding agents to particular base sequences and thereby produce new drugs that might prove effective clinically and complement efforts in the triple helix antigene area. Recent advances in our understanding of molecular recognition between ligands and nucleic acids, together with the advent of new DNA sequencing and footprinting methodology and molecular modelling, permit the design of sequence-selective agents (Lown 1988; Bailly and Henichart, 1991; Nielsen, 1991).

The concept of lexitropsins, or information-reading molecules

Double helical DNA has two channels of information: the major groove, generally preferred by control proteins because of the higher informational content of the major groove, and the minor groove, generally preferred by antibiotics presumably because it represents a vulnerable site of attack in a competing organism's genetic material. In terms of the design of sequence-reading molecules, while intercalators show little, if any, base preference, groove-binding agents of natural origin often display strict sequence-recognizing properties. Natural substances including histones, and the oligopeptide antiviral antibiotics netropsin and distamycin (Zimmer and Wahnert, 1986), which are minor groove specific, provided the initial lead compounds for our studies in this area. Netropsin, known to recognize predominantly $(AT)_n$ sequences (Zimmer and Wahnert, 1986) offered advantages as a lead compound in view of the detailed information available from the X-ray crystallographic analysis of a netropsin-oligomer complex by Dickerson and coworkers (Kopka et al., 1985). Examination of the X-ray data suggested that the main contributors to molecular recognition consisted of (1) hydrogen bonds from the amide NHs to the floor of the groove, (2) electrostatic interactions between the cationic termini and the negatively charged phosphate residues and (3) the semantophoric or information-reading component is provided by the van der Waals contacts (Fig. 2a). This analysis gave rise to the concept of lexitropsins wherein the netropsin structure is modified to accommodate an appropriate heterocyclic moiety (e.g. imidazole, thiazole, triazole, furan) capable of accepting a hydrogen bond from G-2-NH_2 groups, thereby permitting a change in the base site recognition from T to G (Fig. 2b) (Lown, 1988, 1990).

The base and sequence-selective prototype biscationic lexitropsins synthe-

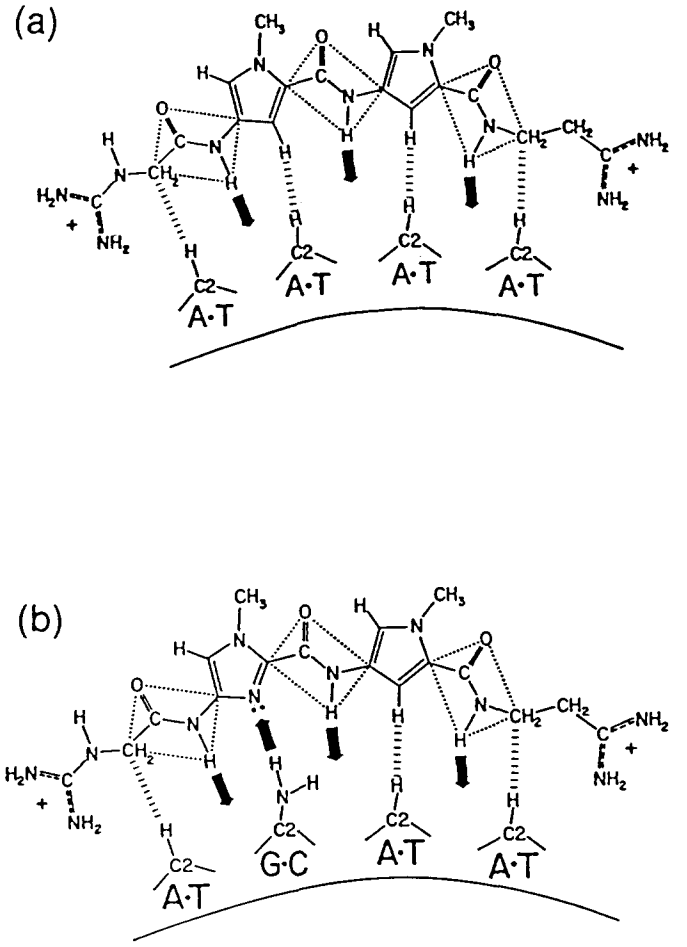


Fig. 2. (a) Bonding components contributing to the molecular recognition and sequence selective binding of netropsin to $(AT)_4$ and (b) prediction of rational alteration of DNA sequence recognition by a prototype lexitropsin.

sized on this basis (Lown, 1989) were evaluated by a number of methods including spectroscopy, complementary strand footprinting, and high field NMR analysis (Lee et al., 1988; 1989a,b,c; Kumar et al., 1990). The consistent conclusion from these extensive studies was that although the strict AT preference of the *N*-methylpyrrole containing lexitropsins steadily decreased as they became progressively imidazole rich, the corresponding capacity to recognize and accept GC sites, while increasing initially, did not reach levels that were anticipated (Burkhardt et al., 1989). This result led to the realization of the importance of electrostatics in the molecular recognition process. Pullman and coworkers pointed out that the highest negative potential in B-DNA exists in the minor groove in AT tracts but on the surface of the major

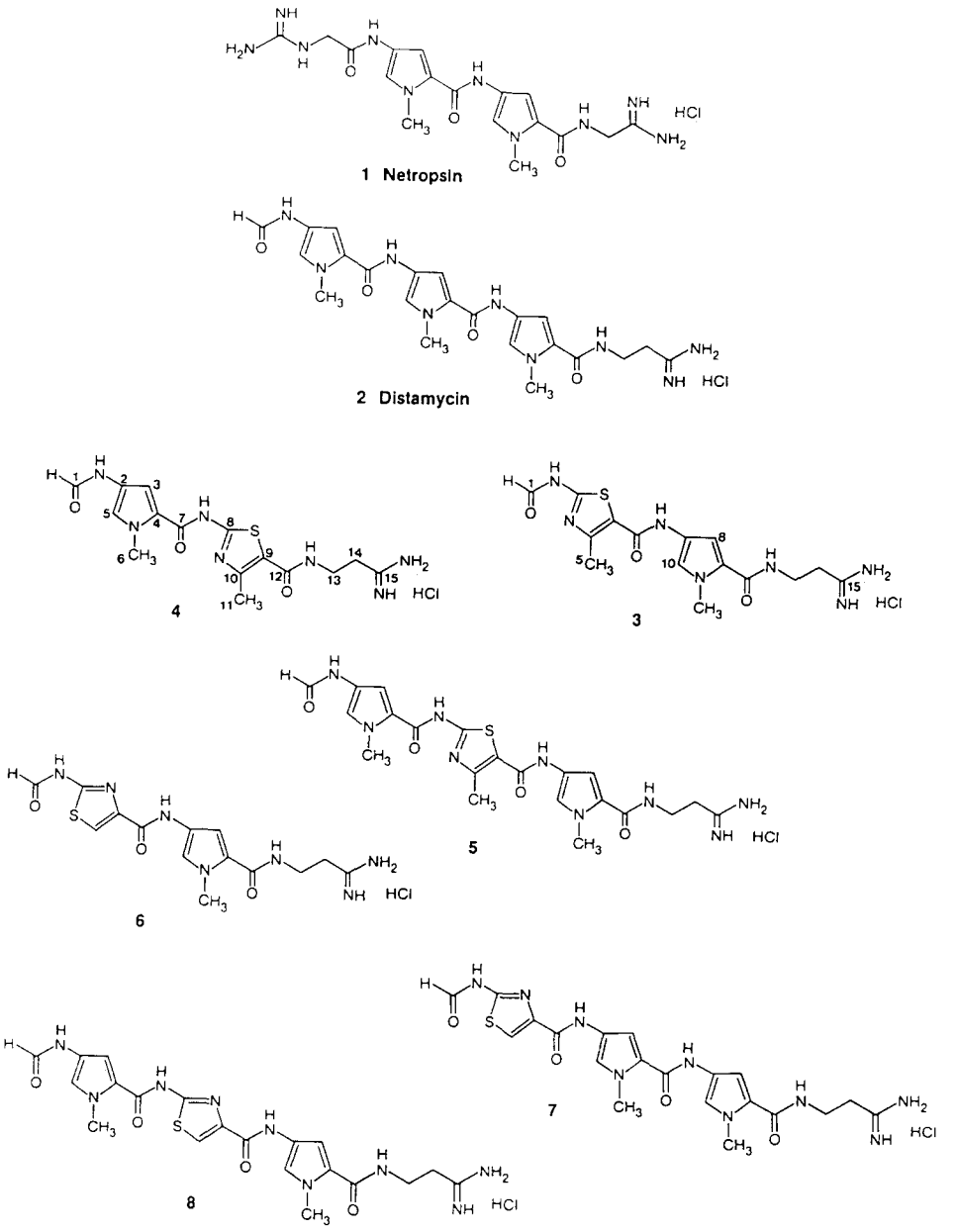


Fig. 3. Structures of natural antibiotics netropsin (1) and distamycin (2) and the novel GC base site avoiding (3, 4, 5) and GC base site accepting (6, 7, 8) thiazole-containing lexitropsins.

groove in GC sequences (Pullman and Pullman, 1981; Lavery et al., 1982, 1985). It follows that polycationic ligands like the prototypes will naturally gravitate to AT sequences regardless of ones ingenuity in designing them to do

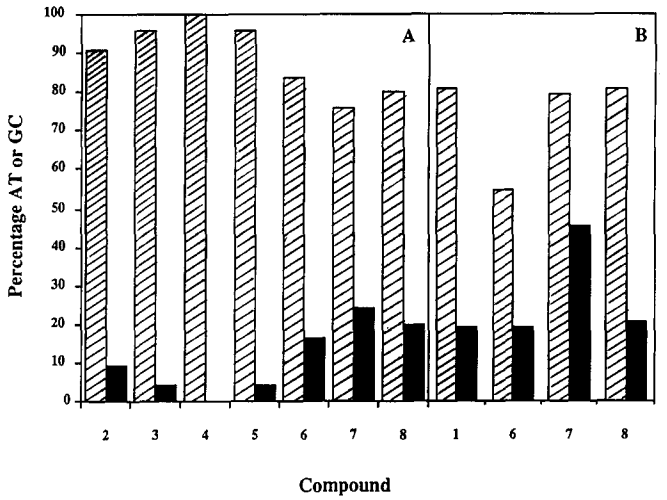


Fig. 4. Percentage of AT (▨) and GC (■) acceptance by netropsin (1) distamycin (2) and thiazole-lexitropsins (3–8) on an *Eco*RI/*Hin*dIII restriction fragment of pBR322 DNA.

otherwise. Accordingly it was then found that monocationic lexitropsins, i.e. based on distamycin, exhibited not only superior GC recognizing capacity but sequence-reading properties in accord with this interpretation (Kissinger et al., 1987).

TABLE 1

Cytotoxicity and antiviral activity of the linked lexitropsins in primary rabbit kidney (PRK) cell cultures

Compound	n[a]	Minimum cytotoxic conc.[b] (μg/ml)	Minimum inhibitory conc.[c] (μg/ml)			
			Herpes simplex virus 1 (IOS)	Herpes simplex virus 2 (G)	Vaccinia virus	Vesicular stomatitis virus
9	0	⩾40	>10	20	7	>40
10	1	⩾10	>4	>4	0.2	>10
11	2	⩾40	>4	>4	>4	>4
12	3	⩾200	⩾200	200	7	>200
13	4	⩾40	>10	>10	0.7	>10
14	5	400	150	70	2	>200
15	6	⩾10	>10	>10	0.2	>10
16	7	⩾100	>40	>40	0.2	>100
17	8	⩾40	>10	>10	0.7	>10
18	9	⩾40	>10	20	2	>40
19	10	⩾40	>10	7	2	>10
20	2	⩾100	>100	>40	20	>100
Tubercidin		⩾1	>0.4	>0.4	>0.1	0.2
(*S*)-DHPA		>400	>400	>400	>70	>70
Ribavirin		>400	>400	300	20	>400
Carbocyclic 3-deazadenosine		>400	>400	400	2	0.7

[a]Number of CH_2 groups in linker; [b]required to cause a microscopically detectable alteration of normal cell morphology; [c]required to reduce virus-induced cytopathogenicity by 50%.

TABLE 2

Cytotoxicity and antiviral activity of the linked lexitropsins against vaccinia virus in different cell cultures

Compound	n[a]	Minimum cytotoxic conc.[b] (μg/ml)				Minimum inhibitory conc.[c] (μg/ml) vaccinia virus					
						PRK			Vero		
		PRK	HeLa	Vero	E_6^{SM}	Expt. 1	Expt. 2	HeLa	Expt. 1	Expt. 2	E_6^{SM}
9	0	40	⩾40	⩾40	100	7	7	20	20	20	2
10	1	40	⩾40	⩾40	⩾40	2	2	2	2	2	0.2
11	2	⩾100	⩾100	⩾200	⩾200	2	2	2	2	7	0.2
12	3	⩾200	⩾400	⩾400	400	20	20	40	70	150	7
13	4	⩾40	⩾400	100	100	2	0.7	1	2	7	0.2
14	5	⩾200	>200	>200	200	7	7	20	20	20	0.7
15	6	⩾40	⩾200	>40	⩾200	0.7	2	2	7	7	0.07
16	7	⩾100	⩾200	>200	⩾100	7	7	20	40	70	0.7
17	8	⩾40	100	⩾40	⩾100	2	2	20	20	>40	0.2
18	9	⩾40	100	100	⩾40	2		20	>40		0.2
19	10	⩾40	⩾40	⩾100	⩾40	2		2	>40		0.2
20	(2)	⩾400	>400	⩾400	>400	300	400	>400	>400	>400	70
21	(2)	⩾4	⩾20	⩾4	>4	>4	>4	>10	>4	>4	>4
Tubercidin		⩾0.4	⩾1	⩾0.4	4	>0.1	>0.1	0.2	0.02	0.02	0.07
(*S*)-DHPA		>400	>400	>200	>400	100	150	70	20	20	100
Ribavirin		>400	⩾400	>400	>400	20	7	20	7	20	70
Carbocyclic 3-deazaadenosine		>400	>400	>200	>400	2	2	0.7	0.2	0.2	2

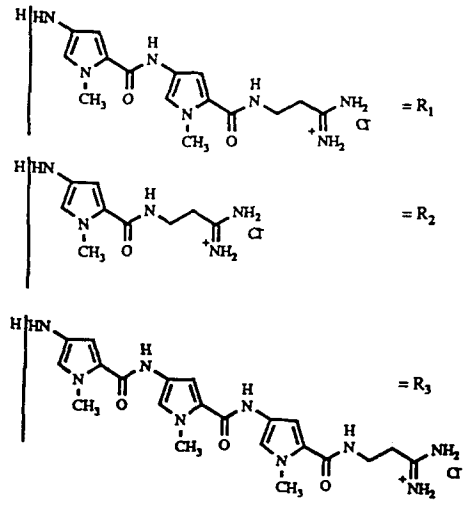
[a,b,c]As Table 1.

TABLE 3

Inhibitory effects of linked lexitropsins on the proliferation of murine leukemia (L1210), murine mammary carcinoma (FM3A), human B-lymphoblast (Raji), and human T-lymphoblast (Molt/4F) cells

Compound	n[b]	ID_{50}[a] (μg/ml)			
		L1210	FM3A	Raji	Molt/4F
1 (Netropsin)		245 ± 92	321 ± 18	139 ± 63	
2 (Distamycin)		27 ± 4.7	31 ± 2.4	24 ± 3.7	
9	0	>100	>100	>100	>100
10	1	28.5 ± 9.7	5.87 ± 2.23	3.39 ± 0.69	2.85 ± 0.64
11	2	>100	47.2 ± 27.8	26.4 ± 2.8	33.8 ± 0.9
12	3	>100	>100	>100	>100
13	4	24.3 ± 8.9	57.8 ± 32.7	13.3 ± 5.6	5.62 ± 0.60
14	5	>100	⩾100	11.1 ± 3.2	5.74 ± 1.85
15	6	3.34 ± 0.27	3.15 ± 0.72	2.14 ± 0.80	1.74 ± 0.28
16	7	10.8 ± 6.6	32.0 ± 9.7	4.36 ± 0.89	3.30 ± 0.99
17	8	4.21 ± 1.52	22.1 ± 11.1	3.24 ± 0.44	2.97 ± 0.49
20	2	>100	>100	>100	>100
21	2	>100	4.29 ± 1.12	41.4 ± 8.4	2.85 ± 0.64

[a]50% Inhibitory dose; [b]number of CH_2 units in linker.



R_1—CO—$(CH_2)_n$—COR_1
R_2—CO—CH_2CH_2—CO—R_2
R_3—CO—CH_2CH_2—CO—R_3

9	R_1—CO—R_1
10	R_2—COCH_2CO—R_1
11	R_1—CO$(CH_2)_2$CO—R_1
12	R_1—CO$(CH_2)_3$CO—R_1
13	R_1—CO$(CH_2)_4$CO—R_1
14	R_1—CO$(CH_2)_5$CO—R_1
15	R_1—CO$(CH_2)_6$CO—R_1
16	R_1—CO$(CH_2)_7$CO—R_1
17	R_1—CO$(CH_2)_8$CO—R_1
18	R_1—CO$(CH_2)_9$CO—R_1
19	R_1—CO$(CH_2)_{10}$CO—R_1
20	R_2—CO$(CH_2)_2$CO—R_2
21	R_3—CO$(CH_2)_2$CO—R_3

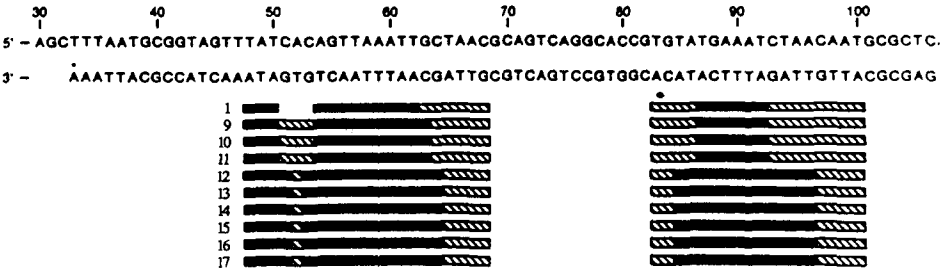


Fig. 5. Structures of linked lexitropsins and summary histogram of areas of DNA protection by these agents of DNase I digestion.

Systematic exploration of the physical characteristics of different types of lexitropsins revealed a number of structural and stereochemical features which contribute to drug design and may be summarized as follows.

- Base site acceptance: incorporation of hydrogen-bond-accepting heterocycles permits the recognition of G sites (Fig. 3) (Lown, 1988, 1990).
- Base site avoidance: incorporation of sterically demanding groups or atoms (e.g. S) directed inwards to the minor groove prevents G site recognition and permits the development of 100% AT selective ligands (Figs. 3 and 4) (Rao et al., 1990).
- Molecular electrostatics: monocationic lexitropsins are permitted to exhibit their intrinsic base and sequence preferential binding in contrast to dicationic ligands (Kissinger et al., 1987).

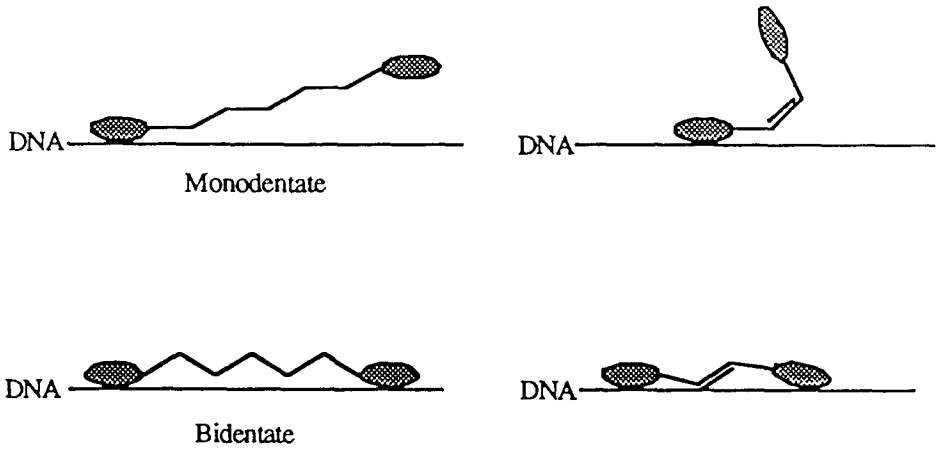


Fig. 6. Mono and bidentate DNA binding of extended linked lexitropsins.

- Stereochemistry: chiral lexitropsins display increased DNA binding when the stereochemistry is appropriate for isohelical recognition (Lee et al., 1989a,c).
- Dynamics: shorter lexitropsins engage in rapid exchange between equivalent recognition sites at rates of approx. 40–60 s^{-1} at ambient temperatures (Kumar et al., 1991).
- Phasing: extended lexitropsins with >5 *N*-methylpyrrole units become out of phase, i.e. there is a lack of dimensional correspondence between the oligopeptide and oligonucleotide sequences. Longer lexitropsins capable of binding to 11 base-pairs may be brought back into phase by the introduction of a linker with appropriate dimensions and properties (Fig. 5) (Kissinger et al., 1990; Lown, 1990; Rao et al., 1991).
- Bidentate vs. monodentate binding: depending on the geometry of the linker, extended lexitropsins may bind both moieties or one at a time in a monodentate exchange or 'operational bidentate' binding mode (Fig. 6) (Kissinger et al., 1990; Lown, 1990; Rao et al., 1991).

One of the major design goals of this work is to produce a lexitropsin capable of recognizing a 15–16-bp sequence which statistically is the minimum length of a unique sequence in the human genome (Helene et al., 1985). Experiments on the uptake and subcellular distribution with spin-labelled lexitropsins in KB human cancer cells revealed fairly rapid uptake, reasonable intracellular stability and concentration in the nucleus (Bailly et al., 1989). The susceptibility of the terminal *N*-formyl group to peptidase action was obviated by the synthesis of appropriate amide isosteres, e.g. the thioamide counterpart (Zimmermann et al., 1991).

Pharmacological properties of lexitropsins

Antiviral and anticancer activity Lexitropsins as a class generally display wide-spectrum antiviral activity in vitro (Tables 1 and 2) as well as cytotoxic action

against a range of human and murine tumor cell lines (Table 3) (Lown et al., 1989). Generally, the longer the lexitropsins and the more strongly they bind to DNA, the more potent are the lexitropsins in terms of cytotoxicity. Correlations exist between the inhibitory activity against two human tumor cell lines Molt/4F and Raji and the DNA binding constants for a series of flexible polymethylene linked bis-netropsins designed to explore the phasing aspect of DNA recognition mentioned above (Lown, 1990). Independent footprinting studies established that for this homologous series a minimum linker length of $(CH_2)_3$ is required to permit bidentate binding to 11 contiguous base-pairs (Fig. 5) (Lown, 1990).

The concept of phasing is a useful one in the design of antivirals when used in conjunction with molecular modelling and molecular mechanics calculations. For example the behavior of a series of rigid 1,2-cycloalkane linked bis-netropsins was examined by force field calculations in order to predict their behavior with respect to isohelicity and closeness of fit within the minor groove (Rao et al., 1991b). This analysis was informative in terms of identifying desirable characteristics in the linker, i.e. length, flexibility to adapt to an induced fit, correct shape to match receptor helicity, and rigidity to promote effective binding. The predictions were then tested experimentally by MPE complementary strand footprinting and by antiviral evaluation. It was concluded from the footprinting experiments (Kissinger et al., 1990; Rao et al., 1991) that, as predicted, *cis*-linked bis-lexitropsins permit only monodentate-DNA binding which is reflected in their relatively lower antivaccinia potency (Rao et al., 1991a). In contrast the *trans*-linked isomers predicted to afford more efficient DNA interaction provided evidence via footprinting of firm bidentate binding (Fig. 6) which is also reflected in a consistently higher antivaccinia potency (Rao et al., 1991a). Also, as predicted, the *trans*-cyclopropane linked structures are the most potent in their antiviral and anticancer activity. Thus the study has provided an understanding of some of the design features that contribute to antiviral potency.

The utility of molecular modelling in the interpretation and visualization of the docking of minor groove binders into their preferred binding sites identified from footprinting is illustrated in Fig. 7. Another illustration of the emerging importance of molecular modelling in the design of antiviral agents is provided by the analogy that exists between pentamidine class of antivirals and the linked bis-lexitropsins. The most potent agent, a minor groove binder, has a distance between the hydrogen bond donor sites of 9.2 Å (Stevens, 1989) which corresponds to the repeat distance between comparable positions in the most potent lexitropsin antiviral of 9.1 Å which permits bidentate binding.

Anti-retroviral activity In addition to the biological properties of the lexitropsins they display marked inhibitory properties against certain enzymes most notably reverse transcriptases. As in the case of the anticancer activity discussed above an homologous series of linked bis-netropsins exhibited inhibitory activity against Moloney leukemic virus reverse

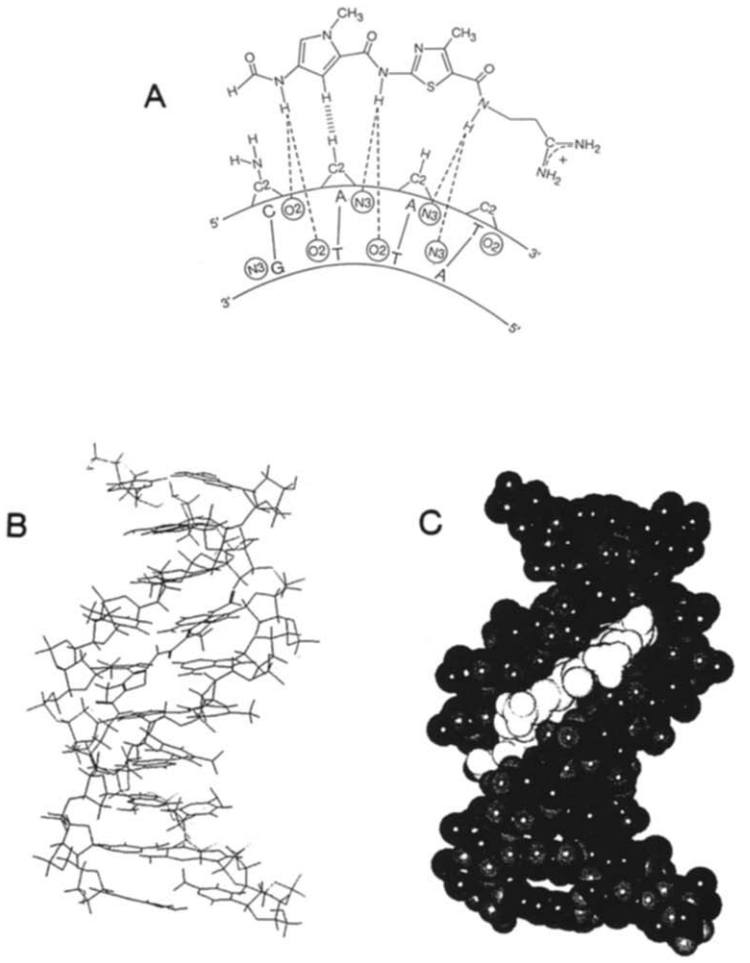


Fig. 7. (A) Orientation of a thiazole-lexitropsin on 5′-CAAT sequence of d-[CGCAATTGCG]$_2$ deduced by ^{1}H-NMR. (B) Skeletal depiction of energy minimized 1:1 complex of ligand:DNA constructed according to NMR-NOE data. To calculate the non-bonded interactions a smooth cut-off of 9.5 Å with a switching function of up to 11 Å was used. A distant dependent dielectric constant of $\varepsilon = 2r$ was used to account for the solvent screening effect. (C) Computer-generated model of the energy minimized 1:1 complex of the ligand:DNA showing location and snug fit in the 5′-CAAT region in the minor groove.

transcriptase which correlates with the DNA binding constants (Fig. 8) (Lown, 1990). As in previous cases the greater inhibitory activity is associated with longer ligands provided the phasing properties permit effective bidentate binding.

Since inhibition of reverse transcriptases is an indicator of possible activity against HIV-1 selected lexitropsins were submitted to the NCI-HIV-1 screen. The first group which consisted of flexible linked bis-netropsins in which moderate activity against HIV-1 was seen in six compounds out of eight. In two

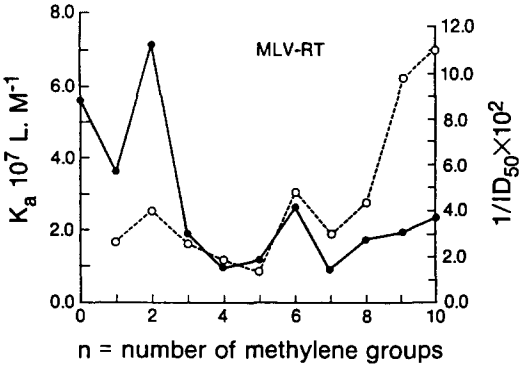


Fig. 8. Correlation between DNA binding constants of linked lexitropsins (K_a solid line) and observed inhibitory properties, expressed in reciprocal ID_{50} values, against Moloney leukemia virus reverse transcriptase.

of the compounds where moderate activity was noted some promotion of the T-lymphocyte cell growth was observed. The second group of compounds consisted of rigid linked bis-netropsins, following the design features deduced from the force field analysis. This group of agents all showed activity with five compounds being designated 'active' and three with moderate activity. In the active compounds the therapeutic index exceeded 500 and the undesired cell growth phenomenon did not appear (Narayanan and Acton, 1991). These encouraging anti-HIV-1 results from examination of only a modest group of lexitropsins serve to support the utility of structurally based drug design in this area and suggest further development to exploit their chemotherapeutic potential. Recent studies indicate that the extended lexitropsins also inhibit HIV-1 reverse transcriptase (Narayanan and Acton, 1991). In this respect they resemble *S*-oligos which in an in vitro anti-HIV assay (Mitsuya and Broder, 1986; Matsukara et al., 1987) exhibited dose-dependent inhibition of T-cell cytotoxicity and also inhibit HIV-1 reverse transcriptase (Matsukara et al., 1987; Majundar et al., 1989) as well as α-oligos (Pauwels et al., 1989). α-Oligonucleotide probes exhibit similar properties (Pauwels et al., 1991).

It is premature however to ascribe the cytotoxic or antiviral properties of these versatile lexitropsins to a particular cellular action.

Topoisomerase inhibition Another instance of the contribution of drug-protein interactions to overall biological potency is that it has been observed that certain DNA minor groove binders inhibit topoisomerases (McHugh et al., 1990). We found recently that a series of linked bis-netropsins inhibit the catalytic activity of isolated topoisomerase II and interfere with the stabilization of the cleavable complexes of topoisomerase II and I in nuclei (Beerman et al., 1991). Dimers with linkers consisting of 0–4 and 6–9 methylene groups (n) were far more inhibitory than netropsin against isolated enzyme and in the nuclear system. The compound with $n = 5$ was uniquely inactive in both assays while the dimer with $n = 10$ inhibited only the isolated enzyme.

Comparison of dimers with fixed linker length ($n = 2$) but varying number of *N*-methylpyrrole residues (from 1 to 3) revealed that the inhibitory properties were enhanced with increasing number of *N*-methylpyrrole units. This is in accord with increased DNA binding and suggests that binding of the ligand to the DNA template contributes to inhibition in the three component template:protein:inhibitor complex.

Overall, drug ability to inhibit catalytic activity of isolated topoisomerase II was positively correlated with calf thymus DNA association constants (Beerman et al., 1991b). In contrast, no such correlation existed in nuclei. However, the inhibitory effects in the nuclear systems were correlated with the association constants for poly(dA-dT). The results indicate that bidentate binding can significantly enhance anti-topoisomerase activity of netropsin related dimeric minor groove binders. However, other factors such as the length of the linker, the number of pyrrole moieties and the nature of the target (isolated enzyme/DNA vs. chromatin in nuclei) also contribute to these activities.

Since the long term objective in lexitropsin research is the development of sequence selective agents we next investigated whether there exists an homology between the sequences recognized by the lexitropsins and the topoisomerase recognition sites. Matrix associated regions (MAR) appear to play a structural role in chromatin and have associated within them several topoisomerase recognition sites (Cockeril and Garrard, 1986; Kaas et al., 1989). In addition there exist certain conserved MAR consensus elements AATATTTTT and ATATTT. Footprinting experiments on a *Pvu*II/*Rsa*I restriction fragment of the heterologous plasmid pLTL-1 revealed that the most potent topoisomerase inhibitors bind to a ATATTTT sequence which is homologous with the MAR consensus elements (Beerman et al., 1991b).

Another instance in which specific sequence recognition of minor groove ligands relates to their biological response is in the case of certain Hoechst 33258 analogues which inhibit topoisomerase II (Beerman et al., 1991). Examination of the cleavage recognition sequence reported for *Drosophila* topoisomerase II revealed the presence of two binding sites for the more potent topoisomerase inhibitors. This sequence, with the ligand binding sites underlined, is ACAATG↓CGCTCATC (the arrow denotes the enzyme cleavage site).

These examples indicate the potential for DNA sequence selective intervention in cellular processes which, taken together with the observed inhibitory activity of extended lexitropsins against viruses especially vaccinia and HIV-1, increase the prospect of eventual development of effective and selective antivirals of a novel structural type.

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