NON-INTERCALATING DNA-BINDERS AS

NOVEL ANTIPARASITIC AGENTS

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Targeting parasitic DNA is attractive and promising:

- transcription of parasite DNA is less complex than in mammal cells

- majority of genes in parasite are regulated by a handful of proteins which bind to very similar DNA promoter regions, which are typically rich in A- and T- nucleotides

- targeting these AT-rich regions with low molecular weight DNA-binding molecules would lead to a down-regulation of parasite genes, thereby inhibiting early steps in cell proliferation

- development of resistance to compounds that target DNA by that mechanism is expected to be less likely compared to compounds that target individual proteins and enzymes
taking a lead from nature’s own DNA-binding molecules such as:

distamycin

new compounds exhibiting in vitro and in vivo activities against parasitic protozoa (P. falciparum, C. parvum)

netropsin

Exerts its action through a mechanism represented by a binding process to DNA with consequent impairment of gene transcription and replication.

This interaction takes place in the DNA minor groove, dictating high affinity for dA-dT rich sequences by involving specific hydrogen bonds directed to relevant functionalities of DNA bases.
Detailed information from X-ray diffraction indicates:

hydrogen bonds from the amide NH groups bridge the strands to the exposed N(3) of adenine and O(2) of thymine residues

methylenes and ring CH's are involved in van der Waals nonbonded contacts with purine and pyrimidine bases
HYDROGEN BONDING INTERACTIONS OF DISTAMYCIN WITH d(AT) SEQUENCES IN THE MINOR GROOVE OF DNA

NMR structure of 2 : 1 complex of distamycin : DNA complex
Besides the TATA box, TAT/ATA motifs exhibit high frequency in the 5’ non-coding regions of eukaryotic genes: a GTATA sequence, present at position -278 from the start of transcription of, e.g., human HLA-DRα gene, is important for the binding of nuclear factors.

Synthetic oligonucleotides employed

GTATA/IFN-γ mer

5’-CTAATGTGCTTCAAGTATATCCCTGTCTAGAAGTCAGATTGGGG-3’
3’-GATTACAGAAGTCTCATATAGGGCACAGATCTTCAGTCTAACC-5’

M-GTATA/IFN-γ mer

5’-CTAATGTGCTTCAAGAGCCCTCCCTGTCTAGAAGTCAGATTGGGG-3’
3’-GATTACAGAAGTCTCGGGAGGGACAGATCTTCAGTCTAACC-5’
Gel retardation assays show binding of nuclear proteins from different cell lines to the GTATA/IFN-γ mer oligonucleotide (B1 - B5 are retarded bands, F is GTATA/IFN-γ 5’ end labelled mer)

Binding of the GTATA/IFN-γ mer (N) and the mutated M-GTATA/IFN-γ mer (M) to the B3 factor(s) from MRN-1 cell line
Effects of distamycin on the binding of the nuclear factor B3 to the GTATA - IFN-γ mer (A) and on the stability of the GTATA - IFN-γ mer (B)

Results of gel retardation assays show that distamycin:
- inhibits the interaction between a nuclear factor and the oligonucleotide
- might alter the binding of transacting factors to AT rich sequences
- would be a prototype capable of modulating the expression of large batteries of genes sharing ATA/TAT rich regulatory sequences
Naturally occurring oligopeptide pyrrole amidine antibiotics

Netropsin
(vs: gram +/-, myco bacteria, Trypanosoma spp, Entamoeba histolytica)

Anthelvencin A (R=H) and B (R=CH₃)
(vs: E. histolytica, Syphacia obvelata, Aspicularis tetraperta, Ascaris suum, Thricuris suis)

Antibiotic TAN-868 A
(vs: bacteria, fungi, some protozoa)

Kikumycin A (R=H) and B (R=CH₃)
(vs: gram +/- bacteria, no fungi and parasites)
Introduced in the medical practice starting since 1937 and 1955, respectively, the diamidine-derived well known antiparasitic drugs affecting DNA synthesis were reported much later (Zimmer & Wählenrt, 1986) to bind to 5′-AATT-3′ sequences in the DNA minor groove in a manner similar as for netropsin (5′-AATT-3′) and distamycin (5′-AAATT-3′)
A reported in vitro antipROTOZOAL activity of:

\[
\text{distamycin}
\]

S. Lee et al. "In vitro sensitivity of \textit{Plasmodium falciparum} to drugs that bind DNA or inhibit its synthesis" J. Parasitology, 79, 780 (1993)

may have its rationale in the observation that:

- the genome of \textit{P. falciparum} is extremely rich (82%) in d(AT) base pairs
- the genome of the human host consists of 59% d(AT)
- during the erythrocytic stage the parasite has a high growth rate (i.e., rate of DNA synthesis) that is more like malignant than normal human cells

\textbf{hence:}

a ligand possessing a specific AT bias could be more inhibitory (toxic) to the parasite than to its human host
Structurally derived analogues

WO 9209574  US 5,670,534  EP 94 00557
WO 93 13739  US 5,472,976

European J. Pharmacol., 267, 143 (1994)
Biochemical Pharmacology, 48, 1583 (1994)
Nucleic Acids Research, 24, 311 (1996)
Antiviral Chemistry and Chemotherapy, 8, 243 (1997)
Anti-Cancer Drugs, 8, 845 (1997)
Pharmacology & Therapeutics, 76, 125 (1997)

\[ X = \text{CONH}_2, \text{HC(NH)NH}_2 \]
ANTIPARASITIC ACTIVITY OF DISTAMYCIN

Expressed as absolute parasitemia (% of infection) in cultures of *Plasmodium falciparum* (ITO 4 strain) exposed (20 hrs) to increased concentrations (µg/ml) of the drug
Expressed as absolute parasitemia (% of infection) in cultures of *Plasmodium falciparum* (ITO 4 strain) exposed (20 hrs) to increased concentrations (µg/ml) of the drug.
ANTIPARASITIC ACTIVITY OF CARBAMOYL ANALOGUES

Expressed as absolute parasitemia (% of infection) in cultures of Plasmodium falciparum (ITO 4 strain) exposed (20 hrs) to increased concentrations (µg/ml) of the drug.

MEN 10400

MEN 10706

MEN 10716
**IN VITRO ANTI-MALARIAL ACTIVITY AND CYTOTOXICITY OF DISTAMYCIN AND DISTAMYCIN ANALOGUES**

<table>
<thead>
<tr>
<th>COMPOUNDS (features)</th>
<th><em>Plasmodium falciparum</em></th>
<th><em>Hep-2</em>&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td></td>
<td>ID&lt;sub&gt;50&lt;/sub&gt; (µM)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ID&lt;sub&gt;50&lt;/sub&gt; (µM)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Distamycin</td>
<td>0.7 - 1.3</td>
<td>32.9</td>
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<tr>
<td><strong>N-formimidoyl analogues</strong></td>
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<tr>
<td>MEN 10568 (3, CONH₂)</td>
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<tr>
<td>MEN 10397 (3)</td>
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<td>26.1</td>
</tr>
<tr>
<td>MEN 10559 (4)</td>
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<td>130</td>
</tr>
<tr>
<td>MEN 10636 (1)</td>
<td>24</td>
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<td><strong>Carbamoyl analogues</strong></td>
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<tr>
<td>MEN 10400 (3)</td>
<td>1.00 - 1.32</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>MEN 10706 (4)</td>
<td>0.4 - 0.7</td>
<td>&gt; 400</td>
</tr>
<tr>
<td>MEN 10716 (5)</td>
<td>0.25</td>
<td>&gt; 400</td>
</tr>
</tbody>
</table>

- **a)** Expressed as cell proliferation
- **b)** Values estimated from the corresponding dose effect curves
### Antiparasitic Activities Against Chloroquine-Sensitive and Chloroquine-Resistant Strains

Values are % parasitemia inhibition, with respect to controls, evaluated after 20 hrs of exposure to the drug.

<table>
<thead>
<tr>
<th></th>
<th>µg/ml</th>
<th>5</th>
<th>1</th>
<th>0.2</th>
<th>0.04</th>
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<tr>
<td>Chloroquine</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<td>MEN 10400</td>
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</tr>
<tr>
<td>Distamycin</td>
<td>100</td>
<td>100</td>
<td>64.7</td>
<td>100</td>
<td>100</td>
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<tr>
<td>MEN 10706</td>
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<tr>
<td>Chloroquine</td>
<td>100</td>
<td>100</td>
<td>85</td>
<td>85</td>
<td>85</td>
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<td>MEN 10706</td>
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<tr>
<td>Distamycin</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Chloroquine</td>
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<td>100</td>
<td>87</td>
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**P. falciparum**, chloroquine-sensitive ITO4 strain

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<tr>
<th></th>
<th>µg/ml</th>
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<th>0.2</th>
<th>0.04</th>
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<td><strong>MEN 10400</strong></td>
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</tr>
<tr>
<td>Chloroquine</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MEN 10400</td>
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<td></td>
</tr>
<tr>
<td>Distamycin</td>
<td>100</td>
<td>100</td>
<td>64.7</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MEN 10706</td>
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</tr>
<tr>
<td>Chloroquine</td>
<td>100</td>
<td>100</td>
<td>85</td>
<td>85</td>
<td>85</td>
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<tr>
<td>MEN 10706</td>
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<tr>
<td>Distamycin</td>
<td>100</td>
<td>100</td>
<td>64.7</td>
<td>64.7</td>
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<tr>
<td>MEN 10716</td>
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<td></td>
</tr>
<tr>
<td>Chloroquine</td>
<td>100</td>
<td>76</td>
<td>2.7</td>
<td>1.9</td>
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</table>

**P. falciparum**, chloroquine-resistant K1 strain
ERHOSTROCYTIC CYCLE OF PLASMODIUM FALCIPARUM

**ERYTHROCYTIC CYCLE OF PLASMODIUM FALCIPARUM**

**Synchronized cultures at trophozoites stage**

**Synchronized cultures at rings and trophozoites stages**

Values are % parasitemia inhibition, with respect to controls, evaluated after 24 hrs from beginning of the experiment.
ANALYSIS OF THE ANTIPARASITIC ACTIVITIES OF MEN 10716 AND CHLOROQUINE AT 4 HOURS EXPOSURE

Expressed as absolute parasitemia (% of infection) in cultures of chloroquine-resistant *Plasmodium falciparum* (K 1 strain) exposed (4 hrs) to increased concentrations (µg/ml) of the drug.
SYNTHESIS OF THE PYRROLE BACKBONES

1) HNO₃, Ac₂O
2) SOCl₂, THF

1) β-Aminopropionitrile, DIPEA
2) Pinner Reaction

H₂, 10% Pd/C

β-Aminopropionitrile, DIPEA

H₂, 10% Pd/C
SYNTHESIS OF N-FORMIMIDOYL ANALOGUES

Reagents and conditions:

i. MeOH, 0.2N NaOH, r.t., 3 h, 95%

ii. MeOH, NaHCO₃, HN=CHNH₂HCl, reflux, 1.5 h, 30%

or

EtOH, NaHCO₃, HN=CHOEt.HCl, r.t., 48 h, 28%
SYNTHESIS OF THE CARBAMOYL UNITY

2 HC≡CCOOCH₃ + CH₃NH₂OH·HCl → NaOH → Toluene Rflx → SO₃ H₂SO₄

H₂NOC

1) SOCl₂
2) NH₄OH
3) NaOH, H⁺

HOOC

1) KMnO₄
2) MeOH, TEA

HOC
SYNTHESIS OF CARBAMOYL ANALOGUES

MEN 10400

MEN 10706

MEN 10716

$\text{DIPEA}$

$n = 1, 2, 3$
# Pharmacological Activities, Toxicity, DNA Binding Properties of Distamycin and Carbamoyl Analogues

<table>
<thead>
<tr>
<th>COMPOUNDS (features)</th>
<th>Cytotoxicity(^a)</th>
<th>Anti-viral(^{a,b})</th>
<th>Anti-malarial(^c)</th>
<th>Acute Toxicity(^d)</th>
<th>c.t. DNA Bind. Const.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ID(_{50}) (µM)</td>
<td>ID(_{50}) (µM)</td>
<td>ID(_{50}) (µM)</td>
<td>LD(_{50}) (mg/kg)</td>
<td>K (\times 10^6) (M(^{-1}))</td>
</tr>
<tr>
<td>Distamycin</td>
<td>33</td>
<td>13</td>
<td>1</td>
<td>147</td>
<td>26.5</td>
</tr>
<tr>
<td>Carbamoyl analogues</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEN 10400 (3)</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>1.2</td>
<td>___</td>
<td>2.0</td>
</tr>
<tr>
<td>MEN 10706 (4)</td>
<td>&gt; 400</td>
<td>27</td>
<td>0.6</td>
<td>235</td>
<td>3.0</td>
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<tr>
<td>MEN 10716 (5)</td>
<td>&gt; 400</td>
<td>9</td>
<td>0.25</td>
<td>333</td>
<td>11</td>
</tr>
</tbody>
</table>

\(^a\) HEP-2 cells  
\(^b\) Herpes simplex virus-1 (HF)  
\(^c\) P. Falciparum, chloroquine-sensitive (ITO-4)  
\(^d\) i.v. administration in mice
potent and rapid antiparasitic action on chloroquine-resistant strain of *P. falciparum* (100% inhibition after 4 hrs at 1 μg/ml)

low cytotoxicity (ID$_{50}$ >400 μM) and acceptable acute toxicity in mice (DL$_{50}$ 333.3 mg/Kg compared to 147.1 mg/Kg for distamycin)

high metabolic stability (presence of the drug after 24 hours following intravenous injection in rats)

NO ORAL BIOAVAILABILITY
In vivo activity of MEN 10716 (i.v.) on Saimiri monkey infected with *P. falciparum*
In vitro antiparasitic activity of MEN 10716 against *Cryptosporidium parvum*

CaCo2 cell cultures (2 x 10^6 cells 70% confluent) inoculated with *C. parvum* sporozoites
MEN 10716 dissolved in DMSO added to the culture at the time of infection and removed 1 hr later by washing
Infected cells were identified by Giemsa staining 24 hrs post infection
Activity of MEN 10716 against Cryptosporidium parvum in immuno-suppressed BALB/C mice

Mice inoculated orally with $10^5$ C. parvum oocysts

Oocyst shedding began between day 5 and 8 post injection and continued during several weeks

Mice were treated with the compound dissolved in drinking water at conc. 5 $\mu$g/ml, and were estimated to drink between 2 and 5 ml/day

Cryptosporidium oocysts were detected in the stools using both Zeil Niessel staining and immunofluorescence

Marked reduction in oocyst shedding after one week of treatment and oocysts could not be detected after 2 weeks of treatment; mice remained parasite free during additional 4 weeks after treatment
By using “clinically old” pentamidine and berenil, medical parasitology was serendipitously exploiting a very sophisticated tool to kill parasites

Growing knowledge of parasite genomic sequences, when matched with the established basis of DNA-sequences recognition by low molecular weight molecules, might result in the rational discovery of novel, effective, antiparasitic drugs

NAXOSPHARMA
creative chemistry & biotech