COMMUNICATION

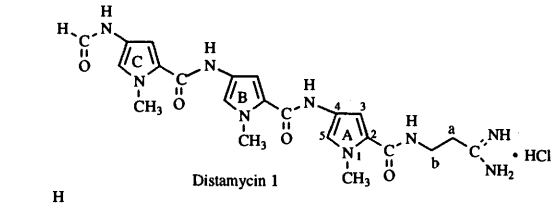
N-Formimidoyl analogues of distamycin

Giovanna Di Pietro,[a] Giuseppe Giannini,[a] Elisabetta M. Iafrate,[a] Edith Monteagudo,[a] Mariella Dentini,[b] Fabio Animati,[a] Paolo Lombardi*[,a] and Federico Arcamone[a]

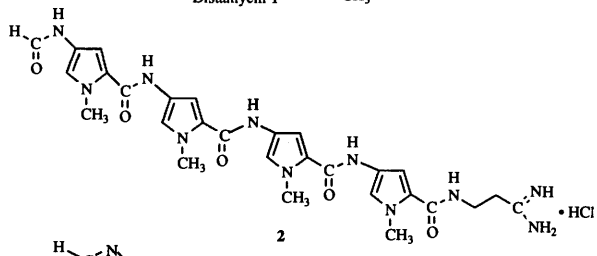
[a] *Menarini Ricerche Spa, Via Tito Speri 10, 00040 Pomezia, Roma, Italy*
[b] *Dipartimento di Chimica, Università 'La Sapienza', P. le A. Moro 5, 00185, Roma, Italy*

Novel *N*-formimidoyl analogues of distamycin, bearing a second positive charge (3–5) or a single positive charge (6) at the N-terminus, were synthesised and assayed for their DNA affinity and anti-herpes activity.

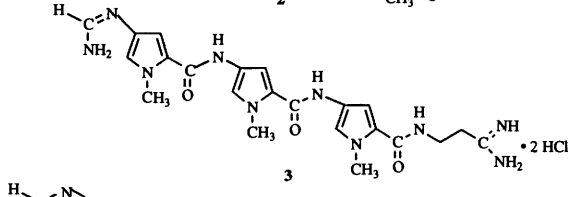
Distamycin, **1**, a pyrrole–amidine oligopeptide isolated from the mycelium of *Streptomyces distallicus*,[1] and a number of synthetic analogues thereof, most notably compounds with a higher number of pyrrole-derived units such as **2**, exhibit a selective inhibition of the multiplication of different viruses (Vaccinia, Herpes simplex, Rous sarcoma virus)[2] by binding in the minor groove of double helical DNA preferentially to dA–dT rich sequences, thus interfering with both replication and transcription.[3] These regions bear a high electrostatic negative potential that seems to play a key role in the drug–DNA recognition and drug–DNA affinity.[4] Therefore, in the course of our studies aimed at the investigation of structure–activity relationships and at the evaluation of the antiviral properties and sequence-specific DNA binding activities of novel distamycin derivatives,[5] we have prepared new analogues either bearing an additional amidine group at the N-terminus (**3–5**), in order to investigate the effect of a second positive charge on the DNA affinity and bioactivity, or bearing a primary amide group at the C-terminus and an amidine group at the N-terminus (**6**), in order to ascertain the influence of the position of the positive charge on the said properties.



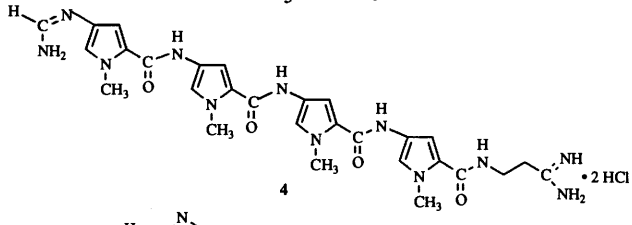
Distamycin 1



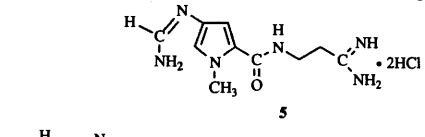
2



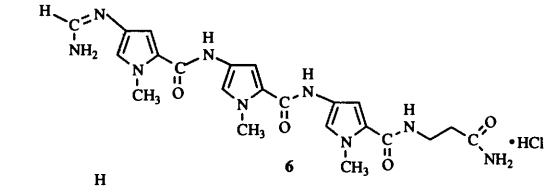
3



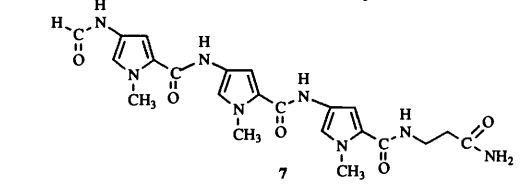
4



5



6



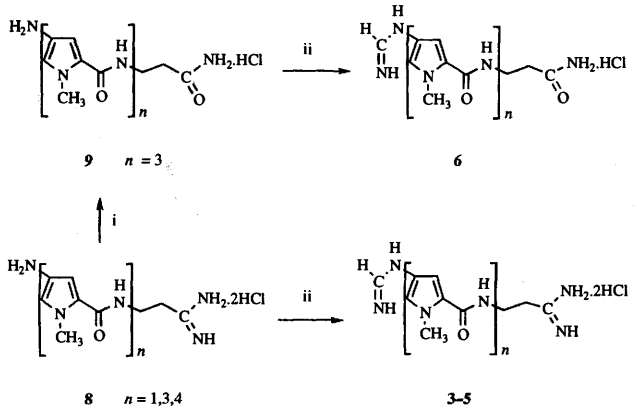
7

Synthesis

Compounds **3–5**, with different number of residues of 1-methyl-4-amino-2-pyrrolecarboxylic acid, were obtained (Scheme 1) from the appropriate amidinopeptide of general structure **8**[6] by condensation either with formamidine hydrochloride or with ethyl formamidate hydrochloride.[7] The condensation with amidine proved to be better than that with amidate ester, yielding the amidinium products in shorter reaction times. Compound **6** was obtained similarly from intermediate peptide **9**.

HPLC and ^{1}H NMR analysis

The analysis of the formamidinium derivatives was complicated by the observation of side chain rotational isomers *Z* and *E*.[8] These isomers could be separated by HPLC under acidic conditions (Fig. 1) but they re-equilibrated to the original mixture at a pH-dependent rate: under acidic conditions the equilibrium took 1 h to reach completion, whereas at pH 7 it was established within a few minutes. The pH dependence of the isomerisation rate indicated that equilibration occurred through a neutral formamidine intermediate by rotation about a C–N single bond. The ^{1}H NMR spectra of the formamidinium derivatives showed duplication of the majority of the signals. In compound **5**, for instance, particularly informative for the assignment were the resonances at δ_H 11.50 and 11.85, with coupling constants of 5 and 12 Hz, corresponding to H^b, and the multiplets at δ_H 8.00 and 8.20, relative to H^c. The NMR data were interpreted in terms of interconverting *Z* and *E* formamidinium isomers, the major form being assigned the *Z* configuration on the basis of the H^b, H^c coupling constants ($J_{b,c}$ value is larger for the *E* isomer than for the *Z* isomer) and NOE data. ^{1}H NMR 2D chemical shift correlation and NOE spectroscopy were used to assign all the



Scheme 1 *Reagents and conditions:* i, MeOH, 0.2 mol dm^{-3} NaOH, RT, 3 h, 95%; ii, MeOH, $NaHCO_3$, HN=$CHNH_2$·HCl, reflux, 1.5 h, 30% or EtOH, $NaHCO_3$, HN=CHOEt·HCl, RT, 48 h, 28%

significant resonances for the new compounds **3**, **4**, **5** and **6** (Spectroscopic data section).

DNA binding properties

Circular dichroism allowed the comparative evaluation of DNA binding properties of the new substances.[9] Titration of the *N*-formimidoyl derivatives with calf-thymus DNA (58% AT) resulted in the appearance of a new CD band between 300 and 350 nm, that directly reflected bound drug molecules, except for the monopyrrole derivative **5**. The monitoring of the observed ellipticity (Θ_{obs}) at the maximum of the induced band, as a function of calf-thymus DNA concentration (c_p) in the presence of a fixed concentration of ligand (c_o), has allowed the determination of the bound drug concentration (c_b) at each point of the titration. The binding parameters—the intrinsic binding constant (K_o) and the binding stoichiometry (r_b) (number of molecules bound per nucleotide base)—were determined by the Scatchard method from the known values of c_b, c_o and c_p. The apparent affinity constants (K_{app}), considered as a measured of the binding affinity, were calculated by the product $K_o \times r_b$ (Table 1, A).

Biological activity

The products were evaluated for their cytotoxicity, expressed as cell proliferation in Hep2 cells, and for their anti-herpes activity, expressed as reduction of viral cytopathic effect on the same cell line infected with Herpes simplex (HSV 1 strain HF). In all experiments average data were plotted as dose effect curves from which ID_{50} values, *i.e.* the drug concentrations (μmol dm^{-3}) required to reduce a given biological effect by 50%, were estimated (Table 1, B).

Discussion

The DNA binding constants (K_{app}) showed that the introduction of a second positively charged amidine group at the N-terminus of the structure did not affect significantly the relative affinity towards calf-thymus DNA when compared to distamycin (entries 1 and 5) and to homologous distamycin, **2** (entries 2 and 6). On the other hand, the shift of the amidine group from the C-terminus to the N-terminus seemed to affect the stoichiometry of binding rather than the intrinsic affinity (entries 1 and 7), since **6** exhibited a r_b value half of that of **1**, but a rather close K_o value. Moreover, the presence of a second, non-delocalized, positively charged group as in des-formyl distamycin, **8** (n = 3) and homologous des-formyl distamycin **8** (n = 4), resulted in somewhat lower affinity towards calf-thymus DNA (entries 3 and 4). As a whole, the picture supported the hypothesis that the electrostatic contribution, albeit important for the formation of the distamycin (analogues)–DNA complex, does not represent an obligatory requisite for DNA binding affinity. Rather, the present results

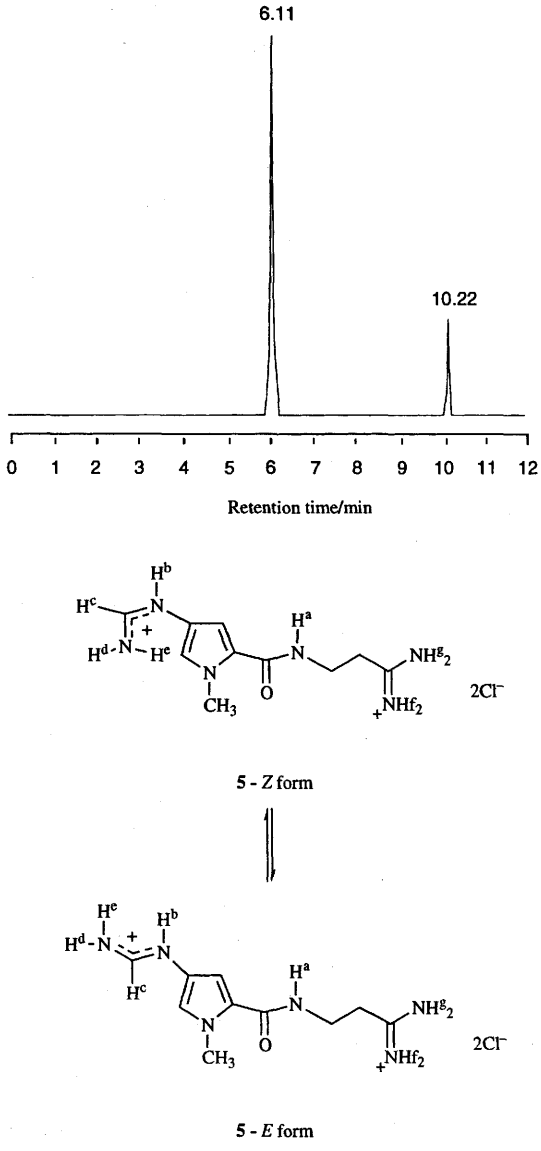


Fig. 1 Chromatographic separation of *Z* and *E* forms of formamidinium derivative **5**. *Conditions:* LiChrospher 100-RP 18 column, 250 × 4 mm; water–acetonitrile 95:5 (+0.1% trifluoroacetic acid) as eluent; room temp.; flow-rate, 0.5 ml min^{-1}; UV detection at 254 nm.

further confirm that this process is dominated by hydrogen bonding and van der Waals contacts,[10] hence by the number of pyrrolecarboxamido units (entries 1 and 2, 5 and 6). The monopyrrole derivative **5**, notwithstanding it features two amidine groups, did not bind appreciably to DNA (entry 8), whereas the uncharged pyrrole oligopeptide **7**, the product from the mild basic hydrolysis of distamycin,[1a] does significantly (entry 9).

A different picture emerges from the analysis of the biological activities. All DNA-binding compounds showed comparable cytotoxicities and anti-herpes properties, with the exception of **6** (entry 7) and **7** (entry 9) which had a K_{app} of the same order of magnitude as that of distamycin, but were devoid of any biological effect. Thus, the presence of the positive charge on the C-terminal chain seems to be fundamental for the exhibition of antiviral activity, whereas the N-formyl group, a typical structural feature of distamycin, is not (entries 3–6).

Spectroscopic data

^1H NMR ([$^2H_6$]DMSO, 300 MHz) data of the formamidinium derivatives **3**, **4**, **5** and **6**; *J* values given in Hz.

Compound 3. δ_H 2.65 (t, *J* 6, α-CH_2), 3.50 (q, *J* 6, β-CH_2), 3.80–3.90 (m, N1-CH_3 rings A, B, C), 6.95–7.25 (m, H-3, H-5

Table 1 DNA binding properties (A) and biological activities (B) of distamycin **1** and distamycin analogues **2–8**

			A				B	
Entry		*n* of pyrroles	λ_{max}/ cm^{-1}	K_o/ $dm^3\ mol^{-1}$	r_b	K_{app}/ $dm^3\ mol^{-1}$	Cytotoxicity ID_{50}/μmol dm^{-3}	Viral cytopathic effects ID_{50}/μmol dm^{-3}
1	**1**	3	326	2.65×10^7	0.106	2.80×10^6	32.9	11.2
2	**2**	4	329	1.25×10^8	0.194	2.43×10^7	24.5	3.6
3	**8**	3	328	1.16×10^7	0.044	5.02×10^5	22.0	9.1
4	**8**	4	331	6.53×10^6	0.186	3.84×10^5	21.6	3.6
5	**3**	3	323	8.22×10^6	0.130	1.07×10^6	17.3	26.1
6	**4**	4	330	9.43×10^7	0.215	2.03×10^7	130.0	26.0
7	**6**	3	323	2.00×10^7	0.057	1.14×10^6	>400	>400
8	**5**	1			(no binding)		>400	>400
9	**7**	3	325	9.35×10^6	0.022	7.20×10^5	>400	>400

rings A, B, C), 8.15–8.25 (m, H^e), 8.25 (t, *J* 6, NH^a), 8.80 (d, *J* 16, H^e *Z*), 8.82 and 8.95 (two br s, NH^f_2 and NH^g_2), 9.55 (d, *J* 15, H^e *E*), 9.60 (d, *J* 6, H^d *E*), 9.65 (d, *J* 6, H^d *Z*), 9.95 (s, NH-4 ring A), 10.00 (s, NH-4 ring B), 11.40 (d, *J* 5, H^b *Z*), 12.00 (d, *J* 13, H^b *E*).

Compound 4. δ_H 2.62 (t, *J* 6, α-CH_2), 3.50 (q, *J* 6, β-CH_2), 3.80–3.90 (m, N1-CH_3 rings A, B, C, D), 6.98–7.28 (m, H-3, H-5 rings A, B, C, D), 8.10 (m, H^e), 8.22 (t, *J* 6, NH^a), 8.60 and 9.00 (two br s, NH^f_2 and NH^g_2), 8.75 (d, *J* 16, H^e *Z*), 9.20 (d, *J* 15, H^e *E*), 9.60 (d, *J* 6, H^d *E*), 9.65 (d, *J* 6, H^d *Z*), 9.90 (s, NH-4 ring A), 9.93 (s, NH-4 ring B), 10.05 (s, NH-4 ring C *E*), 10.15 (s, NH-4 ring C *Z*), 11.45 (d, *J* 5, H^b *Z*), 11.80 (d, *J* 13, H^b *E*).

Compound 5. δ_H 2.70 (t, *J* 6, α-CH_2), 3.54 (q, *J* 6, β-CH_2), 3.80 (s, N1-CH_3 ring A *E*), 3.85 (s, N1-CH_3 ring A *Z*), 6.92 (d, *J* 2, H-3 ring A *E*), 7.02 (d, *J* 2, H-3 ring A *Z*), 7.10 (d, *J* 2, H-5 ring A *E*), 7.17 (d, *J* 2, H-5 ring A *Z*), 8.00 (m, *J* 5, 5, 16, H^c *Z*), 8.20 (m, *J* 15, 13, 6, H^c *E*), 8.40 (t, *J* 5.5, NH^a *E*), 8.50 (t, *J* 5.5, NH^a *Z*), 8.75 (d, *J* 16, H^e *Z*), 8.80 and 9.10 (two br s, NH^f_2 and NH^g_2), 9.25 (d, *J* 15, H^e *E*), 9.60 (d, *J* 6, H^d *E*), 9.70 (d, *J* 5, H^d *Z*), 11.50 (d, *J* 5, H^b *Z*), 11.85 (d, *J* 13, H^b *E*).

Compound 6. δ_H 2.30 (t, *J* 6, α-CH_2), 3.35 (q, β-CH_2), 3.75–3.90 (m, N1-CH_3 rings A, B, C), 6.80 and 7.20 (2 br s, $CONH_2$), 7.00–7.30 (m, H-3, H-5 rings A, B, C), 7.98 (t, NH^a), 8.02 (m, H^c *Z*), 8.22 (m, H^c *E*), 8.82 (d, *J* 16, H^e *Z*), 9.15 (d, *J* 15, H^e *E*), 9.65 (d, *J* 6, H^d *E*), 9.70 (d, *J* 6, H^d *Z*), 9.90 (s, NH-4 ring A), 10.05 (s, NH-4 ring B *E*), 10.15 (s, NH-4 ring B *Z*), 11.52 (d, *J* 5, H^b *Z*), 11.85 (d, *J* 13, H^b *E*).

Acknowledgements

The financial support of this research by A. Menarini (Industrie Farmaceutiche Riunite), Bristol-Myers Squibb (Italy) and Istituto Mobiliare Italiano (Grant No. 53658) is gratefully acknowledged.

References

1 (*a*) F. Arcamone, S. Penco, P. G. Orezzi, V. Nicolella and A. Pirelli, *Nature*, 1964, **203**, 1064; (*b*) F. Arcamone, P. G. Orezzi, W. Barbieri, V. Nicolella and S. Penco, *Gazz. Chim. Ital.*, 1967, **97**, 1097.
2 M. A. Verini and M. Ghione, *Chemotherapy*, 1964, **9**, 144.
3 Ch. Zimmer, K. E. Reinert, G. Luck, U. Wahnert, G. Loeber and H. Thrum, *J. Mol. Biol.*, 1971, **58**, 329; M. L. Kopka, D. Yoon, P. Goodsell, R. E. Pjura and R. E. Dickerson, *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 1376; Ch. Zimmer and U. Wahnert, *Prog. Biophys. Mol. Biol.*, 1986, **47**, 31.
4 B. Pullmann, in *Molecular Basis of Specificity in Nucleic Acid–Drug Interactions*, eds. B. Pullmann and J. Jortner, Kluwer Academic Publishers, Dordrecht, 1990, p. 401.
5 G. Feriotto, A. Ciucci, C. Mischiati, F. Animati, P. Lombardi, P. Giacomini, F. Arcamone and R. Gambari, *Eur. J. Pharmacol.*, 1994, **267**, 143; A. Ciucci, G. Feriotto, C. Mischiati, R. Gambari, F. Animati, P. Lombardi, P. G. Natali, F. Arcamone and P. Giacomini, *Biochem. Pharmacol.*, 1994, **48**, 1583; F. Animati, F. Arcamone, M. R. Conte, P. Felicetti, A. Galeone, P. Lombardi, L. Mayol, L. G. Paloma and C. Rossi, *J. Med. Chem.*, 1995, **38**, 1140; A. Ciucci, S. Manzini, P. Lombardi and F. Arcamone, *Nucleic Acids Res.*, 1996, **24**, 311.
6 S. Penco, S. Redaelli and F. Arcamone, *Gazz. Chim. Ital.*, 1967, **97**, 1110; F. Arcamone, V. Nicolella, S. Pènco and S. Redaelli, *Gazz. Chim. Ital.*, 1969, **99**, 632.
7 J. A. Gautier, M. Miocque and C. C. Farnoux, in *The Chemistry of Amidine and Imidates*, ed. S. Patai, Wiley, New York, 1975, p. 283.
8 R. W. Ratcliffe, K. J. Wildonger, L. Di Michele, A. W. Douglas, R. Hajdn, R. T. Goegehman, J. P. Springer and J. Hirshfield, *J. Org. Chem.*, 1989, **54**, 653.
9 Ch. Zimmer, *Prog. Nucleic Acid Res. and Mol. Biol.*, 1975, **15**, 285.
10 M. Coll, C. A. Frederick, A. H.-J. Wang and A. Rich, *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 8385.

Paper 6/00326E
Received 16*th January* 1996
Accepted 25*th April* 1996