ated from the hemoglobin-hydrogen peroxide interaction. The latent period of the CL, which was found as the time between the initiation of luminol oxidation and the beginning of CL was used for measurement. AOA of serum was determined by the lengthening of the latent period in the model system after addition of 10 μl serum and it was expressed as the concentration of an equivalent ascorbate solution (μM), which was chosen as a reference inhibitor. Results were treated by the Student's t-test and expressed as mean values ± standard mean error.

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DNA-binding properties and cytotoxicity activity of novel aromatic amidines in cultured human skin fibroblasts

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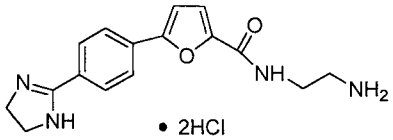
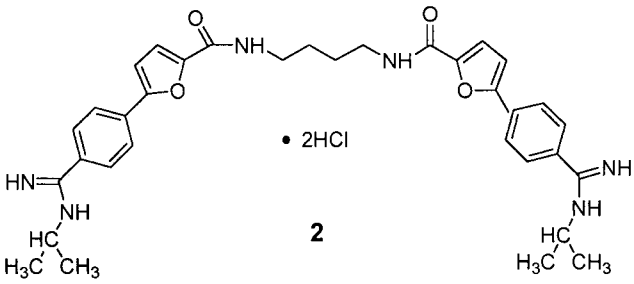
A number of natural and synthetic compounds are known to bind to DNA double helix in a nonintercalative manner [1]. Minor groove binding drugs typically have several aromatic rings, such as pyrrole, furan or benzene connected by bonds possessing torsional freedom [1]. Cationic charges provide affinity for the tunnel of negative potential in the groove and in addition many minor groove ligands possess H-bond donating or accepting atoms [2]. The minor groove binding drugs exhibits a wide spectrum of antimicrobial, antiviral, and antitumour properties [3, 4]. These drugs are thought to exert their biological effects by interfering with the template function of DNA and either blocking gene transcription or inhibiting DNA replication [4, 5]. In the course of our investigations of minor groove binding drugs, new aromatic amidines **1** and **2** were synthesized and tested for DNA-binding properties (Table).

The apparent DNA binding constants (K_{app}) of compounds **1** and **2** to calf thymus DNA, poly(dA-dT) · poly(dA-dT), T4 DNA and poly(dG-dC) · poly(dG-dC) were determined using the ethidium displacement assay [6, 7] and were compared to those of distamycin and netropsin (Table). These data demonstrate that compounds **1** and **2** can bind to the DNAs studied. The association binding constants for T4 coliphage DNA for **1** and **2** gave evidence of their minor-groove selectivity, because the major groove of T4 coliphage DNA is blocked by α-glycosylation of the 5-

Table: Association constants ($K_{app} \times 10^5\ M^{-1}$) of ligands with polynucleotides



1



2

Ligand	Calf thymus DNA	T4 DNA	poly(dA-dT) · poly(dA-dT)	poly(dG-dC) · poly(dG-dC)
Netropsin	8.7	8.3	875	2.5
Distamycin	7.5	6.4	340	2.0
1	2.2	2.6	3.9	1.8
2	1.7	1.6	3.4	1.4

The error for netropsin, distamycin and compounds **1** and **2** is $\pm 0.2 \times 10^5\ M^{-1}$.

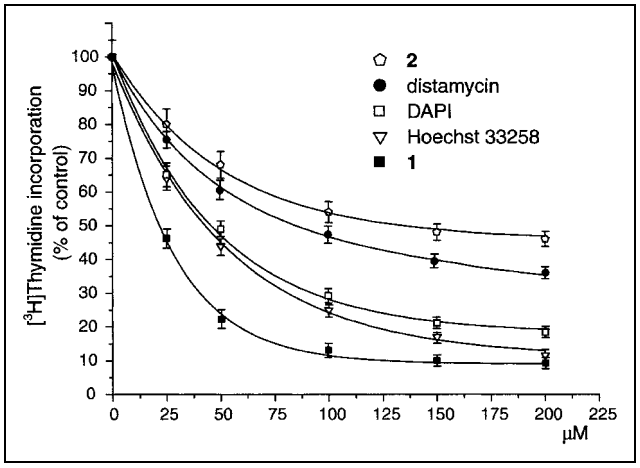


Fig.: DNA synthesis in confluent human skin fibroblasts cultured for 24 h with different concentrations of minor groove binders. Mean values from 3 independent experiments done in duplicate are presented

(hydroxymethyl)cytidine residues. The amidines **1** and **2** bind to AT sequences more weakly than the extensively studied minor-groove binders, such as netropsin and distamycin. However, these compounds show sequence-selectivities. The values of K_{app} of poly(dA-dT) · poly(dA-dT) for **1** and **2** are slightly greater than those for poly(dG-dC) · poly(dG-dC).

All studied minor groove binders inhibited DNA synthesis in cultured human skin fibroblasts, in a dose dependent manner, but with different potency. From the Fig. it is obvious that compound **2** prevented the incorporation of [^{3}H]thymidine to a lower level than distamycin, DAPI and Hoechst 53228 do. However, a clear difference between the behavior of compound **1** and other minor groove binders on DNA synthesis was observed (Fig). Compound **1** caused drastic and fast inhibition. Although this result is not conclusive, it suggests that compound **1** inhibits DNA synthesis in a different manner than the other inhibitors do.

The assumption that the observed structure-activity relationships reflect differences in minor groove binding drug-protein interactions can only be validated by some type of direct measurement. Such extended studies are going on and will be reported in due course.

Experimental

1. Materials

Hoechst 33258, DAPI, netropsin and distamycin were purchased from Sigma Chemical Co. (USA). For the present study, compounds **1** and **2** were synthesized in our laboratory.

1.1. N-(2-aminoethyl)-5-[4-(4,5-dihydro-1 H-imidazol-2-yl)phenyl]-2-furancarboxamide dihydrochloride (**1**)

M.p. (ethanol) 240–242 °C; ^{1}H NMR (DMSO-d_6) 10.77 (br, 1 H), 8.91 (t, 1 H), 8.21 (d, 4 H), 8.4 (d, 4 H), 7.39 (d, 2 H), 7.26 (d, 2 H), 4.02 (s, 4 H), 3.51 (t, 2 H), 2.91 (t, 2 H); ^{13}C NMR (DMSO-d_6) 164.0, 157.6, 152.5, 148.2, 134.3, 129.3, 124.5, 121.1, 115.6, 111.7, 44.3, 43.7, 38.5.
$C_{16}H_{18}N_4O_2 \cdot 2HCl \cdot H_2O$ (389)

1.2. 1,4-Bis[2-[5-(4-[(N-isopropyl)amidinophenyl]furyl]-2-carboxamido]]butane dihydrochloride (**2**)

M.p. (ethanol) 232–234 °C; ^{1}H NMR (DMSO-d_6) 9.70 (br, 2 H), 9.56 (br, 2 H), 9.25 (br, 2 H), 8.95 (t, 2 H), 8.12 (d, 4 H), 7.94 (d, 4 H), 7.33 (d, 2 H), 7.20 (d, 2 H), 4.14 (m, 2 H), 3.33 (m, 4 H), 1.81 (t, 2 H), 1.58 (t, 4 H), 1.30 (d, 12 H). ^{13}C NMR (DMSO-d_6) 161.1, 157.3, 152.5, 148.3, 134.0, 128.8, 127.6, 124.3, 115.4, 110.3, 45.1, 38.2, 26.8, 21.2.
$C_{34}H_{40}N_6O_4 \cdot 2HC1 \cdot 3H_2O$ (723)

2. Cytotoxic assay

Normal human skin fibroblasts, obtained by punch biopsy from an 11 year old male donor were maintained in DMEM supplemented with 10% FBS, 2 mmol/l glutamine, 50 µg/ml penicillin, 50 µg/ml streptomycin at 37 °C in a 5% CO_2 in an incubator. The cells were used between the 12th and 14th passages.

To examine the effect of studied drugs on fibroblast proliferation, the cells were seeded in 24 well tissue culture dishes at 1×10^5 cells/well with 1 ml of growth medium. After 48 h ($1.8 \pm 0.1 \times 10^5$ cells/well) plates were incubated with varying concentrations of minor groove binders and 0.5 µCi of [^{3}H]thymidine for 24 h at 37 °C. Cells were rinsed 3 times with PBS, solubilized with 1 ml of 0.1 M sodium hydroxide containing 1% SDS, scintillation fluid (9 ml) was added and radioactivity incorporation into DNA was measured in scintillation counter.

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