Structure-Activity Relationships of Pyrrole Amidine Antiviral Antibiotics. 1. Modifications of the Alkylamidine Side Chain

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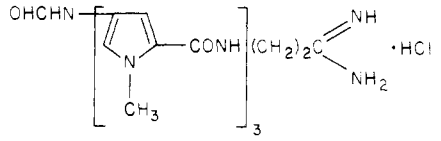
Representatives of three types of side-chain analogues of distamycin A (1) were synthesized. These were tested for cytotoxicity, inhibition of herpes simplex virus (HSV) replication in cultured cells, effects on the synthesis of HSV DNA in isolated nuclei in vitro, as well as on DNA synthesis by purified HSV DNA polymerase. Distamycin A was the most active compound in all three antiviral tests, as well as the most toxic. However, several compounds, in particular the aromatic analogues **15** and **16**, showed no toxicity under the experimental conditions used but were still very active in the three antiviral tests.

Distamycin A (1) is a basic oligopeptide antibiotic



distamycin A (1)

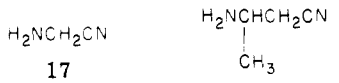
isolated from the fermentation medium of *Streptomyces distallicus*.[1] It is very effective against DNA viruses and certain retroviruses.[2] Distamycin binds to single- and double-stranded DNA molecules,[3] as well as preferentially to some synthetic polynucleotides which are used as templates in a DNA polymerase system.[4] Distamycin A has a high affinity for A-T rich DNA sequences.[2] A second type of binding to G-C pairs in DNA also occurs.[5] Hence, distamycin A is able to protect λ phage DNA from cleavage with the restriction enzyme *EcoR1*.[6] The binding to DNA occurs in the minor groove of DNA.[7] Treatment of herpes simplex virus (HSV) DNA with distamycin A protects it against cleavage with restriction enzymes.[8] The drug has been used clinically in cases of herpes virus infections.[9]

Only a limited amount of information is available on the structural requirements for the antiviral activity of distamycin A. Replacement of the formamide side chain by nitro, amino, acetamide, cyclopentylpropionamide, or *N*-formylglycinamide groups leads to reduction of activity against vaccinia virus.[10,11] When distamycin A (which is a tripeptide) is converted into an analogous recurring tetra- or pentapeptide, the activity against some viruses is increased.[12b,13] At the same time, the cytotoxicity is somewhat reduced, indicating that the antiviral and cytotoxic activities are separable. Replacement of the β-aminopropionamidine side chain by a γ-aminobutyramidine or a *p*-aminobenzamidine side chain causes a sharp reduction in activity against vaccinia virus,[10,12a,c] while an acetamidine side chain causes retention of activity against vaccinia virus and retention of cytotoxicity but considerable reduction of activity against phage T_2.[12c]

Due to the lack of clinically useful antiviral drugs, a thorough investigation into the structure-activity relationships of the known antiviral compounds would be of importance and of possible practical interest. The present and forthcoming publications will deal with the synthesis of novel distamycin analogues and the results of some antiviral tests. This paper deals with analogues in which the natural β-aminopropionamidine side chain is replaced by aminoalkyl or arylamidines and related groups.

Chemistry. We recently described a new total synthesis of distamycin A.[14] The essential novel feature is that it employs as a key intermediate the acid (**2**), which can readily be used for the synthesis of side-chain analogues. We now report on the use of **2** for the synthesis of three types of side-chain analogues of distamycin A.

(a) The first type involved analogues in which the β-aminopropionamidine side chain of the natural product (which, at least formally, is derived from β-aminopropionic acid) was replaced either by an α-aminoacetamidine grouping (compound **12**)[12a] or by a β-amino-β-methylpropionamidine grouping (compound **13**). The replacement of the β-amino acid derived moiety in distamycin A with an α-amino acid derived moiety seemed of some importance in view of the presence of both types of amino acids in nature. This was achieved by condensation of the intermediate acid **2** with aminoacetonitrile (**17**) in the



17 **18**

presence of both dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole to give the nitrile **3**, which with hydrochloric acid in ethanol gave the nitroamidine hydrochloride **10a**, previously synthesized by an alternative route.[12a] Reduction of the nitro group with hydrogen over a palladium catalyst gave the free amine **11**, which without isolation was converted into the desired formamide **12**[12a] by reaction with formic acid in DCC (Scheme I). Compound **13** was prepared from the intermediate acid **2** by condensation with β-amino-β-methylpropionitrile (**18**) to give the nitrile **5**, which following the steps described in Scheme I gave the distamycin homologue **13**. The NMR and mass spectra of compounds **12** and **13** fully support the structures indicated (see Experimental Section).

(b) The second type involved analogues with an aminobenzamidine side chain. These were prepared in order to establish a possible role of electronic effects on the antiviral activity. The synthetic route followed was identical with the one described in type a. The appropriate aminobenzonitrile was used in the condensation reaction with the acid **2** to give, after further reactions parallel to the ones described above (Scheme I), the required *p*- (**14**),[12a] *m*- (**15**), and *o*-aminobenzamidine (**16**).

(c) The third type involved analogues in which the β-aminopropionamidine side chain was replaced by a β-aminoalkylnitrile chain. These were prepared in order to verify the requirement of an amidine side chain for antiviral activity. Two compounds were prepared. The known[14] nitronitrile **4** was catalytically reduced to the aminonitrile **7**, which with formic acid in DCC (Scheme I) gave the desired formamidonitrile **8**. The formamidonitrile **9** was prepared in the same fashion from the nitronitrile **5**.

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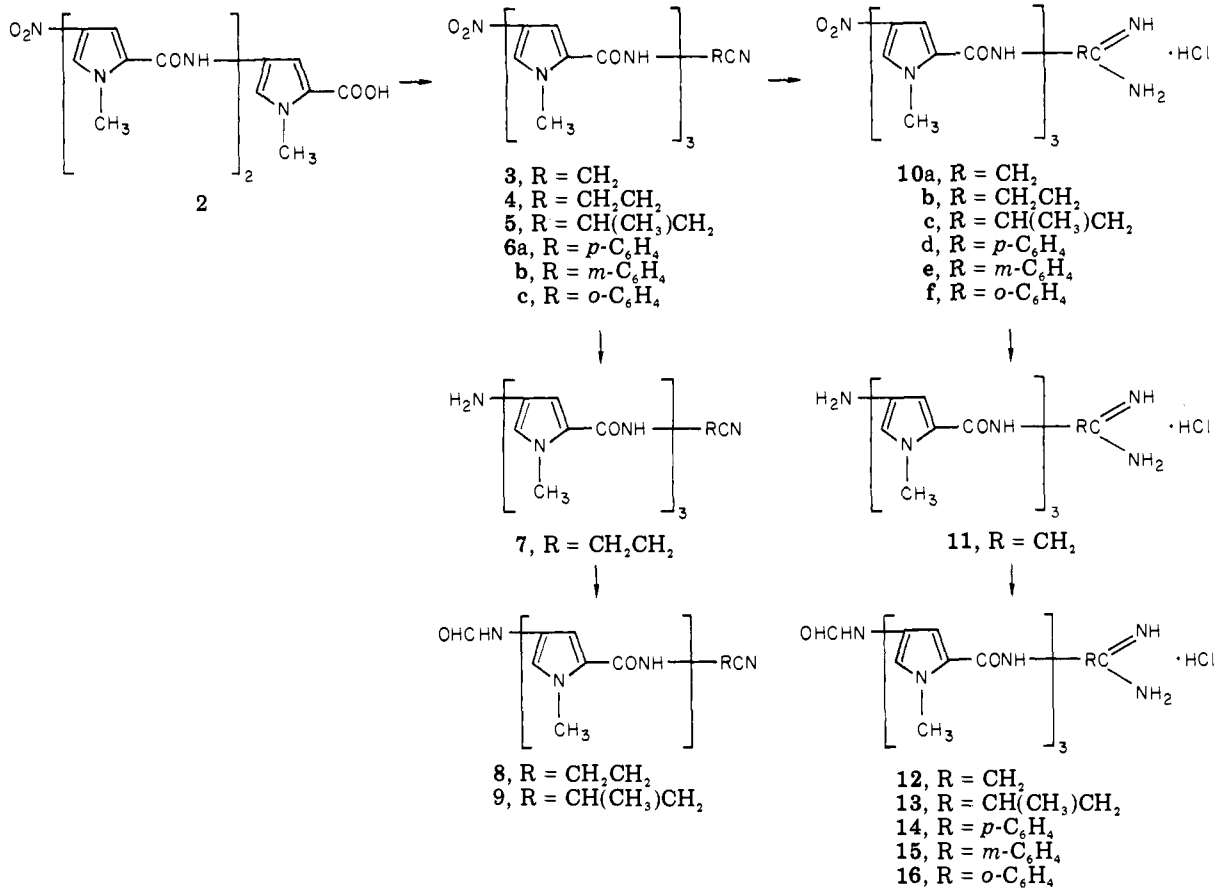
Scheme I



In addition to the final synthetic product in each reaction sequence, we also submitted for testing the respective intermediate nitro compounds **10a–f**. Compounds **10a**, **10b**, and **10d** have previously been reported,[12b,14] though not tested in our systems.

Virology. The antiviral activity of distamycin A and its derivatives was studied in cell cultures infected with HSV type 1. Previous studies showed that distamycin A inhibits the replication of HSV in cultured cells.[15] The present investigation was done using four different techniques: (1) Toxicity of the different compounds to growing cells. Only nontoxic compounds or toxic compounds at nontoxic concentrations were further tested. In the latter case, the highest noncytotoxic concentration was used to study the antiviral activity. (2) Inhibition of virus replication in infected cells. The compounds were added to the infected cultures after virus adsorption, and the virus progeny was determined 20–24 h after infection. (3) The efficacy of distamycin derivatives as inhibitors of viral DNA biosynthesis in isolated nuclei was tested (cf. ref 16 and 17). (4) The effect of the distamycin derivatives on the activity of herpes simplex virus DNA polymerase under in vitro conditions.[18] Details of these assay systems are presented under the Experimental Section.

Results

Cytotoxicity of Distamycin Derivatives. The results revealed that distamycin A (1) and the nitro derivative **10a** were toxic to the cultured cells at a concentration of 100 μg/mL but not at 25 or 50 μg/mL, respectively. All other compounds had no toxic effects on the cells at 200 μg/mL. The distamycin derivatives were solublilzed in 10% Me_2SO in water.

Inhibition of HSV Replication in Cultured Cells. All the distamycin derivatives were tested for their ability to inhibit HSV replication at a drug concentration of 200 μg/mL, except 1 and **10a** which were tested at 25 and 50 μg/mL, respectively. The results in Table I show that distamycin A (1) inhibited virus replication by 99.7%, while compounds **10a,c,d**, **15**, and **16** inhibited the virus

Table I. Toxicity of and Plaque Inhibition by Distamycin Derivatives

no.	max nontoxic concn, μg/mL[a]	% inhibn of virus progeny[b]
1	25	99.7
13	>200[c]	48.0
12	>200	61.0
14	>200	76.4
15	>200	97.1
16	>200	96.7
8	>200	65.5
9	>200	84.6
10a	50	98.1
10b	>200	86.5
10c	>200	93.7
10d	>200	94.8
10e	>200	97.2
10f	>200	96.9

[a] Toxic effect is regarded as cell death within 2 days of incubation of cultured cells with the drug at 37 °C. The maximal nontoxic concentration is defined here as the nontoxic drug concentration whose twofold increase causes toxicity. [b] The titer of virus progeny produced in untreated infected cells and in cells treated with each of the distamycin derivatives was determined by plaque assay on BSC-1 cells. The virus progeny yielded by infected cells is taken as 100% and the yield of virus from infected treated cells is related to the yield from untreated infected cells. All compounds were tested at a concentration of 200 μg/mL except 1 and **10a**, which were tested at 25 and 50 μg/mL, respectively. [c] The designation ">200" indicates that at 400 μg/mL the drug was not toxic.

yield between ca. 94 to 98%. These results indicate that distamycin A, the parent molecule, in effect has the highest inhibitory activity.

The distamycin homologues **12**, **13**, and **4** had a much lower activity, which was similar to that of the nitriles **8** and **9**. The nitro derivative **10b** had a lower activity than the rest of the nitro precursors.

Effect of Distamycin Derivatives on the Synthesis of HSV DNA in Isolated Nuclei in Vitro. In this study, the effect of different concentrations of the distamycin derivatives was determined. Distamycin A (**1**), at a concentration of 200 μg/mL, had the most marked effect (95% inhibition). The homologue **13** inhibited 86% of the in vitro DNA synthesis (not shown), whereas the homologues **12**, **15**, and **16**, as well as the nitrile **8**, inhibited about 65–70% of DNA synthesis. The lowest inhibitory activity was that of the C-1 homologue **14** and the nitrile **9** (42% inhibition or less). At a higher concentration (400 μg/mL), all these derivatives had a stronger inhibitory effect but were still weaker than distamycin A. At lower concentrations, the compounds had a reduced inhibitory capacity, except the nitrile **9** which, however, showed no dose response, being of the same inhibitory activity at concentrations ranging from 25 to 300 μg/mL. None of the nitro precursors **10a–f** had any effect on DNA synthesis in isolated nuclei (not shown).

It is of interest that some derivatives with a low inhibitory effect on virus replication were inhibitory to viral DNA synthesis in isolated nuclei.

Effect of Distamycin Derivatives on DNA Synthesis by HSV DNA Polymerase. The effect of the different compounds on HSV DNA synthesis by the viral DNA polymerase under in vitro conditions resembled that obtained with isolated infected nuclei. Distamycin A (**1**) was the best inhibitor of the viral DNA polymerase activity; the meta-substituted aromatic homologue **15** was the next best derivative. This compound also inhibited HSV replication in infected cells (Table I). It is of interest that the nitro compounds **10b** and **10c** had a marked inhibitory effect on DNA synthesis in the isolated nuclei (not shown).

Discussion and Conclusions

Distamycin A (**1**) is the most active compound in all three tests, as well as the most toxic one. The nitro derivative **10a** is the second most active in one of the tests, as well as the second most toxic compound in the series. However, it seems possible to separate toxicity from antiviral activity. Thus, two of the aromatic homologues (**15** and **16**), as well as several of the nitro precursors (**10c–f**), show no toxicity under the experimental conditions used, yet cause >90% plaque inhibition. This separation between antiviral activity and cell toxicity, which is of crucial importance in any antiviral chemotherapeutic research, needs further experimental support.

Some of the compounds tested (i.e., distamycin A and the aromatic analogues **15** and **16**) show high activity in all three tests. Others, however, show only selective activity. Thus, the isopropyl homologue **13** is very active in the inhibition of DNA synthesis and of DNA polymerase activity but is only slightly active in the plaque-inhibition test. The nitro derivatives (e.g., **10a–f**) are all active in the plaque-inhibition test but show no activity in the DNA synthesis test, and variable activity in the DNA polymerase test.

Experimental Section

Chemistry. Unless otherwise stated, the following spectroscopic methods were followed. UV measurements were made for solutions in EtOH or DMF. IR spectra were taken in KBr pellets. ^{1}H NMR data were determined in Me_2SO-d_6 solvent with sodium 3′-(trimethylsilyl)tetradeuteriopropionate as external standard. TLC was performed on 0.3-mm silica gel plates, which were developed with Ehrlich reagent [2% (*N*,*N*-dimethylamino)-benzaldehyde in 6 N HCl] or by irradiation at 254 nm. Mass spectra (MS) were obtained by direct inlet at 80 eV; in several cases the technique of field desorption was used.

***N*-Methyl-4-[*N*-methyl-4-(*N*-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamidoacetonitrile (3).** The acid **2**[14] (400 mg, 0.97 mmol) was dissolved in dimethylformamide (DMF; 7 mL). Aminoacetonitrile (**17**; 109 mg, 1.94 mmol) was added, followed by the addition of 1-hydroxybenzotriazole (262 mg, 1.94 mmol) and dicyclohexylcarbodiimide (DCC; 200 mg, 0.97 mmol). The reaction mixture was stirred for 1 h at 0 °C and then overnight at room temperature, filtered from the precipitate which is gradually formed, and water slowly added. The precipitate formed was filtered off and crystallized from MeOH to give **3**: yield 280 mg (56%); mp 270 °C; NMR δ 3.89, 3.95, 3.98 (N-CH_3 groups), 7.02, 7.09, 7.31, 7.33, 8.15 (aromatic H's), 8.65, 9.68, 10.01 (amide H's); UV λ_{max} (DMF) 297 nm (ε 36 800); IR ν_{max} 3400, 1650, 1520, 1300 cm^{-1}; MS *m/e* 452 (M^+, 18), 400 (0.8), 370 (11), 275 (100). Anal. ($C_{20}H_{20}N_8O_5 \cdot H_2O$) C, H.

***N*-Methyl-4-[*N*-methyl-4-(*N*-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamidoacetamidine Hydrochloride (10a).** The above described acetonitrile **3** (950 mg, 2.11 mmol) was dissolved in absolute EtOH (60 mL). The solution was cooled to 0 °C, stirred, and dry hydrochloric acid was bubbled through it for 1 h. The solution was kept at 0 °C for additional 12 h and the EtOH was then evaporated. The dry residue was washed with ether. The residue was dissolved in absolute EtOH (12 mL). The solution was cooled to 0 °C, stirred, and dry ammonia was bubbled through it for 1 h. After the bubbling was stopped, the solution was stirred at room temperature for 12 h. The EtOH was evaporated and the residue was crystallized from MeOH to give **10a**: yield 760 mg (70%); mp 214–218 °C; NMR δ 3.88, 3.89, 3.98 (N-CH_3 groups), 7.10, 7.11, 7.26, 7.27, 7.6, 8.03 (aromatic H's); UV λ_{max} (DMF) 296 nm (ε 36 000); IR ν_{max} 3400, 3100, 1640, 1580, 1430, 1300 cm^{-1}; MS *m/e* 452 ($M^+ - NH_3$, 13), 408 (2), 395 (7), 370 (28), 275 (100) [lit.[12a] mp 220 °C (with 3.5 molecules of water and 0.5 molecule of ethanol) without spectral data].

***N*-Methyl-4-[*N*-methyl-4-[*N*-methyl-4-(formylamido)-pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamidoacetamidine Hydrochloride (12).** The above described nitropyrrole derivative **10a** (0.69, 1.19 mmol) was dissolved in DMF (13 mL) and was reduced at atmospheric pressure with 10% Pd on charcoal (0.2 g). The mixture was filtered. The intermediate amine **11**, presumably obtained, was not purified due to instability. Formic acid (55 mg, 1.8 mmol) was added, and then DCC (247 mg, 1.19 mmol) in DMF (10 mL) was gradually added under nitrogen to the solution of **11**, kept at 0 °C for 1 h. The reaction mixture was stirred at room temperature overnight and filtered, and the filtrate was evaporated. The residue obtained was crystallized from MeOH to give **12**: yield 160 mg (27%); mp 188–192 °C; NMR δ 3.88 (N-CH_3 groups), 6.87, 7.12, 7.2 (aromatic H's), 8.16 (formyl H, aliphatic amide H), 9.77, 9.84 (3 amide H's); UV λ_{max} (EtOH) 236 nm (ε 27 000), 302 (35 000); IR ν_{max} 3160–3400, 1640, 1570, 1430, 1400 cm^{-1}; MS *m/e* (field desorption) 438, 412, 384 [lit.[12a] mp 203–207 °C (from butanol; crystallizing with 0.5 molecule of butanol) without spectral data].

***N*-Methyl-4-[*N*-methyl-4-(*N*-methyl-4-nitropyrrole-2-carboxamide)pyrrole-2-carboxamido]pyrrole-2-carboxamido-β-methylpropionitrile (5).** This nitrile was obtained from acid **2** and β-aminobutyronitrile (**18**) as described for the acetonitrile homologue **3**: 76% yield; mp 240 °C; NMR δ 1.4 (CH_3, d, *J* = 6 Hz), 2.72 (2 H), 3.88, 3.92, 4.02 (N-CH_3 groups), 7.08, 7.12, 7.23, 7.3, 7.69, 8.12 (aromatic H's), 8.01, 9.84, 10.12 (amide H's); UV λ_{max} (DMF) 295 nm (ε 27 700); IR ν_{max} 3300–3600, 1660, 1650, 1300; MS *m/e* 480 (M^+, 19), 441 (3.7), 413 (15), 370 (10), 275 (100). Anal. ($C_{22}H_{24}N_8O_5 \cdot 3H_2O$) C, H.

***N*-Methyl-4-[*N*-methyl-4-[*N*-methyl-4-(formylamido)-pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido-β-methylpropionamidine Hydrochloride (13).**

This amidine was obtained from the nitrile **5** via the nitroamidine **10c** as described above for the conversion of **3** into **12** via **10a**. Compound **10c** was obtained in 76% yield from **5**: mp 224–229 °C; NMR δ 1.15 (3 H, d, J = 6 Hz), 2.72 (2 H), 3.22 (1 H, m) 3.85, 3.88, 4.0 (3 N-CH_3 groups), 7.02, 7.11, 7.14, 7.56, 7.82, 8.12 (aromatic H's), 892 (amidine H's), 8.16, 9.82, 10.22 (amide H's); UV λ_{max} (EtOH) 238 nm (ε 30100), 300 (35600); IR ν_{max} 3200–3600, 1650, 1560, 1300 cm^{-1}; MS m/e 480 ($M^+ - NH_3$, 17), 413 (20), 395 (7), 370 (2), 275 (100). Anal. ($C_{22}H_{27}N_9O_4 \cdot HCl \cdot 2H_2O$) C, H, N, Cl.

Amidine 13: obtained in 16% yield from **10c**; mp 203–207 °C; NMR δ 1.32 (3 H, d, J = 7 Hz), 3.91 (N-CH_3 groups), 7.02, 7.97 (aromatic H's), 8.22 (formyl H), 8.36 (aliphatic amide H), 9.02 (amidine H's), 9.89, 10.12 (amide H's); UV λ_{max} (EtOH) 243 nm (ε 27600), 303 (ε 33200); IR ν_{max} 3050–3500, 1660, 1530, 1440, 1390 cm^{-1}; MS m/e 478 ($M^+ - NH_3$, 10%), 450 (3%), 435 (3%), 411 (20%).

***N*-Methyl-4-[*N*-methyl-4-(*N*-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamidobenzonitrile. Para Isomer 6a.** This compound was prepared (following the procedure described for the acetonitrile **3**) from the acid **2** (500 mg, 1.2 mmol) in DMF (13 mL) and *p*-aminobenzonitrile (213 mg, 1.8 mmol), 1-hydroxybenzotriazole (324 mg, 2.4 mmol), and DCC (247 mg, 1.2 mmol): yield 538 mg (84%); mp 212–214 °C; NMR δ 3.9, 4.0 (N-CH_3 groups), 6.9, 7.1, 7.3, 7.7, 7.9, 8.0 (aromatic H's); UV λ_{max} (DMF) 292 nm (ε 23400); IR ν_{max} 3400, 1700, 1630, 1540, 1420 cm^{-1}; MS m/e 223 (34), 145 (5), 96 (100) [lit.[12a] mp 198–201, without spectral data]. Anal. ($C_{25}H_{22}N_8O_5 \cdot 2H_2O$) C, H, N.

Meta Isomer 6b. The preparation of this isomer from the acid **2** and *m*-aminobenzonitrile parallels that of the para isomer **6a**: yield 73%; mp 224–228 °C; NMR δ 3.9, 4.0 (N-CH_3 groups), 6.9, 7.1, 7.22, 7.55, 7.8, 7.9 (aromatic H's); UV λ_{max} (DMF) 298 nm (ε 23000); MS m/e 514 (M^+, 0.8), 494 (4), 487 (3), 413 (3), 370 (100), 275 (23). Anal. ($C_{25}H_{22}N_8O_5 \cdot 2H_2O$) C, H, N.

Ortho Isomer 6c. The preparation of this isomer from **2** and o-aminobenzonitrile parallels that of the para isomer **6a**: yield 81%; mp 220–224 °C; NMR δ 3.9, 4.0 (N-CH_3 groups), 6.8, 7.1, 7.3, 7.5, 7.64 (aromatic H's); UV λ_{max} (DMF) 237 nm (ε 22300), 302 (ε 23700); MS m/e 514 (M^+, 0.6), 487 (2.5), 270 (100), 275 (100). Anal. ($C_{25}H_{22}N_8O_5 \cdot 2H_2O$) C, H, N.

***N*-Methyl-4-[*N*-methyl-4-(*N*-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamidobenzamidine Hydrochloride. Para Isomer 10d.** The above described *p*-benzonitrile **6a** (1 g, 1.94 mmol) was dissolved in absolute EtOH (40 mL). The reaction procedure followed was the one described above for **10a** from **3**: yield 690 mg (62%); mp 235–239 °C; NMR δ 3.9, 4.0 (N-CH_3 groups), 7.0, 7.2, 7.3, 7.4, 7.7 (aromatic H's), UV λ_{max} (EtOH) 242 nm (ε 28000), 300 (ε 34400); IR ν_{max} 3300, 1680, 1640, 1550, 1370 cm^{-1}; MS m/e 442 (100), 413 (100), 398 (25), 395 (25), 370 (42), 275 (100) [lit.[12a] mp 230–234 °C (crystallizing with one molecule of water) without spectral data].

Meta Isomer 10e. The preparation of this isomer from **6b** parallels that of the para isomer **10d** from **6a**: yield 62%; mp 270 °C; NMR δ 3.9, 4.0 (N-CH_3 groups), 7.0, 7.2, 7.3, 7.4, 7.5, 7.7, 8.2 (aromatic H's); UV λ_{max} (EtOH) 238 nm (ε 23200), 302 (ε 28400); IR ν_{max} 3400, 1650, 1550, 1420, 1300, cm^{-1}; MS m/e 438 (29), 424 (100), 412 (100), 395 (64), 362 (100). Anal. ($C_{25}H_{25}N_9O_5 \cdot HCl$) C, H, N.

Ortho Isomer 10f. The preparation of this isomer from **6c** parallels that of the para isomer **10d** from **6a**: yield 59%; mp 270 °C; NMR δ 3.88, 3.91, 4.03 (N-CH_3 groups), 6.95, 7.0, 7.16, 7.3, 7.69 (aromatic H's); UV λ_{max} (EtOH) 236 nm (ε 27200), 298 (ε 32500); IR ν_{max} 3100–3500, 1660, 1540, 1300 cm^{-1}; MS m/e 442 (100), 428 (10), 414 (100), 413 (100), 395 (26), 370 (22), 275 (100). Anal. ($C_{25}H_{25}N_9O_5 \cdot HCl \cdot H_2O$) C, H, N.

***N*-Methyl-4-[*N*-methyl-4-[*N*-methyl-4-(formylamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamidobenzamidine Hydrochloride. Para Isomer 14.** The above described nitropyrrole derivative **10d** (1.2 g, 2.11 mmol) was dissolved in DMF (15 mL) and was reduced at atmospheric pressure with 10% Pd on charcoal (0.4 g). The mixture was filtered, and formic acid (146 mg, 3.16 mmol) and DCC (430 mg, 2.11 mmol) were added. The reaction procedure and workup followed were those described above for compound **12**. Compound **14** (263 mg, 22% yield) was obtained as crystals: mp 185–190 °C; NMR δ 3.73 (3 N-CH_3 groups) 6.83–7.33 (10 aromatic H's), 8.07 (formyl H), 8.7 (amide H's); UV λ_{max} (EtOH) 246 nm (ε 24000), 310 (ε 29000); IR ν_{max} 3200–3500, 3100, 1640, 1570, 1430 cm^{-1}; MS m/e 440 (10), 410 (50), 368 (50), 273 (100); MS m/e (field desorption) 438, 412, 384 [lit.[12] mp 232–235 °C (crystallizing with two molecules of water) without spectral data]. Anal. ($C_{26}H_{27}N_9O_4 \cdot HCl \cdot 2H_2O$) C, H, N.

Meta Isomer 15. The preparation of this isomer from **10e** parallels that of the para isomer **14** from **10d**: yield 27%; mp 195–199 °C; NMR δ 3.81 (3 N-CH_3 groups), 6.87–6.94, 7.03, 7.18 (10 aromatic H's), 8.2 (formyl H), 9.87, 9.90, 10.02 (amide H's); UV λ_{max} (EtOH) 235 nm (ε 19800), 289 (14400); IR ν_{max} 3100–3500, 2920, 1600–1650, 1430, 1400 cm^{-1}; MS m/e 440 (5), 411 (100), 412 (96), 396 (47), 395 (100), 384 (47), 383 (100), 369 (95), 368 (100); MS m/e (field desorbtion) 490, 438, 410. Anal. ($C_{26}H_{27}N_9O_4 \cdot HCl \cdot H_2O$) C, H, N.

Ortho Isomer 16. The preparation of this isomer from **10f** parallels that of the para isomer **14** from **10d**: yield 18%; mp 196–200 °C; NMR δ 3.9, 3.92 (3 N-CH_3 groups), 6.92, 6.98, 7.06, 7.23 (10 aromatic H's), 8.17 (formyl H), 9.8, 10.04 (amide H); UV λ_{max} (EtOH) 246 nm (ε 20200), 293 (ε 22300); IR ν_{max} 3500, 1690, 1540, 1460, 1400 cm^{-1}; MS m/e 490 (6), 467 (14), 455 (13), 440 (19), 411 (69), 393 (100), 383 (69), 368 (69); MS m/e (field desorbtion) 486, 468, 440, 410, 382. Anal. ($C_{26}H_{27}N_9O_4 \cdot HCl \cdot H_2O$) C, H, N.

***N*-Methyl-4-[*N*-methyl-4-[*N*-methyl-4-(formylamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido-β-propionitrile (8).** The known[14] intermediate **4** (1 g, 2.15 mmol) was dissolved in DMF (30 mL) and was reduced with hydrogen at atmospheric pressure over 10% Pd on charcoal (0.3 g). After the reduction was complete, the catalyst was filtered. The amine **7** presumably obtained was not isolated. Formic acid (144 mg, 3.07 mmol) and DCC (443 mg, 2.15 mmol) were added to the solution, which was stirred for 1 h at 0 °C and then overnight at room temperature, filtered, and then water slowly added. The precipitate formed was chromatographed on silica gel (90 g), with chloroform–MeOH (9:1) as elution mixture. On crystallization with ethyl acetate–MeOH, the nitrile **8** was obtained: yield 375 mg (38%); mp 225–229 °C; NMR δ 3.40, 3.81 (CH_2 groups) 3.83 (N-CH_3 groups), 6.98, 7.11, 7.18 (aromatic H's), 8.17 (formyl H and aliphatic amide H), 9.83, 9.97 (aromatic amides H's); UV λ_{max} (EtOH) 241 nm (ε 16800), 300 (ε 21800); IR ν_{max} 3270, 2940, 2220, 1680, 1670, 1550, 1430, 1410 cm^{-1}; MS m/e 464 (M^+), 438 (45), 410 (27), 392 (27), 368 (9), 342 (100). Anal. ($C_{22}H_{24}N_8O_4 \cdot H_2O$) C, H, N.

***N*-Methyl-4-[*N*-methyl-4-[*N*-methyl-4-(formylamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido-β-butyronitrile (9).** This nitrile was obtained in 22% yield from intermediate **5** following the procedure described above for the β-propionitrile **8**: mp 241–245 °C; NMR δ 1.2 (C-CH_3 group, d, J = 4.5 Hz), 2.7 (CH_2), 3.82, 3.85, 3.95 (N-CH_3 group), 6.92, 7.0, 7.12, 7.14 (aromatic H's), 8.06, 8.2 (formyl H, aliphatic amide H), 10.75, 10.87 (aromatic amide H's); UV λ_{max} (EtOH) 238 nm (ε 17000), 303 (ε 20500); IR ν_{max} 3260, 2220, 1670, 1630, 1600, 1570, 1550, 1400 cm^{-1}; MS m/e 478 (M^+, 27), 449 (5), 411 (64), 398 (100), 366 (31). Anal. ($C_{23}H_{27}N_8O_4$) C, H, N.

Virology. Cytotoxicity. The drug to be tested was added at the desired concentration (5–200 μg/mL) to BSC-1 cells in small petri dishes, 48 h after seeding. The cells were observed under a light microscope after a further 24, 48, and 72 h of incubation at 37 °C to determine the degree of cell destruction by the drug.

Inhibition of Virus Replication. Tests for virus replication were performed using monolayers of BSC-1 monkey kidney cells grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. BSC-1 cell monolayers (4×10^6 cells/milk bottle) were infected with the HF strain of herpes simplex virus (HSV) type 1 at a multiplicity of injection of 10 plaque-forming units (pfu) per cell. The drug to be tested was added at the time of infection, and the bottles were incubated for 20–24 h at 37 °C. The cells were then scraped into the medium and disrupted by ultrasonic treatment. A sample of the infected cell homogenate was used for preparing tenfold dilutions for titration by the plaque assay. BSC-1 monolayers in petri dishes were infected with 0.5 mL of each tenfold dilution in medium. After 3-h adsorption at 37 °C, the virus was removed and the cells were overlaid with a

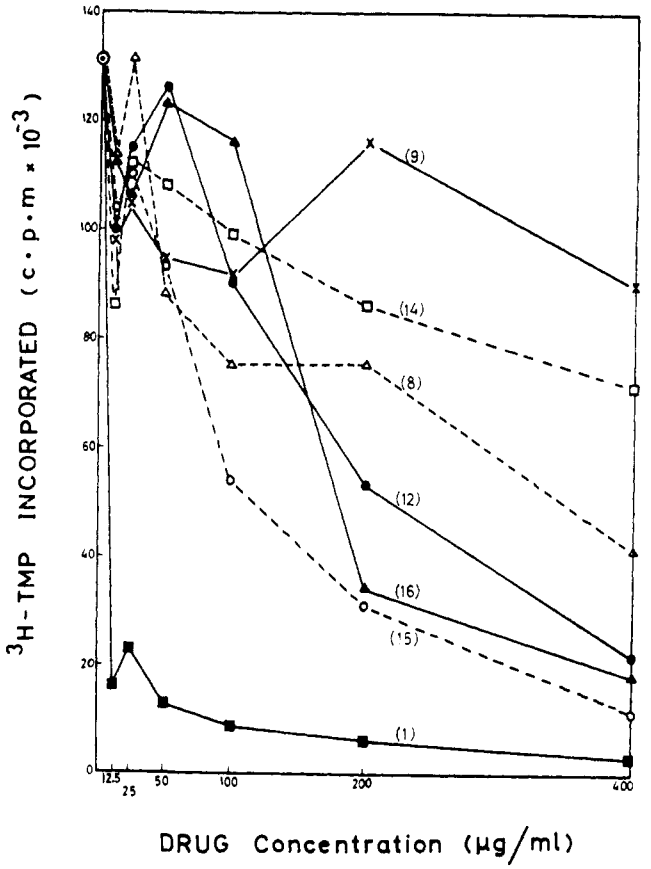


Figure 1. Incorporation of [^{3}H]thymidine triphosphate (^{3}H-TTP) into the DNA of nuclei of cells infected by herpes simplex virus as a function of drug concentration.

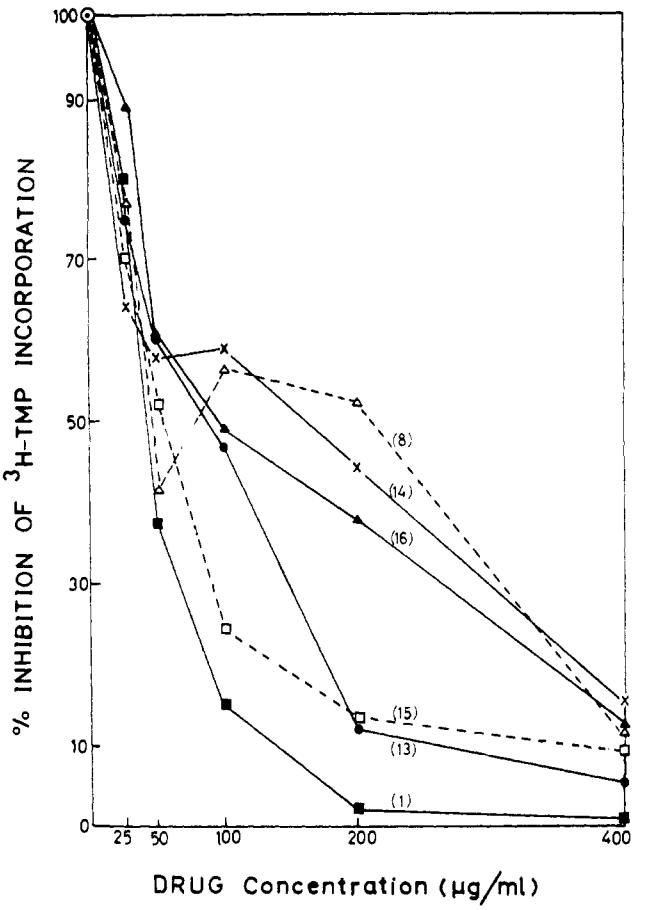


Figure 2. Percent inhibition of [^{3}H]thymidine triphosphate (^{3}H-TTP) incorporation into DNA by herpes simplex virus DNA polymerase as a function of drug concentration.

layer of agar in double-strength medium. After incubation at 37 °C in 5% CO_2 for 72 h, the agar was covered with 20% formalin for 30 min and removed with a spatula, and the cells were stained with 0.1% gentian violet for a few minutes. The number of plaques in each plate were counted, and plaque reduction was determined by comparison of the number of plaques obtained in the presence and absence of the drug.

Inhibition of DNA Synthesis in Isolated Nuclei in Vitro. Milk bottles containing monolayers of BSC-1 cells were infected with HSV for 20 h, which is the duration of the growth cycle of the virus. The medium was then discarded, and the infected cells were scraped into reticulocyte standard buffer (RSB = 0.01 M Tris-HCl, pH 7.7; 0.01 M KCl; 0.15 M $MgCl_2$; 1×10^{-3} M dithiothreitol), homogenized in a glass Dounce homogenizer, and centrifuged for 2 min at 800 rpm in a PR-2 refrigerated centrifuge. The nuclear pellet was washed in phosphate buffer (80 mM potassium phosphate, pH 7.4; 0.25 M sucrose; 1×10^{-3} M dithiothreitol) and resuspended at a concentration of 4×10^6 nuclei/100 μL in the same buffer. The buffers contained 0.4 mM CaCl.

DNA synthesis by the DNA polymerase in isolated nuclei in vitro was performed[16] using a reaction mixture (600 μL) containing 8×10^6 nuclei in 80 mM potassium phosphate buffer, pH 7.4; 250 mM sucrose (RNase free); 6 mM $MgCl_2$; 0.4 mM $CaCl_2$; 1 mM dithiothreitol; 0.04 mM each of dATP, dGTP, and dCTP; 0.002 nM [^{3}H]TTP; 1 mM ATP; 5 mM phosphoenol pyruvate; 15 μg/mL pyruvate kinase; and 5 mM ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA). The amount of [^{3}H]TTP incorporated after 60 min of incubation at 37 °C in the presence of the drug was determined according to the trichloroacetic acid (TCA) precipitable radioactivity in each sample.

Inhibition of HSV DNA Polymerase Activity in Vitro. BSC-1 cells were infected with HSV and incubated for 12 h at 37 °C. The cells were scraped from the bottles, and the nuclei and cytoplasm were separated by Dounce homogenization of the cells followed by centrifugation. The viral DNA polymerases were isolated from the nuclear extracts by precipitation with 70% (w/v) ammonium sulfate and centrifugation in sucrose gradients.[19] The viral enzymes were assayed in the presence of 250 mM concentrations of KCl. The reaction mixtures contained 2 mM $MgCl_2$; 0.05 mM (each) dGTP, dATP, and dCTP; 0.01 mM TTP; 3.75 μCi of [^{3}H]TTP (specific activity 50 Ci/mmol, The Radiochemical Centre, Amersham, England); 500 μg/mL activated calf thymus DNA; 10 mM Tris-HCl, pH 8.1; and 0.5 μg/mL bovine serium albumin.[20] Inhibition of the DNA polymerase activity was determined by assay in the presence and absence of the drug.

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Correlation of Biological Activity and High-Pressure Liquid Chromatographic Retention Index for a Series of Propranolol, Barbiturate, and Anthranilic Acid Analogues

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The antiarrhythmic activity of propranolol analogues, the inotropic activity of propranolol analogues, the anti-inflammatory activity of anthranilic acids, the hypnotic activity of barbiturates, and the inhibition of cell division by barbiturates were correlated with either octanol–water partition coefficients or with high-pressure liquid chromatographic retention indices. The retention index, which was a scale based on the relative retention of the drug and a series of C_3–C_{23} 2-ketoalkanes, was found to give higher correlations with biological activity than was found between octanol–water partition coefficients and biological activity. Only in the case of the anthranilic acids was the retention index found to give the lower correlation.

The use of octanol–water partition coefficients (log *P*) in quantitative structure–activity studies, as pioneered by Hansch, has become a standard method in drug design studies. Numerous workers have realized that there is a close parallel between the retention of drugs on reverse-phase high-pressure liquid chromatographic (LC) columns and the octanol–water partition coefficients and they have tried to link this correlation to biological activity in two distinct ways. The more common approach to the problem has been to use high-pressure LC as a tool to obtain estimates of octanol–water partition coefficients,[1-4] which can then be used in structure–activity studies. The other approach to the problem has been to relate the retention time of drugs on reverse-phase high-pressure LC columns directly to biological activity.[5,6]

In most of the studies where high-pressure LC has been used as a technique to estimate log *P*, various types of "octanol-like" reverse-phase high-pressure LC columns have been used with mobile phases that were largely water.[1-3] These methods have several advantages over the classical shake-flask method of obtaining log *P* values; in that only extremely small quantities of the drug are needed, the drug doses do not need to be extremely pure, and the method is much faster. However, because these methods have been restricted to mobile-phase systems with a high water content, very lipophilic drugs may not be detected due to extremely long retention times. Even if different mobile-phase systems were used, each column mobile-phase system must be calibrated with a number of compounds with known log *P* values.

As indicated earlier, the other basic approach to the problem is to relate high-pressure LC retention times directly to biological activity. It is of considerable importance to note that "octanol-like" reverse-phase high-pressure LC systems have been shown to give inferior correlations with biological activity than commercially available C-18 reverse-phase systems.[6] One of the objectives of the present study was to test the hypothesis that C-18 reverse-phase high-pressure LC systems not only give better correlations with biological activity than the "octanol-like" systems but that it might also be a better model for biological interactions than the direct octanol–water partitioning model.

One of the advantages that the classical log *P* measurement system has over all of the previous high-pressure LC systems is that the classical system provides a *single*, continuous scale for the measurement of the lipophilicity of all drugs. In contrast, most of the previous high-pressure LC measurements of drug lipophilicity used scales that are unique to each column–solvent system that was used. Recently, a retention index scale suitable for use with reverse-phase high-pressure LC systems has been introduced.[7] The retention scale is based on the relative retention times of a series of 2-ketoalkanes (C_3–C_{23}). A given column–solvent combination is calibrated by chromatographing the 2-keto standards and relating the logarithm of the observed capacity factor with the defined retention indices (2-butanone = 400, etc.) in a linear manner.

It has been shown that the retention index of a given drug is fairly constant even when large changes in the composition of the mobile phase were made.[7] It was also found that acetonitrile could be substituted for methanol in the mobile phase, and nearly identical retention indices were obtained for the drugs. Because of these properties, the retention index scale could provide a single, uniform scale for the measurement of the lipophilicity of drugs.

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