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Synthesis and Antiviral Activity of Three Pyrazole Analogues of Distamycin A

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The synthesis of three new monopyrazole analogues of the antiviral compound distamycin A is reported. Suitably protected 4-amino-1-methylpyrrole-2-carboxylic acid and 3-amino-1-methylpyrazole-5-carboxylic acid derivatives were chosen as starting materials. The construction of the trimeric polyamide framework was accomplished by assembly of the monomeric precursors under condensing conditions by analogy with our previous methodology, although with significant improvements in some pivotal steps. After chromatographic purification and spectroscopic characterisation, the analogues were assayed for antiviral activity. Compounds **7a–c** inhibited vaccinia virus at a concentration similar to or lower than distamycin A and the related antibiotic netropsin. Analogues **7b** and **7c** exhibited an antiviral effect comparable to those of distamycin A and netropsin against HSV-1 and HSV-2, whereas their antiviral activity against several other viruses including HIV-1 and HIV-2 was somewhat lower. The cellular toxicity of **7a–c** toward different host cell types proved to be of similar magnitude or lower than those of distamycin A and netropsin.

Distamycin A (DA Fig. 1) is a microbial natural product which displays an exciting spectrum of antiviral and antitumour activities. Since its discovery and structural characterisation more than three decades ago,[1,2] a vast number of structural analogues have been designed and described in the literature. Some of these have also displayed useful inhibitory effects against several microorganisms of clinical interest. Recent progress in the pharmacology and chemistry of DA and its congeners has been amply reviewed.[3–6] Our laboratory has focused attention mainly on the antiviral properties of such derivatives.[7–9]

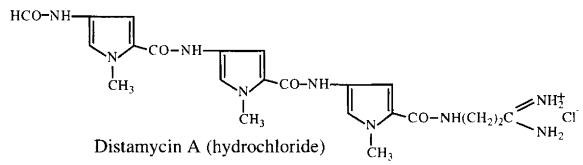

Fig. 1.

The earliest modifications of DA mainly involved the *N*-terminal acyl function and the aliphatic side chain and did not lead to much improvement in antiviral potency. More recent studies reported the preparation of certain dimeric DA analogues linked together through their *N*-terminal amino group by dibasic acyl residues. Such derivatives exhibit enhanced antitumour properties.[10,11] Other interesting oncolytic analogues include miscellaneous polypyrrole carboxamide conjugates with selected alkylating moieties.[12,13] After we had developed our new synthesis of DA,[7] we devoted our efforts to the preparation of DA analogues modified on the pyrrole and amidine nitrogens.[11,12] However, these compounds showed little promise with regard to increased antiviral effects.

It is generally assumed that DA exerts its principal action by binding preferentially to AT-rich regions in the minor groove of helical DNA.[14,15] In order to explore the mechanisms of interaction between DNA and various xenobiotics it is essential to gain insight into the nature of the affinity sites of selected DNA fragments and the properties of the bonds to an attached molecule. In this context, a number of modern powerful techniques, i.e., spectroscopic methods such as CD, NMR and Raman spectroscopy have been extremely useful. In addition, methods involving biochemical techniques, especially

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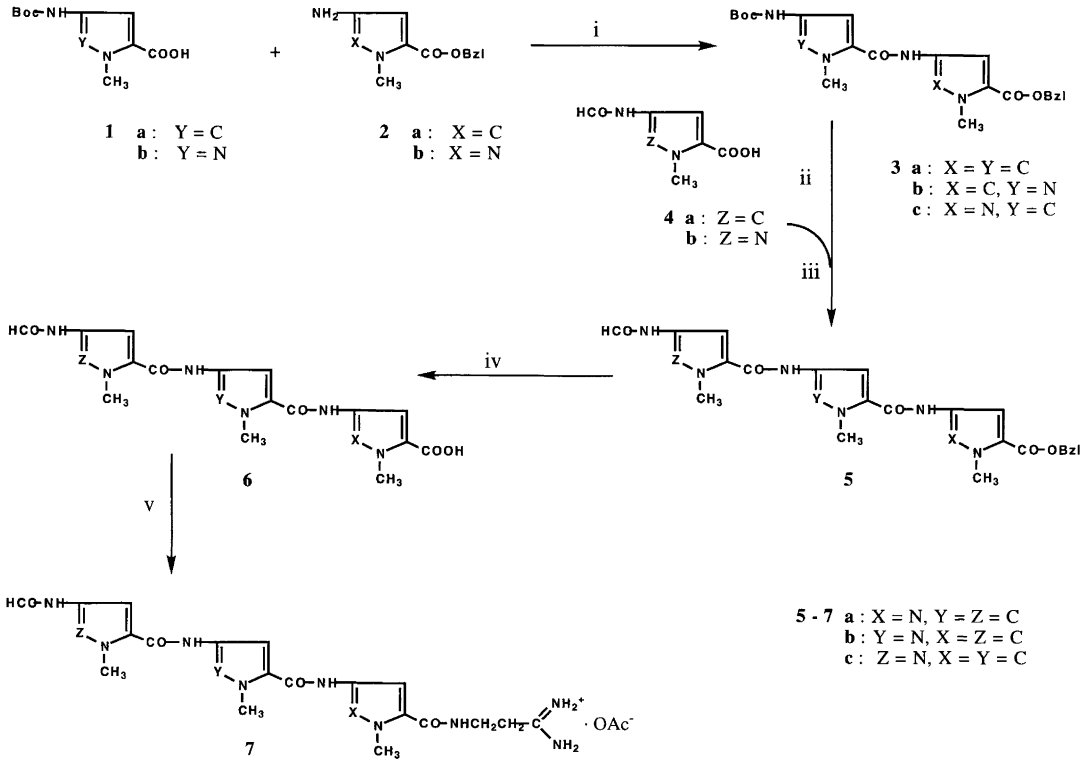
DNA footprinting and affinity cleavage of specific DNA sequences using appropriate DNA conjugates as targeting vehicles, have also been employed to elucidate different aspects of the binding mode of various DA-derived structures to the DNA framework. Recent investigations indicate that the exchange of other heterocyclic nuclei for pyrrole could alter this selectivity and also favour the recognition of various GC sequences. One way to change this DNA affinity might be to incorporate imidazole moieties into the DA structure.[16–20] A wide range of such DA- and netropsin-related analogues have now been synthesized and examined with respect to relevant biological and pharmacological properties but, in general, they possessed lower antiviral activity than DA itself. Furthermore, analogous polycarboxamide derivatives containing pyridine,[17,18] triazole,[21] isooxazole[22] and thiazole rings[23,24] have been synthesized and subjected to a battery of biological evaluations, but so far, little has been published about their antiviral and antitumour activities.

To our knowledge, there exists only one report dealing with pyrazole-substituted analogues related to polypyrrole carboxamide antibiotics.[25] Although a bispyrazole carboxamide derivative carrying an alkylating function exhibited significant oncolytic activity, only scant information is available regarding the specific influence of a pyrazole substitution on the intrinsic biological properties of such compounds. In order to elucidate similar aspects, we have now prepared three novel DA analogues with a pyrazole moiety in each of the three possible positions and studied their antiviral and antitumour activities.

Results and discussion

Synthetic chemistry. The multistep synthesis of DA analogues **7a–c** from known precursors is outlined in Scheme 1. Although it generally follows our earlier synthetic route,[7] some crucial reaction steps had to be further optimized to achieve acceptable yields. In particular, it is of vital importance to accomplish the formation of the various amide linkages in high yields without interfering side products. The original acid chloride approach[2] is still frequently employed in modern work with DA analogues[21,25,26] but it is not always compatible with the use of acid-labile protective groups such as the *tert*-butoxycarbonyl function.[27] However, several recent synthetic studies have clearly demonstrated that carbodiimide-mediated couplings often offer a versatile route for constructing sensitive amide derivatives related to DA. Especially dicyclohexylcarbodiimide (DCC), with or without additives, has been widely used for such purposes,[16,18,23] with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) as a viable alternative when an extractive isolation of the reaction product was required.[7,8]

An interesting paper has appeared which claimed that phosphonitrilic dichloride cyclic trimer[28] constitutes a



Scheme 1. Reagents: i, DCC–DMAP, CH_2Cl_2. ii, TFA, CH_2Cl_2. iii, **a**: DCC–DMAP, CH_2Cl_2–DMF. **b**: TBTU–Et_3N, DMF. **c**: EDC, DMF. iv, H_2 (Pd–C). DMF. v, **a**: 1: HOSu–DCC, DMF. 2: $NH_2(CH_2)_2C(=NH)NH_2 \cdot 2HBr$–$NaHCO_3$, aq. dioxane, **b** and **c**: $NH_2(CH_2)_2C(=NH)NH_2 \cdot 2HBr$, TBTU–$Et_3N$, DMF.

convenient and economical coupling reagent in DA chemistry, whereas other reports outline the use of diethyl cyanophosphonate for this purpose.[29] A totally different approach exploits the reaction of trichloroacetylpyrroles with the appropriate amino compound to obtain pyrrole carboxamides in high yields.[30] Other previously used condensing agents of high synthetic potential will also be discussed in the following sections.

Preparation of dimeric DA precursors. The condensation of **1b**[31] and **2a**[7] using DCC as the dehydrating agent in the presence of catalytic amounts of 4-dimethylaminopyridine (DMAP), afforded dimer **3b** in satisfactory yield. The reaction was usually completed overnight, thus resembling a previously described, similar coupling involving **2a**.[7] The analogous reaction between **1a**[7] and **2b**,[31] however, required more than 4 days to go to completion. This relative retardation in the formation of **3c** is presumably due to the lower nucleophilicity of the amino function of **2b** in comparison with that of **2a**. The use of EDC as the coupling reagent with DMF as the reaction medium did not give rise to significant amounts of **3c**, whereas the corresponding known EDC-mediated coupling of **1a** and **2a** furnished **3a** in good yield.[7] It is also worth mentioning that the pyrazole acid **1b** appeared to be even more reactive than **1a** in these amidations. Compounds **1b** and **2b** were thus readily converted into the corresponding bispyrazole carboxamide dimer in about 90% yield in only 4 h using the above DMAP-catalysed DCC coupling.

The TFA-mediated removal of the Boc group in **3a–c** furnished the corresponding amino analogues des-Boc-**3** in essentially quantitative yield after a convenient work-up. The crude dimeric amines were used directly in the synthesis of the trimeric carboxamides **5a–c**.

Assembly and deprotection of trimeric DA intermediates. When amine des-Boc-**3c** was coupled to **4a**[7] using DCC–DMAP in CH_2Cl_2–DMF, the trimer **5a** was obtained in excellent yield. Separate experiments showed that the presence of DMAP as the catalyst was essential for accomplishing a good yield. DCC alone gave rise to a mixture from which the desired **5a** could be isolated in only about 30% yield. The corresponding EDC-mediated condensation was even less satisfactory. The preparation of the isomeric trimer **5b** required a more reactive condensing agent than EDC or DCC–DMAP. Treatment of **4a** and des-Boc-**3b** with a small excess of DCC in CH_2Cl_2–DMF in the presence of DMAP gave a complex mixture from which ca. 20% of **5b** could be isolated after a laborious work-up. The corresponding coupling using EDC produced no detectable amounts of **5b**. Other investigators have also used carbonyl-1,1-diimidazole (CDI) to couple DA intermediates.[24,32] A preliminary attempt employing CDI in dry DMF afforded only an intractable product mixture even after prolonged reaction times. Obviously, the lower nucleophilicity of the pyrazole amine function in des-Boc-**3b**, as in the case of **2b** discussed above, reduces its reactivity compared with the analogous pyrrole derivatives.

Our search for more efficient coupling reagents to accomplish this sluggish condensation led us to consider the 1,1,3,3-tetramethyluronium compounds. This novel class of reagents has proved to be extremely useful in 'difficult' peptide couplings and many analogues are now also commercially available.[33] Indeed, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) smoothly converted **4a** and des-Boc-**3b** into the desired trimer **5b**. With the related coupling agent (1*H*-benzotriazol - 1 - yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) in DMF, analogous DA amide bond formations have been achieved.[26] By treating a solution of equimolar amounts of the acid and the amino compound and a small excess of TBTU in dry DMF with 2 equiv. of Et_3N at ambient temperature, the reaction was generally complete after a few days. As only water-soluble reaction products are formed by TBTU, the work-up of the highly insoluble trimer is greatly facilitated by this method. The third trimer, **5c**, was readily obtained from **4b** and des-Boc-**3a** using the convenient EDC procedure.

The hydrogenolytic deprotection of benzyl esters **5a–c** was conveniently accomplished with Pd–C in DMF and provided the corresponding acids **6a–c** in essentially quantitative yields.[7,8] Yields and properties of compounds **5** and **6** are given in Table 1.

Attachment of the amidine side chain. The transformation of acids **6a–c** into the desired DA analogues **7a–c** was effected by the direct attachment of the β-aminopropionamidine moiety under dehydrating conditions. In our original approach we employed a two-step strategy in which we first prepared the corresponding succinimidyl ester of the appropriate trimeric acid. This activated species was then incubated with the readily available β-aminopropionamidine dihydrobromide in aqueous dioxane under buffered conditions to give the desired product in fair overall yield.[7] This methodology also proved successful in the preparation of **7a**, but appeared less satisfactory for obtaining acceptable yields of **7b** and **7c**. This is probably due to the limited solubility of the corresponding *N*-hydroxysuccinimide esters in water–dioxane mixtures. The syntheses of **7b** and **7c** were instead conveniently achieved by direct TBTU-mediated coupling of the aliphatic amidine moiety with the appropriate acids **6b** and **6c**, respectively, in dry DMF in the presence of Et_3N. Yields and spectroscopic data are given in Table 2. A very pure product was obtained after a practical work-up involving preparative liquid chromatography (PLC) using a volatile buffer. This novel practical approach is characterized by its simplicity as well as its efficiency and is the best procedure so far for accomplishing this pivotal step in our synthesis.

Biology. Compounds **7a**, **7b** and **7c** were not particularly toxic to different cell types (i.e., ESM, HEL, CEM,

Table 1. General properties of trimeric compounds **5a–c** and **6a–c**.

No.	Yield (%)[a]	M.p. °C	Solvent for recrystallisation	^{1}H NMR [DMSO-d_6, δ (ppm) rel. TMS, 270 MHz][b]
5a	99	239–241 (decomp.)	CH_2Cl_2[c]	10.68 (br s, 1 H), 10.08 (br sign., 1 H), 9.96 (br s, 1 H), 8.12 (d, 1 H), 7.37–7.50 (complex sign., 5 H), 7.35 (d, 1 H), 7.21 (d, 1 H), 7.14 (s, 1 H), 7.13 (d, 1 H), 6.92 (d, 1 H), 5.34 (s, 2 H), 4.05 (s, 3 H), 3.84 (s, 3 H), 3.83 (s, 3 H)
5b	90	237–238 (decomp.)	CH_2Cl_2-acetone (4:1)[c]	10.63 (br s, 1 H), 10.43 (br s, 1 H), 10.11 (br sign., 1 H), 8.12 (d, 1 H), 7.54 (d, 1 H), 7.35–7.47 (complex sign., 5 H), 7.41 (s, 1 H), 7.33 (d, 1 H), 7.04 (d, 1 H), 7.02 (d, 1 H), 5.26 (s, 2 H), 4.05 (s, 3 H), 3.88 (s, 3 H), 3.86 (s, 3 H)
5c	83	237–239 (decomp.)	CH_2Cl_2[d]	10.75 (br s, 1 H), 10.47 (br s, 1 H), 9.99 (br s, 1 H), 8.23 (d, 1 H), 7.49 (d, 1 H), 7.39–7.46 (complex sign., 5 H), 7.33 (s, 1 H), 7.29 (d, 1 H), 7.11 (d, 1 H), 6.97 (d, 1 H), 5.25 (s, 2 H), 4.02 (s, 3 H), 3.86 (s, 6 H)
6a	>95	*e*	*f*	10.58 (br s, 1 H), 10.09 (br sign., 1 H), 9.96 (br s, 1 H), 8.13 (d, 1 H), 7.35 (d, 1 H), 7.21 (d, 1 H), 7.11 (d, 1 H), 7.05 (s, 1 H), 6.93 (d, 1 H), 4.02 (s, 3 H), 3.85 (s, 6 H)
6b	>95	*e*	*f*	12.27 (br s, 1 H), 10.62 (br s, 1 H), 10.41 (br s, 1 H), 10.10 (br sign., 1 H), 8.12 (d, 1 H), 7.48 (d, 1 H), 7.43 (s, 1 H), 7.33 (d, 1 H), 7.04 (d, 1 H), 6.90 (d, 1 H), 4.05 (s, 3 H), 3.87 (s, 3 H), 3.84 (s, 3 H)
6c	>95	*e*	*f*	10.80 (br s, 1 H), 10.46 (br s, 1 H), 9.77 (br s, 1 H), 8.23 (d, 1 H), 7.33 (s, 1 H), 7.28 (d, 1 H), 7.09 (d, 1 H), 6.46 (d, 1 H), 4.02 (s, 3 H), 3.85 (s, 3 H), 3.82 (s, 3 H)

[a]Crude. [b]Main conformer. Weak signals indicating a minor conformer (~8%) were largely obscured in the spectra. [c]These compounds tenaciously retained solvents and moisture despite prolonged heating under high vacuum at elevated temperatures (+50–60 °C). No satisfactory elemental analyses were obtained. [d]Anal. ($C_{25}H_{25}N_7O_5$). C, H, N. [e]Not recorded. [f]Used without purification.

MOLT-4 or L1210), although they reduced the viability of the CEM cells to such an extent that protective effects on HIV cytopathicity could not be correctly evaluated at concentrations higher than 5–10 μg ml^{-1}. Antiviral effects at concentrations that were ≥10 times lower than the cytotoxic concentrations were observed with **7a** against HSV-1, with **7c** against VZV, and with all three compounds (**7a–c**) against vaccinia virus and TK$^-$ HSV-1 (Table 3). Compounds **7a–c** inhibited vaccinia virus at a concentration of 1–2 μg ml^{-1}, which is 3.5 to 7 times lower than the concentration at which netropsin inhibits vaccinia virus. This is reminiscent of the anti-

Table 2. Yields and spectroscopic properties of DA analogues **7a–c**.

No.	Yield (%)[a]	UV (EtOH) λ_{max} nm (log ε)	^{1}H NMR [CD_3OD, δ (ppm) rel. TMS, 270 MHz][b]
7a	69 (59)	303 (4.49) 234 (4.50)	8.13 (s, 1 H, HCO), 7.29 (d, 1 H, pyrrole H), 7.18 (d, 1 H, pyrrole H), 7.10 (s, 1 H, pyrazole H), 6.98 (d, 1 H, pyrrole H), 6.88 (d, 1 H, pyrrole H), 4.05 (s, 3 H, NCH_3), 3.92 (s, 3 H, NCH_3), 3.90 (s, 3 H, NCH_3), 3.69 (t, 2 H, CH_2), 2.72 (t, 2 H, CH_2), 1.89 (s, 3 H, CH_3COOH)
7b	95 (45)	296 (4.46) 235 (4.45)	8.14 (s, 1 H, HCO), 7.28 (d, 1 H, pyrrole H), 7.25 (d, 1 H, pyrrole H), 7.17 (s, 1 H, pyrazole H), 6.94 (d, 1 H, pyrrole H), 6.92 (d, 1 H, pyrrole H), 4.08 (s, 3 H, NCH_3), 3.92 (s, 3 H, NCH_3), 3.89 (s, 3 H, NCH_3), 3.65 (t, 2 H, CH_2), 2.71 (t, 2 H, CH_2), 1.90 (s, 3 H, CH_3COOH)
7c	79 (47)	302 (4.49) 234 (4.50)	8.25 (s, 1 H, HCO), 7.28 (d, 1 H, pyrrole H), 7.19 (s, 1 H, pyrazole H), 7.16 (d, 1 H, pyrrole H), 6.99 (d, 1 H, pyrrole H), 6.89 (d, 1 H, pyrrole H), 4.07 (s, 3 H, NCH_3), 3.91 (s, 3 H, NCH_3), 3.88 (s, 3 H, NCH_3), 3.64 (t, 2 H, CH_2), 2.71 (t, 2 H, CH_2), 1.89 (s, 3 H, CH_3COOH)

[a]Crude, from **6**. Values in parentheses refer to PLC-purified product. [b]See Table 1, footnote *b*.

Table 3. Antiviral and antitumour activity of compounds **7a**, **7b** and **7c**.

		IC_{50} ($\mu g\ ml^{-1}$)[a]				
Virus	Cell	**7a**	**7b**	**7c**	Distamycin[b]	Netropsin
HSV-1	ESM	40	70	40	70	70
HSV-2	ESM	≥400	≥400	>40	70	>100
Vaccinia	ESM	1	2	2	0.2	7
VSV	ESM	>400	>400	>40	>200	≥100
TK^- HSV-1	ESM	20	1	2		7
VZV	HEL	20	20	3	5	10
TK^- VZV	HEL	20	12	7	7	15
CMV	HEL	>50	>50	27	10	>20
HIV-1	CEM	>8.4	>7.2	>4.8		
HIV-2	CEM	>8.4	>7.2	>4.8		
	ESM (Morphology)	≥400	≥400	≥100		≥100
	HEL (Growth)	>50	50	30	20	>20
	CEM (Growth)	>50	>50	32		112
	MOLT-4 (Growth)	>50	>50	>50	28	172
	L-1210 (Growth)	31	16	>50	27	112

[a] 50% Inhibitory concentration, or concentration required to reduce virus-induced cytopathicity of CEM, Molt-4 and L1210 cell growth by 50%, or minimum concentration required to alter microscopic appearance of ESM cell morphology. [b] For distamycin the IC_{50} values against HSV-1, HSV-2, vaccinia virus and VSV refer to data obtained in primary rabbit kidney cells (refs. 11 and 12).

vaccinia virus activity that has been observed with distamycin/netropsin derivatives in previous reports.[11,12,16] In addition, compounds **7b,c** inhibited TK^- HSV-1 at a concentration of 1–2 $\mu g\ ml^{-1}$. This concentration is significantly lower than the concentration required to inhibit wild-type HSV-1 or HSV-2. As a rule, one may postulate that the TK^- HSV strains, while being resistant to viral TK-dependent drugs such as acyclovir, become more sensitive to other antimetabolites acting at the thymidylate synthase step,[34] or any other step in DNA biosynthesis, as is the case here for the minor-groove DNA duplex binders.

Compounds **7a–c** were inactive against various other viruses (i.e., vesicular stomatitis, Coxsackie B4, polio-1, reo-1, Sindbis and Semliki forest) at the highest concentrations tested (200 $\mu g\ ml^{-1}$).

Experimental

Melting points were measured with a Gallenkamp melting point apparatus and are uncorrected. The required pyrrole- and pyrazole-precursors were obtained as described previously.[7,31] All solvents used as reaction media were of analytical grade unless otherwise stated and were dried over molecular sieves (4 Å, activated at 320°C/0.01 mmHg for 10–15 h). TLC analysis was performed on 0.25 mm thick precoated UV-sensitive silica plates (Merck Precoated TLC plates silica 60 F_{254}) and the mobile phases used were CH_2Cl_2–Me_2CO 4:1 (A), CH_2Cl_2–MeOH 9:1 (B), EtOAc–Me_2CO–AcOH–H_2O 5:3:1:1 (C) and $CHCl_3$–EtOH–H_2O 100:50:4 (D). TLC spots were visualized by inspection under UV-light at 254 mm or, preferentially, after brief heating, by exposure to Cl_2 followed by dicarboxidine spray.[35] UV spectra were recorded with a Shimadzu UV-160A spectrophotometer. The preparative LC purifications were carried out on a Delta Prep 3000 (Waters) using the detector Spectromonitor III (LDC). Preparative chromatography was carried out using silica gel 60 (70–230 mesh). ^1H NMR spectra were recorded with a JEOL JNM-EX270 instrument. The NMR signals were tentatively assigned by comparison of chemical shifts and peak multiplicities. Elementary analyses of selected compounds were carried out by Mikro Kemi AB, Uppsala, Sweden. Satisfactory microanalyses (C ± 0.4, H ± 0.3, N ± 0.3) were obtained unless otherwise stated. Yields, melting points and spectroscopic data of all trimeric compounds are collected in Tables 1 and 2.

Synthesis of protected dimers **3a–c**. *General procedure.* Crude benzyl 4-amino-1-methylpyrrole-2-carboxylate (**2a**) or benzyl 3-amino-1-methylpyrazole-5-carboxylate (**2b**) (obtained as described earlier) and 1.0 equiv. of the appropriate acid **1a** or **1b** were dissolved in CH_2Cl_2 (15 ml g^{-1}) together with DMAP (0.1 equiv.). The resulting solution was treated dropwise with DCC [10% excess dissolved in CH_2Cl_2 (5 ml g^{-1})] at ambient temperature with vigorous stirring. The formation of dimers **3a** and **3b** was generally complete in less than 1 h, whereas **3c** required more than 3 days [TLC (A)]. The white solid consisting of dicyclohexylurea (DCU) was filtered off and rinsed repeatedly with small portions of cold CH_2Cl_2. The combined filtrate and washings were taken to dryness *in vacuo* at room temperature, affording a crispy brownish residue which was dissolved in ether (400 ml g^{-1}) and washed consecutively with 1 M $KHSO_4$, 1 M $NaHCO_3$ and brine (3 × 100 ml g^{-1} each). The ether extract was treated with

decolourizing carbon, dried with $MgSO_4$, and evaporated at room temperature to furnish the desired crude dimer as a crispy yellow foam. This crude product was chromatographed on silica using CH_2Cl_2–acetone 10:1 as the eluent. The chromatographed material was further purified by crystallisation from a suitable solvent mixture to furnish an analytical specimen.

*Benzyl 4-(3-*tert*-butoxycarbonylamino-1-methylpyrazole-5-carbamido)-1-methylpyrrole-2-carboxylate (***3b***).* Prepared from **1b** and **2a** according to the general method outlined above. The yield of crude **3b** was 75%. A chromatographed sample was recrystallized from *n*-hexane–Et_2O to give an analytical specimen as broad white needles, m.p. 141–142°C. TLC (A) showed one spot. ^1H NMR ($CDCl_3$): δ 8.03 (br s, 1 H), 7.76 (br s, 1 H), 7.31–7.43 (complex sign, 6 H), 6.90 (br s, 1 H), 6.82 (d, 1 H), 5.28 (s, 2 H), 4.11 (s, 3 H), 3.91 (s, 3 H), 1.51 (s, 9 H). Anal. ($C_{23}H_{27}N_5O_5$) C, H, N.

*Benzyl 3-(4-*tert*-butoxycarbonyl-1-methylpyrrole-2-carbamido)-1-methylpyrazole-5-carboxylate (***3c***).* Synthesized from **1a** and **2b** as outlined above, thus furnishing crude **3c** in 67% yield. An analytical specimen was obtained by recrystallisation from Et_2O–CH_2Cl_2 as white needles; m.p. 179.5–180.5°C (decomp). TLC (A) gave one spot. ^1H NMR ($CDCl_3$): δ 8.09 (br s, 1 H), 7.34–7.43 (complex sign, 5 H), 7.29 (s, 1 H), 6.98 (br s, 1 H), 6.49 (br s, 1 H), 6.32 (br s, 1 H), 5.30 (s, 2 H), 4.09 (s, 3 H), 3.90 (s, 3 H), 1.50 (s, 9 H). Anal. ($C_{23}H_{27}N_5O_5$) C, H, N.

*Benzyl 3-[4-(4-formamido-1-methylpyrrole-2-carbamido)-1-methylpyrrole-2-carbamido]-1-methylpyrazole-5-carboxylate (***5a***). (a) Deprotection of* **3c**. The Boc-group of crude **3c** (486 mg, 1.07 mmol) was removed with TFA as described earlier to give the crude amine des-Boc-**3c** in almost quantitative yield as a white solid (373 mg). TLC (B) gave one spot.

(b) Preparation of **5a** *by DCC coupling.* The above crude des-Boc-**3c** (373 mg, 1.05 mmol), acid **4a** (213 mg, 1.26 mmol) and DMAP (78 mg, 0.05 equiv.) were dissolved together in CH_2Cl_2 (5 ml) containing DMF (2 ml). The solution was then treated dropwise with a large excess of DCC (1.18 g, 5.7 mmol) dissolved in dry CH_2Cl_2 (2 ml) with rapid stirring and ice-cooling under dry nitrogen. The mixture was stirred at room temperature for 48 h, whereupon TLC indicated that the coupling was complete. The DCU was filtered off and the filtrate was evaporated under reduced pressure. The residue was dissolved in a small amount of DMF and filtered to remove insoluble by-products. The filtrate was evaporated and the yellow residue was rinsed with CH_2Cl_2–petroleum ether (1:1), to remove DCU and other impurities, and dried *in vacuo*. Crude **5a**, obtained as a yellow solid, was pure enough for the next step. TLC (B) showed that the crude product contained only traces of impurities. The analytical sample was obtained by chromatography and crystallization as above (Table 1).

*Benzyl 4-[3-(4-formamido-1-methylpyrrole-2-carbamido)-1-methylpyrazole-5-carbamido]-1-methylpyrrole-2-carboxylate (***5b***). (a) Deprotection of* **3b**. Crude **3b** (150 mg, 0.33 mmol) was treated with CH_2Cl_2–TFA by analogy with **3c**, thus providing the corresponding chromatographically pure amine des-Boc-**3b** in essentially quantitative yield as a white solid foam. TLC (B) gave essentially one spot.

(b) Preparation of **5b** *by TBTU coupling.* The above crude des-Boc-**3b** (120 mg, 0.33 mmol), acid **4a** (60 mg, 0.35 mmol) and TBTU (128 mg, 0.4 mmol) were dissolved together in dry DMF (2 ml). The resulting solution was treated dropwise with Et_3N (81 mg, 0.8 mmol) with rapid stirring and ice-cooling under dry conditions. The stirring was continued for 4 days at ambient temperature. As TLC (B) indicated minor amounts of remaining starting materials, more TBTU (32 mg, 0.10 mmol) and Et_3N (20 mg, 0.2 mmol) were added. After a further 2 days, TLC showed complete reaction and the brownish mixture was quenched in ice-cold 0.5 M $KHSO_4$ (40 ml) with rapid stirring. The fine-grained tan precipitate was collected by centrifugation, rinsed repeatedly with ice–water and dried *in vacuo*. This crude product, consisting of essentially pure **5b** was further purified by chromatography using CH_2Cl_2–CH_3OH (10:1) as the eluent. Recrystallization of the chromatographed material gave an analytical sample (Table 1).

*Benzyl 4-[4-(3-formamido-1-methylpyrazole-5-carbamido)-1-methylpyrrole-2-carbamido]-1-methylpyrrole-2-carboxylate (***5c***). (a) Deprotection of* **3a**. Crude **3a** (904 mg, 2.0 mol) was deprotected in CH_2Cl_2–TFA essentially as described above to give the crude amine des-Boc-**3a** in almost quantitative yield as a yellow solid (758 mg), TLC (B) gave one spot.

(b) Preparation of **5c** *by carbodiimide coupling.* The above crude des-Boc-**3a** (758 mg, 2.0 mmol) and **4b** (338 mg, 2.0 mmol) were dissolved in DMF (10 ml). The mixture was treated with EDC (430 mg, 2.24 mmol) in small portions with thorough mixing. The resulting brown solution was stirred at 40°C overnight under argon and then added dropwise to ice-cold 1 M $KHSO_4$ (100 ml) with rapid stirring. The light brown fine-grained precipitate was collected and thoroughly washed in turn with small portions of ice–water. After drying *in vacuo*, the slightly brown powder was repeatedly washed with ether. The resulting crude **5c** obtained as a pale yellow powder was essentially pure by TLC (B). Recrystallization furnished an analytical specimen as white crystals (Table 1).

*3-[4-(4-Formamido-1-methylpyrrole-2-carbamido)-1-methylpyrrole-2-carbamido]-1-methylpyrazole-5-carboxylic acid (***6a***).* Crude **5a** (537 mg, 1.06 mmol) was hydrogenated under H_2 (1 atm) in DMF (20 ml) in the presence of 5% Pd–C (100 mg). When the reaction was complete [TLC (D), 2–5 h] the catalyst was removed by filtration. The filtrate was taken to dryness at room temperature and the residual solid was subjected to a conventional work-up

providing crude **6a** as a light yellow solid to be used in the subsequent coupling (Table 1).

*4-[3-(4-Formamido-1-methylpyrrole-2-carbamido)-1-methylpyrazole-5-carbamido]-1-methylpyrrole-2-carboxylic acid (**6b**).* Crude **5b** (170 mg, 0.35 mmol) was hydrogenated in DMF (5.0 ml) in the presence of Pd–C catalyst (70 mg) essentially as described for the preparation of **6a**. After similar work-up, crude **6b** was obtained as a white solid suitable for the next step (Table 1).

*4-[4-(3-Formamido-1-methylpyrazole-5-carbamido)-1-methylpyrrole-2-carbamido]-1-methylpyrrole-2-carboxylic acid (**6c**).* Crude **5c** (379 mg, 0.75 mmol), was dissolved in DMF (8 ml) and was deprotected as usual to furnish crude **6c** after analogous work-up. The product was sufficiently pure for the next step (Table 1).

DA analogue **7a**. *(a) Preparation of the succinimidyl ester of* **6a**. A solution of crude **6a** (207 mg, 0.50 mmol) and *N*-hydroxysuccinimide (85 mg, 0.73 mmol) in DMF (2.0 ml) was treated dropwise with DCC (185 mg, 0.90 mmol) in dry DMF (1.0 ml) with rapid stirring and ice-cooling under N_2. The resulting brown solution was stirred at room temperature overnight (20 h) and the precipitated DCU was filtered off and rinsed with DMF. The filtrate was evaporated at room temperature and reduced pressure and the resulting semisolid residue was dissolved in acetone (3.0 ml). The turbid solution was slowly filtered into cold ether (37 ml) through glass wool with rapid stirring to furnish a fine-grained precipitate. After stirring at room temperature for 1 h, and chilling to $-20\,^{\circ}C$ for 3 h, the precipitate was collected by filtration and rinsed with cold ether. The crude succinimidyl ester was obtained as a pale yellow powder. The yield was 264 mg (quant.) and TLC (D) indicated that the crude ester contained only traces of DCU and was suitable for the coupling step below.

(b) Coupling step. The above crude active ester (264 mg, 0.5 mmol) in dioxane (5.0 ml) was slowly added to a solution of β-aminopropionamidine dihydrobromide[36] (373 mg, 1.5 mmol) and $NaHCO_3$ (126 mg, 1.5 mmol) in dioxane–water 1:3 (20 ml) with vigorous stirring at room temperature. The mixture was stirred overnight at room temperature to complete the reaction. The pH of the mixture was adjusted to 4.5 with dilute HBr (Br_2-free) and the solvent was removed at room temperature. The residue was redissolved in EtOH (15 ml) and chromatographed on silica. Non-polar impurities were eluted with EtOH (99%) and then the title compound was obtained by continued elution with EtOH–0.01 M aq. HBr (5:1). The chromatographed material containing some inorganic impurities was dissolved in a mixture of EtOH–water (1:1) (30 ml). After concentration at room temperature to about 5 ml, the remaining solution was left in the cold overnight. The yellow precipitate formed was collected by filtration and rinsed with cold water. After drying *in vacuo*, crude **7a** (190 mg) was obtained as a light yellow powder. TLC (C) indicated that the crude product contained only traces of impurities. Pure compound was obtained by PLC (Table 2).

DA analogue **7b**. Crude **6b** (103 mg, 0.25 mmol), β-aminopropionamidine dihydrobromide (95 mg, 0.38 mmol) and TBTU (96 mg, 0.30 mmol) were dissolved in DMF (3 ml) and the mixture was treated dropwise with TEA (138 mg, 1.36 mmol) with vigorous stirring and ice-cooling under N_2. After the addition of TEA, the mixture was stirred at room temperature overnight, whereafter TLC indicated that the coupling was complete. DMF was removed by evaporation at room temperature and the oily residue was dissolved in ethanol (about 1.5 ml) and was then slowly added dropwise to water (10 ml) with rapid stirring. The solution was concentrated until a precipitate appeared. The resulting solution was kept in the cold overnight and the fine grained solid was collected by centrifugation and rinsed with cold water. After drying *in vacuo*, 132 mg of crude **7b** were obtained as a white solid. TLC (C) indicated that the crude product contained minor amounts of unchanged amidine and other impurities. The crude product was purified by PLC according to the procedure outlined below (Table 2).

DA analogue **7c**. Crude **6c** (153 mg, 0.37 mmol), β-aminopropionamidine dihydrobromide (138 mg, 0.55 mmol) and TBTU (130 mg, 0.4 mmol) were dissolved in dry DMF (3.0 ml). The resulting mixture was treated dropwise with TEA (161 mg, 1.6 mmol) with rapid stirring and ice-cooling under N_2. The reaction mixture was then stirred at room temperature overnight, whereafter TLC (C) indicated essentially complete reaction. The solvent was evaporated under reduced pressure and the residue was dissolved in 50% ethanol (15 ml). The pH was adjusted to 4.5 with dilute HBr, and the solution was concentrated to about a third of its volume at room temperature under reduced pressure and allowed to stand in the cold overnight. The resulting precipitate was collected by filtration, rinsed with small portions of cold water and dried *in vacuo*. The crude, dried product weighed 100 mg. Concentration of the aqueous filtrate and washings to one third of the volume and chilling overnight afforded a second crop of pure **7a** (62 mg). TLC (C) showed that the crude product contained only traces of impurities. A pure sample was prepared by PLC as described below (Table 2).

Preparative PLC purification of the crude distamycin A analogues **7a–c**. The crude material obtained as above was dissolved in 0.1 M aq. NH_4Ac buffer (pH = 4.5) containing up to 50% EtOH (about 20 mg ml^{-1}). After filtration through a 2 μm filter, the clear solution was applied to a preparative LC column, Spherisorb C_{18}, 10 μm (250 × 10 mm). The compound was eluted using a mobile phase consisting of 0.1 M aq. NH_4Ac buffer (pH = 4.5)–EtOH gradient with the concentration of EtOH increasing from 0 to 14% over 2 h at a flow rate of 3.0 ml min^{-1}.

The procedure required about 2 h and up to 40 mg of crude compound could be purified in each run. The recovery of pure products, obtained as acetates, was generally higher than 80%. The purity of the chromatographed sample was, after repeated lyophilization from water, carefully assayed by analytical HPLC,[37] TLC, UV and ^{1}H NMR spectroscopy. Only traces of impurities could be detected in the purified specimens (Table 2).

Biological assays. Antitumour (cytostatic) activity assays were carried out as described previously.[11,12] The methods for measuring antiviral activity, as well as the sources of the virus stocks used have also been described previously.[38,39] The abbreviations used are HSV-1 (herpes simplex virus type 1, strain KOS), HSV-2 (herpes simplex virus type 2, strain G), VSV (vesicular stomatitis virus), TK$^-$ HSV-1 (thymidine kinase-deficient HSV-1, strain B2006), VZV (varicella zoster virus, strain OKA), TK$^-$ VZV (thymidine kinase-deficient VZV, strain YS-R), CMV (cytomegalovirus, strain AD-169), HIV-1 (human immunodeficiency virus type 1, strain III_B/BRU/LAI), HIV-2 (human immunodeficiency virus type 2, strain ROD), ESM (embryonic skin-muscle) fibroblasts, HEL (human embryonic lung) cells, CEM and MOLT-4, representing two human T-4 lymphocyte cell lines and L1210, corresponding to murine leukemia cells.

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