The Synthesis of Radiosensitizers Designed to Bind to the Minor Groove of Duplex DNA

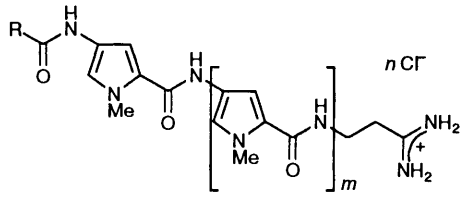
John Parrick,*[a] Manuchehr Porssa[a] and Terence C. Jenkins[b]
[a] *Chemistry Department, Brunel University, Uxbridge, Middlesex UB8 3PH, UK*
[b] *Cancer Research Campaign Biomolecular Structure Unit, The Institute of Cancer Research, Sutton, Surrey SM2 5NG, UK*

The synthesis is described of novel compounds, **17**, **21**, **25** and **27**, containing in their molecular architecture both a terminal nitroarene component and an oligopeptide similar to that in the antibiotic distamycin, which is known to bind into the minor groove of duplex DNA. It is hoped that the compounds may concentrate around DNA in cells and so potentiate the action of the nitroarene as a radiosensitizer.

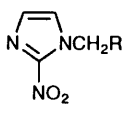
The presence of hypoxic cells in experimental murine tumours can be demonstrated[1] and oxygen-deficient cells in human tumours are thought to be a cause of the long-term failure of some radiotherapy procedures.[2] Indeed, it is known that hypoxic cells require approximately three times the radiation dose necessary to kill the same proportion of oxic cells.[3,4]

During the past 20 years much effort has been devoted to attempts to develop a drug which has the property of sensitizing hypoxic cells to killing by X-rays but has no such effect on oxic cells.[5] Such chemicals are called hypoxia-selective radiosensitizers and several are currently in clinical trials but have not shown a clear beneficial effect,[6,7] though there is clear evidence that the compounds are effective from *in vitro* and *in vivo* experiments.[8] The critical damage to the cells caused by X-rays is known to be at DNA.[5]

In an attempt to produce radiosensitizers that are targetted to DNA we chose to use molecular structures similar to those found in two antitumour antibiotics, netropsin **1**[9] and distamycin **2**[10,11] as the DNA-binding fragment of our proposed compounds since both **1** and **2** are known to undergo non-intercalative association with duplex DNA by binding in the minor groove. Several studies confirm that the curvature of



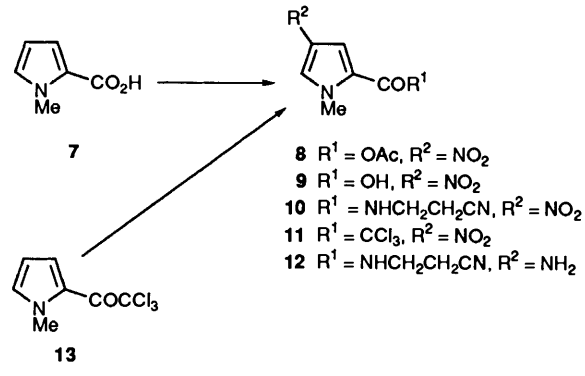
1 R = $CH_2NHC(NH_2)_2^+$, *m* = 1, *n* = 2
2 R = H, *m* = 2, *n* = 1



3 R = CH(OH)CH_2N⬡
4 R = CH(OH)CH_2OMe
5 R = CH(OH)CH_2N△
6 R = $CONHCH_2CH_2OH$

these ligands is essentially isohelical with the DNA minor groove thereby facilitating complexation.[12] For the radiosensitizer part of the target compounds we used the heterocycles furan and imidazole because their nitro derivatives are known to have good radiosensitizer properties in experimental tumours.[3,5,8] Several, nitroimidazoles, *e.g.* pimonidazole **3**, misonidazole **4**, RSU 1069 **5**, and etanidazole **6** are, or have been, in clinical trials. Preliminary molecular modelling suggested that such derivatives would retain the necessary shape complementarity with the DNA minor groove and that, in certain cases, favourable non-bonded interaction with the host molecule may be enhanced.

The initial synthetic target was the diamide **14**, both because **14** was a model structure and potentially useful as a starting point for the synthesis of netropsin-like structures incorporating a benzene ring in place of the terminal pyrrole nucleus. 1-Methylpyrrole-2-carboxylic acid **7** or its esters are useful starting materials for the synthesis of netropsin or its analogues,[13–15] and the nitro acid **9** has been obtained by the nitration of commercially available **7** with a mixture of nitric acid in acetic anhydride.[11,16,17] In our hands, this reaction (carried out at −25 °C) yielded the novel anhydride **8** (Scheme 1), which was readily hydrolysed to the acid **9**. Conversion of

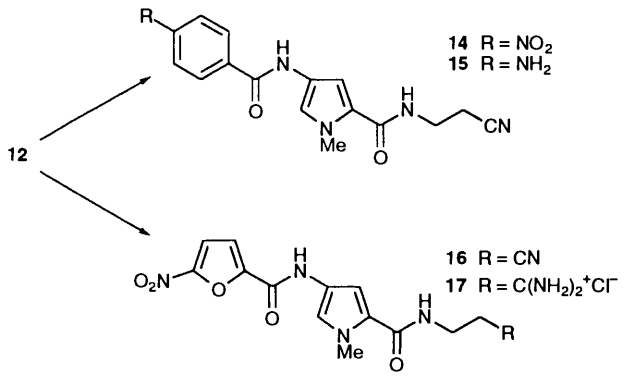


7
8 R^1 = OAc, R^2 = NO_2
9 R^1 = OH, R^2 = NO_2
10 R^1 = $NHCH_2CH_2CN$, R^2 = NO_2
11 R^1 = CCl_3, R^2 = NO_2
12 R^1 = $NHCH_2CH_2CN$, R^2 = NH_2
13

Scheme 1

this acid into the corresponding acid chloride (not isolated) and treatment with 2-cyanoethylamine gave the known amide **10**,[11] which was also obtained from the anhydride **8**, though in poor yield. A better route to **10**, particularly for larger scale work, was found[18,19] to be from 1-methyl-2-trichloroacetylpyrrole **13**[18] which was nitrated[18,19] to produce **11** and, subsequently, treated with 2-cyanoethylamine to give **10**. Catalytic hydrogenation of **10** gave an unstable aromatic amine **12** (not isolated) which yielded **14** on reaction with *p*-nitrobenzoyl chloride. The nitro group was selectively reduced to give the amine **15** thus, potentially facilitating the preparation of extended netropsin analogues containing a benzene ring.

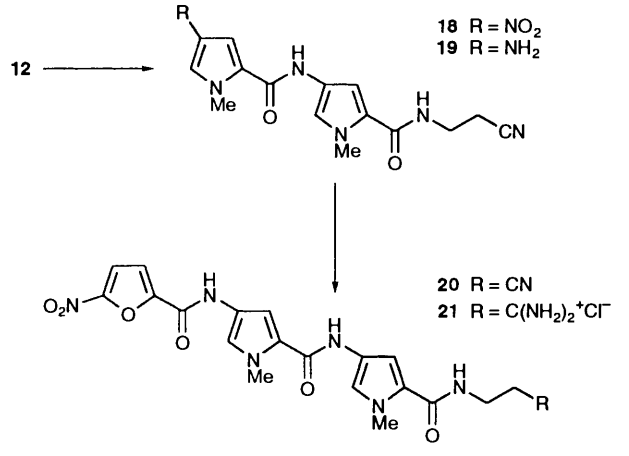
Our attention now turned to the synthesis of compounds containing nitroaromatic systems more likely to be effective as radiosensitizers. 2-Nitrofurans were among the first (and most potent) nitroheteroaromatic systems to be investigated[20] and are still of interest.[21] Treatment of the crude amine **12** with



Scheme 2

5-nitro-2-furoyl chloride afforded the required cyano diamide **16** (Scheme 2), and the corresponding amidine hydrochloride **17** was then easily obtained by the Pinner reaction. A contribution to the binding of the synthetic analogues of netropsin to the minor groove of duplex DNA is thought to involve hydrogen-bonding interaction of the amidine group to either thymine (O-2) or adenine (N-3) base acceptor atoms at the floor of the DNA minor groove.[12,22] Thus, the amidines would be expected to bind more strongly to DNA than the corresponding cyanides.

The cyano triamide **20** was obtained in a similar way by reduction of **10** to the crude amino nitrile **12**, formation of the diamide **18** (Scheme 3) by treatment of **12** with ketone **11**,

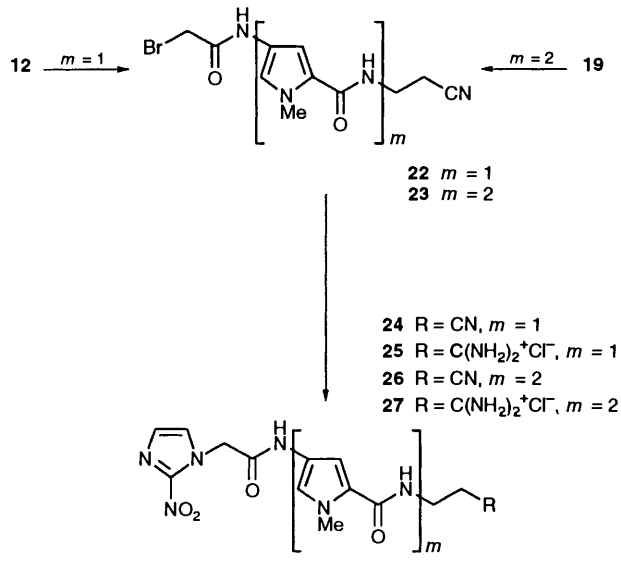


Scheme 3

selective reduction of the nitro group in **18**, and final reaction of the resultant amine **19** (without isolation) with 5-nitro-2-furoyl chloride to give **20**. The cyanide was then converted into the amidine hydrochloride **21** by the general procedure.

Of particular interest to us were oligopeptides of the netropsin or distamycin type containing the 2-nitroimidazole unit as the radiosensitizer function because of their potential importance in the radio- and chemo-therapy of cancer.[23] In addition, 2-nitroimidazoles are known to selectively bind in hypoxic cells.[1] In this case, the amide carbonyl function had to be separated from the 1-position of the imidazole ring by a methylene group because of the ready hydrolysis of 1-acylimidazoles. In order to achieve this structural feature, **12** was treated with bromoacetyl bromide in the presence of diisopropylethylamine to give the bromoacetyl derivative **22** and this was used to alkylate the anion of 2-nitroimidazole, in the presence of a crown ether,[24] to give the cyano diamide **24** (Scheme 4). The corresponding amidine hydrochloride **25** was obtained readily from **24**.

In an analogous manner, the cyano triamide **26** was obtained



Scheme 4

by reduction of the nitro diamide **18** to give the amine **19** which, without isolation, was acylated with bromoacetyl bromide to produce **23** (Scheme 4). Alkylation of 2-nitroimidazole with **23** gave the cyanide **26** and addition of ammonia proceeded smoothly to yield the amidine hydrochloride, **27**.

The biological properties and DNA-binding behaviour of these novel oligopeptides ('lexitropsins') will be described elsewhere.

Experimental

Melting points were determined on an Electrothermal Digital apparatus and are uncorrected. IR spectra were obtained as KBr discs on a Perkin-Elmer 1420 spectrometr. The ^{1}H NMR spectra were recorded on a Varian CFT-20 (80 MHz) spectrometer in [$^2H_6$]-DMSO (unless otherwise stated), with tetramethylsilane as internal reference; J values are recorded in Hz. Low-resolution electron-impact mass spectra were obtained at 70 eV using a modified MS-902 spectrometer. Low-resolution FAB spectra and the accurate mass measurements were provided by the SERC Mass Spectrometry Service, Swansea. Elemental analyses were carried out by MEDAC Ltd, Brunel University. Silica gel 60 (May and Baker, 40–60 μm) was used for column chromatography, while for TLC silica gel 60A (Whatman, 250 μm thick layer) was employed. All solvents were redistilled prior to use.

1-Methylpyrrole and 1-methylpyrrole-2-carboxylic acid were obtained from Aldrich Chemicals Co. The identity of known compounds was established by comparison of their spectroscopic properties with those in the literature.

Nitration of 1-*Methylpyrrole*-2-*carboxylic Acid* **7**.—A mixture of acetic anhydride (7.6 cm^3) and nitric acid (70%; 1.6 cm^3) was added slowly with stirring, to a cooled (−25 °C) suspension of 1-methylpyrrole-2-carboxylic acid **7** (2 g, 16 mmol) in acetic anhydride (12 cm^3). After a further 0.5 h of stirring at −25 °C, the reaction mixture was poured into ice–water, and extracted with chloroform. The extract was dried (Na_2SO_4) and evaporated under reduced pressure to give an off-white solid which crystallised from aqueous methanol to yield 1-(1-*methyl*-4-*nitropyrrol*-2-*yl*)-2-*oxabutane*-1,3-*dione* **8** (1.2 g, 35%), m.p. 170–173 °C; ν_{max}/cm^{-1} 1820 and 1735 (C=O) and 1540 and 1360 (NO_2); δ($CDCl_3$) 2.31 (3 H, s, COMe) 3.99 (3 H, s, NMe), 7.44 (1 H, d, J 1.8, 3-H) and 7.65 (1 H, d, J 1.8, 5-H); m/z (%) 212 (M^+, 6), 170 (100), 153

(35) and 140 (21) (Found: C, 45.2; H, 3.7; N, 13.15. $C_8H_8N_2O_5$ requires C, 45.3; H, 3.8; N, 13.3%).

General Procedure for the Nitroaryl Diamides **14**, **16** *and* **18**.—A methanolic solution of 3-(1-methyl-4-nitropyrrole-2-carboxamido)propiononitrile **10**[10,18] was reduced with hydrogen at atmospheric pressure and room temperature in the presence of palladium on charcoal (10% w/w) for 3 h. The catalyst was filtered off and the filtrate evaporated under reduced pressure. The resultant crude amine was dissolved in dimethylformamide and stirred with the appropriate acyl chloride (1.2 mol equiv.) for 30 min and then treated with ice–water to give a solid.

3-[1-*Methyl*-4-(4-*nitrobenzenecarboxamido*)*pyrrole*-2-*carboxamido*]*propiononitrile* **14**. This compound was collected as a solid and crystallised from aqueous ethanol to give the light yellow *title compound* (57%), m.p. 215–217 °C; ν_{max}/cm^{-1} 3300 (NH), 2260 (C≡N) and 1800 and 1700 cm^{-1} (C=O); δ 2.74 (2 H, t, *J* 6.2, CH_2CH_2CN), 3.46 (2 H, q, *J* 6.2, CH_2CH_2CN), 3.84 (3 H, s, NMe), 6.97 (1 H, d, *J* 1.8, 3-H of pyrrole ring), 7.31 (1 H, d, *J* 1.8, 5-H of pyrrole ring), 8.10 (2 H, d, *J* 8.9, 2- and 6-H), 8.32 (2 H, d, *J* 8.9, 3- and 5-H), 8.38 (1 H, br s, exchanged with D_2O, $CONHCH_2$) and 10.59 (1 H, s, exchanged with D_2O, CONH); *m*/*z* (%) 341 (M^+, 40), 311 (M^+ − NO, 11), 272 (13) and 120 (100) (Found: C, 54.6; H, 4.5; N, 19.75. $C_{16}H_{15}N_5O_4 \cdot \frac{1}{2}H_2O$ requires C, 54.85; H, 4.5; N, 20.0%).

3-[1-*Methyl*-4-(5-*nitrofuran*-2-*carboxamido*)*pyrrole*-2-*carboxamido*]*propiononitrile* **16**. This compound crystallised from ethanol as a dark yellow crystalline solid (61%), m.p. 207–210 °C; ν_{max}/cm^{-1} 3380 (NH), 2260 (C≡N) and 1800 and 1660 cm^{-1} (C=O); δ 2.74 (2 H, t, *J* 5.8 Hz, CH_2CH_2CN), 3.41 (2 H, q, *J* 5.8, CH_2CH_2CN), 3.85 (3 H, s, NMe), 6.96 (1 H, d, *J* 1.6, 3-H of pyrrole ring), 7.29 (1 H, d, *J* 1.6, 5-H of pyrrole ring), 7.53 (1 H, d, *J* 3.4, 3-H of furan ring), 7.79 (1 H, d, *J* 3.4, 4-H of furan ring), 8.04 (1 H, br s, exchanged with D_2O, CONH) and 8.39 (1 H, br s, exchanged with D_2O, $CONHCH_2$); *m*/*z* (%) 331 (M^+, 100), 299 (31), 262 (76) and 191 (66) (Found: C, 50.7; H, 3.9; N, 21.1 $C_{14}H_{13}N_5O_5$ requires C, 50.8; H, 4.0; N, 21.15%).

3-[1-*Methyl*-4-(1-*methyl*-4-*nitropyrrole*-2-*carboxamido*)-*pyrrole*-2-*carboxamido*]*propiononitrile* **18**. This crystallised from ethanol to give the yellow diamide (87%), m.p. 246–248 °C (lit.,[11] 244–245 °C).

3-{1-*Methyl*-4-[1-*methyl*-4-(5-*nitrofuran*-2-*carboxamido*)-*pyrrole*-2-*carboxamido*]*pyrrole*-2-*carboxamido*}*propiononitrile* **20**. This compound was prepared from the nitropyrrole **18** in a manner similar to the formation of the diamide **16** from **10** except that the hydrogenation was carried out in a mixture of dimethylformamide and methanol. The product was crystallised from aqueous ethanol to yield brown microcrystals (60%), m.p. 147–149 °C; ν_{max}/cm^{-1} 3360–3300 (NH), 2260 (C≡N), 1700, 1660 (C=O) and 1620, 1530 and 1350 cm^{-1} (NO_2); δ 2.70 (2 H, t, *J* 5.9, CH_2CH_2CN), 3.27 (2 H, m, CH_2CH_2CN), 3.79 (3 H, s, NMe), 3.85 (3 H, s, NMe), 6.88 (1 H, d, *J* 0.9, 3-H of pyrrole ring), 7.04 (1 H, d, *J* 0.9, 5-H of pyrrole ring), 7.15 (1 H, d, *J* 0.9, 3′-H of pyrrole ring), 7.25 (1 H, d, *J* 0.9, 5′-H of pyrrole ring), 7.42 (1 H, d, *J* 3.5, 3-H of furan ring), 7.72 (1 H, d, *J* 3.5, 4-H of furan ring), 8.39 (1 H, br s, exchanged with D_2O, $CONHCH_2$), 9.87 (1 H, s, exchanged with D_2O, CONH) and 10.70 (1 H, s, exchanged with D_2O, CONH); *m*/*z* (FAB-MS) 453 (M^+, 85%), 399 (8); 384 (30) and 262 (100) (Found: C, 52.8; H, 4.3; N, 21.8. $C_{20}H_{19}N_7O_6$ requires C, 53.0; H, 4.2; N, 21.6%).

3-[4-(4-*Aminobenzenecarboxamido*)-1-*methylpyrrole*-2-*carboxamido*]*propiononitrile* **15**.—The nitrophenyl diamide **14** (341 mg, 1 mmol) was dissolved in a mixture of methanol (15 cm^3) and DMF (10 cm^3) and reduced with hydrogen in the presence of 10% Pd/C (220 mg). After 18 h the catalyst was filtered off and the solvents removed under reduced pressure. The residue was treated with acetonitrile and carbon tetrachloride and solid impurities were filtered off. The filtrate was concentrated under reduced pressure and the residue triturated with ether to give a yellow solid which on recrystallisation from ethanol yielded the *title compound* as pale yellow plates (180 mg, 58%), m.p. 90–92 °C; ν_{max}/cm^{-1} 3560 and 3360 (NH and NH_2) and 2260 (C≡N); δ 2.72 (2 H, t, *J* 5.9, CH_2CH_2CN), 3.35 (2 H, q, *J* 5.9, CH_2CH_2CN), 3.79 (3 H, s, NMe), 5.58 (2 H, s, exchanged with D_2O, NH_2), 6.55 (2 H, d, *J* 8.3, 3- and 5-H), 6.88 (1 H, d, *J* 1.2, 3-H of pyrrole ring), 7.18 (1 H, d, *J* 1.2, 5-H of pyrrole ring), 7.61 (2 H, d, *J* 8.3, 2- and 6-H), 8.24 (1 H, t, *J* 5.5, exchanged with D_2O, $CONHCH_2$) and 9.72 (1 H, s, exchanged with D_2O, CONH); *m*/*z* 311 (M^+, 12%), 192 (3) and 120 (100) (Found: C, 55.6; H, 5.9; N, 19.8. $C_{16}H_{17}N_5O_2 \cdot 2H_2O$ requires C, 55.4; H, 6.0; N, 20.1%).

General Procedure for the 4-*Bromoacetamidopyrroles* **22** *and* **23**.—The appropriate nitropyrrole in a mixture of methanol and dimethylformamide was reduced with hydrogen at room temperature in the presence of palladium on charcoal (10%). After removal of the catalyst, the solvent was removed under reduced pressure and the residue dissolved in a small volume of dimethylformamide and treated with diisopropylethylamine (1.2 mol equiv.) in THF. The resultant solution was then cooled (−40 °C) and bromoacetyl bromide (1.2 mol equiv.) in THF was added gradually with stirring. The mixture was allowed to attain room temperature and stirred for a further 3 h. The solvent was evaporated and the residue purified by chromatography.

3-[4-(*Bromoacetamido*)-1-*methylpyrrole*-2-*carboxamido*]-*propiononitrile* **22**. This was obtained by column chromatography on silica gel [methanol–ethyl acetate, 1:9 (v/v)]. Crystallisation of the product from ethanol gave the *title compound* as a pale yellow solid (57%), m.p. 193–195 °C; ν_{max}/cm^{-1} 2260 (CN), 1655 and 1645 (CO), 900 and 820 cm^{-1}; δ 2.70 (2 H, t, *J* 6.5, CH_2CH_2CN), 3.34 (2 H, m, CH_2CH_2CN), 3.78 (2 H, s, NMe), 3.93 (2 H, s, CH_2Br), 6.73 (1 H, d, *J* 1.8, 3-H), 7.11 (1 H, d, *J* 1.8, 5-H), 8.29 (1 H, br s, exchanged with D_2O, $CONHCH_2$) and 10.20 (1 H, s, exchanged with D_2O, CONH); *m*/*z* 314 (M^+, 100%), 312 (M^+, 94), 245 (66), 243 (62) and 123 (64) (Found: 42.2; H, 4.3; N, 17.8. $C_{11}H_{13}BrN_4O_2$ requires C, 42.0; H, 4.1; N, 17.8%).

3-{1-*Methyl*-4-[4-(*bromoacetamido*)-1-*methylpyrrole*-2-*carboxamido*]*pyrrole*-2-*carboxamido*}*propiononitrile* **23**. This was obtained by column chromatography (methanol–chloroform, 1:9) as flocculent yellow needles (51%). It was homogeneous by TLC, R_f 0.52 [methanol–chloroform (1:9)]; m.p. 103–104 °C; ν_{max}/cm^{-1} 2260 (CN), 1710, 1660, 1650 (CO), 1110, 830 and 780; δ 2.70 (2 H, t, *J* 6.6, CH_2CH_2CN), 3.42 (2 H, m, CH_2CH_2CN), 3.79 (3 H, s, NMe), 3.81 (3 H, s, NMe), 3.95 (2 H, s, CH_2Br), 6.86 (2 H, d, *J* 1.2, 3- and 3′-H), 7.14 (2 H, d, *J* 1.2, 5-and 5′-H), 8.21 (1 H, br s, exchanged with D_2O, $CONHCH_2$), 9.77 (1 H, s, exchanged with D_2O, CONH) and 10.17 (1 H, s, exchanged with D_2O, CONH); *m*/*z* (FAB-MS) 436 (M^+, 100%) 434 (M^+, 97), 414 (10), 413 (20), 392 (12), 391 (35), 245 (66) and 243 (85) (Found: M^+, 434.0648. $C_{17}H_{19}BrN_6O_3$ requires *M*, 434.0702).

General Method for 2-*Nitroimidazole Derivatives* **24** *and* **26**.—A mixture of the appropriate 4-bromoacetamidopyrrole, 15-crown-5 (1.2 mol equiv.) and the dry sodium salt of 2-nitroimidazole[24] (1.2 mol equiv.) in acetonitrile (15 cm^3) was stirred at room temperature until the reaction was complete (TLC) which was from 18 to 54 h. The mixture was evaporated under reduced pressure and the residue chromatographed on silica gel with a mixture of ethyl acetate–methanol (9:1) to give a solid.

3-[1-*Methyl*-4-(2-*nitroimidazole*-1-*acetamido*)*pyrrole*-2-*carboxamido*]*propiononitrile* **24**. This was crystallised from ethyl

acetate–methanol to give the *title compound* (83%); R_f 0.32 [chloroform–methanol (9:1)]; m.p. 132–134 °C; ν_{max}/cm^{-1} 3300 (NH), 2260 (C≡N) and 1540 and 1370 (NO_2); δ 2.69 (2 H, t, *J* 6, CH_2CH_2CN), 3.40 (2 H, q, *J* 6, CH_2CH_2CN), 3.76 (3 H, s, NMe), 5.22 (2 H, s, CH_2CONH), 6.73 (1 H, d, *J* 1.7, 3-H of pyrrole ring), 7.03 (1 H, d, *J* 1.7, 5-H of pyrrole ring), 7.16 (1 H, d, *J* 0.5, 4-H of imidazole ring), 7.59 (1 H, d, *J* 0.5, 5-H of imidazole ring), 8.25 (1 H, t, *J* 6, exchanged with D_2O, CONH) and 10.22 (1 H, s, exchanged with D_2O, CONH); *m/z* (FAB-MS) 346 (MH^+, 25%), 345 (M^+, 13), 299 ($M^+ - NO_2$, 10), 155 (72) and 136 (100) (Found: MH^+, 346.1264. $C_{14}H_{16}N_7O_4$ requires *M*H, 346.1264).

3-{1-*Methyl*-4-[1-*methyl*-4-(2-*nitroimidazol*-1-*ylacetamido*)-*pyrrole*-2-*carboxamido*]*pyrrole*-2-*carboxamido*}*propiononitrile* **26**. This was obtained as a bright yellow powder (59%); R_F 0.39 [chloroform–methanol (8:2)]; m.p. 114–115 °C; ν_{max}/cm^{-1} 3350 (NH), 2260 (CN) and 1540 and 1380 cm^{-1} (NO_2); δ 2.70 (2 H, t, *J* 5.7, CH_2CH_2CN), 3.32 (2 H, m, CH_2CH_2CN), 3.79 (6 H, s, 2 × NMe), 5.23 (2 H, s, CH_2CONH), 6.86 (2 H, d, *J* 1.1, 3- and 3′-H of pyrrole rings), 7.16 (2 H, d, *J* 1.1, 5- and 5′-H of pyrrole rings), 7.32 (1 H, d, *J* 0.5, 4-H of imidazole ring), 7.61 (1 H, d, *J* 0.5, 5-H of imidazole ring), 8.25 (1 H, br s, exchanged with D_2O, $CONHCH_2$), 9.80 (1 H, s, exchanged with D_2O, CONH) and 10.27 (1 H, s, exchanged with D_2O, CONH); *m/z* (FAB-MS) 468 (MH^+, 7%), 467 (M^+, 3), 421 ($M^+ - NO_2$,3), 398 (5) and 243 (100) (Found: MH^+, 468.1744. $C_{20}H_{22}N_9O_5$ requires *M*H, 468.1744).

General Procedure for the Amidine Hydrochlorides **17**, **21**, **25** and **27**.—A stream of dry hydrogen chloride gas was passed through a cooled (−70 °C) solution of the appropriate nitrile in dry ethanol for 45 min after which the mixture was allowed to warm to room temperature; it was then stirred for a further 1.5 h. After this the solvent was evaporated under reduced pressure and the residue dissolved in dry ethanol and treated with dry ammonia at room temperature for 45 min. The reaction mixture was stirred for a further 1.5 h and the solvent evaporated under reduced pressure to give a solid.

3-[1-*Methyl*-4-(5-*nitrofuran*-2-*carboxamido*)*pyrrole*-2-*carboxamido*]*propionamidine hydrochloride* **17**. This was obtained as a bright yellow solid (73%), m.p. 248–250 °C; ν_{max}/cm^{-1} 3280 (NH), 1530 and 1350 (NO_2); δ 2.63 (2 H, m, $CONHCH_2CH_2$), 3.37 (2 H, m, $CONHCH_2CH_2$), 3.83 (3 H, s, NMe), 7.0 (1 H, d, *J* 1.6, 3-H of pyrrole ring), 7.28 (1 H, d, *J* 1.6, 5-H of pyrrole ring), 7.64 (1 H, d, *J* 3.3, 3-H of furan ring), 7.75 (1 H, d, *J* 3.3, 4-H of furan ring), 8.30 (1 H, br s, exchanged with D_2O $CONHCH_2$), 8.64 [2 H, br s, exchanged with D_2O, $-C(NH_2)^+$, 8.89 (2 H, br, exchanged with D_2O, $-C(NH_2)^+$] and 10.89 (1 H, br s, exchanged with D_2O, CONH); *m/z* (FAB-MS) 349 (M^+ − Cl, 100%), 303 (M^+ − Cl − No_2, 3), 262 (17) and 113 (8); *m/z* 349.1260 (M^+ − Cl) ($C_{14}H_{17}N_6O_5$ requires *M* − Cl, 349–1261) (Found: C, 43.7; H, 4.45; N, 21.9. $C_{14}H_{17}ClN_6O_5$ requires C, 43.7; H, 4.45; N, 21.85%).

3-{*Methyl*-4-[1-*methyl*-4-(5-*nitrofuran*-2-*carboxamido*)-*pyrrole*-2-*carboxamido*]*pyrrole*-2-*carboxamido*}*propionamidine hydrochloride* **21**. This was obtained as a brown crystalline solid (65%), m.p. 208–210 °C; ν_{max}/cm^{-1} 3500–3120 (NH and NH_2) and 1520 and 1340 cm^{-1} (NO_2); δ 2.56 (2 H, m, $CONHCH_2CH_2$), 3.58 (2 H, m, $CONHCH_2CH_2$), 3.79 (3 H, s, NMe), 3.85 (3 H, s, NMe), 6.92 (1 H, d, *J* 1.6, 3-H of pyrrole ring), 7.09 (1 H, d, *J* 0.9, 3′-H of pyrrole ring), 7.17 (1 H, d, *J* 1.6, 5-H of pyrrole ring), 7.29 (1 H, d, *J* 0.9, 5′-H of pyrrole ring), 7.52 (1 H, d, *J* 3.9, 3-H of furan ring), 7.71 (1 H, d, *J* 3.9, 4-H of furan ring), 7.10–7.80 [8 H, br, exchanged with D_2O, $-C(NH_2)_2^+$ and $2H_2O$ of crystallisation], 8.25 (1 H, br, exchanged with D_2O, $CONHCH_2$), 9.93 (1 H, br s, exchanged with D_2O, CONH) and 10.95 (1 H, br s, exchanged with D_2O, CONH); *m/z* (FAB-MS) 471 (M^+ − Cl, 20%), 455 (M^+ − 16, 3), 399 (13) and 171 (100); *m/z* 471.1741 (M^+ − Cl) ($C_{20}H_{23}N_8O_6$ requires *M* − Cl, 471.1740) (Found: C, 44.4; H, 5.0; N, 20.8. $C_{20}H_{23}ClN_8O_6{\cdot}2H_2O$ requires C, 44.3; H, 5.0; N, 20.65%).

3-[1-*Methyl*-4-(2-*nitroimidazol*-1-*ylacetamido*)*pyrrole*-2-*carboxamido*]*propionamidine hydrochloride* **25**. This was obtained as a dark brown crystalline solid (68%), m.p. 212–214 °C; ν_{max}/cm^{-1} 3400–3150 (NH and NH_2) and 1530 and 1370 cm^{-1} (NO_2); δ 2.66 (2 H, t, *J* 4.7, $CONHCH_2CH_2$), 3.47 (2 H, m, $CONHCH_2CH$), 3.78 (3 H, s, NMe), 5.25 (2 H, s, CH_2CONH), 6.85 (1 H, d, *J* 1.4, 3-H of pyrrole ring), 7.10 (1 H, d, *J* 1.4, 5-H of pyrrole ring), 7.27 (1 H, s, 4-H of imidazole ring), 7.41 [8 H, br, exchanged with D_2O, $-C(NH_2)_2^+$ and $2H_2O$ of crystallisation], 7.68 (1 H, s, 5-H of imidazole ring), 8.33 (1 H, br, exchanged with D_2O, $CONHCH_2$) and 10.74 (1 H, br, exchanged with D_2O, CONH); *m/z* (FAB-MS) 363 (M^+ − Cl, 28%), 324 (10), 289 (30), 171 (65) and 136 (100) (Found: M^+ − Cl, 363.1529. $C_{14}H_{19}N_8O_4$ requires *M* − Cl, 363.1529).

3-{1-*Methyl*-4-[1-*methyl*-4-(2-*nitroimidazol*-1-*ylacetamido*)-*pyrrole*-2-*carboxamido*]*pyrrole*-2-*carboxamido*}*propionamidine-hydrochloride* **27**. This was obtained as a brown crystalline solid (73%), m.p. 230 °C (decomp.); ν_{max}/cm^{-1} 3300 and 1540 and 1370 (NO_2); δ 2.63 (2 H, br t, *J* 5.2, $CONHCH_2CH_2$), 3.32 (2 H, m, $CONHCH_2CH_2$), 3.78 (6 H, s, 2 × NMe), 5.26 (2 H, s, CH_2CONH), 6.91 (2 H, s, 3- and 3′-H of pyrrole rings), 7.17 (2 H, s, 5- and 5′-H of pyrrole rings), 7.33 (1 H, s, 4-H of imidazole ring), 7.42 (4 H, br s, exchanged with D_2O, $-C(NH_2)_2^+$), 7.63 (1 H, s, 5-H of imidazole ring), 8.21 (1 H, br, exchanged with D_2O $CONHCH_2CH_2$), 9.88 (1 H, br s, exchanged with D_2O, CONH) and 10.57 (1 H, br s, exchanged with D_2O, CONH); *m/z* (FAB-MS) 485 (M^+ − Cl, 23%), 469 (3), 438 (2), 324 (45) and 243 (100) (Found: M^+ − Cl, 485.2009. $C_{20}H_{25}N_{10}O_5$ requires M − Cl, 485.2009).

Acknowledgements

We are grateful to the Cancer Research Campaign for support and a studentship (M. P.).

References

1 R. J. Hodgkiss, G. Jones, A. Long, J. Parrick, K. A. Smith, M. R. L. Stratford and G. D. Wilson, *Br. J. Cancer*, 1991, **63**, 119, and references therein.
2 L. H. Gray, A. D. Conger, M. Ebert, S. Hornsey and O. C. A. Scott, *Br. J. Radiol.*, 1953, **26**, 638.
3 T. C. Jenkins in *Chemistry of Antitumour Agents* (ed. D. E. V. Wilman), Blackie and Son, Glasgow, 1990, pp. 342, and references therein.
4 R. S. Bush, *Int. J. Radiat. Oncol. Biol. Phys.*, 1986, **12**, 2047.
5 P. Wardman, *Radiat. Phys. Chem.*, 1987, **30**, 423.
6 J. Overgaard, *Int. J. Radiat. Biol.*, 1989, **56**, 801.
7 S. Dische, *Radiotherapy and Oncol.*, 1985, **3**, 97.
8 G. E. Adams, *Biochem. Pharmacol.*, 1986, **35**, 71; *Radiation Research*, 1992, **132**, 129.
9 A. C. Finlay, F. A. Hochstein, B. A. Sobin and F. X. Murphy, *J. Am. Chem. Soc.*, 1951, **73**, 341.
10 F. Arcamone, S. Penco, P. Orezzi, V. Nicolella and A. Pirelli, *Nature*, 1974, **203**, 1064.
11 J. W. Lown and K. Krowicki, *J. Org. Chem.*, 1985, **50**, 3774.
12 M. L. Kopka, C. Yoon, D. Goodsell, P. Pjura and R. E. Dickerson, *J. Mol. Biol.*, 1985, **183**, 553; *Proc. Natl. Acad. Sci. U.S.A.*, 1985, **82**, 1376; K. E. Rao, D. Dasgupta and V. Sasisekharan, *Biochemistry*, 1988, **27**, 3018.
13 M. Lee and J. W. Lown, *J. Org. Chem.*, 1987, **52**, 5717.
14 K. Krowicki, J. Balzarini, E. de Clercq, R. A. Newman and J. W. Lown, *J. Med. Chem.*, 1988, **31**, 341.
15 F. Arcamone, F. Animati, B. Barbieri, E. Configliacchi, R. D'Alessio, C. Geroni, F. C. Giuliani, E. Lazzari, M. Menozzi, N. Mongelli, S. Penco and M. H. Verini, *J. Med. Chem.*, 1989, **32**, 774.
16 M. Bailer, B. Yagen and R. Mechoulam, *Tetrahedron*, 1978, **34**, 2389.
17 L. Grehn and U. Ragnarsson, *J. Org. Chem.*, 1981, **46**, 3492.
18 E. Nishiwaki, S. Tanaka, H. Lee and M. Shibuya, *Heterocycles*, 1988, **27**, 1945.

19 E. Nishiwaki, H. Lee, T. Matsumoto, K. Toyooka, H. Sakuari and M. Shibuya, *Tetrahedron Lett.*, 1990, **31**, 1299.
20 J. D. Chapman, A. P. Reuvers, J. Borsa, A. Petkau and D. R. McCalla, *Cancer Research*, 1972, **32**, 2630.
21 D. W. Siemann and R. M. Sutherland, *Radiotherapy and Oncol.*, 1992, **24**, 239.
22 K. Kissinger, K. Krowicki, J. C. Dobrowiak and J. W. Lown, *Biochemistry*, 1987, **16**, 665.
23 G. E. Adams and I. J. Stratford, *Biochem. Pharmacol.*, 1986, **35**, 71.
24 A. Long, J. Parrick and R. J. Hodgkiss, *Synthesis*, 1991. 709.

Paper 3/04082H
Received 13*th July* 1993
Accepted 5*th August* 1993