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Synthesis and antimicrobial activity of some netropsin analogues

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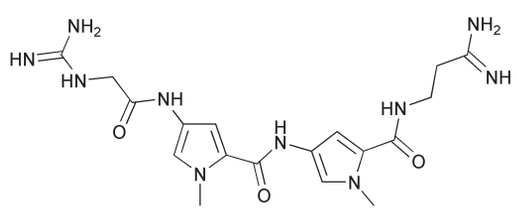
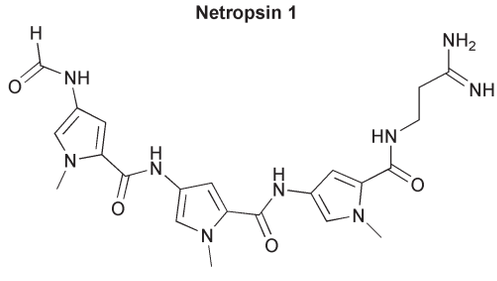
Nine novel lexitropsins were synthesized by linking two netropsin-like moieties through three different dicarboxylic acids; 9,10-dihydro-2,7-phenanthrenedicarboxylic acid; [(3-{[(carboxymethyl)amino]carbonyl}benzoyl)amino]acetic acid and indole-2,5-dicarboxylic acid. The netropsin residues were modified by the use of *N*-isopentylpyrrole, 5-methylthiophene or 5-isopropylthiazole heterocyclic building blocks in place of the usual *N*-methylpyrrole. The compounds were tested against five gram-positive bacteria: *Staphylococcus aureus*, *Streptomyces faecalis*, methicillin resistant *Staphylococcus aureus*, *Enterobacter cloacae*, *Mycobacterium fortuitum*, three gram-negative bacteria: *Klebsiella aerogenes*, *Proteus vulgaris*, *Escherichia coli* and three fungi: *Aspergillus niger*, *Candida albicans* and *Aspergillus nidulans*. Some of the compounds showed significant inhibitory effects on the growth of the microorganisms.

Introduction

Netropsin **1** and distamycin **2** are pyrrole polyamides, known as lexitropsins from their ability to read the base pair sequence of DNA.[1] They are naturally occurring anticancer antibiotics that bind reversibly to the minor groove of double helical B-DNA at regions with at least four consecutive AT base pairs.[2] The high cytotoxicity of the parent compounds prevents them from being used as drugs, but this class of pyrrole polyamides has attracted considerable interest,[1,2] mainly focused on the possibility of modifying and improving their pharmaceutical applications by increasing the sequence selectivity of their binding to DNA.[2]



Netropsin 1



Distamycin 2

Several groups are pursuing ways of improving the antiviral, antifungal and antibacterial activity of netropsin analogues.[3,4] Improved sequence selectivity could be used to target microbial DNA rather than human DNA and in so doing should minimise any associated toxicity to human cells: the increased selectivity is likely to come primarily from an increase in the length of the reading frame and recent results indicate that A/T sequences are useful targets.[3]

The spacing of the hydrogen bonding groups in the minor groove of DNA does not exactly match the separation of the amide NH groups in pyrrole polyamides. To achieve binding to a longer sequence of DNA than that achieved by the natural products, a larger number of heterocyclic units is normally used, and this results in a hydrogen bonding mismatch, with a theoretical loss of affinity and possible loss of selectivity. It has been common, therefore, to use suitable linkers to solve this phasing problem.[5]

Dyatkina *et al.*[3a] recently reported the synthesis of some netropsin analogues which contain two *N*-alkylpyrroles, one on either side of the linker, terminated by amidinium groups. Some of these compounds showed good antibacterial activity. Such compounds may have difficulty reaching a site of infection since the amidinium groups are very basic; pH-partition theory indicates that they may not penetrate lipid membranes very easily.[6–10] In the present study, we have replaced the amidinium group by the less basic dimethylamino and have also replaced the usual *N*-methylpyrrole rings with a range of more lipophilic sub-units, notably *N*-isopropyl or -isopentyl pyrroles and 5-methylthiophene. We have also replaced *N*-methylpyrrole with 5-isopropyl substituted thiazole, which is less basic than the commonly used imidazole and may further increase the compound's ability both to bind to microbial DNA and to reach the site of action.

Results and discussion

Molecular modelling

Molecular modelling studies predicted a number of linkers compatible with the AT sequence, which could be used to extend binding selectivity and affinity for the minor groove. Simulations were performed on a variety of dodecamer–lexitropsin complexes that incorporated different linkers to extend the reading frame of the ligand and the best of these were embodied in structures **3a**–**c**, and **4**–**9**.

We assumed a 1 : 1 binding mode based on our capillary electrophoretic analysis[11] of similar ligands binding to the DNA dodecamer 5′-AAATTATATTAT-3′ (data not shown). Analysis of the minimised average structures of the complexes taken from production runs using this dodecamer and employing periodic boundary conditions revealed that the inner face of the linkers presented to the groove floor suggested compatibility with AT sequences in the same way that pyrrole rings are selective. The indole and dihydrophenanthrene linkers have C–H's in contact with the groove floor that are incompatible with the exocyclic amino group of a guanine, whilst the carbonyl groups of the isophthalamide moiety have the equivalent effect.

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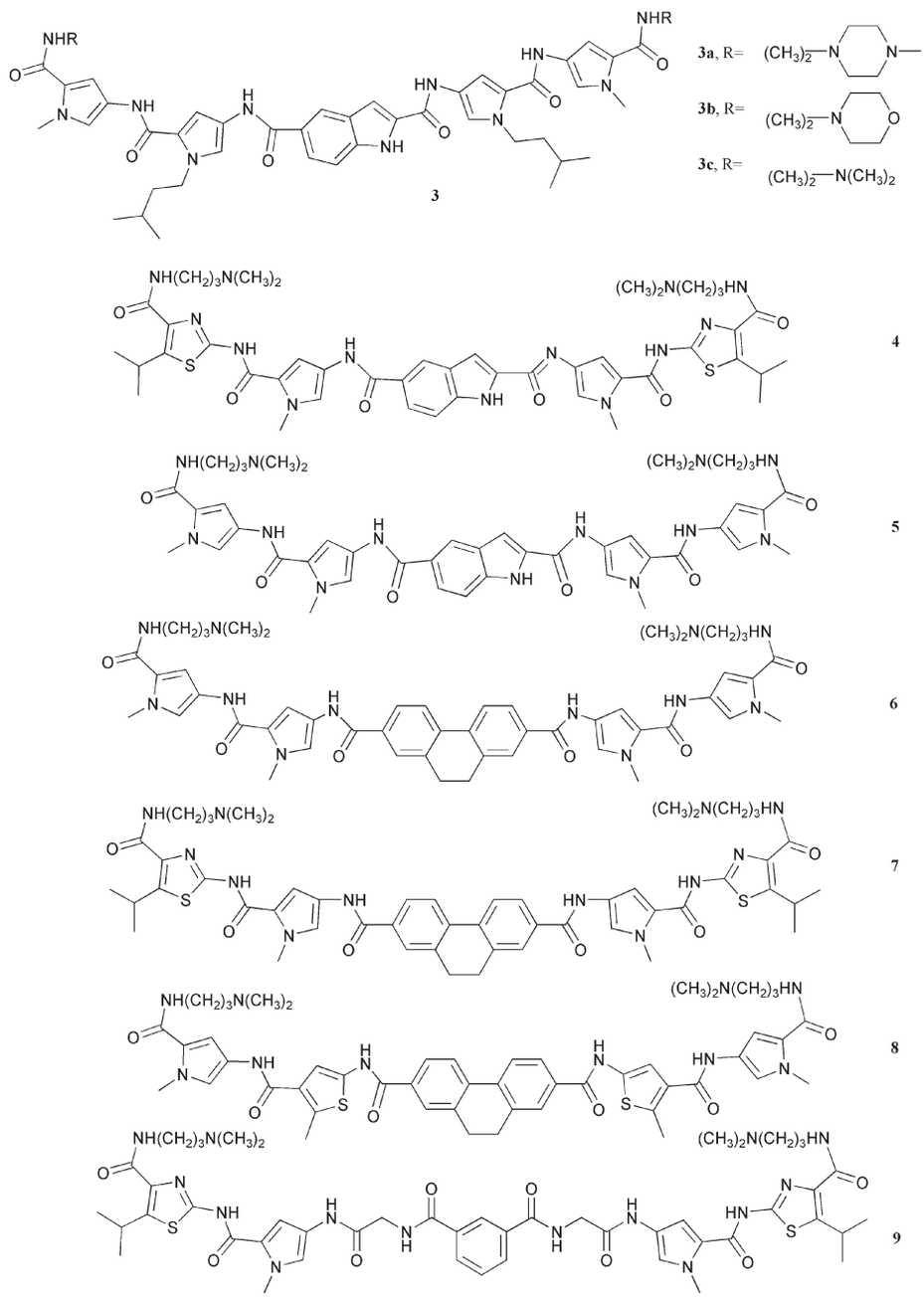


The saturated bridge in the dihydrophenanthrene produces a kink in the ring system that maintains isohelicity with the minor groove of extended polyaromatic systems (Fig. 1). The indole linker has similar dimensions to the benzimidazole systems found in minor groove binders such as Hoechst 33258, and thus complements the groove floor topography (Fig. 2). Rotatable bonds in the isophthalamide group ensure that the extended lexitropsin can adapt to the demands of the minor groove curvature (Fig. 3). The potential for enhanced lipophilic contact between the ligand and the groove walls is evident for compound **9** when considering the position of the isopropyl group and the associated sulfur in the thiazole moiety (Fig. 3). This interaction has recently been confirmed by NMR studies with a related lexitropsin.[7]

Synthesis

1-Isopentyl-4-nitro-1*H*-pyrrole-2-carboxylic acid[12] **10** was converted into the corresponding acid chloride using thionyl chloride, in quantitative yield. The nitro-compounds[5] **11a–c** were reduced using Pd/C/H_2 in methanol and the amine so formed was used immediately. The coupling reactions between the acid chloride formed from **10** and the amines formed from **11a–c** gave the required 'dimers' in high yield; **12a**, 84%; **12b**, 79%; and **12c**, 81%. The final steps in the synthesis of the netropsin analogues were the reduction of the nitro-dimers (again using Pd/C/H_2 in methanol) followed by coupling to 1*H*-indole-2,5-dicarboxylic acid[9,13] **13**, using HBTU as the coupling agent of choice in the presence of *N*-methylmorpholine (NMM) in dimethylformamide (DMF) as solvent. HPLC was used to purify all the final compounds, followed by freeze drying of the appropriate fractions to give the bis-TFA salt of the desired compounds **3a**, **3b**, and **3c** (Scheme 1).

Methyl 2-amino-5-isopropyl-1,3-thiazole-4-carboxylate[14,15] **14** was treated with the acid chloride **15** to give the methyl ester dimer **16** in moderate yield (43%) after purification by column chromatography; the low yield may be attributable to the low nucleophilicity of the amine group of **14**. Base hydrolysis of the methyl ester gave the corresponding carboxylic acid **17** in 96% yield. The tail group 3-aminopropyldimethylamine was attached to the carboxylic acid **17** using HBTU in the presence of NMM to produce **18** in 95% yield.

The nitro-dimer **18** was reduced using Pd/C/H_2 followed by coupling of the amine formed to the appropriate linker, again using HBTU as the preferred coupling agent. The netropsin analogues **4**, **7** and **9** were obtained as bis-TFA salts after

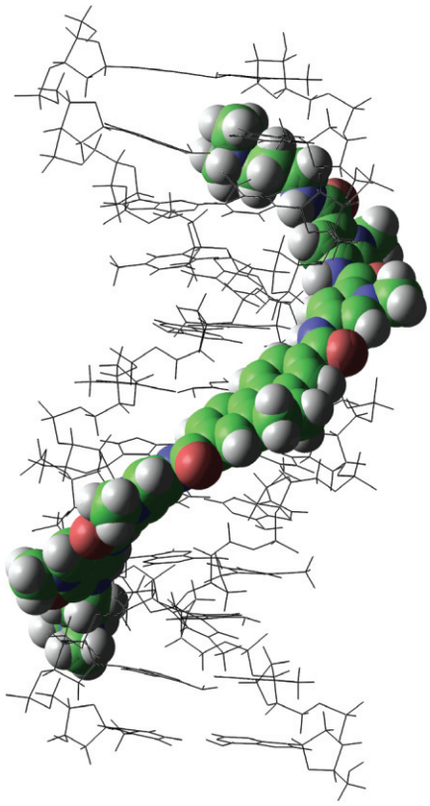


Fig. 1 The minimised average complex of **6** spanning 5′-AAAT-TATATTAT-3′ taken from the last 50 ps of the production run. Explicit water molecules have been removed for clarity. The ligand is displayed as a CPK model to emphasise its isohelicity with DNA.

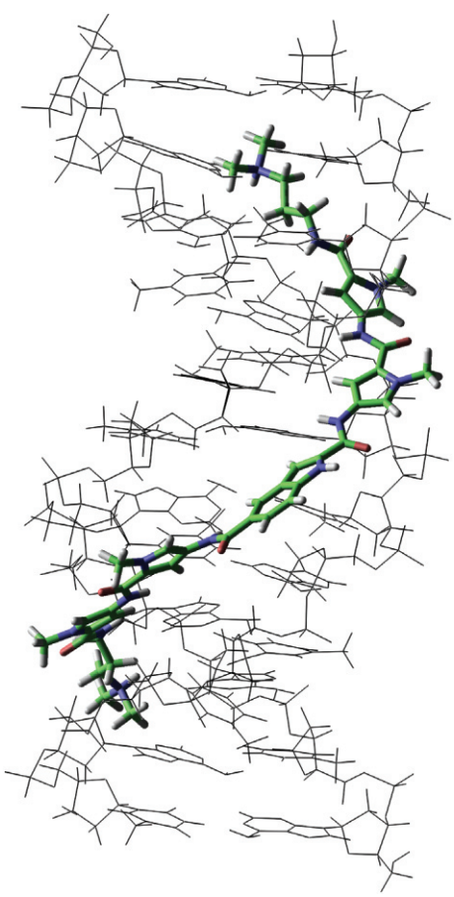


Fig. 2 The minimised average complex of **5** spanning 5′-AAAT-TATATTAT-3′ taken from the last 50 ps of the production run. Explicit water molecules have been removed for clarity.

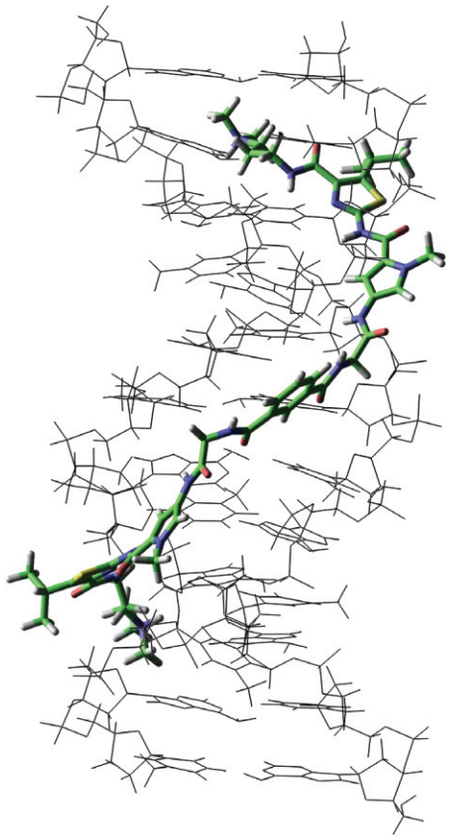
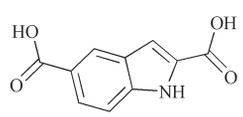


Fig. 3 The minimised average complex of **9** spanning 5′-AAAT-TATATTAT-3′ taken from the last 50 ps of the production run. Explicit water molecules have been removed for clarity.



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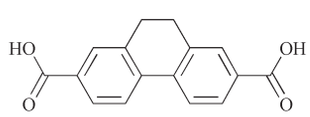

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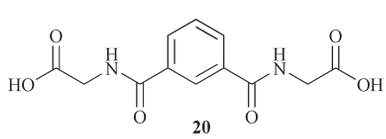

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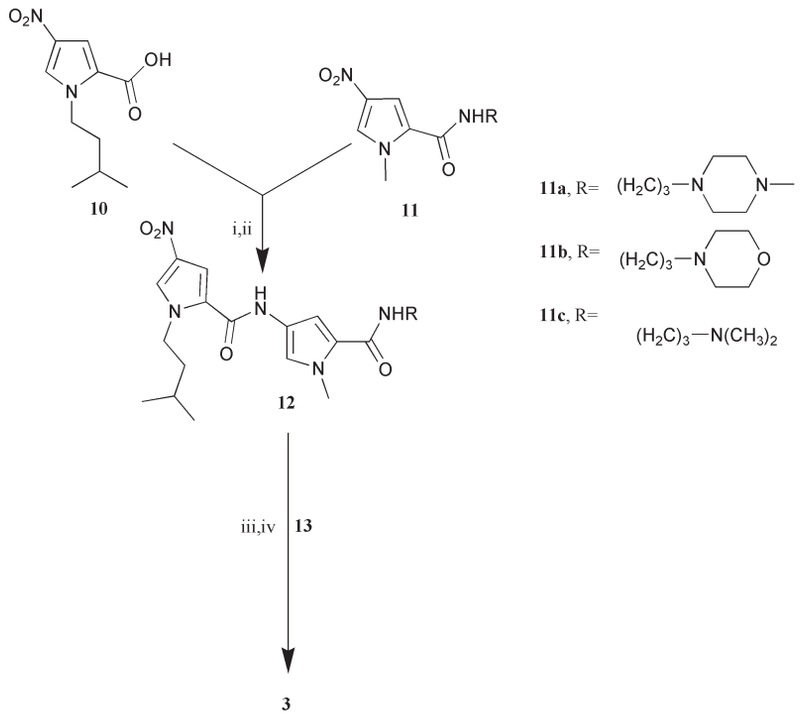
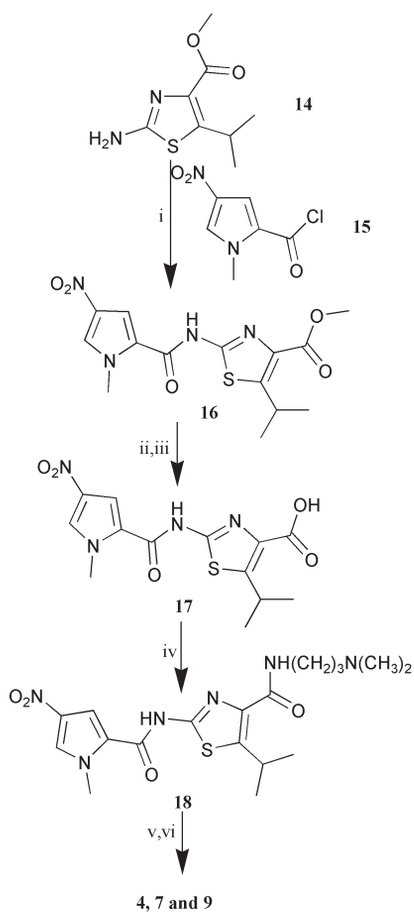
purification by HPLC, which was followed by freeze drying of the fractions containing the desired material (Scheme 2).

Reduction of *N*-[3-(dimethylamino)propyl]-1-methyl-4-{[(1-methyl-4-nitro-1*H*–pyrrol-2-yl)carbonyl]amino}-1*H*–pyrrole-2-carboxamide **21a**,[8] using Pd/C/H_2, produced the amine **21b** (Scheme 3) which was coupled to indole-2,5-dicarboxylic acid **13** and 9,10-dihydro-2,7-phenanthrenedicarboxylic acid[16] **19**, again using HBTU as coupling agent, to produce **5** and **6** respectively (Scheme 3).

2-Methyl-3-thiophenecarboxylic acid[17] **22**, which was prepared in 77% yield according to a standard literature[17] procedure, was nitrated using a mixture of concentrated nitric and sulfuric acids at −5 °C (Scheme 4) to give **23** (67%). Reduction of the nitro-pyrrole **11c** to give **24** was achieved using Pd/C/H_2. Treatment of the carboxylic acid **23** with thionyl chloride gave the corresponding acid chloride, which was

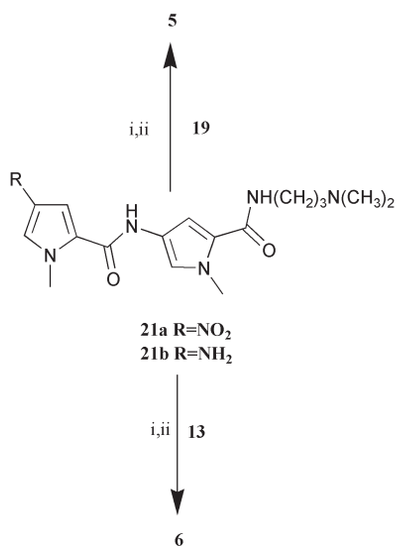


Scheme 1 i) Thionyl chloride; ii) **11**, Pd/C/H_2; iii) **12**, Pd/C/H_2; iv) HBTU/NMM/DMF.



Scheme 2 i) **14** & **15**, NMM, DCM; ii) $NaOH_{aq}$; iii) thionyl chloride; iv) **17** & $NH_2(CH_2)_3N(CH_3)_2$, HBTU/NMM/DMF; v) Pd/C/H_2; vi) HBTU/NMM/DMF, dicarboxylic acid (**13**, **19** and **20**).

coupled to the amine **24** to produce the dimer **25a** (69%). 9,10-Dihydro-2,7-phenanthrenedicarboxylic acid **19** was converted to the corresponding di-acid chloride using thionyl chloride. The nitro-dimer **25a** was reduced as above and coupled to the di-acid chloride in DCM at room temperature to produce **8** in 13% yield, after HPLC purification, as the bis-TFA salt.



Scheme 3 i) Pd/C/H_2; ii) HBTU/NMM/DMF.

Biological activity

Antimicrobial activity was determined using a rapid assay in 96 well plates with resazurin as growth indicator to make minimum inhibitory concentrations easier to determine.[18]

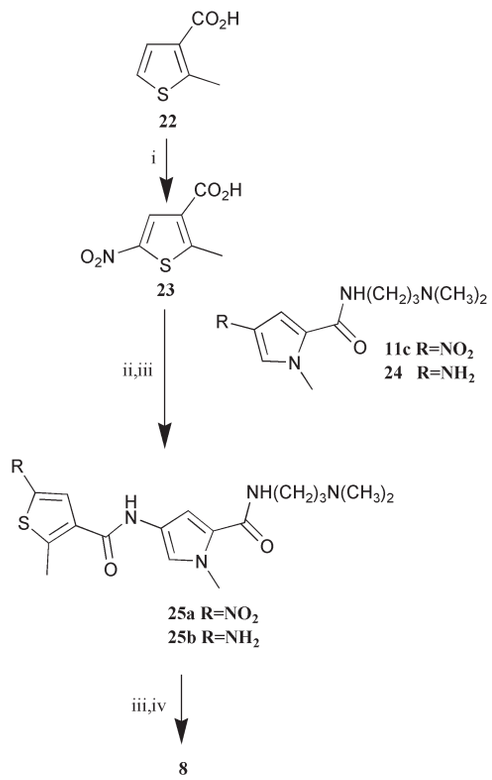
Table 1 summarizes the *in vitro* inhibitory concentrations of the synthesised compounds against five gram-positive bacteria: *Staphylococcus aureus*, *Streptomyces faecalis*, methicillin resistant *Staphylococcus aureus*, *Enterobacter cloacae*, *Mycobacterium fortuitum*, three gram-negative bacteria: *Klebsiella aerogenes*, *Proteus vulgaris*, *Escherichia coli* and three fungi: *Aspergillus niger*, *Candida albicans* and *Aspergillus nidulans.*

It is clear that the presence of the thiazole moiety and the *N*-isopentyl group have a noticeable effect in inhibiting growth of bacteria and fungi under the assay conditions. Compound **4** showed its highest potency against MRSA and *A. niger*, while **7** showed its highest potency against MRSA, *M. fortuitum* and *A. nidulans.* Compound **9** showed its highest potency against *A. niger*, and *K. aerogenes.* Compounds bearing the dimethylamino and *N*-methylpiperazine tail groups showed good activity against the range of microorganisms tested, since **3c**

Table 1 *In vitro* antibacterial and antifungal activity of nine netropsin analogues

	3a	**3b**	**3c**	**4**	**5**	**6**	**7**	**8**	**9**	
MIC/μM	MW 1312.4	MW 1285.6	MW 1202.3	MW 1181.4	MW 1153.1	MW 1090.0	MW 1244.4	MW 1186.3	MW 1256.5	Control
S. aureus NCTC 6571	38.1	19.4	**5.2**	21.1	86.7	11.4	>80.3	84.2	**9.9**	(0.49)A
S. faecalis NCTC775	38.1	>77.7	**5.2**	21.1	43.3	>91.7	**9.9**	>84.2	**9.9**	(0.49)A
MRSA PHLS M1	>76.2	>77.7	>83.2	21.1	>86.7	>91.7	**9.9**	84.2	>149.0	(16.1)A
E. cloacae NCTC 775	76.2	>77.7	83.2	>84.6	>86.7	>91.7	>80.3	>84.2	**9.9**	(4.0)A
M. fortuitum NCTC 10394	**9.5**	>77.7	**5.2**	>84.6	86.7	>91.7	40.1	84.2	79.5	(10.8)S
K. aerogenes WRL CN 345	**19.1**	38.9	**5.2**	>84.6	86.7	>91.7	>80.3	>84.2	**39.8**	(16.1)A
P. vulgaris NCTC4175	>76.2	>77.7	>83.2	>84.6	43.3	>91.7	>80.3	84.2	>79.5	(8.1)A
E. coli NCTC9001	>76.2	>77.7	>83.2	>84.6	>86.7	>91.7	>80.3	>84.2	79.5	(4.0)A
A. niger IMI 1745	**38.1**	>77.7	20.8	**10.6**	86.7	>91.7	>80.3	>84.2	**9.9**	(17.7)I
C. albicans NCPF 3179	76.2	>77.7	>83.2	>84.6	**21.6**	>91.7	>80.3	>84.2	79.5	(35.4)I
A. nidulans CABI 016037	**9.5**	>77.7	**5.2**	>84.6	43.3	>91.7	20.8	>84.2	>79.5	(35.4)I

A: Amoxicillin; S: Streptomycin; I: Itraconazole.



Scheme 4 i) HNO_3/H_2SO_4; ii) **23**, $SOCl_2$; iii) **11c**, $Pd/C/H_2$; iv) **19**, $SOCl_2$.

was active against *S. aureus* and *S. faecalis*, while **3a** and **3c** were active against *A. niger*, *K. aerogenes*, *M. fortuitum*, and *A. nidulans.* The morpholinyl analogue **3b** is less active than its dimethylamino **3c** and piperazine **3a** congeners. This could be due to the oxygen in the terminal morpholine group acting in a repulsive manner to the DNA, or the greater hydrophilicity of the morpholine residue compared to dimethylamino or piperazino. Also, the pK_a of the amine in the morpholino group is significantly lower than the other two congeners, which may affect DNA binding. However, not all compounds containing the preferred structural features were found to be active; compounds **3b** and **6** were inactive. It would not be expected that such a short series of compounds would define a clear structure–activity relationship, however the thiazole and branched *N*-alkyl groups are significant, a conclusion that has been drawn from further work in our group on other distamycin analogues.[4b] It is also notable that the antifungal activity observed in this assay was higher in some cases than that of the control drug, itraconazole, a fact that encourages the further development of these compounds.

Conclusion

Nine novel netropsin analogues were synthesized. Reduction of the nitro group of a pyrrole monomer, followed by coupling to the carboxylic acid of another pyrrole monomer, using HBTU (or acid chloride) gave rise to a dimeric peptide, which was reduced and coupled to a dicarboxylic acid to afford the extended netropsin analogue (Schemes 1–4). The thiazole ester monomer **14**, on the other hand, was coupled to pyrrole carboxylic acid chloride **15** to afford the dimer **16**. This was hydrolysed, coupled to 3-dimethylaminopropylamine to give **18**, which was reduced and coupled to the appropriate linker.

Nine compounds were tested against gram-positive and gram-negative bacteria and fungi. The results are presented in Table 1. Some of the netropsin analogues showed reasonable activity compared to the controls, which were amoxicillin, itraconazole or streptomycin as appropriate. The presence of the *N*-isopentylpyrrole and isopropylthiazole moieties had a significant effect on the inhibition of the growth of the microorganisms.

Experimental

Abbreviations: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; exch, exchangeable; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; HBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HPLC, high performance liquid chromatography; HREIMS, high resolution electron impact mass spectroscopy; HRFABMS, high resolution fast atom bombardment mass spectroscopy; LRESMS, low resolution electrospray mass spectroscopy; NMM, *N*-methylmorpholine; Pd/C, palladium on carbon; TEA, triethylamine; TFA, trifluoroacetic acid. HREIMS and HRFABMS were obtained on a JEOL® JMS-AX505HA mass spectrometer. LRESMS were obtained on a Fisons® VG Platform Benchtop LC-MS. HRLCMS was carried out at the EPSRC National Mass Spectrometry Service Centre, Chemistry Department, University of Wales Swansea. NMR spectra were obtained on a Bruker® AMX 400 spectrometer. HPLC purification of the final compounds was carried out using a Vydac protein and peptide C18 column on a gradient eluting system.[6] IR spectra were run as KBr discs and liquids as films, using a Nicolet® Impact 400D. Column chromatography was performed with silica gel Prolabo® (200–400 mesh).

Antimicrobial assay

Sample dilutions were prepared by dissolving the test sample (2 mg) in sterile water (10 cm^3) to provide a working concentration of 200 μg cm^{-3}. The test wells on each plate (96 well microtitre plate) were initially inoculated with 100 μL of culture medium (for bacteria: Mueller–Hinton Broth and for fungi: Sabourand Broth), chosen for optimum growth of the test organism. The incubation temperature for bacteria was 37 °C and for fungi was 25 °C. A solution of each compound in 100 μL was added to well one of each row of test wells and a series of doubling dilutions were made. When visual evidence of growth was observed after the incubation, an indicator (Resazurin) was added to the wells. Those where growth had occurred produced a distinct colour change from blue to red, allowing the endpoint (mic) to be recorded. All the tests included sterility and growth controls.[18]

Molecular modelling

All experiments were performed using a Silicon Graphics Octane R12000 workstation using the Insight II 2000 graphics interface and Discover 98.0 simulation software (Accelrys Inc., San Diego, CA). The cff-91 all-atom force-field was used for all energy calculations, minimisations and dynamics simulations. Solvent effects were represented by the use of the explicit water SPC model in all calculations. All molecules were constructed using predefined residue libraries in the Biopolymer application of InsightII. Ligands were constructed in-line with the isohelical nature of natural lexitropsins, parameterised and charges assigned using the AM1/ESP method within MOPAC.[19] Structures were docked manually into the minor groove of the target DNA dodecamer until a minimum non-bonded energy was achieved. The ligand–DNA complex was minimised using the Conjugate Gradients algorithm with the terminal base pairs tethered employing a force constant of 10 kcal mol^{-1} until an energy convergence criterion of 1 kcal mol^{-1} $Å^{-1}$ was reached.

Explicit sodium counterions were then included and placed at a distance of 6 Å from each phosphate–oxygen bisector prior to solvation in a box of water of dimensions 35 Å × 55 Å × 35 Å and the solvated complex minimised employing previous conditions. Molecular dynamics simulations using periodic boundary conditions were then performed by integrating Newton's equations of motion using the Verlet-leapfrog algorithm for 100 ps at a simulated temperature of 300 K with a timestep of 2 fs and data capture every 1 ps. High frequency bonds were restrained using the RATTLE algorithm[20] and terminal base pairs were tethered with a force constant of 10 kcal mol^{-1} to prevent fraying throughout all simulations. Production runs were preceded by ramped heating up to the target 300 K in 1 ps stages, followed by an equilibration period of 50 ps. Initial assignment of velocities was according to the Boltzmann–Maxwell distribution and scaling by a single factor every 100 fs to maintain the temperature within 10 K limits. From the production run, 100 structures were sampled at 1 ps intervals and the last 50 structures averaged and minimised as above.

1-Isopentyl-4-nitro-1*H*-pyrrole-2-carboxylic acid[12] (10)

This compound was prepared according to a standard literature procedure[12] in 95% yield, m.p. 154–157 °C [lit.[12] m.p. 154–156 °C].

Methyl 2-amino-5-isopropyl-1,3-thiazole-4-carboxylate[14,15] (14)

This compound was prepared according to a standard literature procedure[14] in 41% yield, m.p. 151–152 °C [lit.[14] m.p. 150–151 °C].

Methyl 5-isopropyl-2-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1,3-thiazole-4-carboxylate (16)

1-Methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid (520 mg, 3.06 mmol) was dissolved in thionyl chloride (10 cm^3). The reaction mixture was heated under reflux overnight. Excess thionyl chloride was removed under reduced pressure to give the acid chloride **15**, which was used without further purification. The acid chloride was dissolved in DCM (20 cm^3, dry), to which NMM (1.214 g, 12.00 mmol) was added. The reaction mixture was cooled to 0 °C and kept under N_2 then methyl 2-amino-5-isopropyl-1,3-thiazole-4-carboxylate solution [(0.612 g, 3.06 mmol) in DCM (20 cm^3, dry)] was added dropwise with stirring over a period of 10 min. The temperature was left to rise to room temperature overnight. DCM (10 cm^3) was added to the reaction mixture then HCl (50 cm^3, dil.) was added and the reaction mixture was then extracted. The organic layer was extracted with brine (50 cm^3), dried ($MgSO_4$) and the solvent removed under reduced pressure to give the crude product. Column chromatography over flash silica gel using ethyl acetate–petroleum ether (1 : 1) as eluent gave the required product as a white crystalline solid (460 mg, 43%), m.p. 118–120 °C (softening). ^{1}H NMR ($CDCl_3$): δ 9.85(1H, br, CONH, exch.); 7.67 (1H, d, J = 1.4 Hz); 7.23 (1H, d, J = 1.4 Hz); 4.11–4.18 (1H, m); 4.08 (3H,s); 3.89 (3H, s); 1.32 (6H, d, J = 6.4 Hz). IR [KBr]: 3557, 3119, 2975, 1712, 1646, 1574, 1509, 1319, 1208 $cm.^{-1}$ HREIMS: Found: 352.08579, calculated for $C_{14}H_{16}N_4O_5S$ 352.08414.

5-Isopropyl-2-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1,3-thiazole-4-carboxylic acid (17)

Methyl 5-isopropyl-2-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)-carbonyl]amino}-1,3-thiazole-4-carboxylate **16** (480 mg, 1.36 mmol) was suspended in ethanolic KOH (0.5 M, 25 cm^3). The reaction mixture was heated under reflux for 4 h, and then cooled to 0 °C. HCl (conc.) was added dropwise with stirring until pH 2. The pale yellow solid obtained was filtered off, washed with deionised water and dried under reduced pressure at 45 °C overnight to give the required material (440 mg, 96%), m.p. 315–319 °C [decomposition]. ^{1}H NMR (DMSO-d_6): δ 12.50–13.20 (1H, br, CO_2H, exch.); 8.28 (1H, d, J = 1.4 Hz); 7.99 (1H, d, J = 1.4 Hz); 4.00–4.07 (1H, m); 3.98 (3H, s); 1.23 (6H, d, J = 6.4 Hz). IR [KBr]: 3189, 3132, 2972, 1668, 1566, 1537, 1512, 1497, 1319, 1231 $cm.^{-1}$ HRFABMS: Found: 337.06396, calculated for $C_{13}H_{13}O_5N_4S$ 337.06067.

N-[3-(Dimethylamino)propyl]-5-isopropyl-2-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl] amino}-1,3-thiazole-4-carboxamide (18)

5-Isopropyl-2-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]-amino}-1,3-thiazole-4-carboxylic acid **17** (250 mg, 0.739 mmol)

was dissolved in DMF (1.0 cm^3, dry) to which NMM (300 μL, dry) was added followed by HBTU (723 mg, 1.91 mmol), at room temperature with stirring. The stirring was continued at room temperature for 10 min. 3-Aminopropyldimethylamine (200 μL, 1.96 mmol) was added dropwise, and stirring was continued at room temperature overnight. The reaction mixture was diluted with ethyl acetate (50 cm^3) and extracted with brine (50 cm^3). The water layer was then extracted with ethyl acetate (2 × 50 cm^3). The combined organic extract was dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The crude product was applied to silica gel column chromatography using methanol–ethyl acetate–TEA 1 : 4 : 0.01 (R_F = 0.2). The product was obtained as a yellow solid after trituration with methanol (296 mg, 95%), m.p. > 230 °C. ^{1}H NMR ($CDCl_3$): δ 7.68 (1H, d, J = 1.4 Hz); 7.62 (1H, s, exch.); 7.55 (1H, d, J = 1.4 Hz); 4.09 (3H, s); 4.37–4.44 (1H, m); 3.43 (2H, q, J = 6.9 Hz); 2.31 (6H, s); 2.45 (2H, t, J = 6.9 Hz); 1.79 (2H, quintet, J = 6.9 Hz); 1.26 (6H, d, J = 6.4 Hz); IR [KBr]: 3131, 2959, 1668, 1551, 1500, 1418, 1310, 1286 cm^{-1} HREIMS: Found: 422.17695, calculated for $C_{18}H_{26}O_4N_6S$ 422.17363.

N^1,*N*^{3}-Bis(2-{[5-({[4-({[3-(dimethylamino)propyl]amino}-carbonyl)-5-isopropyl-1,3-thiazol-2-yl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]amino}-2-oxoethyl)isophthalamide (9)

N-[3-(Dimethylamino)propyl]-5-isopropyl-2-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1,3-thiazole-4-carboxamide **18** (100 mg, 0.236 mmol) was dissolved in methanol (25 cm^3). The solution was cooled to 0 °C under N_2 then Pd/C 10% (86 mg) was added. The reaction mixture was hydrogenated at room temperature and atmospheric pressure for 5 h. The catalyst was removed over kieselguhr and the solvent was then removed under reduced pressure at 50 °C. The amine so formed was dissolved in DMF (2 cm^3, dry), to which [(3-{[(carboxymethyl)amino]carbonyl}benzoyl)amino]acetic acid[7] (33 mg, 0.12 mmol) was added followed by HBTU (270 mg, 0.710 mmol) and NMM (300 μL, dry). An additional amount of DMF (2 cm^3, dry) was added with stirring at room temperature and the stirring was continued for 72 h. The reaction mixture was diluted with ethyl acetate containing 5% methanol (50 cm^3) and extracted with brine. The organic layers were collected, dried over ($MgSO_4$) and the solvents were then removed under reduced pressure. The product was purified by HPLC and the fractions containing the desired material were freeze-dried. The product was obtained as a white solid (25 mg, 16%) with no distinct melting point. ^{1}H NMR [DMSO-d_6]: δ 12.03 (2H, s); 10.08 (2H, s); 9.24 (2H, br, 2 × TFA); 8.93 (2H, t, J = 5.8 Hz); 8.05 (2H, d, J = 1.5 Hz); 7.94 (2H, t, J = 6.0 Hz); 7.61 (1H, t, J = 7.8 Hz); 7.39 (2H, d, J = 1.6 Hz); 7.25 (2H, d, J = 1.6 Hz); 4.21 (2H, quintet, J = 6.9 Hz); 4.06 (2H, d, J = 5.7 Hz); 3.87 (6H, s); 3.30 (4H, m); 3.07 (4H, m); 2.79 (6H, d, J = 4.5 Hz); 1.86 (4H, quintet, J = 7.9 Hz); 1.28 (12H, d, J = 6.9 Hz). IR [KBr]: 1655, 1548, 1467, 1403, 1288, 1202, 1132 cm^{-1} HRLCMS: Found: [M + 2]/2 = 515.2313, calculated for $C_{48}H_{66}N_{14}O_8S_2$ [M + 2]/2 = 515.2309.

The following compounds were prepared similarly:

N^2,*N*^{7}-Bis[5-({[4-({[3-(dimethylamino)propyl]amino}carbonyl)-5-isopropyl-1,3-thiazol-2-yl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]-9,10-dihydro-2,7-phenanthrenedicarboxamide (7)

The product was obtained as a pale yellow solid with no distinct melting point (46 mg, 19%). ^{1}H NMR [DMSO-d_6]: δ 12.11 (2H, s); 10.46 (2H, s); 9.26 (2H, br, 2 × TFA); 8.07–7.91 (6H, m); 7.53 (2H, s); 7.46 (2H, s); 4.20 (2H, quintet, J = 6.9 Hz); 3.92 (6H, s); 3.33 (4H, m); 3.07 (4H, m); 2.97 (2H, s); 2.96 (2H, s); 2.80 (12H, d, J = 4.1 Hz); 1.87 (4H, quintet, J = 7.7 Hz); 1.27 (12H, d, J = 6.9 Hz). IR [KBr]: 1660, 1548, 1468, 1284, 1199, 1132, 832, 800, 721 cm^{-1} HRESMS: Found: [M + 2]/2 = 509.2331, calculated for $C_{52}H_{66}N_{12}O_6S_2$ [M + 2]/2 = 509.2329.

N^2,*N*^{5}-Bis[1-isopentyl-5-({[1-methyl-5-({[3-(4-methyl-1-piperazinyl)propyl]amino}carbonyl)-1*H*-pyrrol-3-yl]amino}-carbonyl)-1H–pyrrol-3-yl]-1*H*-indole-2,5-dicarboxamide (3a)

The product was obtained as a white solid (17 mg, 13%) with no distinct melting point. ^{1}H NMR [DMSO-d_6]: δ 11.95 (1H, s); 10.47 (1H, s); 10.27 (1H, s); 10.01 (1H, s); 9.97 (1H, s); 9.60 (2H, br, 2 × TFA); 8.39 (1H, s); 8.13 (2H, br); 7.91 (1H, d, J = 8.7 Hz); 7.61 (1H, d, J = 8.7 Hz); 7.49 (1H, s); 7.43 (2H, s); 7.21 (2H, s); 7.14 (2H, s); 6.98 (2H, s); 4.43 (4H, m); 3.88 (6H, s); 3.29 (4H, m); 3.20–2.73 (20H, m); 1.80 (4H, m); 1.66–1.55 (6H, m); 0.96 (12H, d, J = 6.5 Hz). IR [KBr]: 1676, 1647, 1584, 1533, 1443, 1404, 1200, 1136 cm^{-1} HRESMS: Found M + H = 1084.6578, calculated for $C_{58}H_{82}N_{15}O_6$ M + H = 1084.6567.

N^2,*N*^{5}-Bis[1-isopentyl-5-({[1-methyl-5-({[3-(4-morpholinyl)-propyl]amino}carbonyl)-1*H*-pyrrol-3-yl]amino}carbonyl)-1*H*-pyrrol-3-yl]-1*H*-indole-2,5-dicarboxamide (3b)

The product was obtained as a pale yellow solid (15 mg, 12%), with no distinct melting point. ^{1}H NMR [DMSO-d_6]: δ 11.95 (1H, s); 10.47 (1H, s); 10.28 (1H, s); 10.02 (1H, s); 9.99 (1H, s); 9.66 (2H, br, 2 × TFA); 8.39 (1H, s); 8.24 (2H, t, unresolved); 7.88 (1H, d, J = 8.7 Hz); 7.49 (1H, s); 7.43 (2H, s); 7.23 (2H, s); 7.14 (2H, s); 7.02 (2H, s); 4.43 (4H, m); 4.06 (4H, m); 3.89 (6H, s); 3.71 (4H, t, J = 4.6 Hz); 3.49 (4H, m); 3.31 (4H, m); 3.19 (8H, m); 1.94 (4H, m); 1.66–1.57 (6H, m); 0.98 (12H, d, J = 6.5 Hz). IR [KBr]: 1677, 1646, 1533, 1443, 1403, 1198, 1134 cm^{-1} HRESMS: Found M + H = 1058.5927, calculated for $C_{56}H_{76}N_{13}O_8$ M + H = 1058.5934.

N^2,*N*^{5}-Bis[5-({[5-({[3-(dimethylamino)propyl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]amino}carbonyl)-1-isopentyl-1*H*-pyrrol-3-yl]-1*H*-indole-2,5-dicarboxamide (3c)

The product was obtained as a pale yellow solid (36 mg, 30%) with no distinct melting point. ^{1}H NMR [DMSO-d_6]: δ 11.95 (1H, s); 10.47 (1H, s); 10.28 (1H, s); 10.02 (1H, s); 9.99 (1H, s); 9.39 (2H, br, 2 × TFA); 8.39 (1H, s); 8.22 (2H, t, unresolved, 2 × CONH); 7.91 (1H, d, J = 8.7 Hz); 7.61 (1H, d, J = 8.7 Hz); 7.49 (1H, s); 7.43 (2H, s); 7.23 (2H, s); 7.13 (2H, s); 7.01 (2H, s); 4.43 (4H, m); 3.89 (6H, s); 3.31 (4H, m); 3.14 (4H, m); 2.86 (12H, d, J = 3.8 Hz); 1.91 (4H, m); 1.66–1.55 (6H, m); 0.98 (12H, d, J = 6.5 Hz). IR [KBr]: 1677, 1647, 1583, 1533, 1443, 1404, 1199, 1135 cm^{-1} HRESMS: Found M + H = 974.5719, calculated for $C_{52}H_{72}N_{13}O_6$ M + H = 974.5723.

N^2,*N*^{5}-Bis[5-({[4-({[3-(dimethylamino)propyl]amino}carbonyl)-5-isopropyl-1,3-thiazol-2-yl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]-1*H*-indole-2,5-dicarboxamide (4)

The product was obtained as a pale yellow solid (32 mg, 23%) with no distinct melting point. ^{1}H NMR [DMSO-d_6]: δ 12.15 (1H, s), 12.09 (1H, s); 11.95 (1H, s); 10.52 (1H, s); 10.32 (1H, s); 9.37 (2H, br, 2 × TFA, exch); 8.35 (1H, s); 7.99 (2H, m); 7.85 (1H, m); 7.54 (2H, m); 7.46 (2H, m); 3.93 (6H, s); 3.23 (4H, m); 3.07 (4H, m); 2.81 (6H, s); 2.80 (6H, s); 2.08 (1H, m); 1.85–1.92 (4H, m); 1.3 (12H, s, isopropylMe$_2$); IR [KBr]: 3415, 2960, 2362, 1654, 1549, 1467, 1398, 1287, 1200, 1134 cm^{-1} HRESMS: found M + H = 954.4200, calculated for $C_{46}H_{60}N_{13}O_6S_2$ M + H = 954.4230.

N^2,*N*^{5}-Bis[5-({[5-({[3-(dimethylamino)propyl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]-1*H*-indole-2,5-dicarboxamide (5)

The product was obtained as a pale yellow solid with no distinct melting point (58 mg, 42%). ^{1}H NMR [DMSO-d_6]: δ 10.41 (1H, s); 11.91 (1H, s); 10.21 (1H, s); 9.96 (1H, s); 9.93 (1H, s); 9.26 (2H, br, 2 × TFA, exch); 8.33 (1H, s); 8.15 (2H, t, unresolved); 7.82 (1H, s); 7.53 (1H, m); 7.43 (1H, s); 7.33 (2H, m); 7.18 (2H, s); 7.12 (2H, s); 7.10 (2H, s); 6.97 (2H, s); 3.90 (3H, s); 3.88 (3H, s); 3.83 (6H, s); 3.24 (4H, m); 3.07 (4H, m);

2.80 (6H, s); 2.79 (6H, s); 1.82–1.90 (4H, m); IR [KBr]: 3420, 2363, 1678, 1640, 1578, 1540, 1436, 1403 cm.$^{-1}$ HRESMS: found M + H = 862.4467, calculated for $C_{44}H_{56}N_{13}O_6$ M + H = 862.4471.

1*H*-Indole-2,5-dicarboxylic acid[9,13] (13)

This compound was prepared according to a standard literature procedure[9,13] in 80% yield, m.p. 284–287 °C (softening) [lit.[9,13] m.p. 290–295 °C].

9,10-Dihydro-2,7-phenanthrenedicarboxylic acid[8,13,16] (19)

This compound was prepared according to a standard literature procedure[8,13,16] in 24% overall yield, m.p. > 300 °C [lit.[8,13,16] m.p. ~ 350 °C].

***N*^{2},*N*^{7}-Bis[5-({[5-({[3-(dimethylamino)propyl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]-9,10-dihydro-2,7-phenanthrenedicarboxamide (6)**

This compound was prepared according to a standard literature procedure[9] in 17% yield (as a pale yellow solid) with no distinct melting point.

1-Isopentyl-*N*-[1-methyl-5-({[3-(4-methyl-1-piperazinyl)amino}-carbonyl)-1*H*-pyrrol-3-yl]-4-nitro-1*H*-pyrrole-2-carboxamide (12a)

1-Methyl-*N*-[3-(4-methyl-1-piperazinyl)propyl]-4-nitro-1*H*—pyrrole-2-carboxamide[8] **11a** (209 mg, 0.676 mmol) was dissolved in methanol (25 cm^3) to which Pd/C 10% (182 mg) was added at 0 °C under N_2 with stirring. The reaction mixture was hydrogenated for 3 h at room temperature and atmospheric pressure. The catalyst was removed over kieselguhr and the solvent was then removed under reduced pressure to give the amine, which was used without further purification. 1-Isopentyl-4-nitro-1*H*-pyrrole-2-carboxylic acid (153 mg, 0.676 mmol) was dissolved in thionyl chloride (4 cm^3) and heated under reflux for 3 h. The excess thionyl chloride was removed under pressure at 50 °C and the acid chloride was dissolved in DCM (5 cm^3). The amine was dissolved in DCM (10 cm^3) to which NMM (200 μL) was added followed by the acid chloride at room temperature with stirring. The stirring was continued at room temperature overnight. KOH (5 cm^3, 10%) was added and the mixture was then extracted. The organic layer was collected, dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to give the crude product, which was purified by flash column chromatography using silica gel and 1:2:0.1 methanol–ethyl acetate–TEA. The product was obtained as glassy yellow material (R_F = 0.45), (278 mg, 84%) with no distinct melting point. ^{1}H NMR [DMSO-d_6]: δ 10.21 (1H, s); 8.23 (1H, d, J = 1.8 Hz); 8.05 (1H, t, J = 5.4 Hz); 7.54 (1H, d, J = 1.8 Hz); 7.18 (1H, d, J = 1.8 Hz); 6.82 (1H, d, J = 1.8 Hz); 4.43 (2H, t, J = 7.4 Hz); 3.81 (3H, s); 3.21 (2H, q, J = 6.6 Hz); 2.32–2.28 (10H, m); 2.15 (3H, s); 1.66 (4H, quintet, J = 6.7 Hz); 1.55–1.47 (1H, m); 0.90 (6H, d, J = 6.5 Hz). IR [KBr]: 2951, 2805, 1642, 1575, 1532, 1506, 1437, 1312 cm.$^{-1}$ HRFABMS: Found 488.29707, calculated for $C_{24}H_{38}N_7O_4$ 488.29853.

The following compounds were prepared similarly:

1-Isopentyl-*N*-[1-methyl-5-({[3-(4-morpholinyl)propyl]amino}-carbonyl)-1*H*-pyrrol-3-yl]-4-nitro-1*H*-pyrrole-2-carboxamide (12b)

The product was obtained as a yellow powder (re-precipitated from ethyl acetate–n-hexane) (R_F = 0.45), (670 mg, 79%), m.p. 155–158 °C.

^{1}H NMR [$CDCl_3$]: 7.64 (1H, d, J = 1.7 Hz); 7.60 (1H, s); 7.22 (1H, unresolved triplet); 7.18 (1H, d, J = 1.7 Hz); 7.07 (1H, d, J = 1.7 Hz); 6.66 (1H, d, J = 1.6 Hz); 4.43 (2H, t, J = 7.5 Hz); 3.93 (3H, s); 3.77 (2H, t, J = 4.6 Hz); 3.49 (2H, q, J = 5.6 Hz); 2.52 (6H, m); 1.80–1.58 (5H, m); 0.97 (6H, d, J = 6.5 Hz). IR [KBr]: 1647, 1589, 1513, 1399, 1309, 1252, 1114 cm.$^{-1}$ HRFABMS: Found 475.26789, calculated for $C_{23}H_{35}N_6O_5$ 475.26689.

***N*-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]-1-isopentyl-4-nitro-1*H*-pyrrole-2-carboxamide (12c)**

The product was obtained as a yellow powder (R_F = 0.20) (347 mg, 81%), m.p. 148–151 °C. ^{1}H NMR [DMSO-d_6]: δ 10.22 (1H, s); 8.23 (1H, d, J = 1.7 Hz); 8.13 (1H, t, J = 5.5 Hz); 7.54 (1H, d, J = 1.7 Hz); 7.19 (1H, d, J = 1.6 Hz); 6.81 (1H, d, J = 1.6 Hz); 4.45 (2H, t, J = 4.6 Hz); 3.81 (3H, s); 3.21 (2H, q, J = 4.6 Hz); 2.34 (2H, t, J = 4.6 Hz); 2.20 (6H, s); 1.67–1.57 (4H, m); 1.55–1.45 (1H, m); 0.88 (6H, d, J = 6.5 Hz). IR [KBr]: 1665, 1642, 1599, 1528, 1499, 1425, 1312 cm.$^{-1}$ HRFABMS: found 433.25630, calculated for $C_{21}H_{33}N_6O_4$ 433.25633.

***N*-[3-(Dimethylamino)propyl]-1-methyl-4-{[(2-methyl-5-nitro-3-thienyl)carbonyl]amino}-1*H*-pyrrole-2-carboxamide (25a)**

The product was obtained as a yellow solid (R_F = 0.4) (440 mg, 69%), m.p. 180–183 °C. ^{1}H NMR [DMSO-d_6]: 10.27 (1H, s); 8.52 (1H, s); 8.11 (1H, t, J = 5.6 Hz); 7.23 (1H, d, J = 1.8 Hz); 6.84 (1H, d, J = 1.8 Hz); 3.81 (3H, s); 3.20 (2H, q, J = 6.7 Hz); 2.76 (3H, s); 2.39 (2H, t, J = 4.5 Hz); 2.25 (6H, s); 1.65 (2H, quintet, J = 7.1 Hz). IR [KBr]: 1655, 1621, 1573, 1530, 1437, 1326, 1267 cm.$^{-1}$ HRFABMS: Found 394.15481, calculated for $C_{17}H_{24}N_5O_4S$ 394.15490.

2-Methyl-3-thiophenecarboxylic acid[16] (22)

Ethyl 2-methyl-3-thiophenecarboxylate[16] (3.420 g, 20.09 mmol) was dissolved in ethanol (5 cm^3) to which was added sodium hydroxide solution (3.214 mg, 80.362 mmol) in water (10 cm^3). The reaction mixture was heated under reflux for 2 h. The volume was reduced to half under reduced pressure at 40 °C and the residue was cooled with ice water. Dilute HCl was added dropwise with stirring until pH 2. The white solid material was filtered off, washed with distilled water and then dried under reduced pressure at 50 °C to give the required material as a pale yellow solid (2.185 g, 77%), m.p. 115–117 °C [lit.[16] m.p. 116–117 °C].

2-Methyl-5-nitro-3-thiophenecarboxylic acid (23)

A mixture of concentrated nitric acid (10 cm^3, sp.gr. 1.42) and concentrated sulfuric acid (6 cm^3) was mechanically stirred in a round-bottomed flask and cooled to (−10 °C) by a dry-ice methanol bath. The temperature was kept below (−5 °C) while 2-methyl-3-thiophenecarboxylic acid **22** (996 mg, 7.01 mmol) was added in small portions. The reaction mixture was stirred at the same temperature for 15 min and was then poured over ice water. The solid material that precipitated was filtered off, washed with distilled water and dried under reduced pressure at 50 °C to give a light brown solid (883 mg; 67%), m.p. 177–180 °C. ^{1}H NMR [DMSO-d_6]: δ 13.35 (1H, br); 8.11 (1H, s); 2.76 (3H, s). IR [KBr]: 1706, 1543, 1514, 1457, 1335, 1257 cm.$^{-1}$ HREIMS: Found 186.99431, calculated for $C_6H_5NO_4S$ 186.99393.

***N*^{2},*N*^{7}-Bis[4-({[5-({[3-(dimethylamino)propyl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]amino}carbonyl)-5-methyl-2-thienyl]-9,10-dihydro-2,7-phenanthrenedicarboxamide (8)**

N-[3-(Dimethylamino)propyl]-1-methyl-4-{[(2-methyl-5-nitro-3-thienyl) carbonyl]amino}-1*H*–pyrrole-2-carboxamide (100 mg, 0.254 mmol) was dissolved in methanol at 0 °C with stirring to which Pd/C 10% (58 mg) was added under N_2. The reaction mixture was hydrogenated for 2 h at room temperature and atmospheric pressure. The catalyst was removed over kieselguhr and the solvent was removed under reduced pressure. The amine so formed was dissolved in DCM (5 cm^3) to which NMM (0.1 cm^3) was added. The carboxylic acid (34 mg,

0.127 mmol) was suspended in thionyl chloride (3 cm^3) and heated under reflux for 2 h. Excess thionyl chloride was removed under reduced pressure and the acid chloride was dissolved in DCM (5 cm^3). This was then slowly added to the amine solution at room temperature with stirring, which was continued at room temperature overnight. The volatile material was removed under reduced pressure and the crude product was purified by HPLC. Fractions containing the required material were collected and freeze-dried to give the product as light brown solid (40 mg, 13%) with no distinct melting point. ^{1}H NMR [DMSO-d_6]: δ 11.58 (1H, d, J = 4.1 Hz); 9.97 (1H, s); 9.30 (1H, br); 8.18–7.88 (8H, m); 7.21 (2H, s); 7.11 (2H, d, J = 2.5 Hz); 5.93 (1H, d, J = 2.5 Hz); 3.83 (6H, s); 3.27 (4H, q, J = 6.2 Hz); 3.07 (4H, m); 2.95 (4H, t, J = 14.0 Hz); 2.79 (12H, d, J = 3.8 Hz); 2.56 (6H, s); 1.86 (4H, quintet, J = 6.6 Hz). IR [KBr]: 1680, 1640, 1576, 1535, 1437, 1304, 1197, 1133 cm^{-1}. HRESMS: Found M + H = 959.4060, calculated for $C_{50}H_{59}N_{10}O_6S_2$ M + H = 959.4055.

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