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Aust. J. Chem. **2004**, *57*, 1073–1077

The Synthesis and Testing of α-(Hydroxymethyl)pyrroles as DNA Binding Agents

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The α-(hydroxymethyl)pyrroles **16a** and **16b** were prepared and shown to be cytotoxic against the P388 cancer cell line. Ethyl 5-hydroxymethyl-1*H*-pyrrole-2-carboxylate **18** was inactive, demonstrating that an α-(hydroxymethyl)pyrrole group alone is not sufficient for activity. Compound **16b** has been shown to bind to DNA with reasonable affinity.

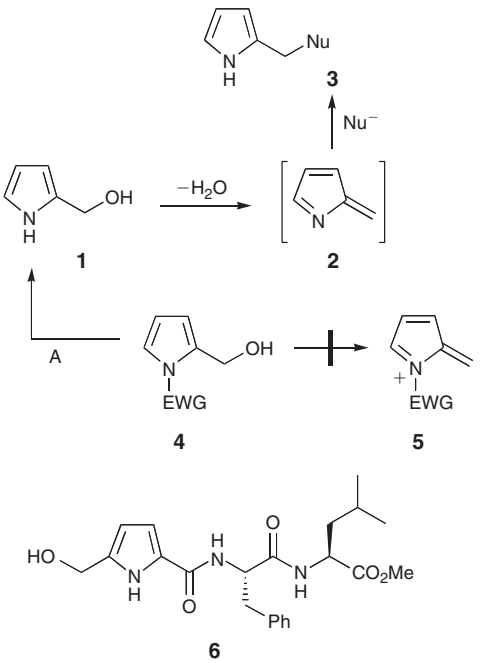
Manuscript received: 30 July 2004.
Final version: 21 September 2004.

Introduction

α-(Hydroxymethyl)pyrroles of type **1** are an important class of π-excessive heterocycle that are particularly susceptible to nucleophilic substitution due to the intermediacy of the non-aromatic azafulvene **2** (Scheme 1).[1–6] This inherent reactivity has been utilized by nature in the biosynthesis of (hydroxymethyl)bilane, a key intermediate to vitamin B12 and the like,[2] and also by medicinal and organic chemists. For example, we recently reported some α-(hydroxymethyl)pyrroles, substituted on nitrogen with an electron-withdrawing group (EWG), as inhibitors of serine proteases, see general structure **4**.[3]

We postulate that that the introduction of an EWG on nitrogen, as in **4**, would suppress the formation of the corresponding azafulvenium species **5**, and hence render the system more stable to nucleophilic displacement.[4,5] The reactive pathway (A in Scheme 1) could then be switched on by removing the EWG in a controlled manner. Our idea was to choose an EWG that resembled the P1 residue[7] of a serine protease substrate, so that the enzyme would accept it as a substrate and catalyze its removal (see step A in Scheme 1).[3] The thus liberated azafulvene could then inactivate the enzyme by covalent attachment to an active site residue, represented by Nu^- in Scheme 1. We also recently reported a related series of α-(hydroxymethyl)pyrrole-based protease inhibitors that contain a C2 peptide-based acyl group, for example **6**.[4] These compounds mimic an extended peptide conformation that is found in natural substrates of these proteases.[4,7]

We now report the results of initial work on targeting DNA by appending a bicyclic aromatic substituent, which is known to bind to DNA,[8] onto an α-(hydroxymethyl)pyrrole. Compounds of this type have the potential to alkylate DNA and hence act as anticancer agents.[9] Examples of hydroxymethylpyrrole-based DNA alkylating agents are known and these include **7**,[10] **8**,[11] and **9**[11] (Scheme 2);



Scheme 1.

however, none of these have the hydroxymethyl substitutent at the optimum α-position for azafulvene formation.[1] An α-(hydroxymethyl)pyrrole moiety is, however, found in a series of metabolites, isolated from the Atlantic sponge *Mycale micracanthoxea*, that displays moderate activity against five cancer cell lines.[12] These compounds possess an acyl side chain, of variable length and unsaturation; for representative examples see **10a**, **10b**, Scheme 2. While these derivatives displayed only moderate activity against the P388 murine leukaemia cell line, they do suggest that an α-(hydroxymethyl)pyrrole moiety might provide a suitable scaffold for the generation of more potent compounds with

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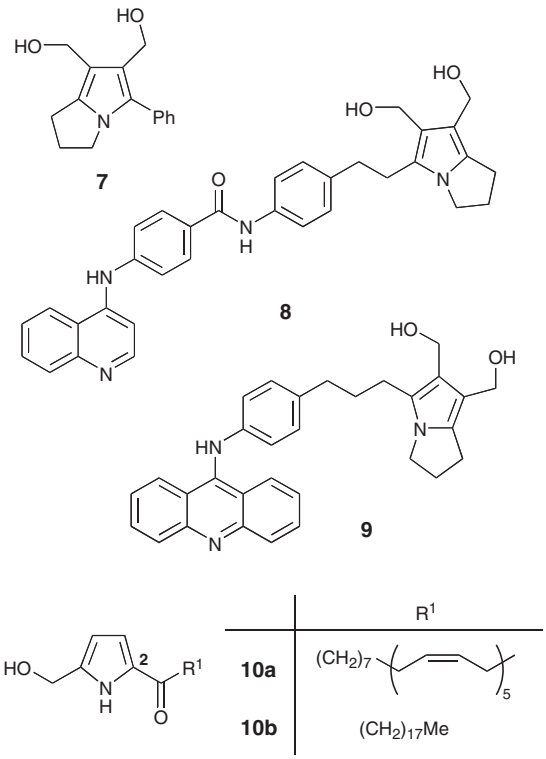
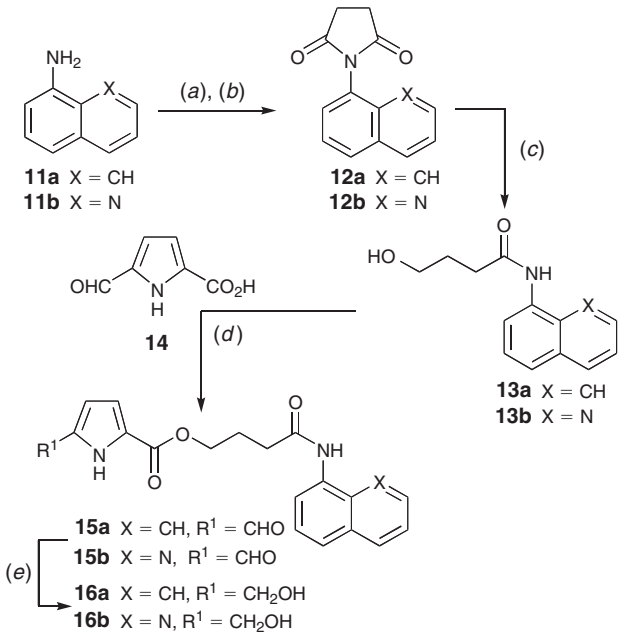
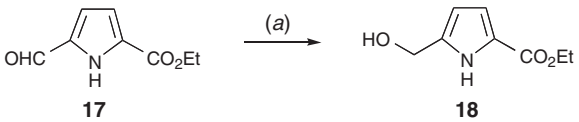


Scheme 2. Hydroxymethylpyrrole-based antitumour agents.



Scheme 3. Reagents and conditions: (*a*) Succinic anhydride, PhMe, reflux, 15 min; (*b*) CH_3CO_2Na, Ac_2O, 70–80°C, 4.5 h (**12a**, 86% over two steps), (**12b**, 79% over two steps); (*c*) $NaBH_4$, 6 : 1 Pr^iOH/H_2O, room temp., 17 h (**13a**, 71%), **13b**; (*d*) BOP-Cl, Et_3N, CH_2Cl_2, room temp., 64 h, **15a**, **15b**. (*e*) $LiBH_4$, THF, −78°C, 1 h, then room temp., 1 h (**16a**, 18% over two steps from **13a**), (**16b**, 11% over three steps from **12b**).



Scheme 4. Reagents and conditions: (*a*) $LiBH_4$, THF, −78°C, 1 h, then room temp., 1 h (76%).

the judicious choice of functionality at C2. We now report the synthesis and in vitro testing, against P388 cancer cells, of compounds in which the aliphatic chain of **10** is replaced by a bicyclic aryl group (naphthalene or quinoline) which is attached to the α-(hydroxymethyl)pyrrole via an ester linker. Aryl groups of this type are known to facilitate non-covalent binding to DNA.[8]

Results and Discussion

The synthesis of the substituted α-(hydroxymethyl)pyrrole **16a** is outlined in Scheme 3. The amine **11a** was treated with succinic anhydride in refluxing toluene, followed by the addition of sodium acetate in acetic anhydride[13] to give the succinimide **12a** in 86% yield over two steps. The succinimide **12a** was then reduced with sodium borohydride in 6 : 1 propan-2-ol/water[14] to give **13a** in 71% yield. The alcohol **13a** was coupled with the pyrrole acid **14**,[15] in the presence of BOP-Cl,[16] to give aldehyde **15a**, which was finally reduced with lithium borohydride to give **16a** in 18% yield over two steps. A heterocyclic ring system known to interact with DNA[8] was also appended to the α-(hydroxymethyl)pyrrole moiety in an analogous fashion, using quinolin-8-ylamine **11b** as shown in Scheme 3. In particular, **11b** was treated with succinic anhydride, followed by sodium acetate/acetic anhydride, to give the succinimide **12b** in 79% yield over two steps. Reduction of **12b** with sodium borohydride gave the alcohol **13b**, which was subsequently coupled to **14** to give **15b**. This was then reduced with lithium borohydride to give the desired product **16b**. The simple α-(hydroxymethyl)pyrrole ester **18**, required as a control in the assays, was prepared by reduction of the formylpyrrole ester **17**[15] with lithium borohydride as outlined in Scheme 4.

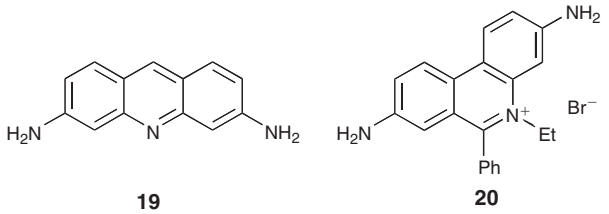
Table 1. P388 cancer cell line cytotoxicity assay results

Compound	ID_{50} [μM]
16a	468
16b	314
18	>739
19	9.4

P388 Cancer Cell Line Cytotoxicity Assay

Compounds **16a**, **16b**, **18**, and **19** were assayed against the P388 cancer cell line,[17] and the resulting ID_{50} values are given in Table 1. The hydroxymethylpyrrole **18** was assayed to determine if an α-(hydroxymethyl)pyrrole moiety alone is sufficient for activity. The tricyclic heterocycle, 3,6-diaminoacridine (Scheme 5, compound **19**), was assayed as a representative intercalator to compare its cytotoxicity to that of **16a** and **16b**.

From the results in Table 1 it is apparent that both **16a** and **16b** are cytotoxic, with ID_{50} values of 468 and 314 μM,



Scheme 5. Assay compounds.

respectively. However, **18** displayed minimal activity, indicating that a hydroxymethylpyrrole moiety alone is not sufficient for cytotoxicity and that an aromatic substituent enhances activity. As might be expected, all compounds proved to be less active than the known and potent intercalator **19**.

Deoxyribonucleic Acid Binding Assay

We next chose to test the ability of the most active compound **16b** to bind to DNA since this represents an important component of the proposed mechanism of action of this case of compound. The method reported by Baguley et al.[18] was used to determine the DNA binding activity of **16b** relative to ethidium bromide (Scheme 5, compound **20**), by measuring the competition for DNA binding sites. The assay used relies on measurement of the fluorescence emitted by **20** upon binding to DNA. The addition of a second DNA binding agent (**16b** in this case) results in a reduction in fluorescence, due to displacement of **20** from the genetic material. Using this method, compound **16b** was determined to have a 3000-fold lower affinity for poly[dA–dT] (K 4.6×10^3 M^{-1}) and poly[dG–dC] (K 3.0×10^3 M^{-1}) than **20** (K 1.10^7 M^{-1} for both oligonucleotides). While the binding constant for **16b** was considerably lower than for **20**, it must be noted that **20** has an extremely high affinity for DNA. A separate assay,[11] to determine if **16b** binds to the genetic material by intercalation, could not be performed owing to the insufficient solubility of **16b** in the assay conditions. However, an intercalative binding mode for **16b** to DNA is considered unlikely, as generally a tricyclic ring system is considered the 'minimum' for intercalation, although exceptions do exist.[8,19]

The inherent reactivity of hydroxymethylpyrroles has been put to good use by nature in the biosynthesis of vitamin B12[2] and also medicinal chemists to generate inhibitors of serine proteases.[3–6] This moiety is also found in several natural product-based DNA alkylating agents (see earlier for a discussion), but none of these have the optimum combination of an intercalating substituent and the hydroxymethylpyrrole group at the α-position[1] (see Scheme 2). In this paper we have presented initial studies towards this goal with the development of a general method for the preparation of α-hydroxymethylpyrroles to which is appended an aromatic group that is known to interact with DNA,[8] see **16a** and **16b**. Scope exists to develop the potency of these compounds with optimization of intercalator and the linker chain. Compounds **16a** and **16b** displayed micromolar cytotoxicity towards cancer P388 cell line, but as expected both were less active than the representative and potent intercalator **19**, where this ring system is known to have a very high affinity for DNA. Importantly, an α-(hydroxymethyl)pyrrole moiety alone is not sufficient for activity, where **18** was effectively inactive. Pyrrole **16b** was shown, by a DNA binding assay, to have moderate affinity for oligonucleotides, but at a lower level than the classical intercalator, ethidium bromide—this result demonstrates that compounds of type **16** are able to bind to DNA.

Experimental

All reactions carried out under an argon atmosphere using oven-dried glassware. Analytical TLC was performed on plastic-backed Merck Kieselgel KG60F_{254} silica plates, and visualized using short-wave UV light. Flash chromatography was performed using 230–400 mesh Merck Silica Gel 60 under positive pressure. Petroleum ether refers to the fraction collected between 60–70°C. All solvents were purified and dried according to standard procedures and compounds **14** and **17** were synthesized by literature procedures.[15] All melting points were obtained on an Electrothermal apparatus and are uncorrected. Infrared spectra were obtained using a Shimadzu 8201PC series FTIR interfaced with an Intel 486 PC operating Shimadzu's HyperIR software. Spectra were obtained in KBr (diffuse reflectance method). ^{1}H NMR spectra were obtained on a Varian Inova spectrometer, operating at 500 MHz. Coupling constants (J) are quoted in Hz. ^{13}C NMR spectra were obtained on a Varian Unity 300 spectrometer, operating at 75 MHz, with a delay (D_1) of 1 s. Chemical shifts are reported in parts per million (ppm) on the δ scale. Two-dimensional NMR experiments, including COSY, HSQC, HMBC, and CIGAR, were obtained on the Varian Inova spectrometer operating at 500 MHz. The HSQC, HMBC, and CIGAR experiments were all obtained with a delay (D_1) of 1 s. Electron-impact (EI) mass spectra were detected on a Kratos MS80 RFA mass spectrometer operating at 4000 V (accelerating potential) and 70 eV (ionization energy) using a source temperature of 200–250°C. Electrospray ionization (ES) mass spectra were detected on a micromass LCT TOF mass spectrometer, with a probe voltage of 3200 V, temperature of 150°C, and a source temperature of 80°C.

General Procedure A: Succinimide Formation from Aromatic Amines

A stirred solution of the aromatic amine (1 equiv.) and succinic anhydride (1 equiv.) in dry toluene (approx. 0.25 M), under an argon atmosphere, was heated at reflux for 15 min. After cooling to room temperature, the solid was collected by filtration and washed with cold petroleum ether. The material was dissolved in a mixture of ethyl acetate (60 mL) and 5% aqueous sodium hydroxide (60 mL), the layers were separated, and the organic phase was extracted with 5% aqueous sodium hydroxide (20 mL). The combined aqueous fractions were acidified to pH ~1 with concentrated hydrochloric acid, and the resultant solid was allowed to stand for 3 h, after which it was collected by filtration, washed with water (10 mL), and dried under reduced pressure. A mixture of the resulting *N*-arylsuccinamic acid (1 equiv.) and anhydrous sodium acetate (0.33 equiv.) in dry acetic anhydride (approx. 0.25 M) was heated to 70–80°C for 4.5 h, under an argon atmosphere. The mixture was cooled to room temperature, poured onto water (60 mL), and extracted with dichloromethane (3 × 20 mL). The combined organic fractions were washed with aqueous saturated ammonium chloride (20 mL), dried ($MgSO_4$), and the solvent was removed by evaporation under reduced pressure. Residual solvent was removed from the desired product by Kugelrohr distillation (up to 140°C). See individual experiments for details.

General Procedure B: Succinimide Reduction/Ring Opening with Sodium Borohydride

To a stirred suspension of the succinimide (1 equiv.) in 6 : 1 propan-2-ol/water (approx. 0.1 M) was added sodium borohydride (2.5 equiv.), and the resulting suspension was stirred at room temperature for 17 h.

The mixture was filtered through Dowex-50W resin and the filtrate evaporated to dryness under reduced pressure. See individual experiments for details.

General Procedure C: Couplings Using Bis(2-oxo-3-oxazolidinyl)phosphinic Chloride

To a stirred suspension of the formylpyrrole acid **14**[15] (1 equiv.), alcohol **13** (1.5 equiv.), and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (2 equiv.) in dry dichloromethane (approx. 0.07 M), at room temperature under an argon atmosphere, was added dry triethylamine (2 equiv.). The resulting suspension was stirred for 64 h, after which it was diluted with ethyl acetate (20 mL), washed with 10% aqueous sodium bicarbonate (10 mL), saturated aqueous brine (10 mL), dried ($MgSO_4$), and the solvent removed by evaporation under reduced pressure. See individual experiments for details.

General Procedure D: Lithium Borohydride Reductions

A stirred solution of the formylpyrrole **15** (1 equiv.), in dry THF (approx. 0.03 M) under an argon atmosphere, was cooled to −78°C (dry ice/acetone). Lithium borohydride (2 equiv.) was added, and the resulting solution was stirred at −78°C for 1 h, then warmed to room temperature and stirred for an additional 1 h. Water (10 mL) was carefully added to quench the reaction. The solution was extracted with ethyl acetate (3 × 10 mL), the combined organic phases were washed with saturated aqueous brine (10 mL), dried ($MgSO_4$), and the solvent was removed by evaporation under reduced pressure. See individual experiments for details.

N-*(Naphthalen-1-yl)pyrrolidine-2,5-dione* ***12a***

Naphthalen-1-ylamine **11a** (1.00 g, 6.98 mmol) was treated with succinic anhydride (699 mg, 6.98 mmol) followed by anhydrous sodium acetate (191 mg, 2.33 mmol) according to general procedure A to give **12a** (1.36 g, 86% over two steps) as a light pink solid, mp 153–154°C (lit.[13] 147–149°C). δ_H (500 MHz, $CDCl_3$, Me_4Si) 2.96–3.09 (4 H, m, CH_2CH_2), 7.33 (1 H, dd, *J* 1.0, 7.3, ArH), 7.53 (4 H, m, ArH), 7.93 (2 H, m, ArH).

N-*(Quinolin-8-yl)pyrrolidine-2,5-dione* ***12b***

Quinolin-8-ylamine **11b** (500 mg, 3.47 mmol) was treated with succinic anhydride (347 mg, 3.47 mmol) followed by sodium acetate (95 mg, 1.16 mmol) according to general procedure A to give **12b** (620 mg, 79% over two steps) as a light brown solid, mp 134–136°C. ν_{max} (KBr/cm^{-1}) 1502.4, 1701.1, 1778.2, 2947.0, 3051.2. δ_H (500 MHz, $CDCl_3$, Me_4Si) 2.97, 3.13 (4 H, 2 m, CH_2CH_2), 7.44 (1 H, dd, *J* 3.9, 8.3, ArH), 7.62 (2 H, m, ArH), 7.93 (1 H, dd, *J* 2.0, 7.8, ArH), 8.20 (1 H, dd, *J* 1.5, 9.8, ArH), 8.87 (1 H, dd, *J* 1.7, 4.2, ArH). δ_C (75 MHz, $CDCl_3$, Me_4Si) 29.0 (2 C, CH_2CH_2), 122.0, 126.2, 129.3, 129.7, 129.8, 136.6, 150.8 (ArC), 176.9 (2 CO_2). *m/z* (EI) 226.0744 (M^+, $C_{13}H_{10}N_2O_2$ requires 226.0742).

4-Hydroxy-N-*(naphthalen-1-yl)butanamide* ***13a***

The succinimide **12a** (1.36 g, 6.04 mmol) was treated with sodium borohydride (571 mg, 15.1 mmol) by general procedure B. The crude product was purified by flash chromatography (ethyl acetate/methanol, 10 : 1), followed by recrystallization from ethyl acetate, to give **13a** (983 mg, 71%) as a white solid, mp 102–104°C (Found: C 73.2, H 6.7, N 6.1. $C_{14}H_{15}NO_2$ requires C 73.3, H 6.6, N 6.1%). ν_{max} (KBr/cm^{-1}) 1508.2, 1541.0, 1652.9, 2950.9, 3274.9. δ_H (500 MHz, CD_3OD, Me_4Si) 2.00 (2 H, m, $CH_2CH_2CH_2$), 2.63 (2 H, t, *J* 7.3, CH_2CO), 3.70 (2 H, t, *J* 6.3, $HOCH_2$), 7.50 (3 H, m, ArH), 7.57 (1 H, d, *J* 7.3, ArH), 7.77 (1 H, d, *J* 8.3, ArH), 7.89 (1 H, m, ArH), 7.98 (1 H, d, *J* 7.8, ArH). δ_C (75 MHz, CD_3OD, Me_4Si) 30.0 ($CH_2CH_2CH_2$), 34.2 (CH_2CO), 62.6 ($HOCH_2$), 123.7, 124.3, 126.7, 127.4, 127.5, 127.8, 129.6, 130.4, 134.4, 135.9 (ArC), 175.7 (CONH). *m/z* (EI) 229.1102 (M^+, $C_{14}H_{15}NO_2$ requires 229.1103).

*4-(Naphthalen-1-ylamino)-4-oxobutyl-5-hydroxymethyl-1*H-*pyrrole-2-carboxylate* ***16a***

Pyrrole acid **14**[15] (100 mg, 0.72 mmol) was coupled to alcohol **13a** (247 mg, 1.08 mmol) using general procedure C. The crude product was purified by flash chromatography (ethyl acetate/petroleum ether, 2 : 1) to give **15a** (95 mg) as an orange solid, which was not purified further. This material was reduced with lithium borohydride (12 mg, 0.54 mmol) by general procedure D and the crude product purified by flash chromatography (ethyl acetate/petroleum ether, 5 : 1, then ethyl acetate), followed by recrystallization from ethyl acetate, to give **16a** (46 mg, 18% over two steps) as a white solid, mp 154–156°C (Found: C 68.2, H 5.8, N 7.8. $C_{20}H_{20}N_2O_4$ requires C 68.2, H 5.7, N 8.0%). ν_{max} (KBr/cm^{-1}) 1500.5, 1531.4, 1654.8, 1685.7, 3282.6, 3433.1. δ_H [500 MHz, $(CD_3)_2SO$, Me_4Si] 2.15 (2 H, m, $CH_2CH_2CH_2$), 2.74 (2 H, t, *J* 7.3, CH_2CO), 4.37 (2 H, t, *J* 6.3, CO_2CH_2), 4.51 (2 H, d, *J* 5.4, $HOCH_2$), 5.15 (1 H, t, *J* 5.9, $HOCH_2$), 6.17 (1 H, m, pyrrole H4), 6.86 (1 H, s, pyrrole H3), 7.60 (3 H, m, ArH), 7.76 (1 H, d, *J* 7.3, ArH), 7.85 (1 H, d, *J* 8.3, ArH), 8.03 (1 H, m, ArH), 8.14 (1 H, m, ArH), 10.05 (1 H, br s, CONH), 11.73 (1 H, br s, pyrrole NH). δ_C [75 MHz, $(CD_3)_2SO$, Me_4Si] 24.9 ($CH_2CH_2CH_2$), 32.5 (CH_2CO), 56.1 ($HOCH_2$), 63.1 (CO_2CH_2), 108.2 (pyrrole C4), 115.7 (pyrrole C3), 121.2 (pyrrole C2) 122.0, 122.9, 125.4, 125.8, 126.0, 126.2, 128.0, 128.3, 133.7, 133.9 (ArC), 139.4 (pyrrole C5), 160.7 (CO_2), 171.6 (CONH). *m/z* (ES) 375.1321 ($M^+ + Na$, $C_{20}H_{20}N_2NaO_4$ requires 375.1321).

*4-(Quinolin-8-ylamino)-4-oxobutyl-5-hydroxymethyl-1*H-*pyrrole-2-carboxylate* ***16b***

The succinimide **12b** (496 mg, 2.19 mmol) was reduced with sodium borohydride (207 mg, 5.48 mmol) using general procedure B. The crude material was purified by flash chromatography (ethyl acetate, then ethyl acetate/methanol, 10 : 1) to give **13b** (335 mg) as a sticky white solid. δ_H (500 MHz, CD_3OD, Me_4Si) 1.99 (2 H, m, $CH_2CH_2CH_2$), 2.68 (2 H, t, *J* 7.6, CH_2CO), 3.67 (2 H, t, *J* 6.3, $HOCH_2$), 7.53 (2 H, m, ArH), 7.61 (1 H, m, ArH), 8.29 (1 H, dd, *J* 2.0, 8.3, ArH), 8.62 (1 H, d, *J* 7.8, ArH), 8.86 (1 H, d, *J* 1.5, 4.4, ArH). Pyrrole acid **14**[15] (135 mg, 0.97 mmol) was coupled to **13b** (335 mg, 1.45 mmol) according to general procedure C. The crude material was purified by flash chromatography (ethyl acetate/petroleum ether, 2 : 1) to give **15b** as an orange solid. This material was reduced with lithium borohydride (19 mg, 0.85 mmol) by general procedure D and the crude material purified by flash chromatography (ethyl acetate/petroleum ether, 4 : 1, then ethyl acetate), followed by recrystallization from ethyl acetate/petroleum ether, to give **16b** (86 mg, 11% over three steps) as a tan solid, mp 118–119°C (Found: C 64.5, H 5.6, N 11.6. $C_{19}H_{19}N_3O_4$ requires C 64.6, H 5.4, N 11.9%). ν_{max} (KBr/cm^{-1}) 1539.1, 1652.9, 1708.8, 2949.0, 3261.4, 3348.2. δ_H (500 MHz, $CDCl_3$, Me_4Si) 2.25 (2 H, m, $CH_2CH_2CH_2$), 2.57 (1 H, br s, OH), 2.71 (2 H, t, *J* 7.1, CH_2CO), 4.39 (2 H, t, *J* 6.3, CO_2CH_2), 4.63 (2 H, s, $HOCH_2$), 6.17 (1 H, t, *J* 3.2, pyrrole 4H), 6.83 (1 H, dd, *J* 2.4, 3.4, pyrrole H3), 7.43 (1 H, dd, *J* 4.4, 8.3, ArH), 7.51 (2 H, m, ArH), 8.14 (1 H, dd, *J* 1.7, 8.1, ArH), 8.74 (2 H, m, ArH), 9.77, 9.85 (2 H, 2 br s, CONH and pyrrole NH). δ_C (75 MHz, $CDCl_3$, Me_4Si) 24.2 ($CH_2CH_2CH_2$), 33.7 (CH_2CO), 56.7 ($HOCH_2$), 62.5 (CO_2CH_2), 107.2 (pyrrole C4), 114.9 (pyrrole C3), 116.3, 121.2, 126.8, 127.4, 133.6, 136.4, 137.2, 147.4 (ArC), 121.4 (pyrrole C2), 137.8 (pyrrole C5), 160.5 (CO_2), 170.3 (CONH). *m/z* (EI) 353.1362 (M^+, $C_{19}H_{19}N_3O_4$ requires 353.1376).

*Ethyl 5-hydroxymethyl-1*H-*pyrrole-2-carboxylate* ***18***

Formylpyrrole ester **17**[15] (150 mg, 0.90 mmol) was reduced with lithium borohydride (39 mg, 1.79 mmol) using general procedure D. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 1 : 1) to give **18** (115 mg, 76%) as a white solid; mp 76–78°C (Found: C 57.0, H 6.6, N 8.2. $C_8H_{11}NO_3$ requires C 56.8, H 6.6, N 8.3%). ν_{max} (KBr/cm^{-1}) 1502.4, 1683.7, 2993.3, 3286.5. δ_H (500 MHz, $CDCl_3$, Me_4Si) 1.35 (3 H, t, *J* 7.1, CH_2CH_3), 4.31 (2 H, q, *J* 7.2, CH_2CH_3), 4.69 (2 H, s, $HOCH_2$), 6.11 (1 H, t, *J* 3.2, pyrrole H4), 6.84 (1 H, dd, *J* 2.4, 3.9, pyrrole H3), 9.51 (1 H, br s, pyrrole NH). δ_C [75 MHz, $(CD_3)_2CO$, Me_4Si] 14.1 (CH_2CH_3), 57.0 ($HOCH_2$), 59.6

(CH_2CH_3), 108.0 (pyrrole C4), 115.2 (pyrrole C3), 122.4 (pyrrole C2), 138.7 (pyrrole C5), 160.7 (CO_2). *m/z* (EI) 169.0739 (M^+, $C_8H_{11}NO_3$ requires 169.0738).

P388 Cancer Cell Line Cytotoxicity Assay

These assays were carried out according to the literature method of Alley et al.,[17] using stock solutions of **16a** (20 mg mL^{-1} in 3 : 1 methanol/dimethylformamide), **16b** and **18** (10 mg mL^{-1} in methanol), and **19** (1 mg mL^{-1} in 3 : 1 methanol/dimethylformamide).

*Determination of the Binding Constant of **16b** to DNA*

Binding constants of 4.6×10^3 M^{-1} for poly[dA–dT] and 3.0×10^3 M^{-1} for poly[dG–dC] were calculated for **16b** and 1.10^7 M^{-1} for ethidium bromide **20** according to the literature method.[18,19] The end point of the assay was based on 20% displacement of **20** by **16b** due to the low solubility of **16b** in the assay medium.[20]

Acknowledgments

The authors thank Professor Bruce Baguley (Auckland Cancer Society Research Centre) for performing the DNA binding assays, and Gill Ellis (Marine Natural Products Group, Department of Chemistry, University of Canterbury) for carrying out the P388 cytotoxicity assays. Financial support from Bright Futures Top Achievers Doctoral Scholarship (D.C.M.) and the Royal Society of New Zealand Marsden Fund is also gratefully acknowledged.

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