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Design and synthesis of a DNA-crosslinking azinomycin analogue

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Received 23rd June 2005, Accepted 2nd August 2005
First published as an Advance Article on the web 5th September 2005

The azinomycins are potent antitumour antibiotics that are able to crosslink DNA, but are relatively unstable and unlikely to progress as therapeutic candidates. A prototype analogue **4** with more clinical potential has been designed and synthesised and incorporates the epoxide function of the azinomycins and a nitrogen mustard. Two further analogues **5** and **6** that can alkylate DNA but cannot crosslink the duplex have also been synthesised. Compound **4** crosslinks DNA efficiently at nM concentrations. Compounds **4–6** were submitted to the NCI 60 cell line screen and have similar antitumour activity, although **4** is slightly less active than the non-crosslinking compounds. These observations will be important in the design of further azinomycin analogues with antitumour activity.

Introduction

The azinomycins (**1–2**, Fig. 1) are potent antitumour antibiotics isolated from *Streptomyces griseofuscus*[1] that bind to DNA and are able to crosslink at 5′-GXC or 5′-GXT sequences.[2] The densely functionalised aziridine moiety of the azinomycins has attracted significant synthetic interest,[3] resulting in the recent total synthesis of azinomycin A.[4] Studies of the biological mode of action of the azinomycins have been less in number and have focussed on the ability of the compounds to crosslink DNA[2,5,6] and their sequence selectivity.[2] These studies have also been the subject of review.[3,7] Alcaro and Coleman have carried out an extensive series of modelling studies, which complement their experimental data and suggest that crosslinking of the duplex is not accompanied by intercalation of the naphthoate moiety.[8,9] Compound **3** is also a natural product,[10] forming the "left hand" epoxide function of the azinomycins and is able to alkylate DNA.[11] The ability of this compound to intercalate into DNA has been the subject of some controversy,[11–13] although the potent antitumour activity of the compound suggests that crosslinking may not be required for activity, or, at the least, that compounds **1** and **2** and compound **3** exert their effect through different mechanisms.

Azinomycins A and B are relatively unstable compounds. In the total synthesis of azinomycin A, the final product was not stable and completion was noted from spectral and chromatographic studies following the final deprotection reaction.[4] This lack of stability suggests that the natural products are unlikely to progress as therapeutic agents and indicates a requirement for more clinically useful analogues. Several groups have previously reported analogues that are close in structure to the natural products and have also contributed to our understanding of their mechanism of action.[14–19] In this paper, we describe the first analogues of the azinomycins based upon a design that incorporates a more therapeutically viable nitrogen mustard moiety in place of the aziridine. The synthesis of the prototype compound **4** was completed along with monoalkylators **5** and **6** (Fig. 2). The non-alkylator **7** was designed as a control. All three alkylating compounds had good activity in the NCI 60 cell line screen. Compound **4**, which is able to crosslink DNA, displayed lower activity than the monoalkylators in a number of cell lines, suggesting that crosslinking is not required for and may be detrimental to antitumour potency for these compounds.

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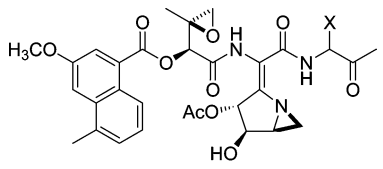
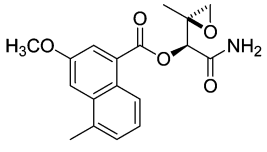
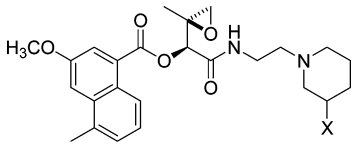


1 Azinomycin A X = H
2 Azinomycin B X = CHO

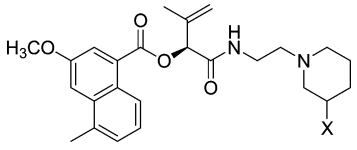


3

Fig. 1



4 X = Cl
5 X = OH



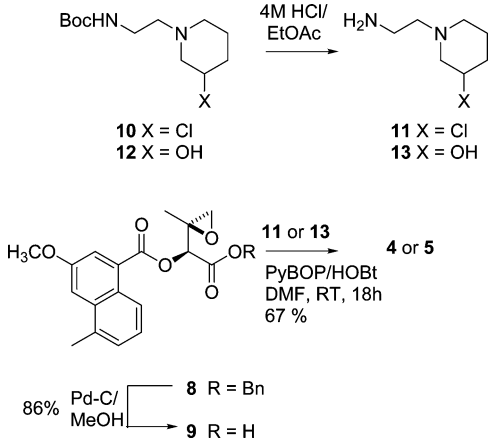
6 X = Cl
7 X = OH

Fig. 2

DOI: 10.1039/b508908e

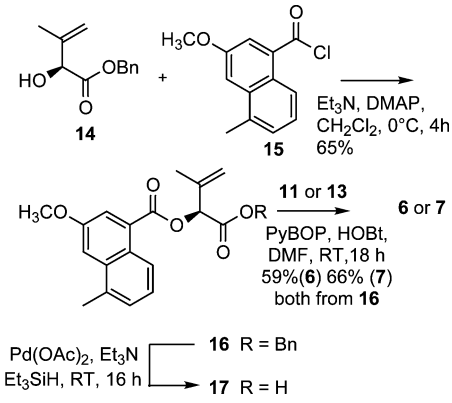
Synthesis of 4–7

The benzyl ester **8** was synthesised in a stereoselective manner[20] and converted to the free acid **9** by hydrogenation (Scheme 1). The most convenient approach to the mustard derivative was through direct reaction with the racemic free amine mustard **11**,[21] Boc-deprotected by exposure to dry HCl in EtOAc immediately prior to coupling. The coupling reaction was mediated by PyBOP–HOBt and gave the target compound **4** in 67% yield as an inseparable mixture of diastereomers. For compound **5**, which can monoalkylate the duplex through the epoxide but cannot crosslink DNA, the free acid **9** was coupled in a similar fashion with the racemic alcohol **13**.



Scheme 1 Synthesis of **4** and **5**.

The alkene **17** was derived from an intermediate in the stereoselective synthesis of **8**.[20] Thus, the alkene **14**[20] was reacted with acid chloride **15** to give the benzyl ester **16**. This compound was carefully deprotected using $Pd(OAc)_2$, Et_3SiH and Et_3N[22] to give the free carboxylic acid **17** (extended reaction times led to concomitant reduction of the double bond). Coupling of **17** with either **11** or **13** gave compounds **6** and **7** respectively (Scheme 2).



Scheme 2 Synthesis of **6** and **7**.

DNA crosslinking

The azinomycins have been shown to crosslink DNA,[2,5,6] which may contribute to their biological activity, although the non-crosslinking analogue **3** has been shown to maintain potent activity. Compound **4** can imitate the natural product with suitably positioned nitrogen-mustard and epoxide groups and crosslinks the linear plasmid pBR322 (Fig. 3). Crosslinking is observed initially at concentrations as low as 300 nM and the DNA is fully crosslinked at 10 μM concentration. This compares well with the di-epoxide and azinomycin analogues designed and synthesised by Shipman and co-workers.[14,23] Compounds **5–7** showed no evidence of DNA crosslinking (data not shown).

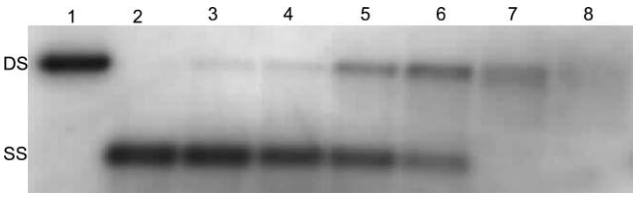


Fig. 3 DNA crosslinking by **4**. Autoradiograph of agarose gel after 1 h incubation of pUC18. DS = Double stranded DNA, SS = single stranded DNA. Lane 1 non-denatured DNA, lane 2 denatured DNA, lane 3–8 0.1, 0.3, 1.0, 3.0, 10, 50 μM **4**.

Cytotoxicity

In an initial screen for cytotoxicity using the U2OS osteosarcoma cell line, compounds **4–6** all had reasonable antitumour activity (around 40 μM) whereas compound **7** was inactive. As a consequence, the alkylating compounds were submitted to the NCI 60 cell line screen. The results are shown in Table 1 along with those in the same screen for compound **3**. The average GI50 gives an indication of the overall activity of each of the agents. These values (**4** 12.3 μM, **5** 2.08 μM, **6** 5.12 μM) are in the low μM range and suggest that the compounds are reasonably active cytotoxic agents. Interestingly, in all cell lines compound **4**, which can crosslink DNA, is less active than **5**, the monoepoxide and in most it is less active than the monoalkylating mustard **6**. This is particularly evident in the non-small cell lung cancers (*i.e.* A549/ATCC to NCI H522), renal cancers (786-0 to UO31) and cancers of the central nervous system (SF-268 to U251). All three compounds are significantly less potent in the 60 cell line screen than the monoepoxide compound **3**.

Discussion

The antitumour antibiotics azinomycins A and B (**1** and **2**) are unstable and unlikely to proceed into the clinic, but can act as lead compounds from which to develop potentially useful new molecules. The natural products have been shown to crosslink DNA at a 5′-GXC or GXT sequence[2,5] and to have potent antitumour activity, although the cytotoxicity data available are limited, presumably due to the lack of stability of the compounds. Compound **3** is also a natural product and a metabolite of the extended agents.[10] Although it cannot crosslink DNA, it retains potent antitumour activity and, as demonstrated here, has activity against a range of tumour cell lines. In previous studies, Terashima and co-workers have also demonstrated that non-crosslinking intermediates, on the synthetic route to a benzyl protected azinomycin B analogue, have good antitumour activity.[15] Interestingly, Hartley *et al.*[14] designed and synthesised a simplified azinomycin A analogue (also made by Terashima *et al.*[24]) and showed that while the compound could crosslink DNA, it had similar activity *in vitro* to a non-crosslinking, monoalkylating analogue. In this paper, we describe the first synthetic analogues of the azinomycins that were designed with a view towards enhancing the therapeutic utility of the compounds. Nitrogen mustards are known, clinically useful DNA alkylating agents. Piperidine-based mustards have been used in the synthesis of intercalator–alkylator conjugates that have broad spectrum antitumour activity.[22] The mustard moiety is more stable and easier to handle than the densely-functionalized system of the azinomycins, but positions the alkylating function relative to the epoxide in a similar fashion to the natural products. Preliminary modelling studies using Coleman *et al.*'s methodology[12] suggested that compound **4** could alkylate both through the epoxide and the mustard, at a similar GXC sequence. The synthesis of **4** was relatively straightforward and benefited from the ready availability of the protected piperidine mustard, previously used by us in other studies.[13] It was found that the most effective route to the final product involved coupling of the intact mustard after Boc-deprotection in dry acid, rather

Table 1 Antitumour activity (GI50/μM) of compounds **3–6** in the NCI 60-cell line screen[a]

	3	4	5	6
CCRF-CEM	0.01	3.26	2.95	3.24
HL-60(TB)		6.98	2.94	12.1
K562	0.028	8.33	2.93	5.16
MOLT-4	0.01	3	2.15	3.3
RPMI-8226	0.25	4.18	2.1	3.43
SR		2.02	1.58	2.34
A549/ATCC	1.41	31.1	3.05	8.28
EKVX	2.75	17.5	3.39	11.4
HOP-62	0.045	100	1.58	3.45
HOP-92	0.21	13.8	2.18	4.12
NCI-H226	1.12			
NCI-H23	0.068			
NCI-H322M	2.57	17.9	5.58	8.07
NCI-H460	0.74	13	2.62	6.26
NCI-H522	1.54	7.77	1.62	5.1
COLO 205	0.43	13.8	2.98	6.08
HCC2998		8.25	1.61	4.27
HCT-116	0.016	100	1.63	12.8
HCT-15			0.39	0.049
HT29	0.22	11	3.4	7.26
KM12	1.48	14.5	2.67	9.3
SW-620	0.01	5.26	1.29	2.76
SF-268	0.079	16.6	3.03	11.9
SF-295	0.44	13.1	2.26	4.35
SF-539	0.44	6.27	1.7	0.45
SNB-19	1.77	14.3	3	5.63
SNB-75	1.9		2.54	1.2
U251	0.023	7.82	1.59	2.63
LOX IMVI		3.62	1.4	2.84
MALME-3M	4.16	14.8	4.81	7.51
M14	0.15	100	1.44	1.75
SK-MEL-2	1.17	18.6	1.81	7.81
SK-MEL-28	1.81	11.6	4.47	8.48
SK-MEL-5	0.19	1.56	1.21	1.99
UACC-257	1.12			
UACC-62	0.15	14.7	1.66	4.41
IGROV1	0.78	12.1	1.78	4.21
OVCAR-3	0.34	18.6	1.86	10.2
OVCAR-4	4.57	21.5	4.74	20.9
OVCAR-5	0.45	16.1	2.76	11.1
OVCAR-8	0.13	44.5	3.59	32.9
SKOV-3	1.94	19.7	4.37	17.2
786-0	0.031	100	2.19	4.48
A498	2.45	17.7	0.39	0.19
ACHN	0.01	1.17	0.26	1.78
CAKI-1	0.31	10.3	2.33	5.66
RXF 393	0.6	17.5	1.77	19.1
SN12C	0.26	6.76	1.46	3.76
TK10	0.68	23.4	2.78	12.1
UO-31				
PC–3	0.14	11.2	3.53	7.96
DU-145	0.32	4.49	1.66	5.15
MCF7	0.01	14.7	1.91	4.62
NCI/ADRRES	0.015	11.1	2.26	12.8
MDA-MB-231/ATCC		14	1.65	11
HS 578T	1.55	17.5	1.99	3.69
NMDA-MB-435	1.15	14.3	1.78	7.62
BT-549	2.29	12.5	2.37	4.55
T-47D	1.74	23	2.34	10.8

[a] GI50: 50% Growth inhibition, concentration of drug resulting in a 50% reduction in net protein increase compared with control cells (http://dtp.nci.nih.gov/branches/btb/ivclsp.html)

than coupling of the alcohol and then chlorination, the latter giving lower yields of the final product due to reaction of the chloride ion with the epoxide. Compound **5** could be synthesised similarly from reaction of the naphthalene-epoxide fragment with the alcohol **13** and compounds **6** and **7** from the novel naphthalene alkene **9** and either the mustard or the alcohol. As expected, compound **4** was a potent DNA crosslinking agent (Fig. 2), while compounds **5** and **6** did not crosslink the duplex. The concentration at which 100% of the DNA was crosslinked (10 μM) by **4** compared favourably to that previously observed with other azinomycin analogues. These include a diepoxide compound (1 μM)[23] (although Shipman and co-workers recently described more active crosslinkers[25]) and a synthetic analogue of the full natural product (100 μM),[14] although a direct comparison was not made. The ability of the compounds to unwind closed-circular supercoiled DNA was also examined, as a preliminary screen for intercalation,[13] but the results were equivocal, with changes in the mobility of the DNA that could have stemmed from effects other than intercalation (data not shown). The cytotoxicity data for all three new agents and the monoalkylator **3** are shown in Table 1. All three compounds demonstrate an overall decrease in activity compared with the natural product **3** suggesting that the structural change of the introduction of the piperidine is deleterious to the efficacy of these agents. This could be an effect of the introduction of the basic nitrogen of the piperidine, which is protonated at physiological pH and may impair cell uptake. However, compound **5**, with the monoexpoxide, is of low μM potency in almost all lines and represents an interesting starting point for the development of further analogues. Most striking is the comparison of the crosslinking compound **4** with the mono-alkylating agents **5** and **6**. In almost all cell lines, the mono-alkylators are more potent than the crosslinkers, suggesting that crosslinking is not a necessary target in azinomycin analogues for effective biological activity. This key observation will be of importance in designing further analogues of the azinomycins and suggests that compound **3** is as valid a lead molecule as the crosslinking compounds **1** and **2**.

Conclusions

Three compounds have been designed and synthesised that are modelled upon the natural product azinomycins, but which incorporate a nitrogen mustard in place of the aziridine. Compound **4** is able to crosslink DNA at low concentrations but has less potent antitumour activity than the monoalkylators **5** and **6**. All three compounds were less active than **3**. Further analogues of the azinomycins that can both monoalkyate and crosslink DNA are under investigation in an attempt to generate compounds that will have clinical utility.

Experimental

The synthesis of compounds **10** and **12** will be disclosed elsewhere.[22] All solvents were purchased from VWR (Poole, Dorset) except for anhydrous solvents, which were purchased from Aldrich and used without further purification. ^{1}H and ^{13}C NMR were measured on a Bruker AM400 spectrometer at 400 and 100 MHz respectively. Mass spectra were acquired on a VG Analytical ZAB-SE4F instrument using Fast Atom Bombardment (FAB) techniques at −20 kV Cs^{+}.

(*S*)-2-({[2-(3-Chloropiperidin-1-yl)ethyl]amino}-1-[(2*S*)-2-methyloxirane-2-yl])-2-oxoethyl 3-methoxy-5-methyl-1-naphthoate (4)

Ester **8** (72 mg, 0.17 mmol) and 10% Pd–C (11 mg) were stirred in dry CH_3OH (10 mL) under an H_2 atmosphere for 2 h at RT. The mixture was filtered through celite and the filtrate concentrated *in vacuo* to give crude carboxylic acid **9** as a colourless oil (49 mg, 86%). This was dissolved in dry DMF (9 mL) and stirred at 0 °C. Compound **11** (0.06 mL, 0.37 mmol), Et_3N (0.052 mL, 0.37 mmol), HOBt (27 mg, 0.20 mmol) and PyBOP (93 mg, 0.18 mmol) were then added and after the mixture had been allowed to warm to RT, it was stirred for 18 h. Toluene (10 mL) and EtOAc (16 mL) were added and the resulting solution was washed successively with 5% aq. HCl (16 mL), H_2O (16 mL), satd. aq. $NaHCO_3$ (16 mL) and brine (16 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a brown oil. Flash column chromatography (10–20%

CH_3OH–CH_2Cl_2) provided **4** as a yellowish-brown oil (47 mg, 67%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.54 (1H, m, ArH), 7.91–7.87 (1H, m, ArH), 7.58–7.56 (1H, m, ArH), 7.36–7.31 (2H, m, ArH), 3.97 (1H, s, H-2), 3.40 (2H, m, –CH_2–), 3.33 (3H, s, OCH_3), 3.28 (2H, t, NCH_2–), 3.21 (2H, t, –CH_2N), 3.11 (1H, d, J = 4.4 Hz, H-4), 2.98 (1H, d, J = 4.8 Hz, H-4), 2.67 (3H, s, Ar–CH_3), 2.51 (2H, m, –CH_2–), 2.22 (1H, m, –CHCl), 1.63 (4H, m, 2 × CH_2), 1.48 (3H, s, CH_3). δ_C(400 MHz; $CDCl_3$) 169.23, 166.42, 157.51, 135.82, 128.83, 126.11, 124.77, 109.32, 79.78, 77.57, 59.61, 56.81, 53.65, 37.44, 24.83, 20.75, 20.14, 18.42, 17.55, 13.95. FABMS m/z (M^+), 474. Anal. Calcd. $C_{25}H_{31}ClN_2O_5$ C, 63.22; H, 6.58; N, 5.90%. Found C, 63.30; H, 6.51; N, 5.98%.

(*S*)-2-({[2-(3-Hydroxypiperidin-1-yl)ethyl]amino}-1-[(2*S*)-2-methyloxirane-2-yl])-2-oxoethyl 3-methoxy-5-methyl-1-naphthoate (5)

Ester **8** (89 mg, 0.17 mmol) and 10% Pd–C (14 mg) were stirred in dry CH_3OH (10 mL) under an H_2 atmosphere for 2 h at RT. The mixture was filtered through celite and the filtrate concentrated *in vacuo* to give crude carboxylic acid **9** as a colourless oil (49 mg, 0.15 mmol). This was dissolved in dry DMF (6 mL) and stirred at 0 °C. Compound **13** (0.02 g, 0.14 mmol), Et_3N (0.02 ml, 0.14 mmol), HOBt (17 mg, 0.13 mmol) and PyBOP (57 mg, 0.11 mmol) were then added and after the mixture had been allowed to warm to RT, it was stirred for 18 h. Toluene (10 mL) and EtOAc (16 mL) were added and the resulting solution was washed successively with 5% aq. HCl (16 mL), H_2O (16 mL), satd. aq. $NaHCO_3$ (16 mL) and brine (16 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a brown oil. Flash column chromatography (10–20% CH_3OH–CH_2Cl_2) provided **5** as a yellowish-brown oil (25 mg, 60%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.56 (1H, t, ArH), 7.92 (1H, dd, J = 2.4 Hz, ArH), 7.59–7.57 (1H, m, ArH), 7.36–7.30 (2H, m, ArH), 3.99 (3H, s, OCH_3), 3.92 (1H, s, H-2), 3.61 (2H, m, –NCH_2–), 3.38 (2H, m, CH_2N–), 3.35 (3H, s, CH_3), 3.13 (1H, d, J = 4.4 Hz, H-4), 3.02 (1H, d, J = 4.8 Hz, H-4), 2.84 (2H, m, –CH_2–), 2.68 (3H, s, Ar–CH_3),2.84 (1H, m, –CHCl), 2.52 (2H, m, –CH_2–), 2.03 (2H, m, –CH_2–), 1.51 (3H, s, CH_3), (1.28 (3H, s, –CH_2–). δ_C(400 MHz; $CDCl_3$) 169.39 (C=O), 166.32 (C=O), 157.51 (ArC), 135.80 (ArC), 134.74 (ArC), 129.72 (ArC), 128.64 (ArC), 126.04 (ArCH), 124.78 (ArCH), 123.21 (ArC), 109.25 (ArCH), 79.83, 77.58, 67.86, 56.13, 53.09, 33.77, 30.22, 24.00, 20.13, 18.30, 17.55. FABMS m/z 457(M^+ + H^+) Anal. Calcd. $C_{25}H_{32}N_2O_6$ C, 65.77; H, 7.07; N, 6.14%. Found C, 65.72; H, 7.01; N, 6.19%.

(2*S*)-Benzyl-2-(3-methoxy-5-methyl-1-naphthoyloxy)-3-methylbut-3-enoate (16)

To a stirred solution of triethylamine (0.16 ml, 1.15 mmol), DMAP (9 mg, 0.073 mmol) and allyl alcohol **14** (156 mg, 0.76 mmol) in dry DCM (5 ml) at 0 °C under N_2 was added a solution of acid chloride **15** (187 mg, 0. mmol) in dry DCM (5 ml) dropwise. The reaction mixture was stirred at 0 °C for 4 h and then water (20 ml) was added. The organic layer was separated and the aqueous layer extracted with DCM (3 × 10 ml). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a brown oil. Flash chromatography (10% EtOAc–hexane) provided alkene ester **16** (198 mg, 65%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 8.63–8.61 (1H, m, ArH), 7.91 (1H, d, J 2.4 Hz, ArH), 7.48 (1H, s, ArH), 7.36–7.31 (7H, m, ArH), 5.77 (1H, s, H-2), 5.33 (1H, br s, =CH_2), 5.31–5.26 (2H, m, CO_2CH_2Ph), 5.18 (1H, br s, =CH_2) 3.96 (3H, s, OCH_3), 2.68 (3H, s, Ar–CH_3), 1.90 (3H, s, CH_3). δ_C (100 MHz; $CDCl_3$) 168.36 (C=O), 166.47 (C=O), 155.90 (ArC), 137.85 (ArC), 135.31 (ArC), 134.32 (ArC), 133.09 (ArCH), 128.69 (ArCH), 128.64 (ArCH), 128.56 (ArC), 127.63 (ArC), 126.86 (ArC), 124.99 (ArCH), 123.88 (ArCH), 121.89 (ArCH), 108.30 (ArCH), 76.70 (C-2), 67.64 (CO_2CH_2Ph), 55.52 (O CH_3), 53.42 (C-3), 52.16 (C-4), 20.09, 18.79. FABMS m/z [405 (M + H)$^+$, 56%], [428 (M + Na)$^+$, 4%, fragments [289, 26%], [216, 9%], [199, 100%].

(*S*)-1-({[2-(3-Chloropiperidin-1-yl)ethyl]amino}carbonyl)-2-methylprop-2-en-1-yl 3-methoxy-5-methyl-1-naphthoate (6)

A solution of $Pd(OAc)_2$ (3 mg, 0.013 mmol), Et_3SiH (0.088 ml, 0.55 mmol) and Et_3N (0.038 ml, 0.27 mmol) in dry CH_2Cl_2 (1 ml) was stirred at 23 °C under N_2 for 15 min. A solution of **16** (111 mg, 0.27 mmol) in 2 ml dry dichloromethane was added dropwise. The mixture was stirred at RT overnight before quenching by the addition of sat. aq. NH_4Cl (5 ml). The organic layer was separated, extracted with Et_2O (3 × 5 ml) and the combined organic extracts were washed with brine (10 ml), dried ($MgSO_4$), filtered through a pad of celite and concentrated in *vacuo* to give **17** (40 mg, 0.13 mmol). This compound was dissolved in dry DMF (7.8 ml), the solution was stirred at 0 °C and **11** (0.052 g, 0.32 mmol), Et_3N (0.044 ml, 0.32 mmol), HOBt (23 mg, 0.17 mmol) and PyBOP (80 mg, 0.15 mmol) were added. The mixture was allowed to warm to RT and stirred for 18 h. Toluene (10 ml) and EtOAc (16 ml) were added and the resulting solution was successively washed with 5% hydrochloric acid (16 ml), H_2O (16 ml), sat. aq. $NaHCO_3$ (16 ml) and brine (16 ml). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a brown oil. Flash column chromatography (10–20% CH_3OH–CH_2Cl_2) provided **6** as a yellow–brown oil (33 mg, 59%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.55 (1H, m, ArH), 7.88 (1H, d, J = 2.4 Hz, ArH), 7.57 (1H, m, ArH), 7.32 (2H, m, ArH), 5.68 (1H, s. H-2), 5.63 (1H, br. NH), 5.32 (1H, m, =CH_2), 5.19 (1H, m, =CH_2), 3.97 (3H, s, OCH_3), 3.92 (2H, m, –NCH_2–), 3.49 (2H, m, CH_2N–), 3.32 (3H, s, CH_3), 3.15–3.10 (4H, m, –CH_2CH_2–), 2.67 (3H, s, Ar–CH_3), 2.61 (2H, m, –CH_2), 1.86 (2H, m, –CH_2–), 2.07 (2H, m, –CHCl–), 1.82 (3H, s, CH_3), 1.28 (3H, m, –CH_2–). δ_C(400 MHz; $CDCl_3$) 169.21 (C=O), 167.10 (C=O), 157.34 (ArC), 139.56 (=CH_2), 136.98 (ArC), 135.56 (ArC), 133.63 (ArC), 128.63 (ArC), 125.87 (ArCH), 124.03 (ArCH), 122.21 (ArC), 116.95 (=CH_2), 80.33, 77.47, 67.86, 56.13, 53.09, 33.77, 28.78, 24.00, 20.13, 19.42, 17.21. FABMS m/z 441(M^+ + H^+) Anal. Calcd. $C_{25}H_{31}ClN_2O_4$ C, 65.42; H, 6.81; N, 6.10%. Found C, 65.80; H, 6.76; N, 6.13%.

(*S*)-1-({[2-(3-Hydroxypiperidin-1-yl)ethyl]amino}carbonyl)-2-methylprop-2-en-1-yl 3-methoxy-5-methyl-1-naphthoate (7)

A solution of $Pd(OAc)_2$ (3 mg, 0.013 mmol), Et_3SiH (0.088 ml, 0.55 mmol) and Et_3N (0.038 ml, 0.25 mmol) in dry CH_2Cl_2 (1 ml) was stirred at 23 °C under N_2 for 15 min. A solution of **16** (100 mg, 0.27 mmol) in 2 ml dry dichloromethane was added dropwise. The mixture was stirred at RT overnight before quenching by the addition of sat. aq. NH_4Cl (5 ml). The organic layer was separated, extracted with Et_2O (3 × 5 ml) and the combined organic extracts were washed with brine (10 ml), dried ($MgSO_4$), filtered through a pad of celite and concentrated in *vacuo* to give **17** (43 mg, 0.14 mmol). This compound was dissolved in dry DMF (8 ml), the solution was stirred at 0 °C and **13** (0.029 g, 0.21 mmol), Et_3N (0.029 ml, 0.21 mmol), HOBt (25 mg, 0.19 mmol) and PyBOP (86 mg, 0.17 mmol) were added. The mixture was allowed to warm to RT and stirred for 18 h. Toluene (10 ml) and EtOAc (16 ml) were added and the resulting solution was successively washed with 5% hydrochloric acid (16 ml), H_2O (16 ml), sat. aq. $NaHCO_3$ (16 ml) and brine (16 ml). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a brown oil. Flash column chromatography (10–20% CH_3OH–CH_2Cl_2) provided **7** as a yellow–brown oil (38 mg, 66%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.57 (1H, t, ArH), 7.90 (1H, d, J = 2.4 Hz, ArH), 7.57 (1H, d, J = 2.4 Hz, ArH), 7.3 (2H, m, ArH), 5.71 (1H, s. H-2), 5.34 (1H, br. NH), 5.19 (2H, m, =CH_2), 3.99 (3H, s, OCH_3), 3.69 (1H, br s, OH), 3.44 (2H, t, –NCH_2–), 2.92 (1H, m, CH), 3.68 (3H, s, CH_3), 2.74 (2H, m, –CH_2CH_2–), 2.68 (3H, s, Ar–CH_3),

2.31 (2H, m, CH_2), 1.92 (3H, s, CH_3), 1.78 (2H, m, –CH_2–), 1.50 (1H, m, –CHCl–), (1.30 (2H, m, –CH_2–). δ_C(400 MHz; $CDCl_3$) 169.78 (C=O), 168.52 (C=O), 157.53 (ArC), 140.81 (=CH_2), 135.82 (ArC), 134.74(ArC), 129.99 (ArC), 128.06 (ArC), 125.98 (ArCH), 124.77 (ArCH), 123.08 (ArC), 117.36 (=CH_2), 109.09, 79.41, 58.03, 57.99, 56.12, 54.39, 27.43, 20.13, 18.92. FABMS m/z 460($M^+ + H^+$) Anal. Calcd $C_{25}H_{32}N_2O_4$ C, 68.16; H, 7.32; N, 6.36%. Found C, 68.24; H, 7.29; N, 6.33%.

DNA helix crosslinking assay.[26] Linearisation of pUC 18 plasmid DNA

A mixture of pUC 18 plasmid DNA (96 µl, 23 µg), REact 2 buffer (12 µl), and H_2O (9 µl) was vortexed in a sterile eppendorf tube and the restriction enzyme Hind III (3 µl, 30 units) added. The sample was then incubated at 37 °C for 1 h. For the purpose of precipitating the DNA, sodium acetate (12 µl, 3 M), tRNA (1 µl), glycogen (1 µl) and 95% ethanol (396 µl) were added and the sample vortexed and placed on a dry ice–ethanol bath for 10 min. Following centrifugation at 13 000 rpm for 10 min, the supernatant was discarded and the pellet washed once with 70% EtOH (200 µl) and further centrifuged and the supernatant removed. The pellet was lyophilised and the dry DNA pellet resuspended in H_2O (304 µl).

Dephosphorylation of linearised pUC 18 plasmid DNA

Linearised pUC 18 plasmid DNA (80 µl), BAP buffer 10 × (10 µl), H_2O (8 µl) and bacterial alkaline phosphatase (BAP) (3 µl, 450 units) were mixed and the sample incubated at 65 °C for 1 h. The sample was allowed to cool to room temperature and 2 volumes (200 µl) of phenol : chloroform : isoamyl alcohol (1 : 24 : 1) added and the mixture vortexed and centrifuged for 4 min. The aqueous layer (DNA) was removed and 1 volume of H_2O (100 µl) was added to the organic phase and the mixture was vortexed, spun and the aqueous layer removed. The combined aqueous layers was washed with 1 volume of chloroform (300 µl). After pulse spinning, the aqueous layer was removed and the volume made up to 400 µl with H_2O. The DNA solution was aliquoted into four eppendorf tubes each containing 100 µl (~5 µg). Each aliquot was precipitated with 3 M NaOAc (10 µl) and 95% ethanol (330 µl). The precipitation mixture was vortexed and placed in a dry ice–ethanol bath (10 min), spun and the supernatant removed. The DNA pellet was lyophilised and the pellet resuspended in dH_2O (10 µl).

5′-End labelling of linearised and dephosphorylated DNA

Forward reaction buffer 5 × (4 µl), γ^{32}P-ATP (1 µl), H_2O (4 µl) and T4 polynucleotide kinase (1 µl, 5 units) were added to a sterile eppendorf containing a mixture of linearised dephosphorylated pUC 18 plasmid DNA (10 µl, ~5 µg) and the reaction mixture incubated at 37 °C for 1 h. 7.5 M NH_4OAc (20 µl) and 95% EtOH (120 µl) were added. The mixture was cooled, spun, and lyophilised. The pellet was re-suspended in 0.3 M NaOAc, 10 mM EDTA (50 µl) and 95% ethanol (150 µl) and the mixture cooled, spun and lyophilised after which the pellet was washed with 70% cold ethanol (2 × 100 µl). Following removal of supernatant and lyophilisation the labelled DNA was re-suspended in H_2O (40 µl) to give a 125 ng µl^{-1} stock solution. 10 µl (~1000 ng) of this stock solution was further diluted to 100 µl of which 10 µl (~100 ng) was used for each drug reaction lane.

Drug treatment of labelled DNA

To 10 µl (~100 ng) of ^{32}P-radiolabelled DNA was added x µl of a drug dilution (where x is between 1–15 µl) and y µl of TeoA (triethanolamine) buffer to give a final volume of 50 µl. The samples were incubated at 37 °C for the appropriate time and the reactions terminated by addition of equal volumes (50 µl) of stop solution (0.6 M sodium acetate, 20 mM EDTA, 100 µg ml^{-1} tRNA) and the DNA precipitated by addition of 3 volumes of 95% ethanol. After removal of the supernatant, the DNA was dried by lyophilisation. The double strand undenatured control sample was dissolved in 10 µl loading buffer (6% sucrose, 0.04% bromophenol blue) and loaded directly. The drug treated samples and the single strand control were alkali denatured by adding the alkali denaturing buffer (0.25 M NaOH, 0.04% bromophenol blue, 6% sucrose) and the samples loaded directly. Samples were electrophoresed on 20 cm long 0.8% horizontal agarose gels submerged in 1 × TAE buffer at 40 V for 16 h. Gels were then covered with cling film and dried for 2 h at 80 °C onto one layer of Whatman 3 MM paper and one layer of DE81 filter papers on a vacuum connected BIO-RAD gel drier. Autoradiography was performed using Kodak hyper film for 5 h at −70 °C in a cassette with an intensifying screen.

Acknowledgements

We thank Professor Paul J. Smith and Dr Rachel Errington for initial cytotoxicity data for **4–7** in the U2OS cell line and the NCI, Bethesda, Maryland, USA for screening compounds **3–6**. We also thank ULSOP for a Millennium Studentship (MCH) and the London School of Pharmacy Analytical Services for mass spectra and elemental analysis.

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