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Mechanistic and anti-proliferative studies of two novel, biologically active *bis*-benzimidazoles

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Abstract

We have previously synthesised a number of novel head-to-head *bis*-benzimidazole derivatives that are structurally related to the fluorochrome, Hoechst 33258, and which possess strong affinity for A:T sites in the minor groove of duplex DNA. Initial studies revealed these compounds to exhibit potent antiproliferative activity against a range of ovarian cell lines and to inhibit transcription in an *in vitro* setting. In this study, we have examined their cellular behaviour in detail and have shown that two of these compounds (ABA13 and ABA833) potently inhibit the proliferation of a range of human tumour cell lines, and show some specificity towards breast carcinoma cell lines. In most of the cell lines investigated, ABA833 was the more potent of the two compounds. Flow cytometric analysis revealed that ABA13 and ABA833 (50–500 nM) induced an S phase block and increased the pre-G1 population in MCF-7 and MDA 468 human breast cancer cells. An increase in the pre-G1 population of RKO colon carcinoma cells was seen only at 500 nM with ABA833, reflecting the reduced sensitivity of this cell line to the *bis*-benzimidazoles in comparison to the breast cancer cell lines. Mechanistic studies revealed that neither ABA13 or ABA833 act as topoisomerase I (topo I) or topoisomerase II (topo II) poisons in plasmid or kinetoplast DNA (kDNA) relaxation assays, but both compounds do inhibit the catalytic activity of these enzymes. Drug uptake studies showed that reduced sensitivity of MCF-7adr and RKO cells compared with MCF-7 to both ABA13 and ABA833 correlated with a markedly reduced intracellular drug accumulation.

Keywords: *bis*-Benzimidazoles; DNA minor groove binders; Topoisomerase

1. Introduction

In the search for new chemotherapeutic agents, there has long been much interest in compounds that bind in the minor groove of DNA [1–4]. Well-known examples of this class of compounds include the antibiotic, distamycin A, and the fluorochromes, Hoechst 33258 and 4,6-diamidino-2-phenylindole (DAPI). By binding in the minor groove of duplex DNA, compounds are able to interfere with processes vital to a cell's survival, e.g. replication and transcription, by competing with proteins such as transcription factors and topoisomerases.

Hoechst 33258 (National Cancer Institute (NCI) entry number 322921) (Fig. 1) is a head-to-tail *bis*-benzimidazole compound that specifically recognises three consecutive A:T base pairs in the minor groove of B-form DNA [4,5]. This compound was shown to possess activity against L1210 murine leukaemia [6] and several positive studies in various solid tumours led to the compound being entered into phase I clinical trials in humans [7]. However, a subsequent phase II trial against pancreatic carcinoma showed little response [8] and, to date, no more trials have been reported.

We hypothesised that the novel head-to-head *bis*-benzimidazole arrangement would be capable of binding to four consecutive A:T base pairs which would increase the extent of minor groove recognition. The structures of two of these compounds (ABA13 and ABA833) are shown in Fig. 1 and they have been shown

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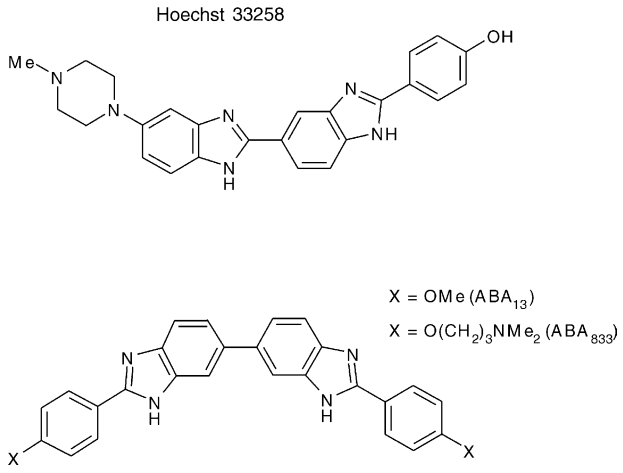

Fig. 1. Chemical structure of Hoechst 33258, ABA13 and ABA833.

by DNA footprinting and X-ray crystallographic methods to bind to DNA in the manner expected [5]. Preliminary studies revealed that these compounds show antiproliferative activity towards a panel of ovarian cell lines [9] with IC_{50} values in the low μM region. Both compounds also showed *in vivo* activity as assessed by the NCI human tumour xenograph hollow fibre assay [9].

In this study, we have investigated the antiproliferative activity of the methoxy (ABA13) and dimethylamine (ABA833) *bis*-benzimidazole derivatives against a wide range of tumour cell lines and assessed their ability to act as inhibitors of topoisomerases I and II (topo I and II).

2. Materials and methods

2.1. Cell Lines

All cell lines were maintained at 37 °C/5% CO_2 in Roswell Park Memorial Institute (RPMI) 1640 medium with 2 mM L-glutamine that had been supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin solution (100 IU/ml pen., 100 μg/ml strep.).

2.2. In vitro *drug sensitivity*

ABA13 and ABA833 were dissolved in dimethyl sulphoxide (DMSO) to yield stock solutions of 10 mM and stored, wrapped in foil, at 4 °C for up to 1 month. The fraction of viable cells remaining after drug treatment was determined by the ability of the cells to metabolise the water-soluble tetrazolium salt, 3-[4,5-dimethylthiazol-2-yl] 2,5 diphenyl tetrazolium bromide (MTT), into a water-insoluble formazan precipitate. Exponentially growing cells were seeded 24 h prior to treatment into sterile flat-bottomed 96-well plates, at varied seeding densities determined by their growth characteristics and the duration of assay. Cells were exposed to serial dilutions of ABA13 or ABA833 for 24, 72 or 96 h. MTT (50 μl of a 2-mg/ml solution) was then added to each well. The plates were incubated for a further 4 h and then the medium and any unconverted MTT was aspirated from the wells. The remaining formazan precipitate was dissolved in 100 μl DMSO and the absorbance read at 570 nm using a Molecular Devices plate reader. IC_{50} values (concentration of compound that produces a 50% reduction in the growth of the cells) were determined from plots of absorbance vs drug concentration. The effect of exposure time on the anti-proliferative effects of ABA13 and ABA833 was investigated by additionally exposing MCF-7, RKO and H630 cells to the drugs for 24 or 72 h.

2.3. Flow cytometry

Cells were seeded into 25-cm^2 flasks, allowed to adhere overnight and then the medium removed and replaced with fresh medium containing varying concentrations of the compounds. Following a 96-h time period, the medium from the flasks was collected and kept on ice and the monolayer of cells detached from the flask by trypsinisation. Cells were resuspended in the medium poured off previously and then pelleted by centrifugation at 1000 rpm for 5 min. The cells were washed twice with 1% FBS/phosphate-buffered saline (PBS) followed by fixation with ice-cold 70% ethanol and storage at −20 °C overnight. Cells were pelleted and washed twice with 1% FBS/PBS and the resultant cell pellet was resuspended in 1 ml PBS and 20 μl RnaseA (10 mg/ml) and 40 μl propidium iodide (2.5 mg/ml) were added. Samples were then incubated at 37 °C for 30 min and then analysed on the Beckman Coulter Epics XL flow cytometer.

2.4. Drug uptake and intracellular localisation

The intracellular localisation of ABA13 and ABA833 in RKO cells was determined by growing cells overnight on sterile coverslips in six-well plates (5×10^4 cells per well). Drugs were added at a final concentration of 100 nM and the incubation carried out for 20 min at 37 °C. Medium was removed from the coverslips and cells washed twice with PBS. Coverslips were then mounted onto slides and viewed under blue light using a Leica DMLB fluorescent microscope.

The time course of drug uptake was determined using MCF-7, MCF-7-adr breast carcinoma cell lines and the RKO colon carcinoma cell line. Cells were trypsinised, resuspended in fresh RPMI 1640 medium and 5×10^5 cells/ml added to a 75-mm tube. The average fluorescence of 10 000 cells was analysed using a Beckman Coulter FACScan flow cytometer. Gating was carried out on these cells and the settings kept the same throughout the experiment to ensure the fluorescence being measured is that of the cells and not any drug

present in the medium. ABA13 or ABA833 was added to the tube to a final concentration of 100 nM at 37 °C and the tube returned to the flow cytometer. The sample was agitated constantly and the change in the mean fluorescence of the cells was measured over a 20-min time interval.

2.5. Kinetoplast DNA decatenation assays

The compounds were also assayed for catalytic inhibition of topo IIα decatenation using kinetoplast DNA (kDNA) as a substrate [10]. This assay was carried out using a kit from TopoGEN (Columbus, OH, USA) according to the manufacturer's instructions with some modifications. Decatenation mixtures (21 μl) were prepared using 2 μl 10× topo II α assay buffer (1× = 50 mM Tris–HCL, pH 8; 120 mM NaCl; 10 mM $MgCl_2$; 0.5 mM adenosine triphosphate (ATP) (added prior to each assay); 0.5 mM dithiothreitol (DTT), 30 μg/ml bovine serum albumin (BSA)), 0.175 μg kDNA, 1 unit topo II and 1 μl drug at the indicated final concentrations. Reaction mixtures were incubated for 30 min at 37 °C then terminated by the addition of 4 μl stop buffer/gel loading dye (5% Sarkosyl, 0.0025% bromophenol blue, 25% glycerol). Reaction products were analysed on a 1% agarose gel containing 0.5 μg/ml ethidium bromide at 100 V. The DNA was visualised under ultraviolet (UV) light and photographed.

2.6. Inhibition of topo I and II: DNA relaxation experiments

Compounds were assessed for their ability to act as topo I or topo IIα poisons using a conventional plasmid relaxation assay [11]. Supercoiled pKMp27 DNA (0.5 μg) was incubated with 4 units of human topo I or II (TopoGEN, Columbus, OH, USA), in relaxation buffer (50 mM Tris (pH 7.8), 50 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM ethylene diamine tetra acetic acid (EDTA)), in the presence of varying concentrations of the test compounds. Reactions were carried out at 37 °C for 1 h and then terminated by the addition of sodium dodecyl sulphate (SDS) to 0.25% and proteinase K to 250 μg/ml. DNA samples were then added to the electrophoresis dye mixture (3 μl) and electrophoresed in a 1% agarose gel containing ethidium bromide (1 μg/ml) at room temperature for 2 h at 120 V. Gels were washed and photographed under UV light.

3. Results

3.1. In vitro *drug sensitivities*

IC_{50} values for ABA13 and ABA833 following a 96-h exposure were determined in a panel of cell lines and the results are shown in Table 1. With the exception of the MCF-7 and SkBr3 cell lines, in which ABA13 and ABA833 exhibit similar potencies, IC_{50} values were consistently lower for ABA833. Three breast cancer cell lines (MCF-7, MDA 468 and ZR-75-1) and one lung cancer line (H838) showed the greatest sensitivity to ABA833. Cross-resistance was observed in the MCF-7 doxorubicin-resistant cell line (MCF-7adr) with resistance factors of 4.3 and 24.1 for ABA13 and ABA833, respectively. Cross-resistance was not observed in the H630 cell line that has been rendered resistant to 5-fluorouracil (5-FU).

Exposure of MCF-7, RKO and H630 cells to the drugs for 24, 72 or 96 h revealed that the antiproliferative response is time- as well as dose-dependent (Fig. 2). 24 h IC_{50} values were the highest, while near maximal effects had been achieved by 72 h.

As previously reported in Ref. [9], these compounds have also been evaluated by the NCI 60 cell line screen and subjected to COMPARE analysis. These data are reproduced in Table 2 and show that neither ABA13 or ABA833 share a pattern of growth inhibition with other anti-cancer agents, including known topisomerase inhibitors. The highest GI_{50} coefficients were 0.682 (ABA13) and 0.661 (ABA833) for MDR Rhod30.

Table 1
Anti-proliferative activity of bis-benzimidazole derivatives

Cell line	IC_{50} values (μM)	
	ABA833	ABA13
Breast		
MCF-7	0.058±0.007	0.075±0.012
MCF-7adr	1.400±0.2 (rf = 24.1)	0.319±0.01 (rf = 4.3)
MDA 468	0.064±0.009	0.389±0.034
ZR-75-1	0.081±0.009	0.192±0.024
SkBr3	0.341±0.003	0.377±0.005
Colon		
RKO	0.269±0.017	0.624±0.025
H630	0.415±0.05	0.898±0.062
R10	0.402±0.011	0.738±0.018
Ovarian		
OAW42	0.139±0.001	0.171±0.004
A2780	0.408±0.018	0.889±0.053
Lung		
H838	0.064±0.001	0.785±0.044
H157	0.346±0.03	0.830±0.057
Prostate		
PC3	0.398±0.017	0.755±0.017

IC_{50} values are the mean of at least three determinations, ±the standard error of the mean (SEM). Resistance factor (rf) is defined as IC_{50} MCF-7adr/IC_{50} MCF-7.

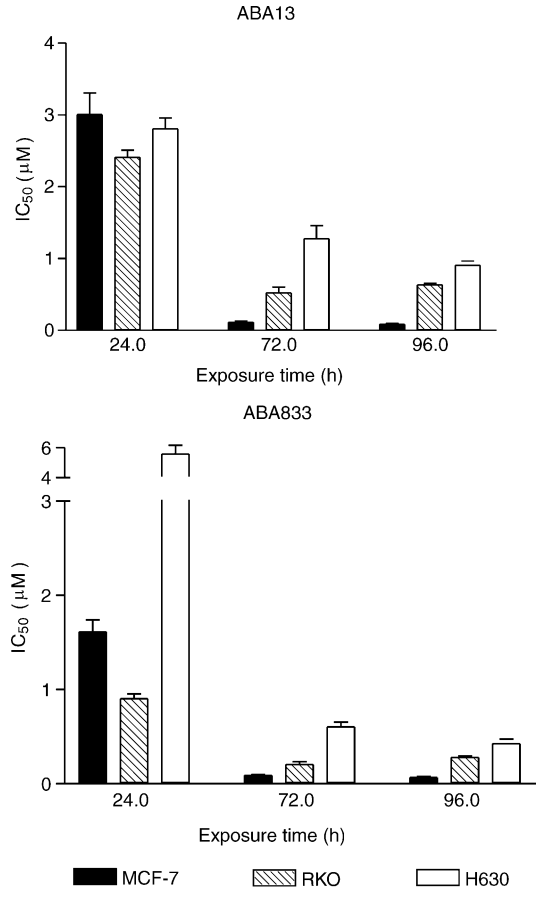

Fig. 2. The effect of exposure time on the IC_{50} values (μM) for inhibition of cell proliferation of MCF-7, RKO and H630 exposed to ABA13 or ABA833. Values are the mean and standard deviation (S.D.) of three determinations.

3.2. Cell-cycle blockade and induction of apoptosis

The effect of the compounds on cell cycle was elucidated by flow cytometry (Fig. 3). Both ABA13 and ABA833 produced a dose-dependent induction of apoptosis in the MDA 468 cell lines, as indicated by the increase in the pre-G1 cell population (Fig. 3a). The effects were more dramatic with ABA833, in line with its lower IC_{50} (Table 1). The increase in the sub-G1 population of MCF-7 cells was modest and only observed at the highest concentration. In both MCF-7 and MDA 468 cells, a S-phase block was also produced. With ABA13, very little effect was seen on the cell cycle of RKO cells at these concentrations, which were below the IC_{50} for this compound (Table 1). ABA833 did induce an increase in the pre-G1 population of RKO cells at a concentration (500 nM), approximately twice the IC_{50} (Table 1).

3.3. Drug uptake and intracellular localisation

As expected, ABA13 was predominantly localised in the nucleus of RKO colon carcinoma cells after 20-min exposure (Fig. 4). Similar results were obtained with ABA833 in RKO cells and with both drugs in MCF-7, MDA468 and H838 cells (data not shown).

Cellular uptake as detected by fluorescent activated cell sorting (FACS) analysis revealed that uptake of ABA13 and ABA833 by MCF-7 cells showed different kinetics (Fig. 5). Initial uptake of ABA833 was more rapid, and a near steady-state intracellular level had been achieved within 8–10 min of exposure (Fig. 5b). Whilst initial uptake of ABA13 was slower (Fig. 5a), intracellular levels continued to rise and by 20 min were approximately twice that of ABA833. Intracellular accumulation of both ABA13 and ABA833 was much reduced in the doxorubicin-resistant MCF-7 variant MCF-7adr and in RKO cells.

Table 2
NCI COMPARE analysis (GI_{50}) for ABA13 and ABA833

Drug	Mean panel log GI_{50}	Pearson correlation coefficient
COMPARE analysis: parent drug ABA13 NSC (699126)		
MDR Rhod30	0.00	0.682
Tamoxifen	−3.60	0.573
Asaley	−3.90	0.547
Pibenzimol	−2.70	0.534
Daunomycin	−4.30	0.530
Teroxirone	−2.60	0.525
BCNU	−3.30	0.525
Melphalan	−3.50	0.513
VM-26	−4.60	0.509
Doxorubicin	−4.60	0.499
Macbecin II	−3.30	0.493
COMPARE analysis: parent drug ABA833 NSC (699127)		
MDR Rhod30	0.00	0.661
Echinomycin	−4.12	0.578
Bruceantin	−3.70	0.472
Chronomycin A3	−4.00	0.464
Deoxydoxorubicin	−3.70	0.430
Pibenzimol	−2.70	0.427
Tetrocarcin	−4.30	0.411
Melphalan	−3.50	0.513
Vinblastine	−5.50	0.399
Actinomycin D	−6.60	0.357
Hexamethylene bisacetamide	−2.20	0.356

BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea.

3.4. Inhibition of topo I and topo IIα activity

Many DNA-binding agents exert their antitumour effect, at least in part, by inhibition of topo I (e.g. camptothecin) or topo II (e.g. doxorubicin). Hence, we examined the *bis*-benzimidazoles for their ability to interfere with the activity of these enzymes.

We first examined the ability of ABA13 and ABA833 to inhibit the decatenation of kinetoplast DNA (kDNA) by human topo IIα. Topo IIα alone produces topoisomers of nicked and circular (relaxed) DNA that are

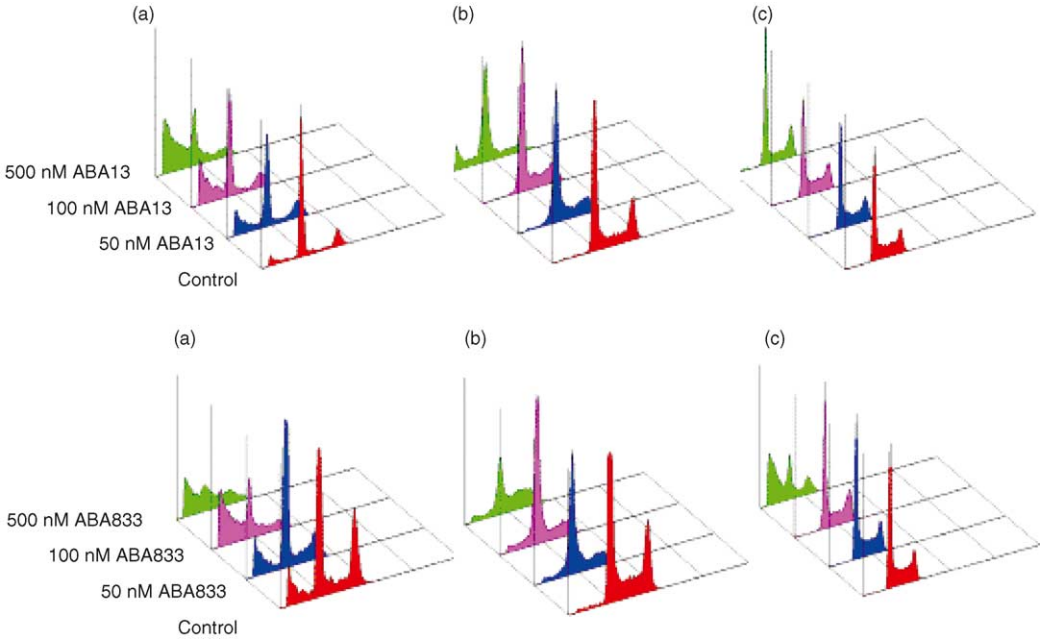

Fig. 3. Flow cytometric analysis of cells exposed to ABA13 and ABA833. Results shown are representative histograms from at least three experiments. Cells were treated for 96 h. (a) MDA 468 cells; (b) MCF-7 cells; (c) RKO cells.

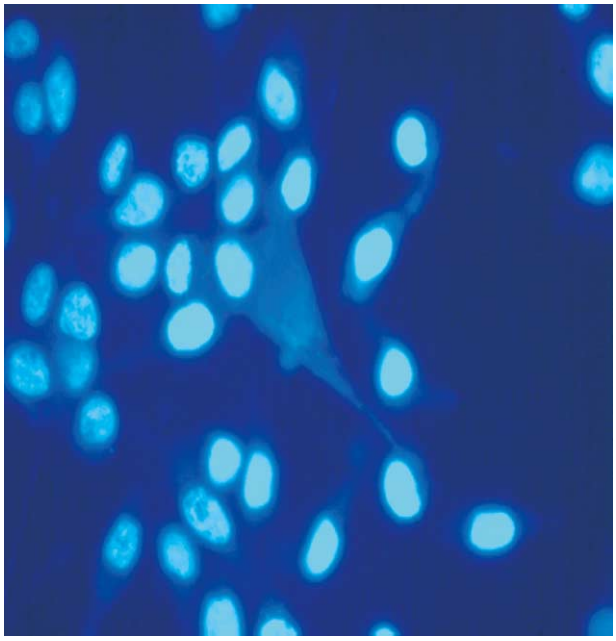

Fig. 4. RKO cells viewed under blue light using a Leica DMLB fluorescent microscope following incubation with ABA13 (100 nM) for 20 min at 37 °C.

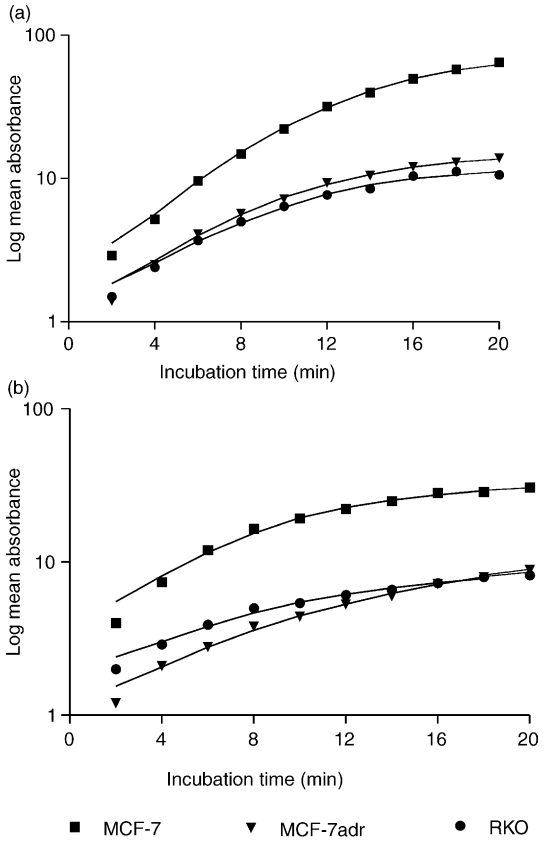

Fig. 5. Uptake of ABA13 (a) and ABA833 (b) (100 nM) by MCF-7, MCF-7adr and RKO cells at 37 °C over a 20-min period. Data are expressed as log mean absorbance versus time.

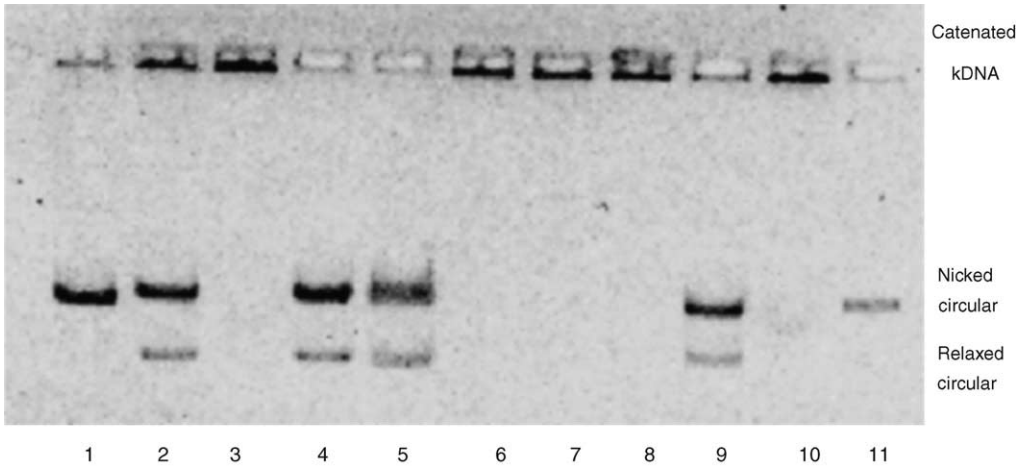

Fig. 6. Effect of the *bis*-benzimidazoles on the decatenation of kinetoplast DNA (kDNA) by topoisomerase II (topo II). Lane 1: linear DNA marker; lane 2: 10 μM camptothecin; lane 3: 10 μM doxorubicin; lane 4: control; lanes 5, 6 and 7: 500 nM, 5 μM and 25 μM of ABA13, respectively; lanes 8, 9, 10: 5 μM, 500 nM and 25 μM of ABA833, respectively; lane 11 = decatenated DNA marker.

resolved into two single bands by the inclusion of ethidium bromide in the agarose gel (Fig. 6, lane 4). An inhibitor of topo II catalytic activity (doxorubicin) completely inhibited the production of nicked and open circular DNA by topo II (Fig. 6, lane 3). The Topo I inhibitor, camptothecin, did not have this effect (Fig. 6 lane 2). 500 nM ABA13 or ABA833 had little effect on the ability of topo II to decatenate kDNA (Fig. 6, lanes 5 and 9), whilst 5 and 25 μM of ABA13 and ABA833 completely inhibited the ability of the enzyme to decatenated kDNA (Fig. 6, lanes 6 and 7 and 8 and 10, respectively).

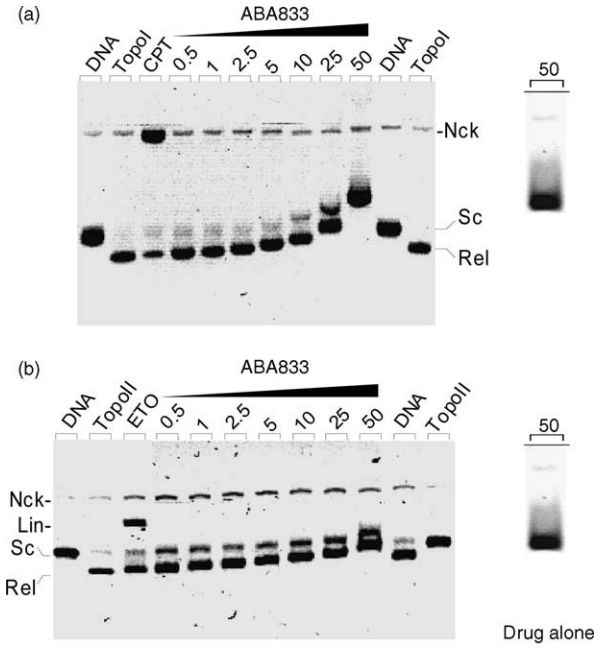

Fig. 7. The effect of ABA833 on the relaxation of plasmid DNA by human topoisomerases I (a) and II (b). Native supercoiled pKMp27 DNA (0.5 μg, lane DNA) was incubated with 4 units of topoisomerase in the absence (lane topo) or presence of ABA833 at the indicated concentration (μM). Camptothecin (CPT) and etoposide (ETO) were used at 20 μM. Also shown is DNA mobility shift induced by the highest concentration of drug in the absence of enzyme. Nck, nicked; Lin, linear; Rel, relaxed; Sc, supercoiled.

The effect of the two compounds on the catalytic activities of purified human topo I and II was investigated using a conventional plasmid DNA relaxation assay [11]. As shown in Fig. 7(a), ABA833 (500 nM–50 μM) alters the electrophoretic mobility of the DNA, but does not poison topoisomerase I, since in contrast to the alkaloid camptothecin, used as a positive control, ABA833 does not promote DNA cleavage by the enzyme. The amount of nicked DNA molecules remains minimal indicating that the drug does not stabilise topo I–DNA covalent complexes. Electrophoretic mobility shift by ABA833 was similar in the absence of enzyme and again there was no evidence of DNA cleavage. Similarly, ABA13 did not affect the catalytic activity of topo I (data not shown). Fig. 7(b) shows that treatment with the known topo II poison, etoposide, results in the production of linear DNA which demonstrates that etoposide stabilises DNA–topo II covalent complexes and hence stimulates double-strand cleavage by the enzyme. Conversely, with ABA833 no band corresponding to linear DNA is detected in the presence or absence of enzyme, implying that this compound does not act as a topo II poison. Treatment with ABA833 did result in a decrease in the amount of relaxed DNA compared with enzyme alone. This suggests the compound does interfere with the catalytic activity of the compound. Similar results were obtained with ABA13 (data not shown).

4. Discussion

We have shown that the novel *bis*-benzimidazole ligand, ABA13 and ABA833, exhibit potent anti-

proliferative effects *in vitro* against a variety of cell lines of different tumour origin (Table 1 and Fig. 2). Some selectivity for breast cancer cell lines (especially MCF-7) is apparent and in most of the cell lines tested, the dimethylamine derivative, ABA833, is more potent than the methoxy compound, ABA13. Differential activity ranged from 2- to 6-fold, although the two compounds were approximately equitoxic in MCF-7, SkBr3 (breast) and OAW42 (ovarian) cells (Table 1). ABA833 was previously reported to be the most potent of the two *bis*-benzimidazoles in a panel of ovarian cancer cell lines [5,9]. This cellular selectivity and higher potency of ABA833 cannot be explained by differences in intracellular drug levels achieved over a 20-min incubation period, at least in MCF-7, MCF-7adr and RKO cells. Intracellular levels of ABA13 exceeded those of ABA833 in MCF-7 cells (Fig. 5), although the compounds were approximately equipotent (Table 1). Uptake of ABA13 was also greater than that of ABA833 in both MCF-7adr and RKO cells, although RKO cells were 2-fold less sensitive to ABA13 than ABA833, whilst in MCF-adr this difference was 6-fold.

Both compounds demonstrated cross-resistance to doxorubicin using the MCF-7adr cell line which overexpresses P-glycoprotein and displays the Multiple Drug Resistance (MDR) phenotype. The reduced intracellular accumulation of ABA13 and ABA833 in this cell line compared with the parental MCF-7 line would be consistent with an enhanced drug efflux via the P-glycoprotein-associated transport mechanism. Reduced nuclear binding of Hoechst 33258 and 33342 in cells displaying the MDR phenotype has previously been described in Ref. [12]. It is probable, therefore, that the reduced uptake for ABA13 and ABA833 described here also reflects a reduced nuclear accumulation. This would be supported by the observation of rapid nuclear localisation by fluorescent microscopy (Fig. 4), which in turn would be predicted from the known DNA binding properties of the drugs. However, whilst the degree of reduction in drug accumulation in MCF-7adr cells was similar for both compounds (Fig. 5), the resistance factor for ABA833 (24.1) was far higher than for ABA13 (4.3) (Table 1). Differences in drug accumulation could explain the differential sensitivity of MCF-7 and MCF-7adr cells to ABA13, but for ABA833, additional factors must be involved. Reduced drug accumulation by RKO cells does correlate with reduced sensitivity in comparison with MCF-7 cells, although RKO cells do not overexpress P-glycoprotein [13].

The higher potency of ABA833 compared with ABA13 is most probably due to its higher affinity for the minor groove of DNA [5,9], reinforcing the belief that it is the DNA binding property of the compounds that confers on them their antitumour activity. In support of this, in an *in vitro* assay both ABA13 and ABA833 were shown to specifically inhibit transcription at a number of A:T sites [5] The fact that ABA833 exhibits a much greater potency than ABA13 for this inhibition of transcription implies their mechanism of action may be partly related to this property. It is possible that the enhanced uptake of ABA13 by MCF-7 cells (Fig. 5) may, in part, compensate for its lower affinity for the DNA minor groove allowing a similar potency to ABA833 in this cell line.

Topo I and II are the molecular targets for a variety of clinically used chemotherapeutic agents [14–16]. DNA topoisomerases are nuclear enzymes capable of resolving the topology of DNA to allow its replication. They do this by covalently binding to DNA to form a topo-DNA cleavable complex as an intermediate [17]. The enzyme then produces a single (in the case of topo I) or a double (in the case of topo II) strand-break in the DNA through which it passes the double helix and finally religates the break [18]. Successive cycles alter the topological state of the double helix sufficiently to allow DNA replication and transcription. Many antitumour agents poison topoisomerases by stabilising this topo–DNA cleavable complex, thus shifting the reaction equilibrium towards cleavage, which leads ultimately to cell death. Topoisomerase poisons include camptothecin, doxorubicin and etoposide. There are also biologically active agents that have been shown to inhibit topo I and II activities without stabilising the cleavable complex, such as merbarone and the quinobenoxazines [19]. These compounds inhibit the catalytic activity of the topoisomerase at a step prior to the formation of the topo–DNA complex. We set out to further elucidate the mode of action of these *bis*-benzimidazoles and demonstrated that, whilst they do not act as topo I or II poisons, they are capable of inhibiting the catalytic activity of both topo I and topo IIα (Figs. 6 and 7). In the NCI COMPARE analysis, ABA13 and ABA833 did not share a pattern of growth inhibition with other reported anticancer agents, including known topoisomerase inhibitors (Table 2) suggesting this may not be their sole mechanism of action. The cell-cycle effects of these compounds include induction of a pre-G1 population (indicative of an apoptotic response) and an S phase block (Fig. 3). The major cell-cycle effect of topo I and II inhibitors is a G2/M block [12,20,21], again supportive of the thesis that these novel *bis*-benzimidazoles have a mechanism of action distinct from classical topo inhibitors. It is likely that the ability of ABA13 and ABA833 to inhibit the activity of topo I and II that we have observed (Figs. 6 and 7) is simply due to blockade of the binding of these enzymes to DNA. It may be a combination of this antitopoisomerase effect and their previously shown ability to inhibit transcription that enables them to potently inhibit cellular proliferation. The nature of the specific site(s) of transcriptional activity affected remains to be determined. It has also been reported that molecules in the head-to-head *bis*-

benzimidazole category may inhibit DNA helicase activity [22].

In conclusion, these novel *bis*-benzimidazoles inhibit the proliferation of a range of human tumour cell lines *in vitro*. Some selectivity towards breast cancer cell lines was observed, and expression of the MDR phenotype was associated with resistance, which could not be solely attributed to reduced drug uptake in the case of ABA833. They do not appear to act as classical topo I or II inhibitors, and their precise mechanism of action remains to be established. Head-to-head *bis*-benzimidazoles represent a novel family of DNA minor groove binders with therapeutic potential and additional structure–activity studies seem warranted.

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