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Original article

DNA-targeting pyrroloquinoline-linked butenone and chalcones: Synthesis and biological evaluation

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ABSTRACT

A series of conjugates of α,β-unsaturated ketone systems, phenyl-butenone and diaryl-propenones (chalcones), with the tricyclic planar pyrroloquinoline nucleus were synthesised and evaluated for their anticancer properties. The aim was to target DNA by butenone and chalcones, and determine the occurrence of interactions with the macromolecule or related functional enzymes. The ability to inhibit cell growth was assayed on three human tumor cell lines, and the capacity to form molecular complexes with DNA was studied by linear flow dichroism (LD). The effect on the activity of the nuclear enzyme DNA topoisomerase II was also investigated.

A noticeable cytotoxic effect was observed for all pyrroloquinoline-conjugated compounds **5** and **7a–c**, particularly against human melanoma cell line JR8 (IC_{50} 1.2–3.3 μM); the unconjugated chalcones (**8a–c**) and **butenone** had a lower or no effect at the tested concentrations. LD experiments confirmed the pyrroloquinoline nucleus as an efficacious carrier for intercalative complexation with DNA. The ability of pyrroloquinoline derivatives to intercalate between base pairs appears to inhibit the relaxation of supercoiled DNA by topoisomerase II, while they induce no significant DNA cleavage. Since the concentrations inhibiting the enzyme appear relatively high with respect to cytotoxicity, the effective intercalation could affect the activity of more DNA processing enzymes and these overall nuclear effects may induce cell death.

1. Introduction

Diaryl-propenone (chalcone) and aryl-butenone derivatives are an important class of natural and synthetic products, which possess a number of interesting biological activities including antimicrobial, antiviral, antimalarial, antileishmanial [1] and antioxidant actions [2]. Butenone and chalcone derivatives have also been shown to exhibit *in vitro* and *in vivo* antitumor activities [3–6] and capacity to inhibit carcinogenesis induced by chemical agents through enhancement of reduced glutathione levels [7,8]. Structural requirements for their cytotoxic activity vary according to the mechanism of action, although the exact mechanism still remains to be established [9]. One advantage of anticancer chalcones and butenones is that they may be free from the problems of mutagenicity and carcinogenicity that are associated with many alkylating agents used in cancer chemotherapy, such as chlorambucil and melphalan [10,11].

Recently, in the area of alkylating drugs, DNA-targeted agents consisting of a polycyclic planar moiety linked to an alkyl or aniline mustard have been shown to be less toxic and more active than clinically useful simple mustards [12–18]. Alkylating pyrroloquinolines also appear to be more active than the reference nitrogen mustards in inducing antiproliferative activity on tested human tumor cell lines [19], confirming the fact that the pyrroloquinoline nucleus (Fig. 1) is a good scaffold or carrier which increases the DNA affinity of reactive molecular groups. Indeed, in these conjugated molecules, the pyrroloquinoline moiety bridged with an aniline ring has larger planarity than pyrroloquinoline alone, and is able to intercalate into double-strand DNA.

On the basis of the above findings, it was of interest to prepare some pyrroloquinoline-linked chalcones having an α,β-unsaturated carbonyl function, which may be regarded as alkylating agents due to the ability of α,β-unsaturated ketones to undergo addition reactions (Michael-type addition) with such biologically important nucleophiles as amines and thiols (Fig. 1) [20,21]. We synthesised one conjugated pyrroloquinoline-phenyl-butenone and some conjugated pyrroloquinoline-diaryl-propenones (chalcones). The ability to inhibit cell growth was evaluated on three human tumor cell lines (HL-60, HeLa, JR8). Interaction with DNA was studied by

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linear flow dichroism experiments and the effect on DNA topoisomerase II activity was examined.

2. Results and discussion

2.1. Chemistry

The general strategy for synthesising new α,β-unsaturated ketones, both butenone derivative **5** and chalcones **7a–c**, characterised by conjugation with the pyrroloquinoline nucleus, was the following:

- synthesis of 9-chloro-pyrrolo[3,2-*f*]quinoline,
- linking of aniline carbonyl (formyl or acetyl) derivatives,
- conventional Claisen–Schmidt aldol condensation to form final conjugated α,β-unsaturated keto compounds.

Scheme 1 shows the synthesis of butenone derivative **5**, for which 9-chloro-pyrroloquinoline **1**, prepared by a multi-step synthesis starting from 5-nitro-indole as previously described [22], was reacted with *p*-dimethoxymethyl-aniline **3** to afford formyl compound **4** by means of an acid-catalysed nucleophilic aromatic displacement reaction. Compound **3** was obtained starting from commercial *p*-nitro-benzaldehyde, which was protected as dimethyl-acetal **2** and then reduced by H_2 and Pd/C 10% as catalyst. Final **5**, in which the tricyclic nucleus is linked to phenyl-butenone through an amine bridge, resulted from Claisen–Schmidt condensation between formyl derivative **4** and acetone in alkaline medium (NaOH 50% w/v) [23]. Compound **5** had an *E*-configuration as confirmed by the coupling constants of 16.3 Hz of doublets at δ 6.66 and 7.57 for α-H and β-H, respectively. As reported by chalcone literature, to yield only *E*-stereoisomers is a prerogative of the Claisen–Schmidt condensation reaction [24].

In Scheme 2, the strategy for synthesis of chalcone derivatives **7a–c** was slightly different with respect to that described above. In this case, nucleophilic substitution of 9-chloro-pyrrolo[3,2-*f*]quinoline **1** [22] with *p*-amino-acetophenone in the presence of HCl as catalyst afforded acetyl-aniline compound **6** [25] as mono-hydrochloride, as the α,β-unsaturated ketone function was in reverse order. The latter furnished final compounds **7a–c** when submitted to Claisen–Schmidt condensation with the appropriate aromatic aldehyde (**a–c**). These aldehydes (pyrrolyl-2-carbaldehyde, thienyl-2-carbaldehyde, *p*-nitro-benzaldehyde) were chosen to give conjugates in which the chalcone moieties were representative of biologically interesting ones [26,27]. Simple similar arylchalcones **8a–c** where chosen as reference compounds, **8a**, **b** were obtained by condensation between acetophenone and aldehydes 2-formyl-pyrrole and 2-formyl-thiophene, and **8c** ((*E*)-3-(4-nitro-phenyl)-1-phenylprop-2-en-1-one) was obtained commercially (Scheme 3).

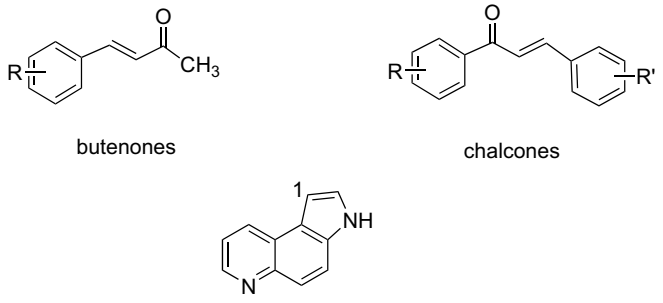


Fig. 1.

The *E*-stereochemistry of the newly synthesised compounds described in Schemes 2 and 3, was assigned by comparing the chemical shifts and coupling constants of olefinic protons with those of similar chalcones described in literature [26,27]. In all cases, both α- and β-proton signals or at least one of them, were clearly distinguishable at chemical shifts in the ranges 7.35–7.75 and 7.85–8.10 δ, with coupling constants ranging from 15 to 16.03 Hz.

2.2. Antiproliferative activity

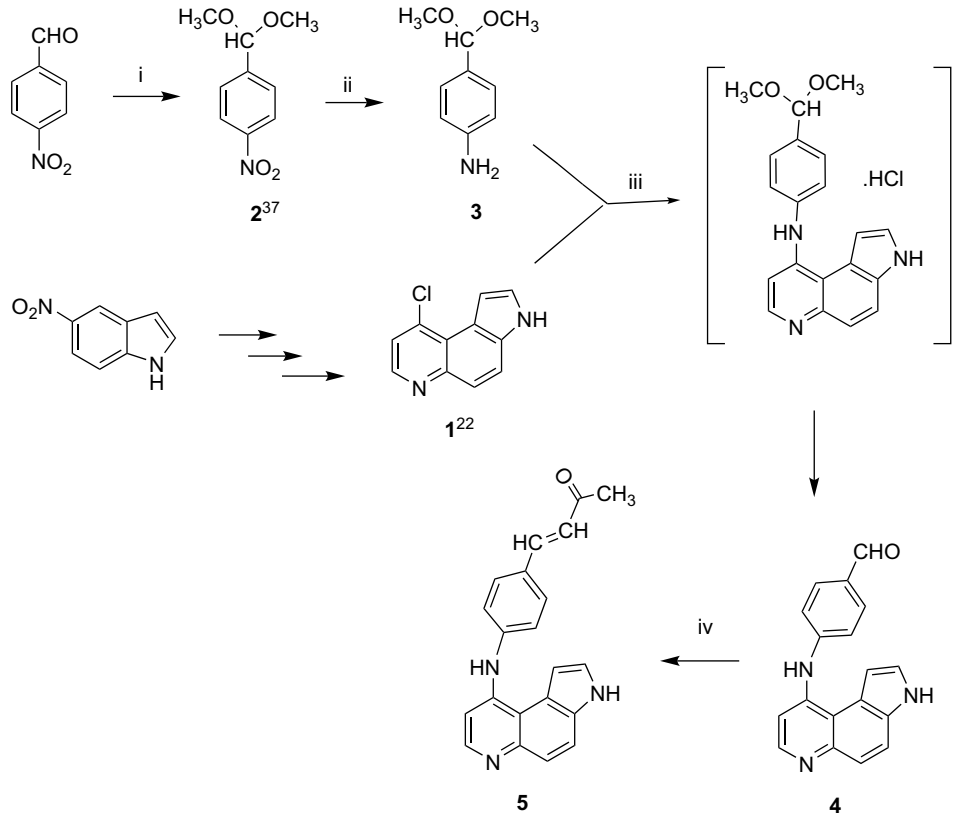
The antiproliferative activity of butenone derivative **5**, chalcones' derivatives **7a–c**, **8a–c** and the known *E*-1-(4′-hydroxyphenyl)but-1-en-3-one [28], simply named **butenone**, was evaluated by an *in vitro* assay on three human tumor cell lines: HL-60 (human myeloid leukemic cells), HeLa (human cervix adenocarcinoma cells) and JR8 (human melanoma cells). The well-known drug ellipticine was taken as reference compound. Results are expressed as IC_{50} values, i.e. the concentration (μM) of compound able to cause 50% of cell death with respect to the control culture, and are listed in Table 1.

Data indicate that both pyrroloquinoline-phenyl-butenone **5** and pyrroloquinoline-diaryl-propenones **7a–c** exert a definite antiproliferative effect on test cell lines, with IC_{50} values in the micromolar range. In particular, JR8 are the most sensitive cells (IC_{50} 1.2–3.3 μM) and, interestingly, their IC_{50} values are comparable to that of ellipticine. On HeLa (IC_{50} 5.3–18.1 μM) and HL-60 (IC_{50} 2.3–5.6 μM) cells, the inhibitory effect was less pronounced and actually lower than that of the reference drug.

Among simple diaryl-propenones **8a–c**, only compound **8c** induces a detectable antiproliferative effect on the most sensitive JR8 and HL-60 cell lines (IC_{50} 6.0 and 7.8 μM, respectively). For both **8a** and **b**, no inhibition was shown up to 20 μM concentration, in the same experimental conditions. These distinct activity profiles are probably due to the specific molecular structure of **8c**, in which the *p*-NO_2-phenyl moiety affects the reactivity of the α,β-unsaturated function, making it more reactive towards addition reactions of nucleophilic groups in the biological environment [20,21]. Lastly, **butenone** was ineffective on all test cell lines.

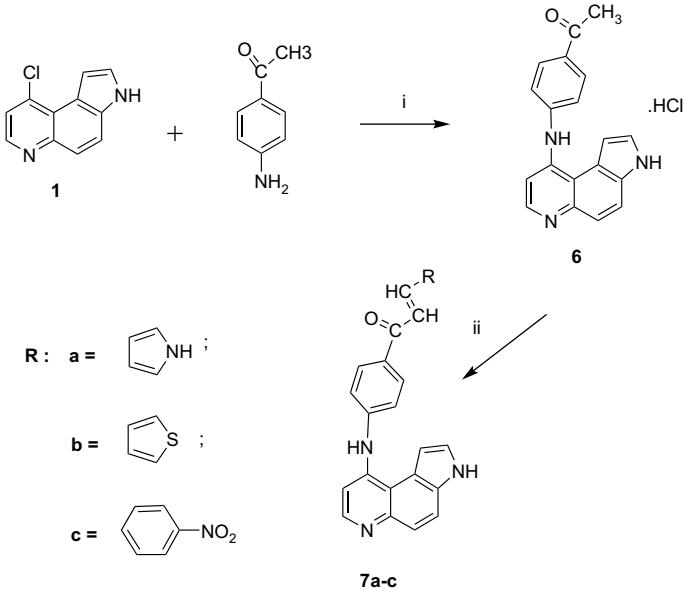
2.3. Interaction with DNA

A previous study, performed on pyrroloquinoline derivatives characterised by an aniline bifunctional mustard linked to the planar tricyclic nucleus, demonstrated the occurrence of an intercalative molecular complex with DNA. Interestingly, the capacity of these compounds to interact with the macromolecule was accompanied by notable enhancement of antiproliferative activity with respect to the alkylating aniline bifunctional mustard [19]. On the basis of these results, we studied the capacity of the new pyrroloquinoline-linked butenone and chalcones to give rise to molecular complexes with the macromolecule. For this purpose, solutions of salmon testes DNA in the presence of **5**, **7a–c** and of chalcones **8a–c** were analyzed by linear flow dichroism (LD). Fig. 2A shows the LD spectra of the macromolecule alone (line a), and of derivatives **5**, **7c** and **8c** (lines b–d, respectively). The DNA spectrum shows the well-known negative dichroic signal at 260 nm, the wavelength at which the macromolecule chromophores absorb (line a) [29,30]. In the spectra obtained after incubation with **5** and **7c** (lines b and c), a further negative signal was observed at higher wavelengths (300–450 nm), where only the chromophore of the added compound absorbs. Similar spectral trends were also obtained for **7a** and **b** (results not shown). Since the small pyrroloquinoline-linked butenone and chalcones cannot become oriented in the flow field, the LD signal which appears at 300–450 nm must be attributed to the formation of a molecular complex



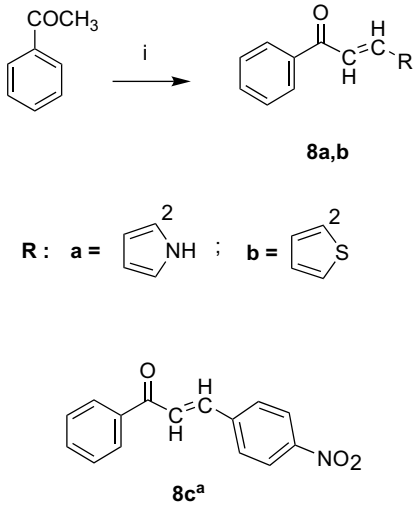
Scheme 1. Reagents and conditions. i: HCl dry gas, anhydrous methanol, refl., 71%; ii: H_2, Pd/C 5%, ethanol, rt, 93%; iii: HCl, methanol, 70°C, 21%; iv: NaOH 50% w/v, methanol, rt, 56%.

with DNA. Their negative signals also indicate that the orientation of the molecular plane of the chromophore is preferentially parallel to the plane of DNA bases [31]. Thus, it may be concluded that complexation with the macromolecule occurs through an intercalative mode of binding of **5** and **7a–c**. In addition, the significant difference in the extent of the induced dichroic signals of lines b and c suggests complexation capacity better for **5** than for **7c**.



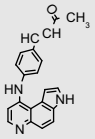
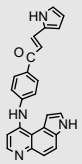
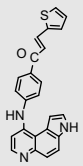
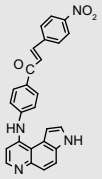
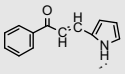
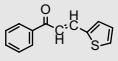
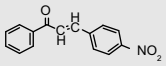
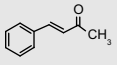
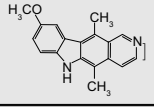
Scheme 2. Reagents and conditions. i: HCl, metahnol, 70°C, 23%; ii: 2-formyl-pyrrole or 2-formyl-thiophene or 4-nitro-benzaldehyde, NaOH 50% w/v, methanol, rt, 17% for 7a, 15% for 7b and 13% for 7c, respectively.

Fig. 2B shows the LD spectra of **5** (line b) and of the drugs ellipticine (line c) and *m*-AMSA (line d), whose intercalative capacity has already been demonstrated by linear dichroism techniques [32,33]. Comparisons between the LD behaviour of compound **5** with those of the two known intercalators confirm the notable capacity of the new pyrroloquinoline conjugate to intercalate between base pairs. Otherwise, the spectrum of **8c** in Fig. 2A (line d) practically overlaps with that of DNA (line a), and the same behaviour was also observed for **8a**, **b** and **butenone** (spectra not shown), indicating the inability of the simple phenyl-butenone and chalcones to form molecular complexes with the macromolecule.



Scheme 3. Reagents and conditions. i: NaOH 50% w/v, methanol, 2-formyl-pyrrole or 2-formyltiophene, rt, 48% for 8a and 43% for 8, respectively. a) From Aldrich

Table 1
Cell growth inhibition in presence of examined compounds.

Compd	Cell lines IC_{50} (μM)[a]			
	Structure	HL-60	HeLa	JR8
5		2.3 ± 0.1	7.0 ± 0.1	1.6 ± 0.2
7a		5.6 ± 0.8	18.1 ± 0.8	3.3 ± 0.2
7b		3.2 ± 0.3	5.3 ± 0.6	2.1 ± 0.2
7c		4.4 ± 0.5	10.4 ± 0.7	1.2 ± 0.2
8a		>20	>20	>20
8b		>20	>20	>20
8c[b]		6.0 ± 0.4	>20	7.8 ± 0.5
Butenone[b]		>20	>20	>20
Ellipticine[c]		0.66 ± 0.02[c]	0.29 ± 0.02[c]	1.1 ± 0.1

[a] Results are reported as means ± standard error (M ± S.E.).
[b] Purchased from Aldrich.
[c] Ref. [41].

2.4. Effect on DNA topoisomerase II activity

DNA topoisomerase II is an enzyme that catalyses changes in DNA topology during essential nuclear functions such as replication and transcription. The key role of this enzyme in cell proliferation makes it an important target for anticancer strategies, and a wide range of antitumor drugs has been shown to influence its catalytic cycle

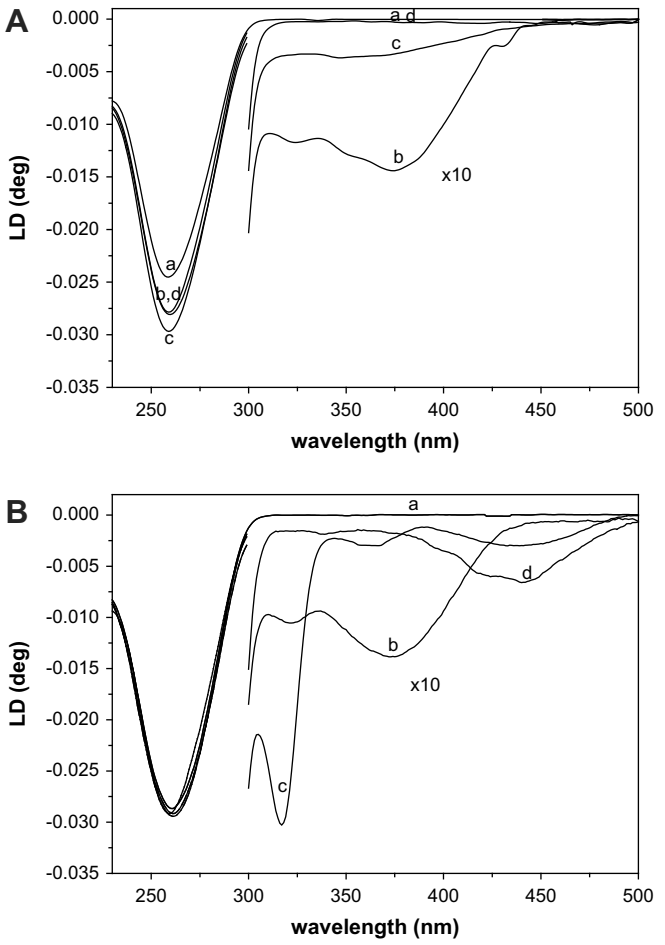


Fig. 2. Linear flow dichroism spectra for (A) DNA alone (line a) and in presence of compounds **5**, **7c** and **8c** (lines b–d), and (B) DNA alone (line a) and in presence of compound **5**, ellipticine and *m*-AMSA (lines b–d).

[34,35]. It has also already been demonstrated that chalcones and chalcone derivatives can interfere with a number of enzymatic activities [9,36]. In view of these considerations, and the fact that the new compounds show the ability to form intercalative complexes with the macromolecule, we examined their capacity to interfere with DNA topoisomerase II. Fig. 3A and B shows the effects of **5** and **7c** on the relaxation of supercoiled pBR322 DNA catalysed by the enzyme. In detail, both conjugates inhibit the enzyme in a dose-dependent manner. Nevertheless, **7c** was more active than **5**, as it completely abolished catalytic activity at 20 μM, a concentration at which **5** was able to exert only a weak effect. For comparison, Fig. 3C shows the effects of **5**, **7c** and **8c**, at the same concentrations. It reveals significant differences between the tested compounds and, in particular, **8c** appears unable to inhibit the enzyme (Fig. 3C). The latter result matches LD data (Fig. 2A) which indicate the inability of **8c** to intercalate. For the cytotoxic **8c**, different cellular target(s) may be suggested, i.e., interactions with other enzymatic proteins, or tubulin polymerisation, likewise to other chalcones [9,28].

Conversely, treatment of supercoiled pBR322 DNA substrate with 50 μM **5** in the absence of the enzyme does induce a gel shift, arising from an alteration in the macromolecular structure, probably due to the effective intercalative process, as demonstrated on salmon testes DNA by linear flow dichroism analysis (see Fig. 2). As regards derivative **7c**, its capacity to inhibit topoisomerase II is already evident at 10 μM. Nevertheless, it should be emphasised that, in this case, the derivative does not perturb

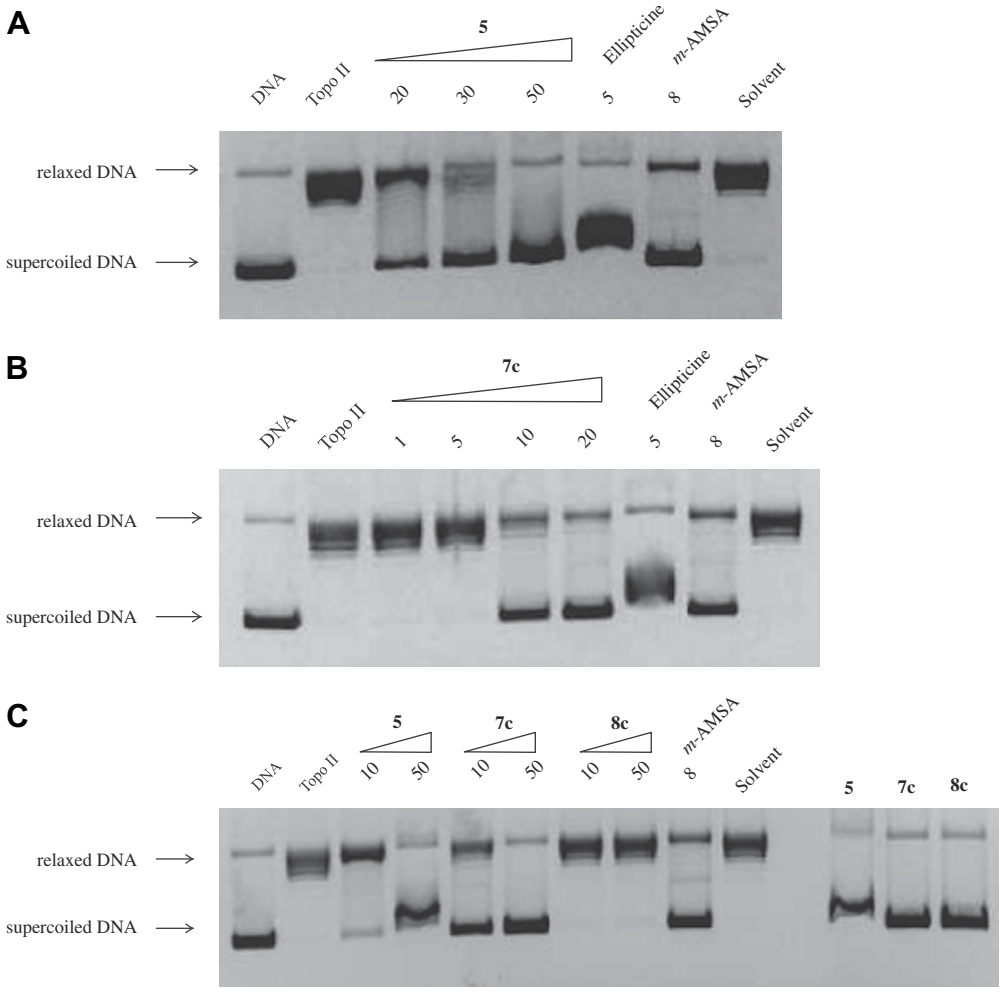


Fig. 3. Effect of compounds **5** (A), **7c** (B) and **8c** (C) on relaxation of supercoiled pBR322 DNA by human recombinant topoisomerase II. Supercoiled DNA (DNA) was incubated without or with topoisomerase II (topo II) in absence and presence of test compounds at indicated concentrations (μM). Solvent alone (Solvent), 5 μM ellipticine and 8 μM *m*-amsacrine (*m*-AMSA) were used as references. In lanes marked **5**, **7c**, and **8c** (C), DNA substrate was incubated with indicated compounds at 50 μM (no enzyme).

the electrophoretic migration of DNA and does in fact induce a dichroic signal lower than that of **5** (Fig. 2A). Topoisomerase II-directed agents can be divided into two classes: poisons, able to stabilise the covalent DNA topoisomerase II or cleavable complex, and inhibitors, which interfere with any of the other steps in the enzyme activity. The occurrence of the covalent complex can be demonstrated experimentally by enzyme-dependent formation of linear from supercoiled DNA. Fig. 4 shows a cleavable complex assay performed in the presence of increasing concentrations of **5** and **7c**, and *m*-AMSA was taken as reference compound. Both derivatives are unable to promote DNA cleavage up to 20 μM concentration, but at 50 μM a weak increase in intensity of the band corresponding to linear DNA appears. In contrast, exposure to 15 μM *m*-AMSA, induces the formation of a high amount of linear DNA.

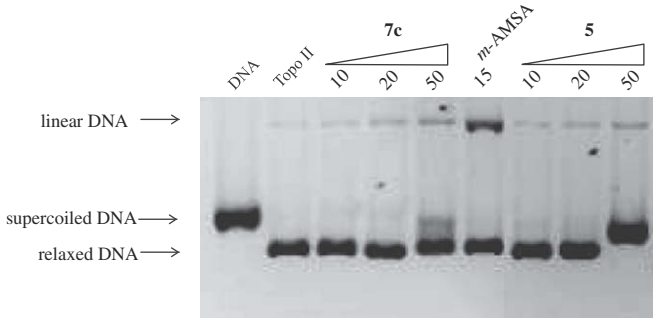


Fig. 4. Effect of compounds **5** and **7c** on the catalytic activity of human recombinant topoisomerase II. Supercoiled pBR322 DNA (DNA) was incubated without or with topoisomerase II (topo II) in absence and presence of test compounds at indicated concentration (μM) and 15 μM *m*-amsacrine (*m*-AMSA) as reference. The DNA cleavage products were separated by agarose gel electrophoresis in the presence of ethidium bromide.

3. Conclusions

Some conjugated structures were obtained by linking the planar pyrroloquinoline nucleus with a phenyl-butenone (**5**) and some diaryl-propenones (chalcones) (**7a**–**c**) through an amine bridge, in order to take advantage of the wider resulting molecular planarity to intercalate into the DNA macromolecule. The antiproliferative effects of the new compounds were investigated. Pyrroloquinoline conjugates **5** and **7a**–**c** show a cytotoxic effect at micromolar concentrations on test tumor cell lines (Table 1). Particularly against human melanoma JR8 cells, the IC_{50} values are comparable to that of ellipticine (IC_{50} 1.2–3.3 μM). LD experiments confirm that pyrroloquinoline nucleus is a good carrier, allowing an effective intercalative process of all conjugated molecules.

The performed experiments on topoisomerase II demonstrate the capacity of new compounds to inhibit the relaxation of supercoiled DNA, while no significant increase in cleavage complex was observed. As their inhibition concentration appears relatively high with respect to the cytotoxicity, we suggest the involvement of others cellular targets in the antiproliferative effect. In this connection, the effective insertion of the linked pyrroloquinolines **5** and **7a–c** between base pairs compromises topo II activity and may also compromise the activity of more DNA processing enzymes and these overall nuclear effects may lead to cell death.

Unconjugated chalcones (**8a**, **b**) and butenone appeared unable to induce any effect at the tested concentrations, whereas **8c** revealed a certain cytotoxic activity on the most sensitive cell lines (JR8 and HL-60). For the latter compound, neither the formation of a molecular complex with the macromolecule nor the inhibition of topoisomerase II was observed. Consequently, the weak cytotoxic effect of **8c** suggests a mechanism of action involving cellular target(s) other than DNA, as already proposed for a number of chalcones [9,28].

Enones are known to act biologically by multi-target mechanisms and also to display poor selectivity in the biological system. Actually, the latter is a drawback for using chalcones and related compounds in anticancer therapy. In this connection, the acquired DNA-targeting ability by the pyrroloquinoline nucleus may partly overcome the problem, indicating that our approach is a useful strategy worth developing in the near future.

4. Experimental section

4.1. Chemistry

Melting points were determined on a Gallenkamp MFB 595 010 M/B capillary melting point apparatus, and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 1760 FTIR spectrometer as potassium bromide pressed disks; values are expressed in cm^{-1}. UV–vis spectra were recorded on a Perkin–Elmer Lambda UV–vis spectrometer, and ^1H NMR and ^{13}C NMR spectra on a Bruker AMX spectrometer at 300.13 MHz for ^1H and 75.04 for ^{13}C, with the indicated solvents; chemical shifts are reported in δ (ppm) downfield from tetramethylsilane as internal reference. Coupling constant values are given in hertz. In the case of multiplets, the chemical shift quoted was measured from the approximate center. Integrals corresponded satisfactorily to those expected on the basis of compound structure. *E*-stereo assignments were made on the basis of chemical shift and coupling constant values. The real purity of *E*-enone derivatives was not evaluated.

Elemental analyses were performed in the Microanalytical Laboratory, Department of Pharmaceutical Sciences, University of Padova, with a Perkin–Elmer Elemental Analyzer Model 240B; results fell in the range $\pm$0.4% of theoretical values. High-resolution mass spectra were obtained with an Applied Biosystems Mariner System 5220 LC/Ms (nozzle potential 250.00). Column flash chromatography was carried out on Merck silica gel (250–400 mesh ASTM); reactions were monitored by analytical thin-layer chromatography (TLC) with Merck silica gel 60 F-254 glass plates. Solutions were concentrated in a rotary evaporator under reduced pressure. Starting materials for the syntheses shown in the schemes, (*E*)-1-(4′-hydroxyphenyl)but-3-en-2-one (**butenone**) and (*E*)-3-(4-nitro-phenyl)-1-phenylprop-2-en-1-one (**8c**) were purchased from Aldrich and Acros Organics.

4.1.1. 1-Dimethoxymethyl-4-nitro-benzene (**2**) [37]

A solution of 2.52 g (16.7 mmol) of *p*-nitro-benzaldehyde in anhydrous methanol (50 mL) was saturated with dry HCl gas and then refluxed for about 1 h. After cooling, 200 mL of a solution obtained by dissolving 15 g of Na_2CO_3 and 1.13 g of hydroxylamine hydrochloride in 300 mL of water, was added, and the mixture was stirred for 30 min at room temperature. It was then extracted with ethyl acetate, and the extracts, washed with 100 mL of NaOH 1 N in water and dried with anhydrous Na_2SO_4, were evaporated at 50 °C under reduced pressure to give a yellow liquid. Yield 71%; rf 0.51 (chloroform/*n*-hexane 1:1); ^1H NMR ($CDCl_3$) δ 3.32 (s, 6H, $2CH_3$), 5.48 (s, 1H, CH), 7.65 (d, 2H, $J_{2,3}$ and $J_{5,6} = 8.7$ Hz, 3-H and 5-H), 8.23 (d, 2H, $J_{2,3}$ and $J_{5,6} = 8.7$ Hz, 2-H and 6-H).

4.1.2. 4-Dimethoxymethyl-phenylamine (**3**)

A solution of 1-dimethoxymethyl-4-nitro-benzene (2.23 g, 11.31 mmol) in ethanol was dropped into a suspension of 10% Pd/C (0.125 g) saturated with H_2 in ethanol (250 mL). The mixture was stirred at room temperature and in hydrogen at atmospheric pressure for 8 h. The catalyst was filtered off and the solvent was evaporated under reduced pressure to give the corresponding amine derivative **5** as a yellow-green liquid. Yield 93%; rf 0.31 (chloroform/*n*-hexane 1:1); ^1H NMR (DMSO-d_6) δ 3.45 (s, 6H, $2CH_3$), 4.87 (bs, 2H, NH_2), 5.92 (s, 1H, CH), 6.58 (d, 2H, $J_{2,3}$ and $J_{5,6} = 8.6$ Hz, 2-H and 6-H), 6.95 (d, 2H, $J_{2,3}$ and $J_{5,6} = 8.6$ Hz, 3-H and 5-H).

4.1.3. 4-(3H-Pyrrolo[3,2-f]quinolin-9-ylamino)benzaldehyde (**4**)

A solution of 9-chloro-3*H*-pyrrolo[3,2-*f*]quinoline [22] (0.480 g, 2.39 mmol) in 50 mL methanol was added to HCl 37%, and then 4-dimethoxymethyl-phenylamine (0.360 g, 2.39 mmol) in 10 mL methanol was added dropwise under stirring. The mixture was heated to 70 °C for more than 30 h without the reaction ending. The reaction was therefore stopped and, after the solvent had evaporated, the residue was chromatographed by flash chromatography (ethyl acetate first and the ethyl acetate/methanol 7:2). A mixture of two end-products was recovered, composed of the dimethoxymethyl derivative and the desired formyl derivative **4**. This mixture was dissolved in methanol (50 mL), added to 10 mL H_2SO_4 2 N, and heated at refluxing for 30 min. After cooling, the solution was made alkaline with $NaHCO_3$ 10% and extracted with ethyl acetate. The extracts, washed with water and dried with anhydrous Na_2SO_4, were evaporated at 50 °C under reduced pressure to afford a yellow crystalline product which was recrystallised from ethanol. Yield 21%; rf 0.62 (ethyl acetate); mp 265–266 °C; ^1H NMR (DMSO-d_6) δ 6.97 (s, 1H, 1-H), 7.16 (d, 1H, $J_{2',3'}$ and $J_{5',6'} = 9.4$ Hz, 2′-H and 6′-H), 7.43 (m, 2H, 8-H and 2-H), 7.75 (m, 3H, 4-H, 3′-H, 5′-H), 7.90 (d, 1H, $J_{4,5} = 8.9$ Hz, 5-H), 8.65 (d, 1H, $J_{7,8} = 4.9$ Hz, 7-H), 9.18 (s, 1H, NH), 9.78 (s, 1H, CHO), 11.7 (s, 1H, pyrrole NH); HRMS *m*/*z* [MH^+] 288.1055.

4.1.4. (E)-4-(4′-(3H-Pyrrolo[3,2-f]quinolin-9-ylamino)phenyl)but-3-en-2-one (**5**)

Formyl derivative **4** (0.240 g, 0.8 mmol) was dissolved in 10 mL of acetone, and NaOH 1 N (5 mL) was added under stirring. After 30 min, water (50 mL) was added and the solution was extracted with ethyl acetate. The extracts, washed with water and dried with anhydrous Na_2SO_4, were evaporated at 50 °C under reduced pressure. The residue was purified by flash chromatography (ethyl acetate/methanol 7:3). Yield 56%; rf 0.6 (ethyl acetate/methanol 7:3); mp 267–269 °C; IR (KBr) 3430 (NH) 1665 (C=O), 1630 (CH=CH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.31 (s, 3H, CH_3), 6.66 (d, 1H, $J_{\alpha,\beta} = 16.30$ Hz, β-H), 7.06 (bs, 1H, 1-H), 7.19 (d, 2H, $J_{3',2'}$ and $J_{5',6'} = 8.6$ Hz, 3′-H and 5′-H), 7.37 (d, 1H, $J_{7,8} = 5.1$ Hz, 8-H), 7.47 (t, 1H, 2-H), 7.57 (d, 1H, $J_{\alpha,\beta} = 16.30$ Hz, α-H), 7.63 (d, 2H, $J_{2',3'}$ and $J_{6',5'} = 8.6$ Hz, 2′-H and 6′-H), 7.68 (d, 1H, $J_{4,5} = 9.0$ Hz, 4-H), 7.86 (d, 1H, $J_{4,5} = 9.0$ Hz, 5-H), 8.58 (d, 1H, $J_{7,8} = 5.1$ Hz, 7-H), 8.74 (bs, 1H, NH), 11.78 (bs, 1H, pyrrole NH); ^{13}C NMR (DMSO-d_6) δ 28 (CH_3), 106 (C-8), 111 (C-9a), 118 (C-2′ and C-6′), 118.5 (C-9b), 119.5 (C-1), 124

(C-3a), 124.6 (C-4′), 125 (C-α), 127 (C-4), 131 (C-3′, C-5′ and C-2), 133 (C-5), 144 (C-β), 146 (C-1′), 146.5 (C-5a), 147 (C-7), 148 (C-9), 198 (C-O); HRMS *m*/*z* [MH$^+$] 328.1332; anal. calcd. for $C_{21}H_{17}N_3O$ (327.379): C, 77.04; H, 5.23; N, 12.84; found: C, 77.31; H, 5.45; N, 12.73.

4.1.5. General procedure for synthesis of pyrroloquinoline substituted chalcones (**7a**–**c**)

A methanol solution of reagents, **6** [25] and 4-nitro-benzaldehyde in an equimolar ratio (0.188 g and 0.084 g, 0.56 mmol), was added to aqueous NaOH 1 M (0.3 mL) and kept under stirring for 12 h at room temperature. A red solid separated, which was collected and recrystallised from methanol.

*4.1.5.1. (E)-1-(4′-(3H-Pyrrolo[3,2-f]quinolin-9-ylamino)phenyl)-3-(1H-pyrrol-2″-yl)prop-2-en-1-one (**7a**).* Yield 17%; mp > 300 °C; rf 0.50 (chloroform/methanol 8:2); IR (KBr) 3382 (NH), 1661 (C═O), 1594 and 974 (CH═CH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.19 (dd, 1H, 4″-H), 6.67 (dd, 1H, 5″-H), 7.02 (d, 1H, J = 2.29 Hz, 1-H), 7.08 (bs, 1H, 3″-H), 7.20 (d, 2H, $J_{3',2'}$ and $J_{5',6'}$ = 8.58 Hz, 3′-H and 5′-H), 7.45 (m, 2H, 2-H and α-H), 7.56 (d, 1H, $J_{8,7}$ = 6.82 Hz, 8-H), 7.70 (d, 1H, $J_{4,5}$ = 8.87 Hz, 4-H), 7.85 (d, 1H, J = 15.26 Hz, β-H), 7.87 (d, 1H, $J_{5,4}$ = 8.87 Hz, 5-H), 7.98 (d, 2H, $J_{2',3'}$ and $J_{6',5'}$ = 8.58 Hz, 2′-H and 6′-H), 8.62 (d, 1H, $J_{7,8}$ = 4.95 Hz, 7-H), 8.93 (s, 1H, NH), 11.71 (bs, 2H, pyrrole 2 × NH); ^{13}C NMR (DMSO-d_6) δ 105.6 (C-8), 111 (C-4″), 113 (C-9a), 115 (C-2″), 116 (C-2′ and C-6′), 117 (C-9b), 119.5 (C-1), 123.7 (C-3a), 124 (C-4′), 124.5 (C-5″), 130 (C-3′ and C-5′), 130.5 (C-4), 131 (C-5), 131.5 (C-α), 132 (C-2), 133 (C-2″), 145 (C- β), 147 (C-5a), 147.5 (C-9), 148.5 (C-7), 149 (C-1′), 186.5 (C-O); HRMS *m*/*z* [MH$^+$] 379.1552; anal. calcd. for $C_{24}H_{18}N_4O$ (378.426): C, 76.17; H, 4.79; N, 14.81; found: C, 76.20; H, 4.59; N, 14.72.

*4.1.5.2. (E)-1-(4′-(3H-Pyrrolo[3,2-f]quinolin-9-ylamino)phenyl)-3-(thiophen-2″-yl)prop-2-en-1-one (**7b**).* Yield 15%; mp 289–290 °C; rf 0.52 (ethyl acetate/methanol 8:2); IR (KBr) 3382 (NH), 1660 (C═O), 1598 and 979 (CH═CH)cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.03 (bs, 1H, 1-H), 7.17 (m, 3H, $J_{3',2'}$ and $J_{5',6'}$ = 8.71 Hz, 3′-H, 5′-H and 4″-H), 7.43 (d, 1H, $J_{8,7}$ = 5.15 Hz, 8-H), 7.46 (dd, 1H, 2-H), 7.55 (d, 1H, $J_{\alpha,\beta}$ = 15.45 Hz, α-H), 7.64 (d, 1H, $J_{3'',4''}$ = 3.22 Hz, 3″-H), 7.70 (d, 1H, $J_{4,5}$ = 8.90 Hz, 4-H), 7.75 (d, 1H, $J_{5'',4''}$ = 4.91 Hz, 5″-H), 7.85 (d, 1H, $J_{\alpha,\beta}$ = 15.45 Hz, β-H), 7.89 (d, 1H, $J_{5,4}$ = 8.90 Hz, 5-H), 8.03 (d, 2H, $J_{3'',2''}$ and $_{5'',6''}$ = 8.71 Hz, 3″-H, 5″-H), 8.64 (d, 1H, $J_{8,7}$ = 5.15 Hz, 7-H), 9.05 (s, 1H, NH), 11.76 (bs, 1H, pyrrole NH); ^{13}C NMR (DMSO-d_6) δ 104.9 (C-8), 116 (C-9a), 117 (C-9b), 119 (C-1), 121 (C-3″); 123 (C-3a), 125 (C-2′ and C-6′), 125 (C-4′), 128.2 (C-4), 128.7 (C-4″), 129.5 (C-5″), 131 (C-5 and C-α), 132.7 (C-2), 133 (C-3′ and C-5′), 135 (C-2″), 140 (C-β), 147 (C-5a), 148.5 (C-9), 148.5 (C-7), 149 (C-1′), 186 (C–O); HRMS *m*/*z* [MH$^+$] 396.1212; anal. calcd. for $C_{24}H_{17}N_3OS$ (395.476): C, 72.89; H, 4.33; N, 10.63, S, 8.11; found: C, 72.68; H, 4.51; N, 10.49; S, 8.08.

*4.1.5.3. (E)-1-(4′-(3H-Pyrrolo[3,2-f]quinolin-9-ylamino)phenyl)-3-(4″-nitrophenyl)prop-2-en-1-one (**7c**).* Yield 13%; mp > 300 °C; rf 0.52 (ethyl acetate/methanol 8:2); IR (KBr) 3381 (NH), 1663 (C═O), 1594 and 974 (CH═CH), 1540 and 1340 (NO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.01 (bs, 1H, 1-H), 7.17 (d, 2H, $J_{3',2'}$ and $J_{5',6'}$ = 8.89 Hz, 3′-H, 5′-H), 7.45 (m, 2H, 8-H and 2-H), 7.72 (d, 1H, $J_{4,5}$ = 8.77 Hz, 4-H), 7.75 (d, 1H, $J_{\alpha,\beta}$ = 15.45 Hz, α-H), 7.88 (d, 1H, $J_{5,4}$ = 8.77 Hz, 5-H), 8.1 (m, 5H, 2′-H, 6′-H, 2″-H, 6″-H, β-H), 8.27 (d, 2H, $J_{3'',2''}$ and $J_{5'',6''}$ = 8.89 Hz, 3″-H and 5″-H), 8.65 (d, 1H, $J_{8,7}$ = 4.91 Hz, 7-H), 9.10 (s, 1H, NH), 11.8 (bs, 1H, pyrrole NH); ^{13}C NMR (DMSO-d_6) δ 105.5 (C-8), 116 (C-9a), 118 (C-9b), 119.5 (C-1), 123 (C-3a), 124.5 (C-2′ and C-6′), 124.7(C-4′), 127 (C-4), 129 (C-2), 130 (C-2″ and C-6″), 131.5 (C-5 and C-α), 133 (C-3′ and C-5′), 140 (C-β), 142 (C-3″ and C-5″), 146.5 (C-5a), 146.5 (C-9), 147 (C-4″), 148.5 (C-7), 149.5 (C-1′), 187 (C-O); HRMS *m*/*z* [MH$^+$] 435.1478; anal. calcd. for $C_{26}H_{18}N_4O_3$ (434.446): C, 71.88; H, 4.18; N, 12.90; found: C, 71.70; H, 4.45; N, 12.78.

4.1.6. General procedure for synthesis of 1-phenyl-propenone-3-substituted derivatives (**8a**, **b**)

The same procedure described above for the synthesis of compounds **7a**–**c** was applied to **8a**, **b**, starting from acetophenone and the appropriate aldehyde in an equimolar ratio (4–8 mmol).

*4.1.6.1. (E)-1-Phenyl-3-(1H-pyrrol-2-yl)prop-2-en-1-one (**8a**).* Yellowish-orange crystalline solid: yield 48%; rf 0.26 (chloroform); mp 134–137 °C (Ref. [37] 138–139 °C); ^{1}H NMR (DMSO-d_6) δ 6.16 (dd, 1H, $J_{3',2'}$ = 2.1 Hz, $J_{3',4'}$ = 3.4 Hz, 3′-H), 6.70 (dd, 1H, $J_{4',NH}$ = 1.14 Hz, $J_{4',3'}$ = 3.4 Hz, 4′-H), 7.10 (bs, 1H, 4-H), 7.35 (d, 1H, $J_{\alpha,\beta}$ = 15.00 Hz, α-H), 7.5 (dt, 1H, 2′-H), 7.52 (d, 2H, $J_{2,3}$ and $J_{6,5}$ = 7.6 Hz, 2-H and H-6), 7.60 (d, 1H, $J_{\beta,\alpha}$ = 15.00 Hz, β-H), 7.95 (dd, 2H, $J_{2,3}$ and $J_{6,5}$ = 8.0 Hz, $J_{4,3/5}$ = 1.33 Hz, 3-H, 5-H); HRMS *m*/*z* [MH$^+$] 198.0903; anal. calcd. for $C_{13}H_{11}NO$ (197.233): C, 79.16; H, 5.62; N, 7.10; found: C, 78.98; H, 5.52; N, 7.15.

*4.1.6.2. (E)-1-Phenyl-3-(thiophen-2-yl)prop-2-en-1-one (**8b**).* Dusty yellowish-green solid: yield 43%; rf 0.2 (*n*-hexane/chloroform 7:3), mp 67–69 °C (Ref. [38] 58–59 °C); ^{1}H NMR (DMSO-d_6) δ 7.20 (dd, 1H, $J_{4',5'}$ = 5.01 Hz, $J_{4',3'}$ = 3.61 Hz, 4′-H), 7.52 (m, 3H, 2-H, 6-H and α-H), 7.65 (dt, 1H, 3′-H), 7.70 (d, 1H, $J_{4,3/5}$ = 3.61 Hz, 4-H), 7.79 (d, 1H, $J_{4',5'}$ = 5.01 Hz, 5′-H), 7.92 (d, 1H, $J_{\alpha,\beta}$ = 15.44 Hz, β-H), 8.08 (dd, 2H, 3-H and H-5); HRMS *m*/*z* [MH$^+$] 215.0472; anal. calcd. for $C_{13}H_{10}OS$ (214.283): C, 72.87; H, 4.70; S, 14.96; found: C, 73.01; H, 4.65; S, 14.82.

4.2. Inhibition growth assay

HL-60 and JR8 were grown in RPMI 1640 (Sigma) supplemented with 15% and 10% heat-inactivated fetal calf serum (Biological Industries), respectively. HeLa were grown in Nutrient Mixture F-12 [HAM] (Sigma) supplemented with 10% heat-inactivated fetal calf serum (Biological Industries). 100 U/mL Penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B (Sigma) were added to both media. The cells were cultured at 37 °C in a moist atmosphere of 5% carbon dioxide in air.

HL-60 cells (4×10^4) were seeded into each well of a 24-well cell culture plate. After incubation for 24 h, various concentrations of the test compounds were added in complete medium and incubated for a further 72 h. HeLa and JR8 cells (4×10^4) were seeded into each well of a 24-well cell culture plate. After incubation for 24 h, the medium was replaced with an equal volume of fresh medium, and various concentrations of the test compounds were added. The cells were then incubated in standard conditions for a further 72 h.

A trypan blue assay was performed to determine cell viability. Cytotoxicity data are expressed as IC_{50} values, i.e., the concentration of the test agent inducing 50% reduction in cell number compared with control cultures.

4.3. Linear flow dichroism

Linear dichroism (LD) measurements were performed on a Jasco J500A circular dichroism spectropolarimeter, converted for LD and equipped with an IBM PC and a Jasco J interface.

Linear dichroism was defined as:

$$LD_{(\lambda)} = A_{\parallel(\lambda)} - A_{\perp(\lambda)}$$

where $A_\parallel$ and $A_\perp$ correspond to the absorbances of the sample when polarised light was oriented parallel or perpendicular to the flow direction, respectively. The orientation was produced by

a device designed by Wada and Kozawa [39] at a shear gradient of 500–700 rpm, and each spectrum was accumulated twice.

Salmon testes DNA was purchased from Sigma and the DNA concentration was determined using an extinction coefficient of 6600 $M^{-1}\,cm^{-1}$ at 260 nm. Aqueous solutions of DNA (1.9×10^{-3} M) in 10 mM TRIS, 1 mM EDTA (pH 7.0) and 10 mM NaCl were used (ETN buffer). Spectra were recorded at 25 °C at [drug]/[DNA] = 0 and 0.02.

4.4. DNA topoisomerase II relaxation assay

The topoisomerase II relaxation assay was performed essentially according to Bailly et al. [40]. Supercoiled pBR322 plasmid DNA (0.25 μg, Fermentas Life Sciences) was incubated with 1 U topoisomerase II (USB) and the test compounds, as indicated, for 60 min at 37 °C in 20 μL reaction buffer consisting of 10 mM Tris-HCl (pH = 7.9), 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg/mL BSA, 1 mM ATP.

The reaction was stopped by adding 4 μL stop buffer (5% SDS, 0.125% bromophenol blue and 25% glycerol), 50 μg/mL proteinase K (Sigma) and incubating for a further 30 min at 37 °C. The samples were separated by electrophoresis on a 1% agarose gel. The gel was stained with ethidium bromide 1 μg/mL in TAE buffer, transilluminated by UV light, and fluorescence emission was visualised by a CCD camera coupled to a Bio-Rad Gel Doc XR apparatus.

4.5. DNA topoisomerase II cleavage reaction

The reaction conditions were the same as for the relaxation assay except that 10 units DNA topoisomerase II were used. The DNA cleavage products were separated by electrophoresis on a 1% agarose gel containing 0.5 μg/mL ethidium bromide in TBE buffer. The gel was transilluminated by UV light and fluorescence emission was visualised by a CCD camera coupled to a Bio-Rad Gel Doc XR apparatus.

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