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A comparative study of antitumor activities and DNA cleavage on a class of dehydroabietylamine derivatives

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A series of novel dehydroabietylamine derivatives containing tricyclic diterpene structures were synthesized. The antitumor activities of these compounds against L02, Hey-1B and HepG2 cells were investigated. Significant activity was discovered for fourteen analogs. Meanwhile these compounds exhibit DNA cleavage activities on plasmid DNA (*Escherichia coli*), which depend on the Schiff base structure and the substituent of the aromatic moiety. Our findings present further information on the relationship between the chemical structure, biological function and DNA cleavage characteristics.

1. Introduction

Many bioactive tricyclic diterpenes, such as dehydroabietylamine, have become an important area of natural product chemistry. Dehydroabietylamine derivatives have been reported to show constant anticancer activity, Schiff bases and their metal complexes are under investigation (Son et al. 2005; Gigante et al. 2003). Anticancer drugs cause cell death through different mechanisms and their cytotoxicity is related to their interactions with DNA (Gu and Wang 2010; Cui et al. 2010). Although dehydroabietylamine derivatives synthesis and associated antitumor activity have been persistently investigated (Savluchinske-Feio et al. 2006; Fonseca et al. 2004; Tashima et al. 2006; Wada et al. 1985; Sepuĭlveda et al. 2005; Faneyte et al. 2004; Cortez et al. 2001; Harper and Elledge 2007), any relationship between DNA cleavage activity and structure of these tricyclic diterpenes derivatives have not been reported so far.

We report the synthesis, DNA cleavage and antitumor activities for newer Schiff bases derived from dehydroabietylamine (White et al. 1997; Dervan and Edelson 2003; Egli and Pallan 2010). The Schiff bases are capable of generating reactive species under alkaline pH conditions to induce DNA damage and inhibitory action against L02 (normal human hepatocyte cell line), HepG2 (human liver carcinoma cell line) and Hey-1B (human ovarian carcinoma cell line) *in vitro* (Heinecke and Melander 2010). These observations attracted our considerable attention due to their importance in the design of probes for DNA structures (Tian et al. 2007; Rossi et al. 2005), because DNA cleavage by natural or designed molecules has long been recognized as a potential way to affect cell proliferation.

2. Investigations, results and discussion

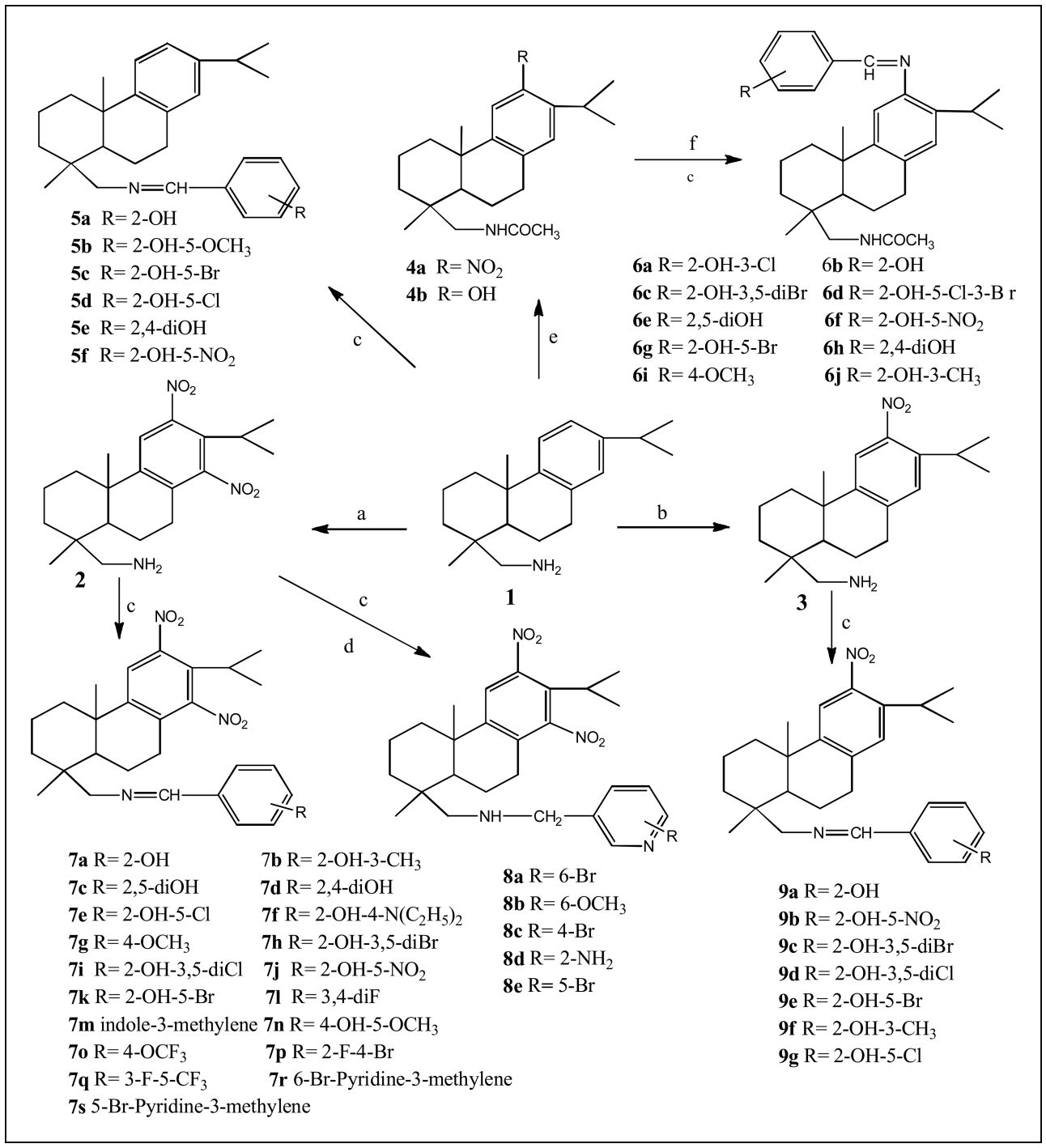
2.1. Synthesis of the compounds

The synthesis of dehydroabietylamine derivatives was performed as outlined in the Scheme. All the synthesized compounds were characterized by IR, MS, and ^{1}H NMR spectra. In FT-IR spectral analysis of the compounds **2**, **3** and **4a** show broad peak in the range $3350 - 3422\,cm^{-1}$ for NH_2, intensity peaks for NO_2 stretching in the range $1500 - 1521\,cm^{-1}$. The ^{1}H NMR spectra of the compounds **2**, **3** and **4a** showed the protons of the aromatic ring appear 7. 08 ppm (s, 1H), 7. 64 (s, 1H) for **2** and 7. 55 ppm (s, 1H) for **3**. Those Schiff bases for compounds showed sharp peaks for nitrile grouping stretching in the range $1580 - 1650\,cm^{-1}$ and the protons of methine proton in Schiff bases is in the range $\delta 8.00 - 8.50$ ppm in ^{1}H NMR for compounds **5**, **6**, **7** and **9**. The mass spectra of all compounds exhibited molecular ion peaks at their respective molecular weight which confirmed their structures.

2.2. Antitumor activity

The antitumor activities of the compounds were determined by the activity of each compound against L02, Hey-1B and HepG2 cells. Initially, the compounds were tested for cytotoxic activity by the MTT assay method. The compounds were tested at different concentrations to find out the fifty percent growth inhibitory doses (IC_{50}) against these cells. Partial compounds showed antiproliferative effects in the range of 10 to 50 μM. Some of the most interesting compounds showed excellent activity at lower concentrations (Table).

Among these compounds, **5a**, **5e – f**, **7a**, **7d – c**, **7m**, **7r – s**, **8d – e and 9a – b** showed potential activities against L02, Hey-1B and HepG2 cells. Apparently, the antitumor activity of these compounds may be related to their functional group (C=N) and substituent groups in the aromatic aldehyde. Compounds with a hydroxyl substituent in the *ortho* position of the phenyl group have shown greater antitumor activity than other compounds without a hydroxyl substituent. The presence of nitro groups on the 12 and 14 position on the aromatic ring is not important for determining the biological activity of the compounds **5a**, **7a** and **9a**. Among these compounds, **7r – s** and **8d – e**



Scheme: Reagents and conditions: (a) H_2SO_4/HNO_3, 10 h, 0–5 °C, 87%; (b) $(CF_3CO_2)O$, HNO_3, 3 h, 0–5 °C, 76%; (c) Aldehyde/EtOH, refulx, 5 h, 85%; (d) $NaHB_4$, r.t. (97%); (e) HNO_3-HAc, 4 h, 0–5 °C, 69% or $N_2H_4H_2O$-EtOH, refulx, 6 h H_2SO_4-$NaNO_2$, 71%; (f) $N_2H_4H_2O$-EtOH, reflux, 6 h, 78%.

showed good cytotoxicity against HepG2 and Hey-1B cancer cells, but substituent groups of Br or OCH_3 in the *ortho* or *para* position of pyridine ring of compounds **8a – c** reduced antitumor activity. This may be because the introduction of these groups decreases the toxicity and chemotherapeutic index (Vlietinck et al. 1995; Montogomery 1959). The results have also demonstrated that the introduction of functional group (N—C) in compound **7r** and **8a** led to less active compounds. The compounds **7l**, **7p – q**, **8a** and **8c** with an electron-withdrawing group on the position of phenyl ring had little or no activity against HepG2 and Hey-1B cancer cells. Meanwhile, compound **5e** displayed antiproliferative effect against Hey-1B cell at concentrations of less than 10 μM. The cytotoxic effect for Hey-1B and PC-3 was evaluated after treated with different concentrations of compound **5e** for 24 h. As indicated in Figs. 1 and 2, the typical morphology of apoptosis such as cells shrinkage and membrane blebbing was observed under an inverted phase-contrast microscopy, compared to untreated control cells. Obviously, compound **5e** induced Hey-1B cell apoptosis in a dose-dependent manner.

2.3. DNA cleavage activity

After treatment with compounds **7l**, **7o**, **7q**, the plasmid DNA has been cleaved completely; compound **8a** with the introduction of a functional group (N—C) showed no effect on DNA cleavage. After treatment with compounds **4a – b**, **5a – f**, **6a – j**, **7e – k**, **7m – n**, **8b**, **9f**, the Form I (supercoiled DNA) can be partially

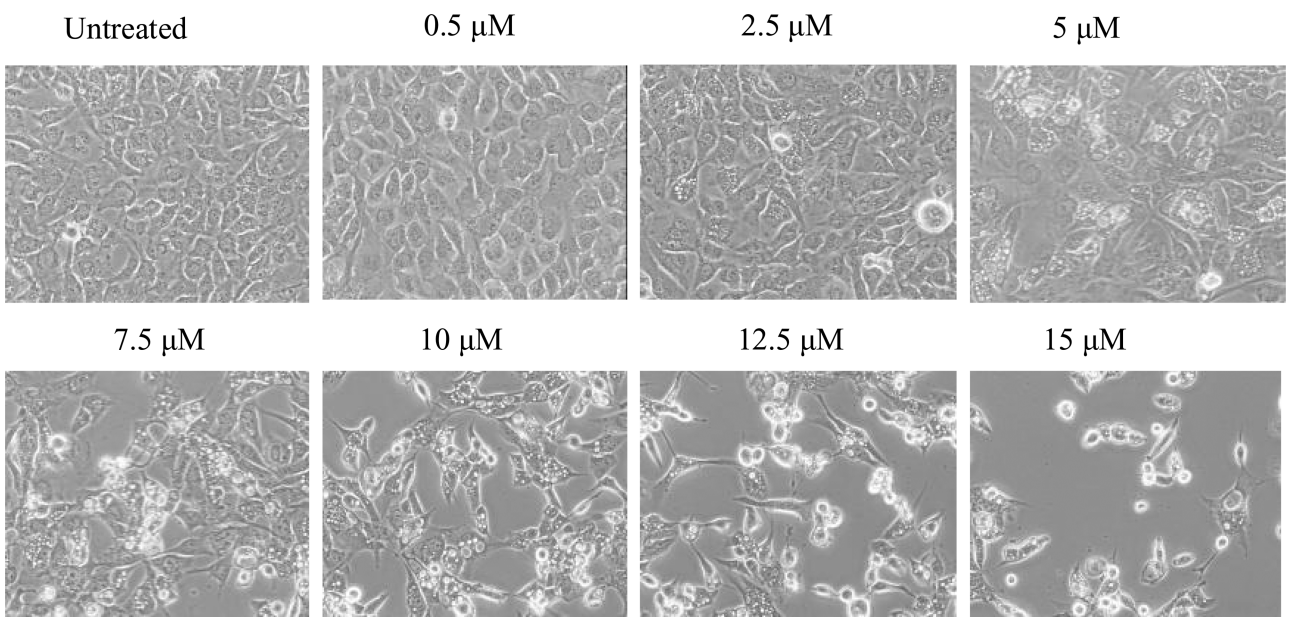


Fig. 1: Compound **5e** induces apoptosis in Hey-1B after treatment for 24 hours.

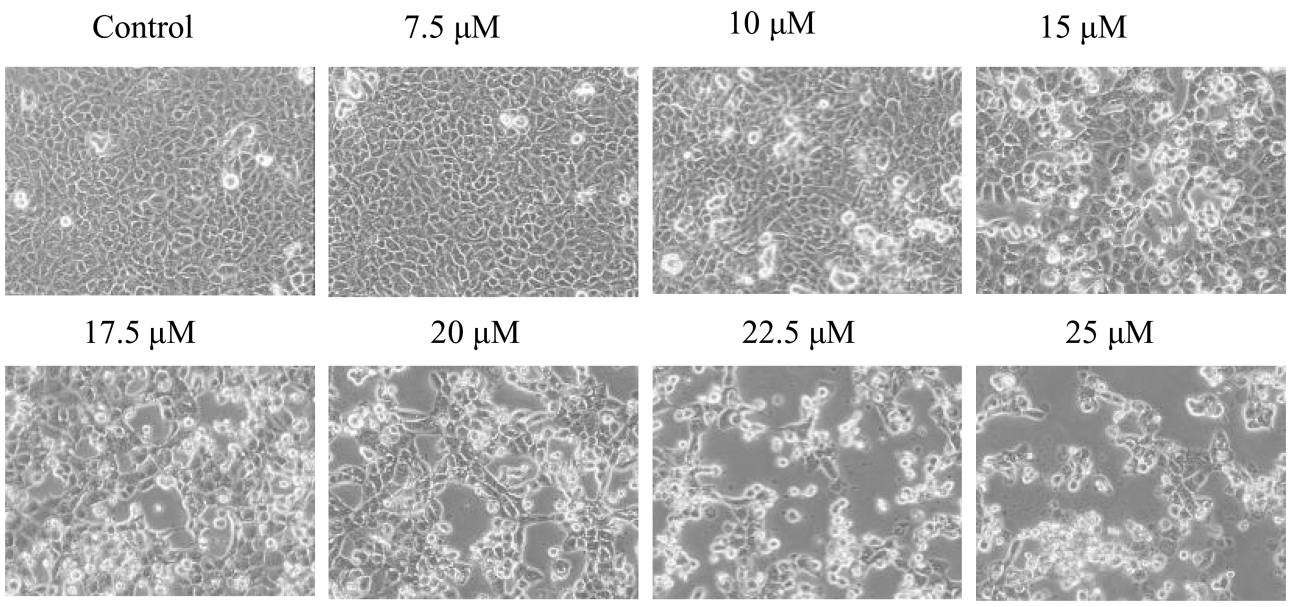


Fig. 2: Compound **5e** induces apoptosis in PC-3 cells after treatment for 24 hours.

Table: IC_{50} of compounds

Compd	L02 IC_{50}(μg/ml)	HepG2 IC_{50}(μg/ml)	Hey-1B IC_{50}(μg/ml)
5a	5.43	8.14	20.33
5d	–	–	2326
5e	5.39	5.02	19.17
5f	7.36	9.22	21.37
7a	5.61	7.67	6.37
7c	5.34	6.64	–
7d	5.57	7.94	–
7m	5.68	6.11	7.40
7r	5.86	6.72	–
7s	5.56	7.28	–
8d	9.99	6.95	19.77
8e	6.58	7.96	–
9a	6.05	5.71	–
9b	5.58	8.44	–

converted to Form II; other compounds can convert Form I into Form II and Form III simultaneously (Fig. 3). We also examined the DNA-cleaving activity of sectional compounds in the presence of metal ions. Apart from compound **9b**, the compounds **5e**, **7a – d**, **9a** with hydroxyl groups on the phenyl ring showed no DNA cleaving activity in the presence of Fe(II) or Fe(III) in comparison with control groups. Fe ions inhibited hydroxyl radical mediated or other radical damage to pBR322 plasmid DNA (Meneghini 1997). Compounds **7l**, **7p – q** with an electron-withdrawing group at the meta position of phenyl ring displayed very good DNA-cleaving activity in the presence of Fe irons, which may imply some form of nucleophilic reaction mechanism for the initiation of cleavage as shown in Figs. 4 and 5 (Taj et al. 2011).

2.4. Conclusion

A series of new dehydroabietylamine derivatives were synthesized to further elucidate the relationships between structure and activity. The substituent group in the Schiff base struc-

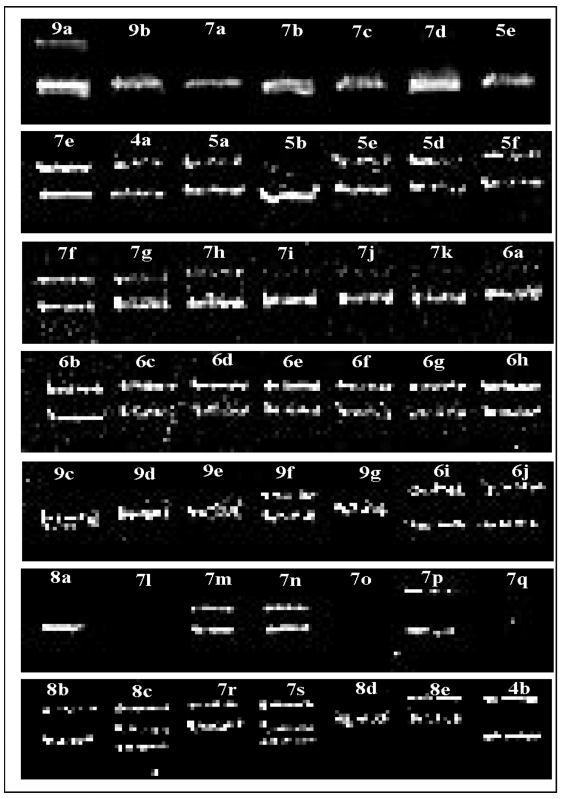


Fig. 3: DNA cleavage activity of compounds FormII (Oc DNA), FormIII (Lin DNA).

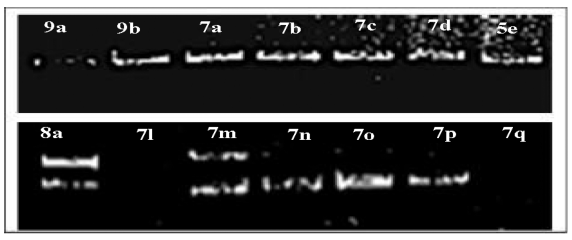


Fig. 4: DNA cleavage activity of compounds in the presence of Fe(II).

ture were associated with antitumor activity and DNA-cleaving activity. The DNA-cleaving activity seemed to increase with an increase in the number of electron-withdrawing groups, which may decrease the electron density of Schiff base structure by an inductive effect. The substrate phosphate activation via phosphate coordinating to benzyl carbocation of Schiff base to form the planar uncle carbon ions structure is proposed for the hydrolytic DNA cleavage in alkaline condition. However, the compounds having better activity against Hey-1B cells were not consistent with DNA-cleaving activity, which demonstrated that compounds inhibiting tumor cells activity was not through the cleavage of DNA.

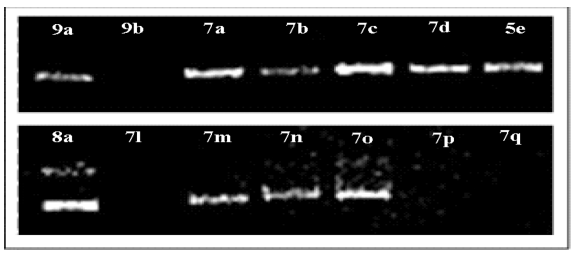


Fig. 5: DNA cleavage activity of compounds in the presence of Fe(III).

3. Experimental

3.1. Chemistry

Dehydroabietylamine was converted into 12,14-dinitro-dehydroabietylamine **2** by using a HNO_3–H_2SO_4 mixture for 10 h at 0 °C and 12-nitro-dehydroabietylamine **3** by $(CF_3CO)_2O$–HNO_3 mixture for 3 h at 0 °C. The ethanol solution of compound **1–3** with different substituted aromatic aldehydes afforded the corresponding substituted the Schiff base **5a–f** (Zhao et al. 2007; Sui et al. 2011), **7a–s** and **9a–g** according to the literature, respectively. Compound **8a–e** is a reduzate from the corresponding Schiff base through $NaBH_4$. Dehydroabietylamine was reacted with nitric acid mixture for 4 h in the presence of acetic anhydride to provide compound **4a**, which was reduced using hydrazine hydrate for 6 h under refluxing to give 12-amino-acetyl dehydroabietylamine. The diazotization of 12-amino-acetyl dehydroabietylamine with H_2SO_4–$NaNO_2$ and hydrolysis reaction gave the compound **4b**. Condensation reaction of 12-amino-acetyl dehydroabietylamine with different substituted aromatic aldehydes provided compounds **6a-6j**.

3.2. Antitumor activity

HepG2, L02 and Hey-1B cells were seeded in 96-well plates, respectively. Cells were diluted in medium to a density of 5×10^4 viable cells per ml. Using a DMSO control group, cells were treated with test compounds (10 μg/ml) the following days, respectively. All cells were incubated and maintained 44 h in a humidified atmosphere at 37 °C and 5% CO_2. MTT was used to assess the viability of cells following treatment. Aliquots of 20 ml of stock MTT solution (5 μg/ml) were added to each well and incubated with the cells for 4 h. Following incubation the medium was removed and 100 ml of DMSO added to solubilize the formazan crystals. The OD value was measured at 570 nm by a microplate reader (Model 550, Bio-Rad, USA). The relative cell viability was calculated as absorbance of compound-treated/absorbance of control.

3.3. DNA cleavage activity

The compounds have been studied using pBR322 supercoiled plasmid DNA (Form I) as a substrate in a medium of 50 μM Tris-HCl/NaCl buffer (pH = 7.4) under physiological conditions. Reactions were carried out for 3 h at 37 °C in 20 μl (total volume) of 50 μM Tris-HCl/NaCl buffer (2.5% DMF), containing 5 μl pBR322 supercoiled DNA (0.25 μg/μl), 50 μM of each dehydroabietylamine derivative or 10 μM Fe irons. The DNA cleavage fragment was analyzed by agarose gel electrophoresis. After electrophoresis, the gels were illuminated with UV light and photographed.

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