

College of Chemical Engineering¹, Nanjing Forestry University, Nanjing; Bozhou Vocational and Technolege College², Bozhou, China; Department of Chemistry³, Cleveland State University, Cleveland, USA; School of Life Sciences⁴, Northeast Normal University, Changchun, China

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

CHAO-XIANG LIU^{1,2}, ZHONG-XIANG LIN¹, XING YU¹, ZHOU LU¹, AI-MIN ZHOU³, YONG-LI BAO⁴

Received February 18, 2013, accepted April 12, 2013

Zhong-Xiang Lin, College of Chemical Engineering, Nanjing Forestry University, Nanjing 210037, P. R. China
linzhongxiang36@gmail.com

Pharmazie 68: 861–865 (2013)

doi: 10.1691/ph.2013.3575

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; Sepulveda 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

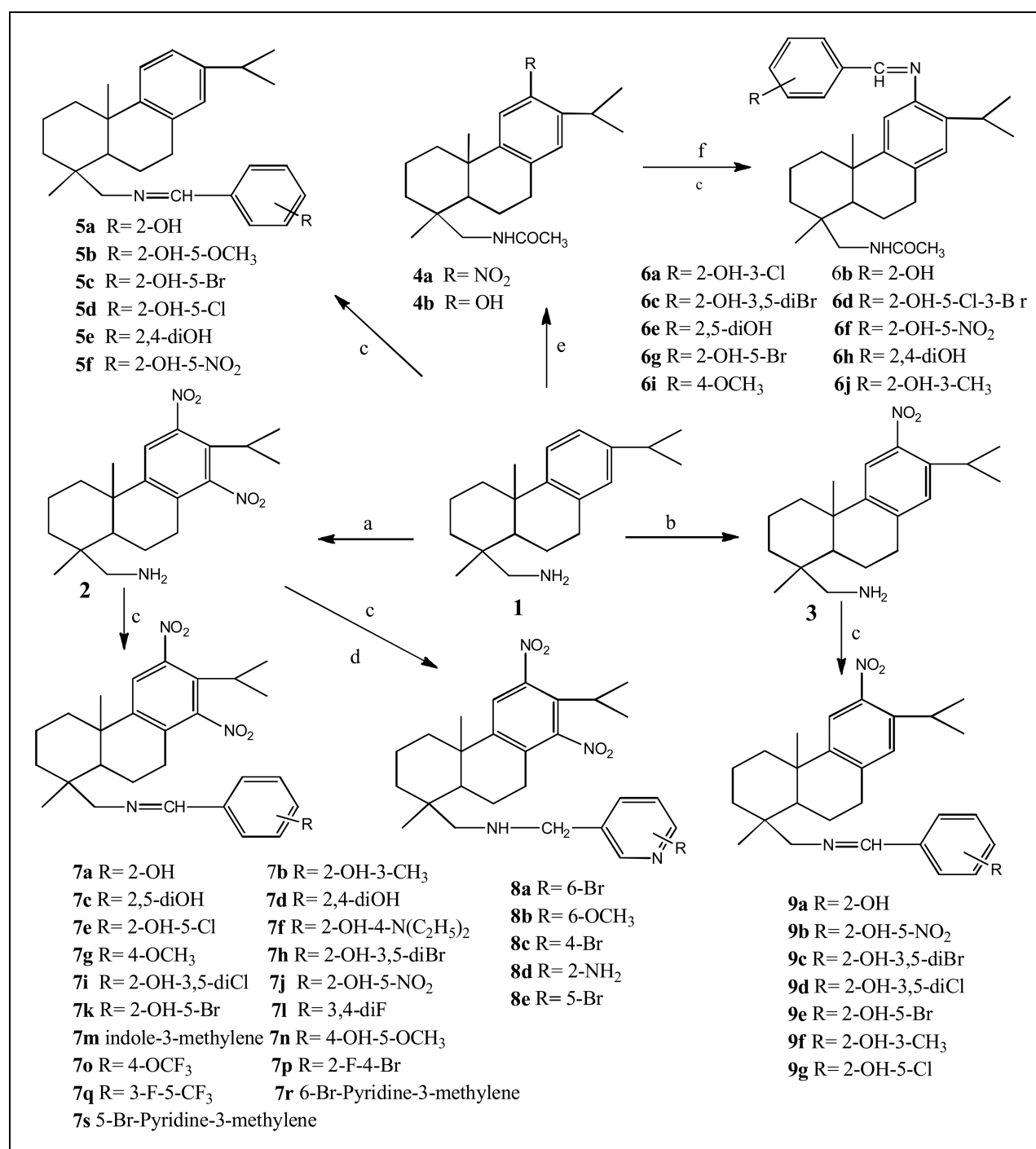
The synthesis of dehydroabietylamine derivatives was performed as outlined in the Scheme. All the synthesized

compounds were characterized by IR, MS, and ¹H 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 NH₂, intensity peaks for NO₂ stretching in the range 1500 – 1521 cm⁻¹. The ¹H 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 8.00 – 8.50 ppm in ¹H 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 (IC₅₀) 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) H₂SO₄/HNO₃, 10 h, 0–5 °C, 87%; (b) (CF₃CO₂)₂O, HNO₃, 3 h, 0–5 °C, 76%; (c) Aldehyde/EtOH, reflux, 5 h, 85%; (d) NaHB₄, r.t. (97%); (e) HNO₃-HAc, 4 h, 0–5 °C, 69% or N₂H₄H₂O-EtOH, reflux, 6 h H₂SO₄-NaNO₂, 71%; (f) N₂H₄H₂O-EtOH, reflux, 6 h, 78%.

showed good cytotoxicity against HepG2 and Hey-1B cancer cells, but substituent groups of Br or OCH₃ in the *ortho* or *para* position of pyridine ring of compounds **8a–c** reduced anti-tumor activity. This may be because the introduction of these groups decreases the toxicity and chemotherapeutic index (Vlietinck et al. 1995; Montgomery 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 concentra-

tions 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

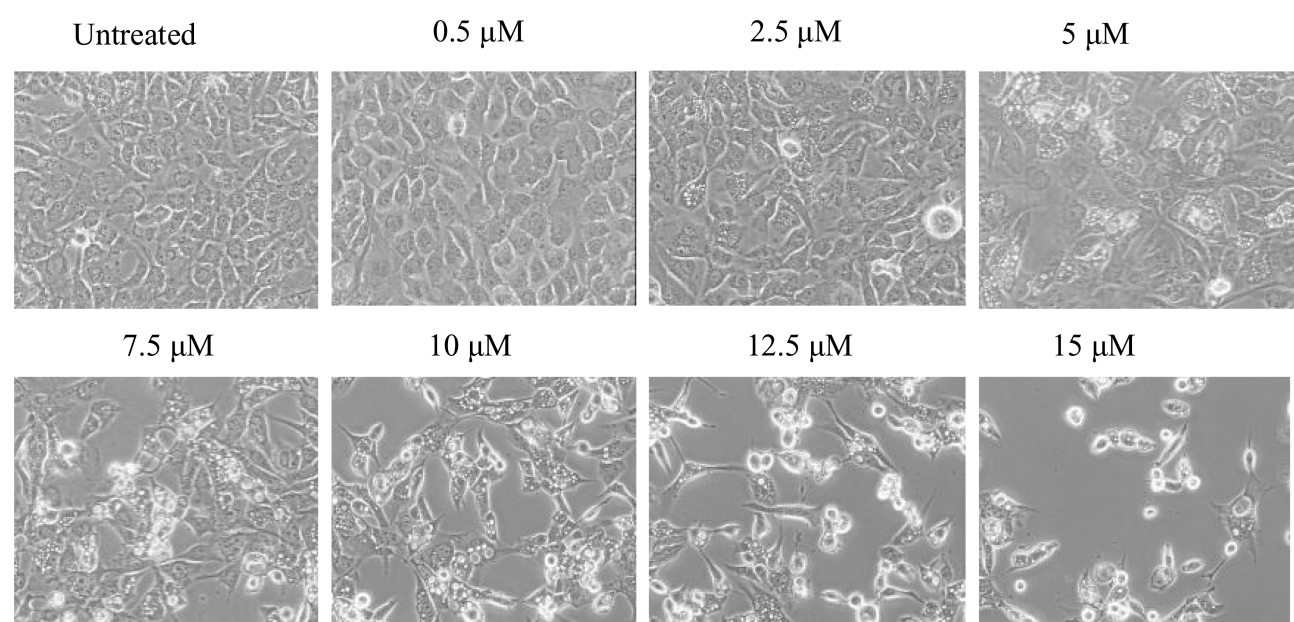


Fig. 1: Compound **5e** induces apoptosis in Hey-1B after treatment for 24 hours.

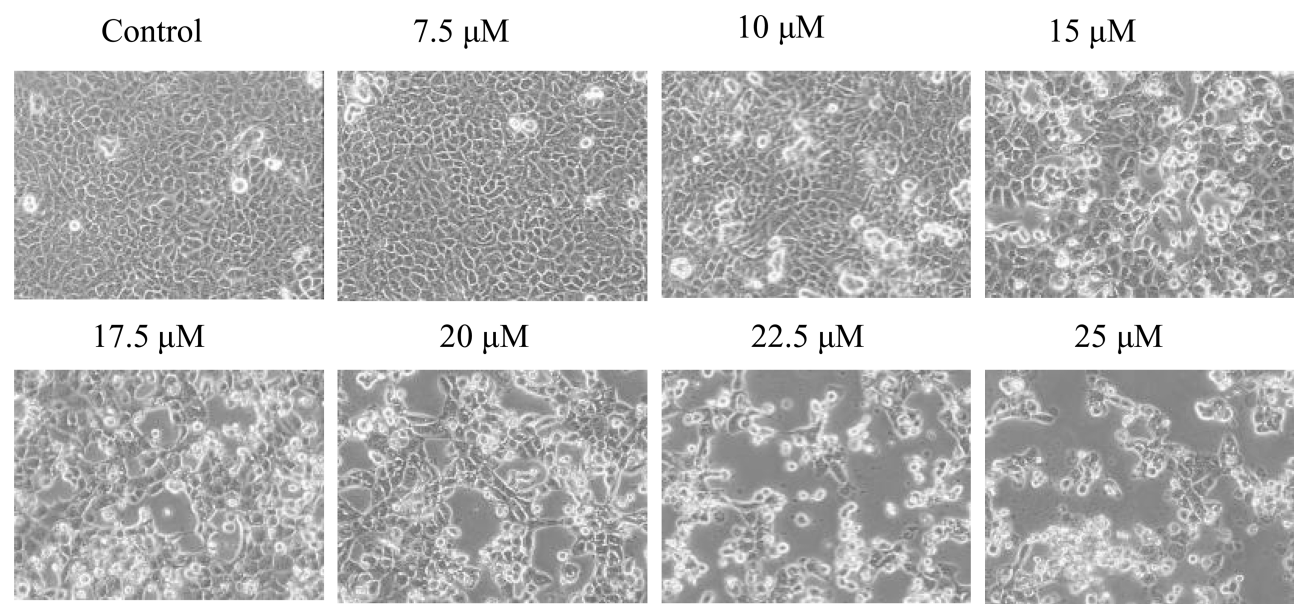


Fig. 2: Compound **5e** induces apoptosis in PC-3 cells after treatment for 24 hours.

Table: IC₅₀ 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 ions, 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-

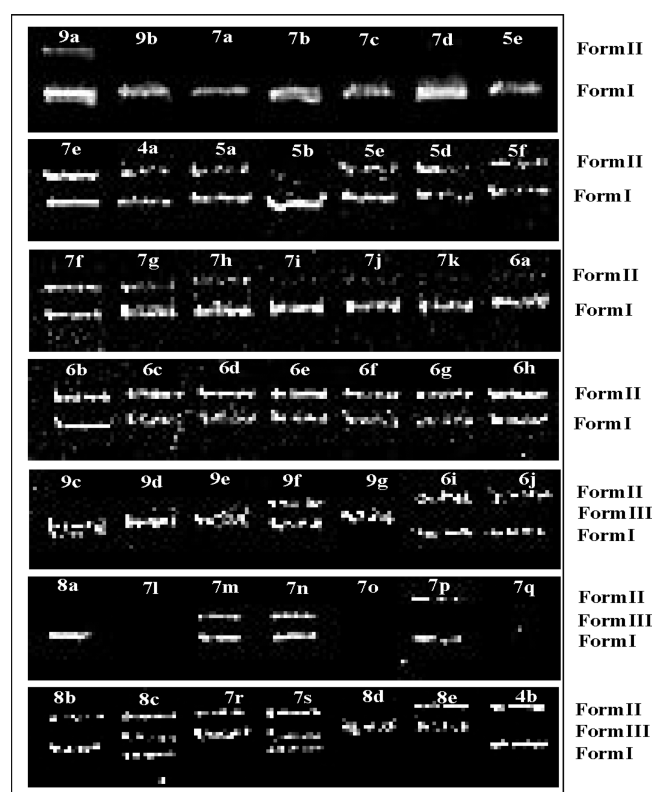


Fig. 3: DNA cleavage activity of compounds Form II (Oc DNA), Form III (Lin DNA).

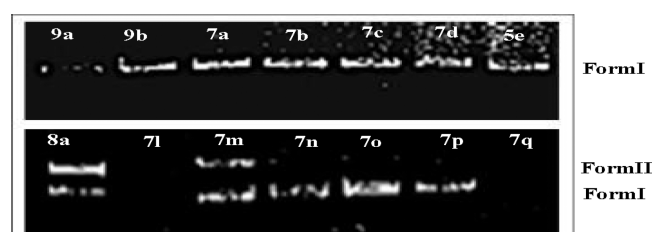


Fig. 4: DNA cleavage activity of compounds in the presence of Fe(II).

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.

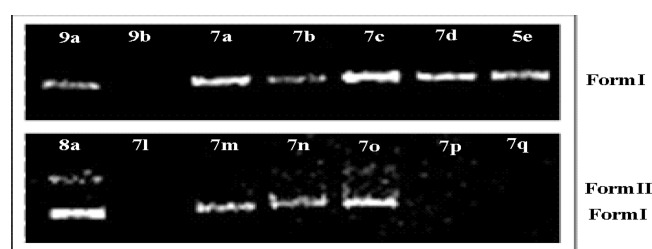


Fig. 5: DNA cleavage activity of compounds in the presence of Fe(III).

3. Experimental

3.1. Chemistry

Dehydroabietylamine was converted into 12,14-dinitro-dehydroabietylamine **2** by using a $\text{HNO}_3\text{--H}_2\text{SO}_4$ mixture for 10 h at 0°C and 12-nitro-dehydroabietylamine **3** by $(\text{CF}_3\text{CO})_2\text{O--HNO}_3$ 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 redazate 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 $\text{H}_2\text{SO}_4\text{--NaNO}_2$ 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 $\mu\text{g/ml}$) 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 $\mu\text{g/ml}$) 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 ($\text{pH} = 7.4$) 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 $\mu\text{g}/\mu\text{l}$), 50 μM of each dehydroabietylamine derivative or 10 μM Fe ions. The DNA cleavage fragment was analyzed by agarose gel electrophoresis. After electrophoresis, the gels were illuminated with UV light and photographed.

Acknowledgements: The generous financial support of the Natural Science Foundation of China (31170536) was gratefully acknowledged.

References

- Cui YM, Yasutomi E, Otani Y, Ido K, Yoshinaga T, Sawada K, Ohwada T (2010) Design, synthesis, and characterization of BK channel openers based on oxidation of abietane diterpene derivatives. *Bioorg Med Chem* 18: 8642–8659.
- Cortez D, Guntuku S, Qin J, Elledge SJ (2001) ATR and ATRIP: Partners in checkpoint signaling. *Science* 294: 1713–1716.
- Dervan PB, Edelson BS (2003) Recognition of the DNA minor groove by pyrrole-imidazole polyamides. *Curr Opin Struct Biol* 13: 284–299.
- Egli M, Pallan PS (2010) Crystal structure of homo-DNA and nature's choice of pentose over hexose in the genetic system. *Curr Opin Struct Biol* 20: 262–275.
- Faneyte IF, Kristel PM, van de Vijver MJ (2004) Multidrug resistance associated genes MRP1, MRP2 and MRP3 in primary and anthracycline exposed breast cancer. *Anticancer Res* 24: 2931–2940.
- Fonseca T, Gigante B, Marques MM, Gilchrist TL, De Clercq E (2004) Synthesis and antiviral evaluation of benzimidazoles, quinoxalines and indoles from dehydroabietylamine. *Bioorg Med Chem* 12: 103–112.
- Gigante B, Santos C, Silva AM, Curto MJ, Nascimento MS, Pinto E, Pedro M, Cerqueira F, Pinto MM, Duarte MP, Laires A, Rueff J, Gonçalves J, Pegado MI, Valdeira ML (2003) Catechols from abietic acid: synthesis and evaluation as bioactive compounds. *Bioorg Med Chem* 11: 1631–1638.
- Gu W, Wang SF (2010) Synthesis and antimicrobial activities of novel 1H-dibenzo[a,c] carbazoles from dehydroabietylamine. *J Med Chem* 45: 4692–4296.
- Harper JW, Elledge SJ (2007) The DNA damage response: ten years after. *Mol Cell* 28: 739–745.

ORIGINAL ARTICLES

- Heinecke CL, Melander C (2010) Analysis of kinamycin D-mediated DNA cleavage. *Tetrahedron Lett* 51: 1455–1458.
- Meneghini R (1997) Iron hemeostasis, oxidative stress and DNA damage. *Free Radic Biol Med* 23: 783–792.
- Montgomery JA (1959) The relation of anticancer activity to chemical structure: a review. *Cancer Res* 19: 447–463.
- Rossi LM, Neves A; Bortoluzzi AJ, Horner R, Szpoganicz B, Terenzi H, Mangrich AS; Pereira-Maia E, Castellano EE, Haase W (2005) Synthesis, structure and properties of unsymmetrical μ -alkoxo-dicopper(II) complexes: biological relevance to phosphodiester and DNA cleavage and cytotoxic activity. *Inorg Chim Acta* 358: 1807–1822.
- Savluchinske-Feio S, Curto MJ, Gigante B, Roseiro JC (2006) Antimicrobial activity of resin acid derivatives. *Appl Microbiol Biotechnol* 72: 430–436.
- Sepulveda B, Astudillo L, Rodríguez JA, Yáñez T, Theoduloz C, Schmeda-Hirschmann G (2005) Gastroprotective and cytotoxic effect of dehydroabietic acid derivatives. *Pharmacol Res* 52: 429–437.
- Son KH, Oh HM, Choi SK, Han DC, Kwon BM (2005) Anti-tumor abietane diterpenes from the cones of *Sequoia sempervirens*. *Bioorg Med Chem Lett* 15: 2019–2021.
- Sui Y.; Liu DS; Hu RH, Chen HM (2011) Discovery of a new type of organic ferroelectric materials in natural biomass dehydroabietylamine Schiff bases. *J Mater Chem* 21: 14599–14603.
- Taj T, Kamble RR, Gireesh TM, Hunnur RK, Margankop SB (2011) One-pot synthesis of pyrazoline derivatised carbazoles as antitubercular, anti-cancer agents, their DNA cleavage and antioxidant activities. *Eur J Med Chem* 46: 4366–4373.
- Tashima T, Toriumi Y, Mochizuki Y, Nonomura T, Nagaoka S, Furukawa K, Tsuru H, Adachi-Akahane S, Ohwada T (2006) Design, synthesis, and BK channel-opening activity of hexahydrodibenzazepinone derivatives. *Bioorg Med Chem* 14: 8014–8031.
- Tian JL, Feng L, Gu W, Xu GJ, Yan SP, Liao DZ, Jiang ZH, Cheng P (2007) Synthesis, crystal structure, magnetic property and nuclease activity of a new binuclear cobat(II) complex. *J Inorg Biochem* 101: 196–202.
- Vlietinck AJ, Van Hoof L, Totté J, Lasure A, Vanden Berghe D, Rwangabo PC, Mvukiyumwami J (1995) Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *J Ethnopharm* 46: 31–47.
- Wada H, Kodato S, Kawamori M, Morikawa T, Nakai H, Takeda M, Saito S, Onoda Y, Tamaki H (1985) Antiulcer activity of dehydroabietic acid derivatives. *Chem Pharm Bull* 33: 1472–1487.
- White S, Baird EE, Dervan PB (1997) On the pairing rules for recognition in the minor groove of DNA by pyrrole-imidazole polyamides. *Chem Biol* 4: 569–578.
- Zhao XP, Song WK, Radbil AB, Radbil BA (2007) Synthesis and biological activity of Schiff bases derived from dehydroabietylamine and benzaldehyde derivatives. *Russian J Appl Chem* 80: 1373–1375.