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Research article

Synthesis, antitumor and DNA cleavage activities of a novel class of dehydroabietylamine derivatives

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ABSTRACT

Previous studies have reported higher biological activity of dehydrorosinamine derivatives. In order to further synthesize novel compounds with higher biological activity, a series of novel compounds containing benzo-azepine structures were synthesized from dehydroabietylamine in good yields in this study. The structures of synthesized compounds were identified by infra red (IR), ^{1}H-NMR, ^{13}C-NMR, and mass spectra (MS) analysis. The antitumor activities of the target compounds against L02 and HepG2 cells were studied. Furthermore, the dehydroabietylamine derivatives were studied on plasmid DNA cleavage activities. The results showed that the synthesized target compound exhibit antitumor and DNA cleavage activities against plasmid DNA (Escherichia coli). Our results further demonstrate the relationship between the chemical structure and biological function of the synthesized compounds.

1. Introduction

A large number of research reports on bioactive natural products based on dehydrorosinamine (**1**), which is widely used in the fields of paper-making, medicine, pesticide and chemical industries, especially in the field of natural product and medicinal chemistry [1, 2, 3]. Due to the unique structure and biological activity, the compound **1** has been used as antibacterial drug, and chiral agents for synthesizing many important derivatives [4, 5, 6]. In particular, natural tricyclic diterpenes are an important class of potential anticancer drugs [7, 8].

Previous research has found that dehydrorosinamine derivatives have a wide range of biological activities, such as antibacterial, antifungal, insecticidal, herbicidal and anti-cancer activities [9, 10]. However, anticancer drugs cause cell death through different mechanisms and their cytotoxicity is related to their interactions with DNA [11, 12, 13]. Modifications of the structures of bioactive natural products are effective methods to find potential compounds with anticancer activity. Recently, synthesis and bioactivities of dehydroabietylamine derivatives are a focus of research in the forest chemical field.

Our group has been interested in the synthesis of Schiff bases and acylamide of dehydroabietylamine derivatives. The cytotoxicities of dehydroabietylamine derivatives against PC-3, Hey-1B, L02 and HepG2 in vitro by the MTT assay were investigated [14, 15]. As a result, these compounds also showed good selectivity towards these cells and DNA cleavage [16].

However, the synthesis, antitumor and DNA cleavage activities for benzo-azepine structures derived from dehydroabietylamine have not yet been reported so far. Therefore, novel benzo-azepine derivatives with potential bioactivities from dehydroabietylamine were designed and synthesized. Through reported or designed synthetic steps, target compounds with higher yields can be obtained [17, 18]. In order to further study their bioactivities, these novel compounds were screened for their possible antitumor activities and DNA cleavage activities.

2. Materials and methods

2.1. General experimental procedures

Infrared spectra (IR) of all compounds were recorded in the film with a Brukers Vector 22 spectrophotometer, and ν_{max} values are given in cm^{-1}. ^{1}H NMR spectra by using Bruker Spectrospin DPX (300 MHz spectrometer) output, MeOD or $CDCl_3$ was used as a solvent and tetramethyl silane (TMS) as an internal standard. δ (ppm) scale and J values as the basis for analyzing the spectrum. Mass spectra (MS) were run by ESI at 70 eV. Elemental analysis was recorded on a carlo Elba modol 1106 analyzer. The syntheses reagents are all commercial reagent grade solvents.

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2.2. Synthetic procedure

2.2.1. E-18-acetylamino-7-hydroxyimidedehydroabietane (3b)

Compound **3a** (3.41 g, 10 mM) and anhydrous sodium carbonate (1.7 g, 16 mM) in 20 mL anhydrous ethanol was cooled to the ice point, then the solution was added to hydroxylamine hydrochloride (0.7 g, 12 mM) dissolved in 5 mL water under stirring. The mixture was refluxed for 6h, and then the solvent was evaporated under hypobaric condition. The crude product was purified by column chromatography by using ether-ethyl acetate (1:1), the result showed that the yellow solid compound yield was (1.63 g, 67.5%), mp 80–82 °C. IR(max, film, cm^{-1}): 3310, 2958, 2928, 2867, 1655, 1555, 1441, 1379, 1287, 959, 826, 720. ^{1}H NMR (300 MHz, δ, $CDCl_3$): 1.05 (3H, s, H-19), 1.11 (3H, s, H-20), 1.20–1.29 (6H, d, J = 13.1 Hz, H-16, 17), 1.25–1.63 (2H, m, H-3), 1.73 (2H, m, H-2), 1.94 (3H, s, CH_3CO), 2.04 (1H, m, H-5), 2.17 (2H, m, H-1), 2.50–2.70 (1H, m, H-6α), 2.84–3.20 (3H, m, H-15, 18), 3.25–3.41 (1H, s, H-6β), 7.21 (1H, m, H-12), 7.72 (2H, m, H-11,14). ^{13}C NMR (75 MHz, δ, $CDCl_3$): 18.17 (C-22), 18.60 (C-2), 22.85 (C-19), 23.11 (C-16 and 17), 23.46 (C-20), 23.78 (C-4), 24.03 (C-10), 33.63 (C-15), 36.13 (C-3), 36.98 (C-6), 37.54 (C-1), 42.02 (C-5), 49.41 (C-18), 122.13 (C-14), 122.98 (C-11), 127.90 (C-12), 128.89 (C-8), 146.48 (C-9), 148.94 (C-13), 155.64 (C-7), 170.53 (C-21). EI-MS m/z: 379.2 $[M + Na]^+$, 357.3 $[M + H]^+$. Molecular formula for $C_{22}H_{32}N_2O_2$: C, 74.12%, H, 9.05%, N, 7.96%, O, 8.98%; Found: C,74.03%, H,8.01%, N,8.03%, O, 9.06%.

2.2.2. 2-[(1S,3R)-3-(acetamidemethyl)-2-carboxyl-1,3-dimethylcyclohexyl]-5-isopropylbenzoicacid (3c)

Compound **3a** (3.42 g, 10 mM) in dioxane (10 mM) was added into the cooled potassium persulfate (6.5 g, 24 mM) in 40% sulfuric acid (25 mL) at 0 °C under stirring. After refluxing for 6 h, the mixture was added to water (200 mL) and dichloromethane (200 mL). The solution was regulated to neutral with a saturated aqueous sodium bicarbonate solution. The dichloromethane solvent was evaporated under hypobaric condition. Purification of crude product by column chromatography via using petroleum ether-ethyl acetate (1:3), and the result showed that the yellow solid compound yield was (2.02 g, 59.0%), mp 109-112 °C. IR (max, film, cm^{-1}): 3393, 2963, 2930, 1722, 1647, 1522, 1383, 1296, 732. ^{1}H NMR (300 MHz, *δ,* MeOD): 1.07 (3H, s, H_3C-3′), 1.30–1.14 (9H, m, H_3CC-1′, C*H*$(CH_3)_2$), 1.38 (2H, m, H-4′), 1.53–1.63 (2H, m, H-5′), 1.77–1.68 (m, 1H, H-6′α), 1.90–1.79 (3H, s, $COCH_3$), 1.92 (1H, m, H-6′β), 2.00 (1H, m, H-2′), 2.98–2.84 (2H, m, CH_2NH), 3.28–3.17 (1H, m, C*H*$(CH_3)_2$), 7.32 (1H, d, J = 11.8 Hz, H-3), 7.50–7.47 (1H, d, J = 11.8 Hz, H-2), 7.60 (1H, s, H-6). ^{13}C NMR (75 MHz, *δ,* $CDCl_3$): 17.93 (C-5′), 19.80 (*C*H_3C-1′), 20.13 ((*C*$H_3)_2$CH), 21.18 ((*C*$H_3)_2$CH), 24.06 (C-3′), 26.20 (*C*H_3C-3′), 31.72 (*C*H_3C-1′), 35.20 (*C*H$(CH_3)_2$), 36.27 (C-4′), 37.78 (C-1′), 38.45 (*C*H_2NH), 43.08 (C-6′), 49.18 (C-2′), 123.97 (C-3), 133.06 (C-6), 135.21 (C-1), 137.10 (C-3), 149.26 (C-5), 152.72 (C-2), 171.86 ($COCH_3$), 177.05 (ArCOOH), 187.90 (HOO*C*C-2′). EI-MS m/z: 388.1 $[M-H]^+$. Molecular formula for $C_{22}H_{31}NO_5$: C, 67.84%, H, 8.02%, N, 3.60%, O, 20.54%; Found: C, 67.75%, H, 8.01%, N,3.51%, O,20.47%

2.2.3. E-7- (4-chlorobenzoxyimine)-18-acetylamino-dehydroabietane (3d)

Compound **3b** (3.75 g, 10 mM) and 4-chlorobenzoyl chloride (2.1 g, 12 mM) were dissolved in acetone (20 mL) at 0 °C. After stirring for 1 h at 0 °C, then the mixture was added to water (20 mL) for 30 min. The solution was adjusted to pH 7 with diluted HCl, extracted with dichloromethane (3×20 mL). The dichloromethane solvent was evaporated under hypobaric condition. The crude product was purified by column chromatography using petroleum ether-ethyl acetate (1:1), to yield colorless solid (2.23 g, 59.5%), mp 99−101 °C. IR (max, film, cm^{-1}): 3398, 3318, 2956, 2929, 1735, 1658, 1436, 1378, 1294,1251, 749. ^{1}H NMR (300 MHz, *δ,* $CDCl_3$): 1.12 (3H, s, H-19), 1.29–1.18 (9H, m, H-16, 17, 20), 1.50–1.33 (2H, m, H-3), 1.63–1.53 (2H, m, H-2), 1.74–2.17 (2H, m, H-1), 1.98–1.91 (3H, s, CH_3CO), 2.04 (1H, m, H-5), 2.25 (1H, m, H-6α), 2.95–2.76 (3H, m, H-15,18), 3.33–3.15 (1H, m, H-6β), 7.30 (1H, m, H-12), 7.60–7.36 (3H, m, H-3′, 11, 14), 8.09–7.84 (2H, m, H-2′, 6′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.11 (C-22), 18.68 (C-2), 23.24 (C-19), 23.41 (C-16 or 17), 23.61 (C-16 or 17), 23.76 (C-20), 24.07 (C-4), 24.43 (C-10), 33.65 (C-15), 36.12 (C-3), 37.4 (C-6), 42.08 (C-1), 45.27 (C-5), 49.39 (C-18), 123.14 (C-14), 124.13 (C-11), 126.52 (C-12), 126.90 (C-6′), 127.26 (C-8), 129.61 (C-4′), 130.50 (C-2′), 131.11 (C-3′), 131.96 (C-7′), 132.76 (C-5′), 139.82 (C-9), 146.75 (C-13), 150.34 (C-7), 163.61 (COAr), 170.51 ($COCH_3$). EI-MS m/z: 495.3 $[M + H]^+$, 466.3, 357.3, 339.3, 298.2, 256.2, 202.1. Molecular formula for $C_{29}H_{35}ClN_2O_3$: C, 70.36%, H, 7.13%, N, 7.16%, N, 5.66%, O, 9.70%; Found: C,70.56%, H,7.01%, N, 7.03%, O, 9.57%.

2.2.4. E-7-(3,5-dinitrophenylaminoimine)-18-acetylamino-dehydroabietane (3e)

2,4-Dinitrophenylhydrazine (0.4 g, 2.02 mM), H_2O (2 mL), C_2H_5OH (5 mL) and H_2SO_4 (3 mL, 98%) were carefully mixed. The cooled mixture was added to compound **3b** (0.60 g, 1.76 mM) in anhydrous ethanol. After filtering to obtain a precipitate, then it was washed 3 times with water to obtein red crystals (0.26 g, 65.0%), mp187-189 °C. IR (max, film, cm^{-1}): 3382, 3306, 2956,1654, 1507, 1085, 758. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 1.27 (3H, s, H-20), 1.40–1.45 (9H, m, H-16, 17, 19), 1.71 (2H, H-3), 1.85–1.90 (2H, H-2), 2.09 (2H, m, H-1), 2.47 (3H, s, H_3CCO), 2.62 (1H, m, H-5), 2.94 (1H, H-6α), 3.29–3.34 (1H, m, H-15), 3.73 (2H, m, H-18), 7.33 (1H, s, H-12), 7.60–7.36 (2H, m, H-3′, 5′, 11, 14), 8.09–7.84 (2H, m, H-4′, 6′). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 18.14 (C-22), 19.00 (C-2), 23.44 (C-19), 23.48 (C-16 or 17), 23.79 (C-16 or 17), 23.91 (C-20), 24.09 (C-4), 33.74 (C-10), 36.38 (C-15), 36.89 (C-3), 37.35 (C-6), 37.62 (C-1), 41.85 (C-5), 49.57 (C-18), 116.63 (C-1′), 123.11 (C-14), 123.19 (C-6), 123.55 (C-4′), 124.41 (C-11), 127.32 (C-12), 128.81 (C-8), 130.02 (C-6′), 139.07 (C-9), 145.88 (C-13), 147.78 (C-7), 151.18 (C-5′), 152.97 (C-3′), 172.44 ($COCH_3$). EI-MS m/z: 522.1 $[M + H]^+$, Molecular formula for $C_{28}H_{35}N_5O_5$: C, 64.47%, H, 6.76%, N, 13.43%, O, 15.34%; Found: C, 64.56%, H 7.79%, N 13.23%,O 15.15%.

2.2.5. 7-hydroxylamine-18-acetylamino-dehydroabietane (3f)

$NaBH_4$ (0.75 g, 20 mM) was added to **3d** (4.95 g, 10 mM) dissolved in anhydrous ethanol (20 mL) in portions. The mixture was stirred for 3 h at room temperature, then adjusted to pH 7 with diluted HCl. The mixture was extracted with dichloromethane (3×20 mL). The dichloromethane solvent was evaporated. The crude product was purified by using petroleum ether-ethyl acetate (1:3), to obtein colorless solid (3.01 g, 60.8%), mp 62−63 °C. IR (max, film, cm^{-1}): 3323, 3106, 2960, 2932, 2873, 1712, 1671, 1559, 1381, 1209, 1158, 722. ^{1}H NMR (300 MHz, *δ,* $CDCl_3$): 1.08 (3H, s, H-19), 1.20–1.33 (9H, m, H-16, 17, 20), 1.41–1.54 (4H, m, H-2, 3), 1.79 (1H, s, H-5), 1.96–1.85 (3H, s, CH_3CO), 1.97–2.17 (2H, m, H-1), 2.16 (2H, m, H-6), 2.63 (1H, m, H-15), 2.91–2.77 (2H, m, H-18), 3.47–3.38 (1H, m, H-7), 7.18 (2H, d, J = 12.1 Hz, H-12), 7.36 (1H, s, H-11), 7.46 (1H, s, H-14). ^{13}C NMR (75 MHz, *δ,* $CDCl_3$): 18.20 (C-22), 18.56 (C-2), 21.76 (s), 23.12 (C-19), 23.47 (C-16 or 17), 23.77 (C-16 or 17), 24.02 (C-20), 28.00 (C-4), 29.96 (C-10), 33.65 (C-6), 36.19 (C-3), 37.58 (C-1), 42.15 (C-5), 51.26 (C-18), 69.54 (C-7), 122.15 (C-14), 122.80 (C-11), 127.91 (C-12), 128.80 (C-8), 143.54 (C-9), 146.50 (C-13), 170.36 ($COCH_3$). EI-MS m/z: 358.1 M^+, 357.1 $[M-H]^+$, 339.1, 326.1. Molecular formula for $C_{22}H_{34}N_2O_2$: C, 73.70%, H, 9.56%, N, 7.81%, O, 8.93%; Found: C,73.81%, H 9.45%, N 7.92%,O 8.81%.

2.2.6. (8R,11aS)-3-isopropyl-8,11a-dimethyl-8-acetamidemethyl-6-oxo-6,7,7,8,9,10,11,11a-octahydro-5H-dibenzo[b,d]azepine (3g)

Compound **3b**(5.01 g, 14 mM) and PPA(50 mL) were carefully mixed. The mixture was kept stirring for 10 min at 100–120 °C, then pumped into 400 mL ice water and adjusted to pH 8 with ammonium hydroxide. The precipitate was filtered off and washed with water 3 times. The crude product was purified by using petroleum ether-ethyl acetate(1:9), to yield pale yellow solid (3.61 g, 72.1%), mp241-242 °C. IR (max, film, cm^{-1}): 3398, 3318, 2956, 2929, 2869, 1735, 1658, 1436, 1294, 1118, 828, 749. ^{1}H NMR (300 MHz, *δ,* $CDCl_3$): 1.09–0.94 (3H, s, H_3CC-8), 1.23

(6H, d, J = 6.9 Hz, (CH_3)$_2$CH), 1.45 (3H, s, H-11a), 1.54 (2H, dd, J = 13.1, 3.1 Hz, H-9), 1.85–1.60 (2H, m, H-10), 1.92 (3H, s, H_3CCO), 2.06–1.99 (3H, m, H-7a, 11), 2.47 (2H, d, J = 7.8 Hz, H-7), 2.85 (1H, qq,CH(CH_3)$_2$), 3.05 (1H, dd, J = 14.2, 6.0 Hz, H-8aα), 3.26 (1H, dd, J = 14.1, 7.6 Hz, H-8aβ), 5.88 (1H, s, HNCOCH_3), 6.68 (1H, s, H-4), 7.00 (1H, d, J = 8.3 Hz, H-2), 7.32 (1H, d, J = 8.3 Hz, H-1), 7.89 (1H, s, HN-Ar). ^{13}C NMR (75 MHz, δ, $CDCl_3$): 18.77 (C-10), 19.19 (CH_3CO), 23.29 (CH_3C-8), 23.46 (CH_3C-11a), 23.66 (C-7), 23.69 (C-11a), 33.17 (CH_3CH), 33.19 (CH_3CH), 35.75 (C-11), 39.61 (CH(CH_3)$_2$), 41.00 (C-8), 43.42 (C-9), 48.17 (C-7a), 50.19 (CH_2NH), 120.41 (C-4), 123.06 (C-2), 128.89 (C-1), 134.49 (C-1b), 138.53 (C-4a), 147.90 (C-3), 170.60 ($COCH_3$), 176.66 (C-6). EI-MS m/z: 358.2 $[M+2H]^+$, 357.2 $[M + H]^+$. Molecular formula for $C_{22}H_{32}N_2O_2$: C, 74.12%, H, 9.05%, N, 7.86%, O, 8.98%; Found: C,74.30%, H, 8.91%, N 8.03%, O, 9.06%.

2.2.7. (6S)-18-acetamino-6-Br-7-oxo-dehydroabietane (3h)

Compound **3a** (5.01 g, 14 mM) in acetic acid (40 mL) was dissolved under stirring. then added to Br_2 (4 g, 5.0 mM). The mixture was stirring for 2 h at room temperature, then pumped into water (400 mL) dissolving sodium bicarbonate (60 g), and extracted with diethyl ether (3×100 mL). The diethyl ether solvent was evaporated, to yield red brown paste (4.61 g, 68.1%), mp 60–61 °C. IR (max, film, cm^{-1}): 3421, 2958, 2930, 1736, 1652, 1436, 1388, 1045, 1037, 744. ^{1}H NMR (300 MHz, δ, $CDCl_3$): 1.08 (3H, s, H-19), 1.26 (6H, m, H-16, 17), 1.59–1.49 (3H, m, H-20), 1.74 (4H, d, J = 14.0 Hz, H-2, 3), 2.00 (3H, s, CH_3CO), 2.29 (1H, d, J = 7.8 Hz, H-15), 2.97 (2H, d, J = 13.7 Hz, H-1), 3.26 (1H, d, J = 14.2 Hz, H-5), 3.26 (1H, d, J = 14.2 Hz, H-5), 3.56 (2H, s, H-18), 4.74 (1H, d, J = 14.3 Hz, H-6), 7.23 (1H, d, J = 10.7 Hz, H-12), 7.47 (1H, d, J = 8.0 Hz, H-11), 7.67 (1H, s, H-14). ^{13}C NMR ($CDCl_3$, δ, 75 MHz): 18.14 (C-2), 18.51 (C-22), 23.39 (C-19), 23.71 (C-16 or 17), 23.77 (C-16 or 17), 23.79 (C-20), 23.82 (C-4), 33.49 (C-10), 35.68 (C-15), 35.93 (C-12), 37.46, 37.71 (C-1), 44.25 (C-5), 49.03 (C-18), 51.28(C-6), 123.61 (C-14), 124.86 (C-11), 130.53 (C-12), 132.61 (C-8), 146.78 (C-9), 153.42 (C-13), 170.42 (C-21), 199.07 (C-7). EI-MS m/z: 364.1 $[M + Na]^+$, 359.1, 342.1 $[M + H]^+$. Molecular formula for $C_{22}H_{30}BrNO_2$: C 62.86%, H 7.19%, N 19.01%, O 7.61%; Found: C, 63.02%, H,7.01%, N, 18.81%, O, 7.82%.

2.2.8. (8R,11aS)-3-isopropyl-8,11a-dimethyl-8-acetamidemethyl-6,7,7,8,9,10,11,11a-octahydro-5H-dibenzo [b,d] azepine (3i)

$LiAlH_4$ (0.051 g, 1.266 mM) was added to compound **3g** (0.301 g, 0.844 mM) in diethyl ether (30 ml) under stirring. The mixture was stirred for 2 h at room temperature, then pumped into NaOH solution (30 mL, 0.1 M), and the extracted with diethyl ether (3×20 mL). The solvent was evaporated, to yield light yellow paste (0.221g, 73.4%); mp110-111 °C. IR (max, film, cm^{-1}): 3318, 3099, 2958, 2930, 2867, 1655, 1560, 1463, 1378, 1093, 805, 699. ^{1}H NMR (300 MHz, δ, $CDCl_3$): 0.98 (3H, s, H_3CC-8), 1.07–1.01 (3H, m, CH_3C-11a), 1.23–1.18 (6H, m, (CH_3)$_2$CH), 1.60 (2H, d, J = 13.4 Hz, H-10), 1.73 (2H, d, J = 12.0 Hz, H-9), 1.80 (2H, m, H-11), 1.87–1.80 (2H, m, H-7), 1.95 (3H, s, CH_3CO), 2.29 (1H, dd, J = 15.4, 11.8 Hz, H-7a), 2.48 (1H, d, J = 10.8 Hz, H-6β), 2.58 (1H, d, J = 7.1 Hz, H-6a), 2.76 (1H, m, CH(CH_3)$_2$), 2.88 (1H, d, J = 19.5 Hz, H-8aα), 3.37–3.22 (1H, m, H-8aβ), 6.48 (1H, dd, J = 5.7, 2.0 Hz, H-4), 6.75–6.63 (1H, m, H-2), 7.30 (1H, d, J = 9.1 Hz, H-1). ^{13}C NMR (75 MHz, $CDCl_3$): δ18.36 (C-10), 20.79 (CH_3C-8), 21.41 ((CH_3)$_2$CH), 22.33 ((CH_3)$_2$CH), 23.55 (CH_3CO), 23.72 (CH_3C-11a), 23.82 (C-7), 28.64 (C-11a), 33.04 (C-8), 37.91 (CH(CH_3)$_2$), 42.30 (C-9), 43.89 (C-11), 49.22 (C-18), 50.04 (C-7a), 61.95 (C-6), 118.28 (C-4), 118.62 (C-2), 127.50 (C-1), 138.88 (C-1b), 146.67 (C-4a), 150.87 (C-3), 170.39 (C=O). EI-MS m/z: 344.2 $[M+2H]^+$, 343.2 $[M + H]^+$. Molecular formula for $C_{22}H_{34}N_2O$: C, 77.14%, H, 10.01%, N, 8.18%, O, 4.67%; Found: C, 77.02%, H,9.89%, N,8.01%, O, 4.82%.

2.2.9. (8R,11aS)-3-isopropyl-8,11a-dimethyl-8-ethylaminomethyl-6,7,7,8,9,10,11,11a-octahydro-5H-dibenzo [b,d]azepine (3j)

$LiAlH_4$ (0.310 g, 7.66 mM) was added to compound **3g** (0.277 g, 0.776 mM) in diethyl ether (30 ml) under stirring. The mixture was stirred for 12 h at room temperature, then pumped into NaOH solution (30 ml, 0.1 mol/L), and extracted with diethyl ether (3×20 mL). The diethyl ether solvent was evaporated, to yield light yellow paste (0.218g, 71.4%), mp 87-88 °C. IR (KBr): 3355, 2962, 2867, 1660, 1609, 1463, 1380, 1261, 1096, 1021, 801, 700. ^{1}H NMR (300 MHz, δ, $CDCl_3$): 1.01–0.94 (3H, m, CH_3C-8), 1.10–1.02 (3H, m, CH_3C-11a), 1.24–1.19 (6H, m, (CH_3)$_2$CH), 1.39 (3H, d, J = 8.1 Hz, CH_3CH$_2$NH), 1.50 (2H, dd, J = 12.6, 4.0 Hz, NCH_2CH$_3$), 1.63 (2H, m, H-10), 1.78–1.69 (2H, m, H-9), 1.83 (1H, d, J = 3.5 Hz, H-11), 1.97–1.84 (2H, m, H-7), 2.04 (1H, d, J = 10.9 Hz), 2.27 (1H, d, J = 11.9 Hz, H-7a), 2.51 (1H, d, J = 11.9 Hz, H-6β), 2.58 (1H, d, J = 7.1 Hz, H-6a), 2.77 (1H, dd, J = 13.8, 6.9 Hz, CH(CH_3)$_2$), 2.89 (1H, m, H-8aα), 3.30 (1H, m, H-8aβ), 6.47 (1H, d, J = 7.3 Hz, H-4), 6.71 (1H, dd, J = 8.3, 2.0 Hz, H-2), 7.30 (1H, d, J = 8.9 Hz, H-1). ^{13}C NMR (75 MHz, δ, $CDCl_3$): 15.28 (C-10), 18.67 (CH_3C-8), 21.41 ((CH_3)$_2$CH), 22.35 ((CH_3)$_2$CH), 23.74 (CH_3C-11a), 23.86 (C-22), 28.64 (C-7), 33.05 (C-11a), 38.05 (C-8), 41.77 (CH(CH_3)$_2$), 38.40 (C-9), 42.34 (C-11), 44.00 (C-18), 45.08 (C-7a), 49.32 (C-21), 61.94 (C-6), 118.23 (C-4), 118.61 (C-2), 127.53 (C-1), 139.44 (C-1b), 146.81 (C-4a), 150.77 (C-3). Molecular formula for $C_{22}H_{36}N_2$: C, 80.43%, H, 11.04%, N, 8.53%; Found: C, 80.28%, H,10.89%, N,8.41%.

2.3. Antitumor activity

HepG2 and L02 cells were cultured in 96-well plates at a density of 5×104 cells per mL. All cells were incubated cultured at 37 °C in a humidified incubator containing 5% CO2. The experiments are divided into the following groups: control group (dimethyl sulphoxide, DMSO), drug group (test compounds 3a-3j, 10 μg/ml). After treatment, 20 μL of MTT solution (5 mg/mL) was added to each well and incubated at 37 °C for 4 h, following which the culture medium was removed and 100 μL of DMSO was added. The absorbance was measured at 570 nm using a microplate spectrophotometer (Model 550, Bio-Rad, USA).

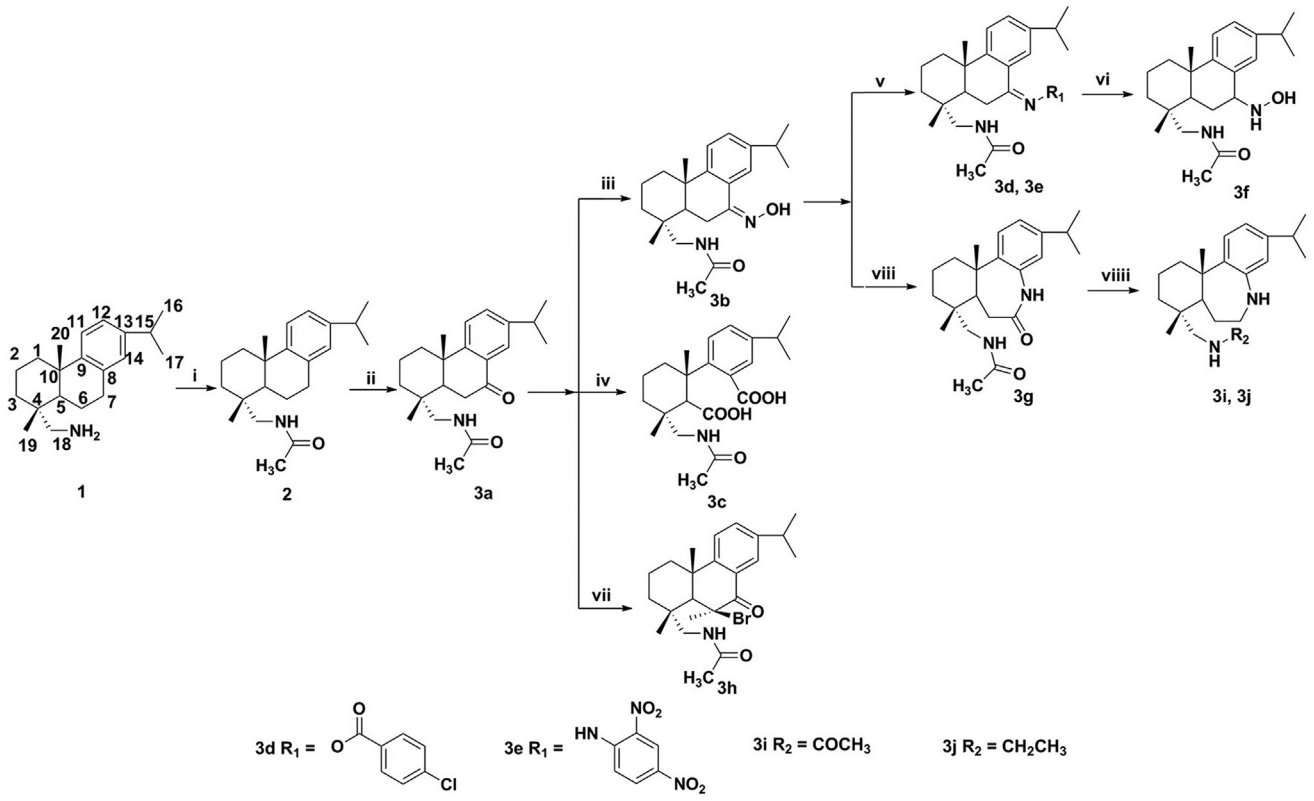
2.4. DNA cleavage activity

The compounds have been studied using pBR322 supercoiled plasmid DNA (Form I) as a substrate in a medium of 50 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 5μl pBR322 supercoiled DNA (0.25 μg/μl), 50 μM of each dehydroabietylamine derivative or 10 μM Fe irons. The DNA cleavage fragmentwas analyzed by agarose gel electrophoresis. After electrophoresis, the gels were illuminated with UV light and then photographed.

3. Results and discussion

3.1. Synthesis

The synthetic routes of the title compounds are outlined in Scheme 1 [15]. Compound **3a** was originated in Laboratory of our research group. With the compound **3a** in hand, we carried out the oxime reaction in the 7-position on ring B in refluxing ethanol to obtain **3b** in the presence of NH2OH·HCl, oxidation reaction in dioxane at 0 °C to obtain **3c** in the presence of K2S2O8, and bromination reaction in the 6-position on ring B in AcOH at room temperature to obtain **3h** in the presence of Br2. Then, we carried out the functional group interconversions necessary to obtain more derivatives. The first strategy was the acylation reaction of **3b** staring from **3a**, which transformed the 2-chlorobenzoyl chloride and 2, 4-dinitrophenylhydrazone group into the OH of oxime gives **3d** and **3e**, respectively. The second tactics was the Beckmann rearrangement of **3b** using polyphosphoric acid at 100 °C to get **3g**, in that an electropositive nitrogen is formed that initiate anti-position alkyl substituent migration simultaneously. Subsequent reduction of **3g** by LiAlH4 in diethyl ether afforded the corresponding product **3i** for 2 h and **3j** for 12 h at room temperature in good yield. Two carbanyl group in compound **3g** can be both reduced to **3j** by extending the time.



Scheme 1. Synthetic route for target compounds. Reagents and conditions: ii.2,4-dinitrophenylhydrazine, C_2H_5OH, H_2SO_4, 0 °C; iii. $NH_2OH{\cdot}HCl$, C_2H_5OH, Na_2CO_3,reflux; iv. $K_2S_2O_8$, dioxane, 40% H_2SO_4, 0 °C; v. 4-chlorobenzoyl chloride, acetone, 0 °C; vi. $NaBH_4$, CH_3CH_2OH, r.t.; vii. Br_2, AcOH, r.t.; viii. PPA, 100 °C; *IX.* $LiAlH_4$, diethyl ether, 2 h or 12h, r.t.

3.2. Bioactivities

The effects of synthetic compounds **3a**-**3j** on the viability of HepG2 and L02 cells were evaluated. The results showed that all compounds had low cytotoxicity (Figure 1). The results demonstrated that compound 3a and 3b displayed a modest cytotoxic activity against the cancer cells. Interestingly, the functional group on ring B plays an important role in their cytotoxicity. carbonyl (3h, 3b and 3a) and lactam (3g) < imine – like side chain (3i and 3j) < oxime ether – like side chain (3d and 3e). The antineoplastic activity of compounds 3d and 3e are higher than 3i and 3j, which is in good agreement that the N-containing group is helpful to bind to the receptor and improve the activity of the compound.

After treatment with compound **3a**, the plasmid DNA has been cleaved completely in Figure 2. Introducing electron-withdrawing group in ortho of carbonyl in compound **3h**, DNA cleavage activity was weakened. It may be steric hindrance of carbonyl group from Br to prevent carbonyl-induced DNA damage. Compound **3b**, **3d** and **3e** can convert partially the Form I (supercoiled DNA) to Form II because of their oxime structure. Compounds **3f**, **3g** and **3i** can convert partially the Form I to Form III, which may imply some form of nucleophilic reaction mechanism for the initiation of cleavage. The results suggest that the

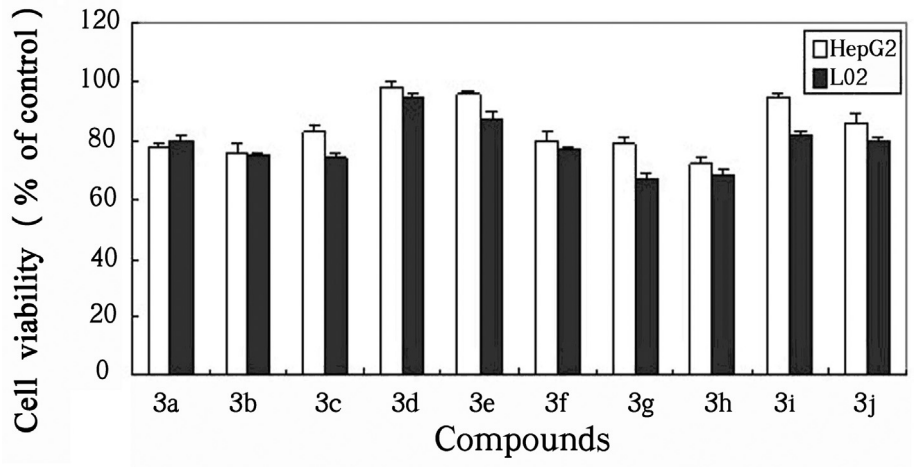


Figure 1. Effect of compounds on cell viability.

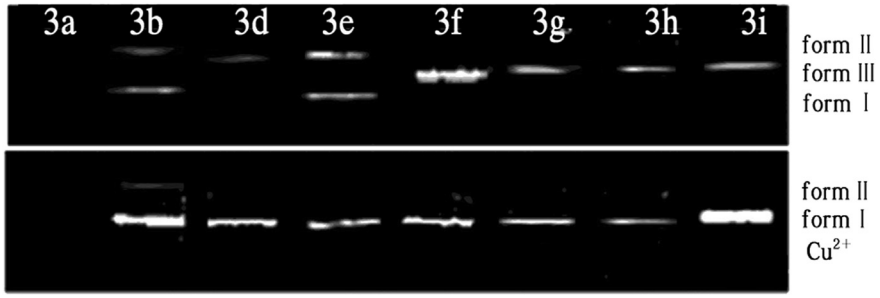


Figure 2. DNA cleavage activity of compounds.

structure and substituent property and the conformation of B-ring have different selective for cleavaging DNA.

In addition, we also examined the DNA-cleaving activity of these compounds in the presence of Cu^{2+} ions. Apart from compound **3a**, other compounds displayed very weak DNA-cleaving activity compared with control groups, which suggest Cu^{2+} ions can inhibit carbonyl group mediated or other radical damage to pBR322 plasmid DNA [18, 19]. Thus, the above results suggest that Cu^{2+} approaching to oxygen and then lowering polarity of carbonyl may be important factors in the mechanism of DNA damage.

4. Conclusion

A series of novel dehydroabietylamine derivatives were synthesized and characterized. Meanwhile, the anti-tumor and DNA cleavage activities of the synthesised compounds were investigated. The results have demonstrated that the functional group on ring B plays an important role in their cytotoxicity. The N-containing group is helpful to bind to the receptor and improve the activity of the compound:carbonyl (3h, 3b and 3a) and lactam (3g) < imine – like side chain (3i and 3j) < oxime ether – like side chain (3d and 3e). The results suggest that the structure and substituent property and the conformation of B-ring have different selective for cleavaging DNA. Searching for new compounds is the highlight of this study. Furthermore, these results continue to encourage us to synthesize more dehydrorosinamine derivatives with the aim of obtaining compounds with more potent biological activity.

Declarations

Author contribution statement

Jincai Li: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Chaoxiang Liu: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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