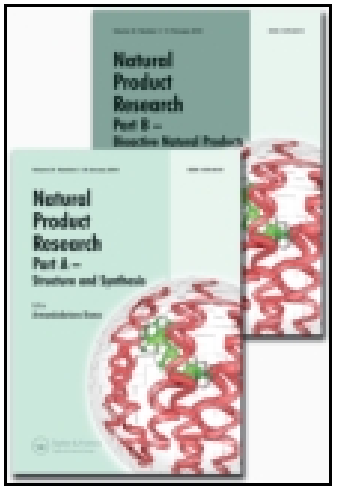

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Synthesis and antitumour activities of a novel class of dehydroabietylamine derivatives

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Structural modification is still a popular and important route in the forest chemical field for finding novel tricyclic diterpenes with more potential bioactivities and broad bioactive spectra. In this study, a series of dehydroabietylamine derivatives containing tricyclic diterpene structures were synthesised through oxidation in the 7th position of ring B and nitrification in the 12th position of ring C using dehydroabietylamine as the starting material. Structures of the synthesised compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, MS and HRMS. The cytotoxicities of these compounds against PC-3 (human prostate carcinoma cell line) and Hey-1B (human ovarian carcinoma cell line) cells by the MTT assay were investigated. The results showed that the presence of a nitro group at 12th position and a carbonyl group at 7th position resulted in an increase of cytotoxic activity. Our findings present more evidence, showing the relationship between the chemical structure and biological function.

Keywords: dehydroabietylamine; antitumour; cytotoxicity

1. Introduction

Cancer remains the second leading cause of death in most countries and as a result, there is a need for more effective compounds. Total synthesis and modification of the structures of bioactive natural products are effective methods to find potential compounds with anticancer activity. Natural tricyclic diterpenes are an important class of potential anticancer drugs. Some tricyclic diterpenes from natural products have been synthesised or modified, and their anticancer activities have been investigated (Gigante et al., 2003; Grace, Jin, Wilson, & Coates, 2006; Son, Oh, Choi, Han, & Kwon, 2005). In particular, functional group modification at C-7 and C-12 positions can be a way to prepare compounds with potential biological interest (González et al., 2010; Li, She, Zhang, Wu, & Pan, 2003; Xiong, Wang, Pan, Sun, & Tu, 2006; Zhang & Lin, 2009). Dehydroabietylamine (Figure 1) is a distinctive synthetic primary amine having a tricyclic diterpene structure which is obtained as part of a mixture of amines prepared by the hydrogenation of rosin acid nitrile. Dehydroabietylamine is widely used in the fields of paper-making, medicine, pesticide and chemical industries (Rao, Song, & He, 2008; Wilkerson, Galbraith, DeLucca, & Harris, 1993). Recently, antitumour and cytotoxic

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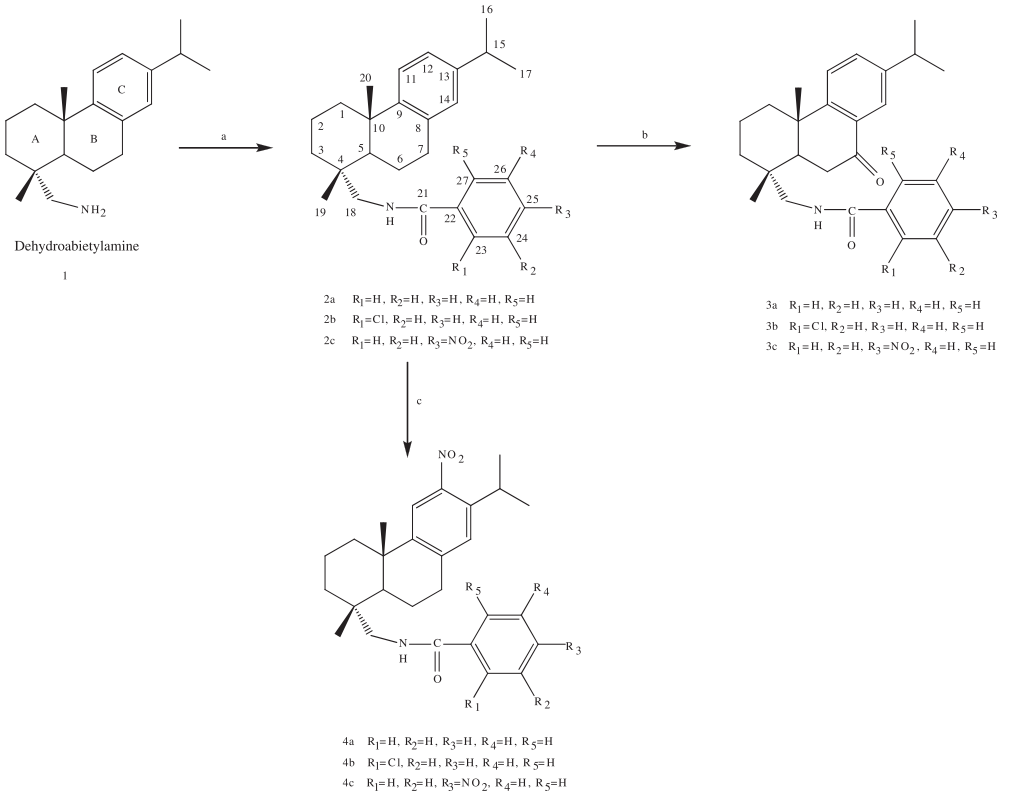

Figure 1. Reagents and conditions: (a) benzoyl chloride and substituted benzoyl chloride, toluene, 80°C, 8 h, (b) CrO_3, AcOH, 60°C, 12 h and (c) $Cu(NO_3)_2$, Ac_2O, 10°C, 12 h.

activities of dehydroabietylamine derivatives are a focus of research in the forest chemical field.

Pursuing our studies in the chemical transformation of dehydroabietylamine, we have been looking at the possibility of preparing new compounds from dehydroabietylamine through the functionalisation of rings B and C (Figure 1). In this communication, a series of C-7 oxidised or C-12 nitrated abietane diterpenes were synthesised from dehydroabietylamine, and their antitumour activities were evaluated against two cultured human-tumour cell lines (PC-3 and Hey-1B) for investigating the influences of functional groups modification on the bioactivities. Among these compounds, **3a**, **4a**, **4b** and **4c** showed good cytotoxicities against PC-3 cancer cells (IC_{50} 5.7–8.2 μg mL^{-1}) and Hey-1B cancer cells (IC_{50} 11.3–16.0 μg mL^{-1}). Our results have revealed that the presence of a nitro group at 12th position and a carbonyl group at 7th position resulted in an increase of cytotoxic activity. These results may provide useful information for the design of anticancer drugs.

2. Results and discussion

2.1. *Chemistry*

To determine the effect of the type and the position of functional groups on bioactivity, a series of novel tricyclic diterpenes with a carbonyl group in the 7th position on ring B or a nitro group in the 12th position on ring C and benzamides with various substituents

in the 18th position were synthesised using dehydroabietylamine as the starting material (Figure 1).

2.1.1. *Synthesis of analogues of N-benzoyl-dehydroabietylamine (**2a**)*

Dehydroabietylamine (compound **1**) was converted to compounds **2a**–**2c** *via* acylation with benzoyl chloride and substituted benzoyl chloride in toluene. The structures of compounds **2a**–**2c** were confirmed by analyses of IR and ^{1}H-NMR spectra. The presence of the characteristic band of amides at 1644–1638 cm^{-1} was observed in the IR spectra of **2a**–**2c**. The formation of amides was easily confirmed by their ^{1}H-NMR spectrum due to a broad one-proton singlet at δ 6.10–6.24 ppm corresponding to the amides N–H. In contrast to the standard spectra of dehydroabietylamine, the chemical shift value of H-18 in **2a**–**2c** changed from δ 1.20–1.21 to δ 3.31–3.53 ppm due to the deshielding effect caused by formation of the amides.

2.1.2. *Synthesis of analogues of N-benzoyl-dehydroabietylamine-7-one (**3a**)*

The oxidation of an olefin to an α,β-unsaturated ketone has been utilised in the synthesis and in the transformation of natural products. Oxidation with transition metals such as complexes of copper and selenium compounds is the main method used in the laboratory. In most cases, chromium (VI) has been the oxidant and varying results have been obtained depending upon the specific chromium (VI) reagent used and the conditions employed. The conditions used most often have been chromium (VI) in acetic acid (Dauben, Lorber, & Fullerton, 1969; Li & Si, 2003). Compounds **2a**–**2c** were converted to **3a**–**3c** *via* oxidation with chromium trioxide in acetic acid. Compounds **3a**–**3c** presented a typical difference in their spectra from their parent compounds **2a**–**2c**. Comparing the IR spectra of compounds **2a** and **3a**, a new absorption appeared at 1685 cm^{-1} for compound **3a**, indicating the presence of a keto group in the 7th position of ring B. In the MS spectrum of compound **3a**, the molecular ion peak was observed at m/z 404.3. The resonance of C-7 changed from δ 30.53 to δ 199.37 ppm by comparing the ^{13}C-NMR spectra of **2a** and **3a**, indicating the formation of carbonyl group. The IR, MS and ^{13}C-NMR spectra of compounds **3b** and **3c** had similar features.

2.1.3. *Synthesis of analogues of N-benzoyl-12-nitrodehydroabietylamine (**4a**)*

Nitration of aromatic rings is a classical organic synthesis reaction. In this study, nitration of dehydroabietylamine derivatives was attempted using acetic anhydride–cupric nitrate since this reagent gives low concentrations of nitronium ion in solution and is more selective than a nitric acid–sulphuric acid mixture (Cambie & Franich, 1971). The presence of the characteristic band of the nitro group at 1644–1638 cm^{-1} was observed in the IR spectra of **4a**–**4b**. The presence of the nitro group in **4a** was confirmed by the downfield shift of H-11 and H-14 to 7.62 and 7.07 ppm, respectively, and by comparison with the ^{1}H-NMR spectra of **2a** (6.88 and 7.17 ppm, respectively) due to the deshielding effect caused by formation of the nitro group. The ^{1}H-NMR spectra of compounds **4b** and **4c** had similar features.

2.2. *Antitumour activities*

The effects of the synthesised compounds (**2a**–**2c**, **3a**–**3c** and **4a**–**4c**) on the *in vitro* growth of two human cancer cell lines PC-3 and Hey-1B were evaluated, and the results were summarised as IC_{50} values in $\mu g\,mL^{-1}$ in Table 1.

Among these compounds, **2c**, **3a**, **4a**, **4b** and **4c** showed potential activities against PC-3 and Hey-1B cancer cells. Apparently, the nitro groups at 12th and 25th positions play an

Table 1. *In vitro* antitumour activities (IC_{50} $\mu g\,mL^{-1}$) of the synthesised compounds.

Compounds	PC-3 IC_{50} ($\mu g\,mL^{-1}$)	Hey-1B IC_{50} ($\mu g\,mL^{-1}$)
2a	62.6	61.6
2b	84.9	129.8
2c	8.7	18.9
3a	5.7	16.0
3b	73.1	86.2
3c	80.9	97.2
4a	8.2	11.3
4b	7.8	15.2
4c	6.2	15.0

important role in cytotoxicity against cancer cells. Compounds **3a** and **3b**, with a carbonyl group in the 7th position on ring B showed a remarkable increase in their cytotoxic activities in comparison with their parent compounds **2a** and **2b**, suggesting the importance of the carbonyl group in biological function.

3. Experimental

3.1. *Chemistry*

All chemicals and solvents were analytical grade, and solvents were purified by general methods before being used. Melting points were determined with a XT6 melting point apparatus and uncorrected. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker DPX 300 spectrometer at 300 and 75 MHz, respectively. The chemical shifts are reported in ppm relative to TMS. IR spectra were recorded on a Nicolet 360 FT–IR spectrometer. Mass spectra were recorded on a Truace DSQ GC–MS. HRMS results were obtained on an ABI MARINER–ESI–TOF. Thin layer chromatography was done using silica gel GF254. Silica gel used for column chromatography was 100–200 mesh.

3.1.1. *Synthesis of N-benzoyl-dehydroabietylamine (**2a**)*

To a solution of dehydroabietylamine (1.43 g, 5 mmol) in toluene (50 mL) benzoyl chloride (0.7 g, 5 mmol) was added. The mixture was stirred at room temperature for 1 h, and heated at 80°C with magnetic stirring for 8 h. The toluene was evaporated *in vacuo*. The resulting crude product was purified by recrystallisation from methanol. Compound **2a** was obtained as white crystals (1.38 g, 71%), m.p. 162–164°C. IR (KBr): 3433, 2925, 1638 and 823. ^{1}H-NMR (δ_H, $CDCl_3$, 300 MHz): 1.01(3H, s, H-19), 1.20–1.24(9H, m, H-16, 17, 20), 1.32–1.84(7H, m, H-1α, 2, 3, 6), 1.95–2.02(1H, m, H-5), 2.31(1H, brd, $J = 12.7$ Hz, H-1β), 2.77–2.97(3H, m, H-7,15), 3.31–3.47(2H, m, H-18), 6.10(1H, s, 22-CONH), 6.88(1H, s, H-14), 6.99(1H, d, $J = 8.2$ Hz, H-12), 7.17(1H, d, $J = 8.2$ Hz, H-11), 7.39–7.51(3H, m, H-24, 25, 26) and 7.71–7.74(2H, m, H-23, 27). ^{13}C-NMR (δ_c, $CDCl_3$, 75 MHz): 18.75(C-19), 18.88(C-2), 19.20(C-6), 24.07(C-16 or C-17), 25.55(C-20), 30.53(C-7), 33.50(C-15), 36.48(C-3), 37.64(C-4), 37.82(C-10), 38.44(C-1), 45.90(C-5), 50.42(C-18), 123.95(C-12), 124.3(C-11), 126.97(C-14 or C-23), 126.97(C-27), 128.61(C-24 or C-26), 131.39(C-25), 134.84(C-8 or C-22), 145.65(C-13), 147.12(C-9) and 167.79(C-21). EI–MS *m/z*: 390.1$[M + H]^+$, 339.0, 269.1, 187.2, 173.1, 133.9. HR–ESI–MS *m/z*: 390.2831 ($[M + H]^+$, $C_{27}H_{36}NO^+$; Calcd 390.2797).

3.1.2. *Synthesis of N-(o-chlorobenzoyl)-dehydroabietylamine (**2b**)*

Compound **2b** was similarly prepared according to the procedure of **2a**. Yield 72%, white crystals, m.p. 146–148°C. IR (KBr): 3437, 2913, 1644 and 820. ^{1}H-NMR (δ_H, $CDCl_3$, 300 MHz): 1.03(3H, s, H-19), 1.20–1.23(9H, m, H-16, 17, 20), 1.33–1.82(7H, m, H-1α, 2, 3, 6), 1.96–2.00(1H, m, H-5), 2.30(1H, brd, $J = 12.8$ Hz, H-1β), 2.77–2.95(3H, m, H-7,15), 3.39–3.42(2H, m, H-18), 6.21(1H, s, 22-CONH), 6.88(1H, s, H-14), 6.99(1H, d, $J = 8.1$ Hz, H-12), 7.16(1H, d, $J = 8.1$ Hz, H-11), 7.28–7.38(3H, m, H-25, 26, 27), 7.64–7.66(1H, m, H-24). ^{13}C-NMR (δ_c, $CDCl_3$, 75 MHz): 18.73(C-2 or C-19), 19.16(C-6), 24.07(C-16 or C-17), 25.42(C-20), 30.34(C-7), 33.49(C-15), 36.41(C-3), 37.59(C-4), 37.63(C-10), 38.44(C-1), 45.81(C-5), 50.77(C-18), 123.90(C-12), 124.22(C-11), 126.96(C-14 or C-26), 127.12(C-27), 130.24(C-24), 131.21(C-22 or C-25), 134.76(C-8), 135.41(C-23), 145.63(C-13), 147.10(C-9) and 166.70(C-21). EI–MS m/z: 424.2$[M + H]^+$, 401.0, 382.9, 330.3, 318.3, 302.3, 274.8, 271.0, 196.3, 151.9. HR–ESI–MS m/z: 424.2516 ($[M + H]^+$, $C_{27}H_{35}ClNO^+$; Calcd 424.2407).

3.1.3. *Synthesis of N-(p-nitrobenzoyl)-dehydroabietylamine (**2c**)*

Compound **2c** was similarly prepared according to the procedure of **2a**. Yield 68%, pale yellow crystals, m.p. 150–151°C. IR (KBr): 3435, 2930, 1641 and 823. ^{1}H-NMR (δ_H, $CDCl_3$, 300 MHz): 1.05(3H, s, H-19), 1.23–1.25(9H, m, H-16, 17, 20), 1.33–1.82(7H, m, H-1α, 2, 3, 6), 1.96–2.03(1H, m, H-5), 2.35(1H, brd, $J = 12.9$ Hz, H-1β), 2.80–3.01(3H, m, H-7, 15), 3.33–3.53(2H, m, H-18), 6.24(1H, s, 22-CONH), 6.92(1H, s, H-14), 7.01–7.03(1H, m, H-12), 7.19(1H, d, $J = 8.2$ Hz, H-11), 7.91(2H, d, $J = 8.7$ Hz, H-23, 27), 8.28(2H, d, $J = 7.8$ Hz, H-24, 26). ^{13}C-NMR (δ_c, $CDCl_3$, 75 MHz): 18.68(C-19), 18.84(C-2), 19.21(C-6), 24.02(C-16 or C-17), 25.45(C-20), 30.38(C-7), 33.46(C-15), 36.47(C-3), 37.59(C-4), 37.97(C-10), 38.36(C-1), 45.84(C-5), 50.78(C-18), 123.71(C-24 or C-26), 124.00(C-12), 124.22(C-11), 126.98(C-14), 128.29(C-23 or C-27), 134.64(C-8), 140.55(C-22),145.73(C-13), 147.00(C-9), 149.40(C-25) and 166.12(C-21). EI–MS m/z: 435.3$[M + H]^+$, 332.1, 302.9, 274.4, 270.5, 120.7. HR–ESI–MS m/z: 435.2625 ($[M + H]^+$, $C_{27}H_{35}N_2O_3^+$; Calcd 435.2648).

3.1.4. *Synthesis of N-benzoyl-dehydroabietylamine-7-one (**3a**)*

A solution of CrO_3 (1 g, 10 mmol) in 20 mL of acetic acid was added dropwise to a solution of **2a** (1.95 g, 5 mmol) in acetic acid. The mixture was stirred at room temperature for 30 min, and heated at 60°C with magnetic stirring for 12 h. The mixture was then poured into water and ice, extracted with chloroform. The combined organic layers were dried over anhydrous sodium sulphate, and the solvent was evaporated under reduced pressure (Li et al., 2003). The crude product was purified by silica gel column chromatography using petroleum ether/acetone (3 : 1, v/v) as the eluent to give compound **3a** (1.11 g, 55%) as white needle-shaped crystals, m.p. 174–176°C. IR (KBr): 3422, 2933, 1685, 1648 and 830. ^{1}H-NMR (δ_H, $CDCl_3$, 300 MHz): 1.10(3H, s, H-19), 1.21–1.24(6H, m, H-16, 17), 1.28(3H, s, H-20), 1.35–1.89(5H, m, H-1α, 2, 3), 2.01–2.07(1H, m, H-5), 2.35(1H, brd, $J = 12.5$ Hz, H-1β), 2.67–2.80(2H, m, H-6), 2.84–2.97(1H, m, H-15), 3.18–3.55(2H, m, H-18), 6.22(1H, s, 22-CONH), 7.27–7.49(5H, m, H-11, 12, 24, 25, 26), 7.70–7.73(2H, m, H-23, 27), 7.83–7.84(1H, m, H-14). ^{13}C-NMR (δ_c, $CDCl_3$, 75 MHz): 18.22(C-19), 18.67(C-2), 23.68(C-16 or C-17), 24.01(C-20), 33.41(C-15), 35.86(C-6), 36.11(C-3), 37.51(C-4), 37.76(C-10), 38.1(C-1), 44.42(C-5), 49.50(C-18), 123.59(C-11), 124.84(C-14), 127.06(C-23 or C-27), 128.40(C-24 or C-26), 130.4(C-12), 131.30(C-25), 132.69(C-8), 134.70(C-22), 146.58(C-13), 153.51(C-9), 168.09(C-21) and 199.37(C-7). EI–MS m/z: 404.3$[M + H]^+$,

390.4, 341.1, 300.8, 279.0, 244.0, 209.3, 191.9. HR–ESI–MS *m/z*: 404.2636 ($[M+H]^+$, $C_{27}H_{34}NO_2^+$; Calcd 404.2590).

3.1.5. *Synthesis of N-(o-chlorobenzoyl)-dehydroabietylamine-7-one (**3b**)*

Compound **3b** was similarly prepared according to the procedure of **3a**. Yield 50%, white crystals, m.p. 163–164°C. IR (KBr): 3442, 2930, 1684, 1644 and 840. ^{1}H-NMR (δ_H, $CDCl_3$, 300 MHz): 1.10(3H, s, H-19), 1.21–1.24(6H, m, H-16, 17), 1.27(3H, s, H-20), 1.41–1.91(5H, m, H-1α, 2, 3), 2.04–2.10(1H, m, H-5), 2.35(1H, brd, $J=12.9$ Hz, H-1β), 2.53–2.76(2H, m, H-6), 2.80–2.94(1H, m, H-15), 3.20–3.51(2H, m, H-18), 6.33(1H, s, 22-CONH), 7.23–7.44(5H, m, H-11, 12, 25, 26, 27), 7.56–7.59(1H, m, H-24), 7.80–7.81 (1H, m, H-14). ^{13}C-NMR (δ_c, $CDCl_3$, 75 MHz): 18.24(C-19), 18.48(C-2), 23.70(C-20), 23.84(C-16 or C-17), 33.48(C-15), 35.91(C-6), 35.99(C-3), 37.52(C-4), 37.73(C-10), 37.80(C-1), 44.47(C-5), 49.86(C-18), 123.55(C-11), 124.83(C-14), 126.88(C-26 or C-27), 129.96(C-24), 130.47(C-12), 130.93(C-22), 132.54(C-8 or C-25), 135.67(C-23), 146.60(C-13), 153.32(C-9), 167.08(C-21) and 198.90(C-7). EI–MS *m/z*: 438.3$[M+H]^+$, 436.3, 426.3, 422.3, 411.1, 383.4, 389.7. HR–ESI–MS *m/z*: 438.0242 ($[M+H]^+$, $C_{27}H_{33}ClNO_2^+$; Calcd 438.2200).

3.1.6. *Synthesis of N-(p-nitrobenzoyl)-dehydroabietylamine-7-one (**3c**)*

Compound **3c** was similarly prepared according to the procedure of **3a**. Yield 52%, pale yellow crystals, m.p. 204–205°C. IR (KBr): 3389, 2925, 1662, 1523, 1345 and 833. ^{1}H-NMR (δ_H, $CDCl_3$, 300 MHz): 1.13(3H, s, H-19), 1.20–1.31(9H, m, H-16, 17, 20), 1.41–1.88(5H, m, H-1α, 2, 3), 2.06–2.10(1H, m, H-5), 2.39(1H, brd, $J=12.8$ Hz, H-1β), 2.57–2.82(2H, m, H-6), 2.85–2.92(1H, m, H-15), 3.25–3.59(2H, m, H-18), 6.49(1H, s, 22-CONH), 7.31(1H, d, $J=8.8$ Hz, H-11), 7.41(1H, dd, $J=8.0$ Hz and 2.0 Hz, H-12), 7.78–7.79(1H, m, H-14), 7.91(2H, d, $J=8.8$ Hz, H-23, 27), 8.24(2H, d, $J=9.2$ Hz, H-24, 26). ^{13}C-NMR (δ_c, $CDCl_3$, 75 MHz): 18.18(C-19), 18.81(C-2), 23.62(C-16 or C-17), 23.97(C-20), 33.35(C-15), 35.86(C-6), 36.10(C-3), 37.51(C-4), 37.81(C-10), 38.23(C-1), 44.24(C-5), 49.58(C-18), 123.58(C-24 or C-26), 123.72(C-11), 124.64(C-14), 128.32(C-23 or C-27), 130.20(C-12), 132.97(C-8), 140.28(C-22), 146.67(C-13), 149.40(C-25), 153.55(C-9), 166.16(C-21) and 199.57(C-7). EI–MS *m/z*: 449.3$[M+H]^+$, 431.3, 367.0, 294.1, 145.9, 104.7. HR–ESI–MS *m/z*: 449.0154 ($[M+H]^+$, $C_{27}H_{33}N_2O_4^+$; Calcd 449.2440).

3.1.7. *Synthesis of N-benzoyl-12-nitrodehydroabietylamine (**4a**)*

Powdered cupric nitrate trihydrate (2.42 g, 10 mmol) was added in portions to a stirred solution of **2a** (1.95 g, 5 mmol) in acetic anhydride (60 mL), and the blue solution was stirred at 10°C for 8 h. The mixture was then poured into water and ice. The pale yellow precipitate was filtered off, washed with water, dried and recrystallised from methanol to give **4a** (1.52g, 70%) as pale yellow flakes (Cambie & Franich, 1971), m.p. 168–170°C. IR (KBr): 3424, 2931, 1648, 1524, 1349 and 825. ^{1}H-NMR (δ_H, $CDCl_3$, 300 MHz): 1.00–1.02(3H, m, H-19), 1.19–1.27(9H, m, H-16, 17, 20), 1.35–1.81(7H, m, H-1α, 2, 3, 6), 1.99–2.14(1H, m, H-5), 2.29(1H, brd, $J=12.3$ Hz, H-1β), 2.70–3.03(3H, m, H-7, 15), 3.18–3.60(2H, m, H-18), 6.17(1H, s, 22-CONH), 7.07(1H, s, H-14), 7.39–7.52(3H, m, H-24, 25, 26), 7.62(1H, s, H-11), 7.72–7.75(2H, m, H-23, 27). ^{13}C-NMR (δ_c, $CDCl_3$, 75 MHz): 18.09(C-19), 18.40(C-2), 18.78(C-6), 23.63(C-16 or C-17), 25.28(C-20), 28.71(C-15), 30.22(C-7), 36.13(C-3), 37.68(C-4), 37.77(C-10), 38.32(C-1), 45.13(C-5), 50.23(C-18), 120.35(C-11), 126.85(C-23 or C-27), 127.99(C-14), 128.67(C-24 or C-26), 131.51(C-25), 134.82(C-22), 139.56(C-13), 141.05(C-8), 148.37(C-12), 149.06(C-9) and

167.74(C-21). EI–MS *m/z*: 435.3$[M+H]^+$, 432.1, 401.1, 294.1, 121.2, 86.8. HR–ESI–MS *m/z*: 435.2759 ($[M+H]^+$, $C_{27}H_{35}N_2O_3^+$; Calcd 435.2648).

3.1.8. *Synthesis of N-(o-chlorobenzoyl)-12-nitrodehydroabietylamine (**4b**)*

Compound **4b** was similarly prepared according to the procedure of **4a**. Yield 74%, pale yellow flakes, m.p. 165–166°C. IR (KBr): 3405, 2929, 1645, 1517, 1345 and 827. ^{1}H-NMR (δ_H, $CDCl_3$, 300 MHz): 1.03(3H, s, H-19), 1.19–1.27(9H, m, H-16, 17, 20), 1.35–1.86(7H, m, H-1α, 2, 3, 6), 2.06–2.17(1H, m, H-5), 2.29(1H, brd, $J=12.9$ Hz, H-1β), 2.72–3.04(3H, m, H-7, 15), 3.20–3.60(2H, m, H-18), 6.25(1H, s, 22-CONH), 7.08(1H, s, H-14), 7.28–7.40(3H, m, H-25, 26, 27), 7.63–7.67(2H, m, H-11, 24). ^{13}C-NMR (δ_c, $CDCl_3$, 75 MHz): 18.04(C-19), 18.40(C-2), 18.75(C-6), 23.61(C-16 or C-17), 25.12(C-20), 28.71(C-15), 30.05(C-7), 36.15(C-4), 37.63(C-3), 38.15(C-10), 38.33(C-1), 44.75(C-5), 50.50(C-18), 120.31(C-11), 127.12(C-26), 127.95(C-14 or C-27), 130.20(C-24), 131.24(C-22 or C-25), 135.35(C-23), 139.54(C-13), 141.17(C-8), 148.42(C-12), 149.11(C-9) and 166.80(C-21). EI–MS *m/z*: 469.3$[M+H]^+$, 427.3, 395.1, 218.1, 155.9, 80.2. HR–ESI–MS *m/z*: 468.8725 ($[M+H]^+$, $C_{27}H_{34}ClN_2O_3^+$; Calcd 469.2258).

3.1.9. *Synthesis of N-(p-nitrobenzoyl)-12-nitrodehydroabietylamine (**4c**)*

Compound **4c** was similarly prepared according to the procedure of **4a**. Yield 69%, pale yellow flakes, m.p. 178–180°C. IR (KBr): 3435, 2928, 1661, 1523, 1348 and 829. ^{1}H-NMR (δ_H, $CDCl_3$, 300 MHz): 1.02–1.03(3H, m, H-19), 1.17–1.26(9H, m, H-16, 17, 20), 1.31–1.87(7H, m, H-1α, 2, 3, 6), 1.99–2.11(1H, m, H-5), 2.31(1H, brd, $J=12.6$ Hz, H-1β), 2.63–3.05(3H, m, H-7, 15), 3.24–3.59(2H, m, H-18), 6.26(1H, s, 22-CONH), 7.16(1H, s, H-14), 7.61(1H, s, H-11), 7.88–7.90(2H, d, $J=9.0$ Hz, H-23, 27), 8.24–8.27(2H, d, $J=8.4$ Hz, H-24, 26). ^{13}C-NMR (δ_c, $CDCl_3$, 75 MHz): 18.10(C-19), 18.36(C-2), 18.72(C-6), 23.65(C-16 or C-17), 25.25(C-20), 28.71(C-15), 30.13(C-7), 36.12(C-3), 37.67(C-4), 37.91(C-10), 38.27(C-1), 45.26(C-5), 50.58(C-18), 120.29(C-11), 123.79(C-24 or C-26), 126.31(C-14), 128.15(C-23 or C-27), 139.62(C-13), 140.42(C-22), 140.98(C-8), 148.3(C-12), 148.98(C-9), 149.46(C-25) and 165.98(C-21). EI–MS *m/z*: 478.3$[M-H]^+$, 436.4, 338.5, 227.4, 156.9. HR–ESI–MS *m/z*: 480.2605 ($[M+H]^+$, $C_{27}H_{34}N_3O_5^+$; Calcd 480.2498).

3.2. *Biological assays*

The biological assays were performed as described by Cui, Fan, Huang, Liu, and Zhou (2009). Hey-1B and PC-3 cells (5000 cells per well) were seeded in 96-well plates. One day after seeding, the cells were treated with the compounds (**2a**–**2c**, **3a**–**3c** and **4a**–**4c**) at various concentrations and incubated for 36 h. After removing the media, each well was refilled with 100 uL fresh media and 10 uL MTT reagent. The cells were incubated under the same conditions for 5 h. About 100 uL of MTT detergent was added to each well, and plates were incubated in the dark overnight at room temperature. The OD value was measured at 490 nm by a LD 400C Luminescence Detector.

4. Conclusions

We have prepared a series of dehydroabietylamine derivatives with a carbonyl group in the 7th position on ring B or a nitro group in the 12th position on ring C and various benzamides substituted in the 18th position. The cytotoxicities of the synthesised compounds against PC-3 and Hey-1B cells were investigated.

The results have demonstrated that the presence of a nitro group in the 12th position on ring C or a carbonyl group in the 7th position on ring B were very important in determining the biological activity of these compounds. Our findings provide new

evidence, showing the relationship between chemical structure and biological function. The information obtained from these studies may be useful for the design of novel chemotherapeutic drugs for cancer (Cui et al., 2009). These results confirm that dehydroabietylamine-type diterpenes do have interesting antitumour properties and encourage us to research targets recognised for these diterpenes in PC-3 and Hey-1B cells; furthermore, these results also encourage us to synthesise additional dehydroabietylamine derivatives with the aim of obtaining compounds with more potent biological activity (González et al., 2010).

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