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Synthesis and antimicrobial activity of *p*-menth-3-en-1-amine amide derivatives

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Abstract A series of *p*-menth-3-en-1-amine amide derivatives were designed and synthesized. These compounds were identified by FT-IR, ESI^+-MS, HRMS, ^1H NMR and ^{13}C NMR. Their antimicrobial activity against gram-positive *Staphylococcus aureus*, gram-negative *Klebsiella pneumoniae* and *Candida albicans* was investigated using the microbroth dilution method. The result indicated that some of these newly synthesized compounds had remarkable antimicrobial activity, and the suitable substituent groups were essential for high antimicrobial activity. Compound 5 g displayed the same antimicrobial activity against *K. pneumoniae* as commercially available antibacterial agents (kanamycin sulfate and rifampicin). The MIC of compound 5 g against *K. pneumoniae* only was 0.44 μg/mL.

Keywords *p*-Menth-3-en-1-amine amide derivatives · Antimicrobial activity · Minimum inhibition concentration · Synthesis

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Introduction

Pathogenic microorganisms could develop drug resistance to antimicrobial medication by some pathways, such as changing the absorption of antibacterial agents and biofilm formation [1]. The development of antibacterial resistance worldwide has become the largest threat to human health during the last few decades due to antibiotic abuse [2–5]. Fighting against the drug-resistant pathogenic microbes has become the most important and challenging problem [6]. Therefore, the new antibacterial agents with novel chemical structure should be developed to help to overcome drug resistance and improve antibacterial activity.

Monoterpenes are widely used as pharmaceuticals and pesticides due to the broad spectrum bioactivity, innovative mechanisms and low toxicity to the human body [7]. Many monoterpenes are known to be active against a variety of pathogenic microorganisms, including gram-positive, gram-negative bacteria and fungi [8–10]. Toxic effects on membranes have been widely used to explain the antimicrobial mechanism of monoterpenes [11]. In fact, monoterpenes will preferentially partition from an aqueous phase into membrane structures due to their lipophilic character [12, 13]. As a result, the fluidity and permeability of membranes have been increased and membrane-embedded proteins have been disturbed. Furthermore, this results in inhibition of respiration and alteration of ion transport processes.

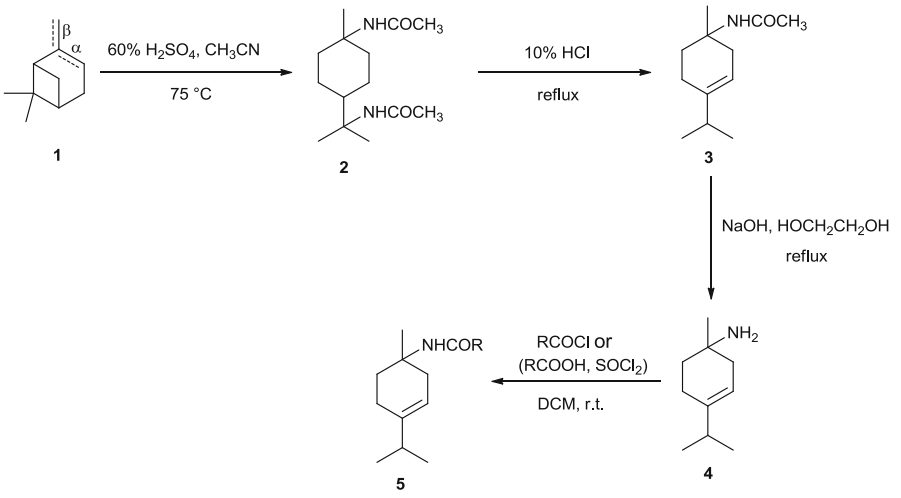
Amide derivatives have become a research focus in the development of medicines and pesticides because of their various bioactivities such as antibacterial, herbicidal, and antitumor activities [14, 15]. In the last three decades, amide derivatives have gained a significant importance in the therapeutic progress against pathogenic microorganisms. For example, lincomycin (an amide compound) and its semi-synthetic chlorinated derivative clindamycin have high biological activity against bacteria; they could be used for the treatment of important protozoal diseases (e.g., malaria) [16]. Based on the above characteristics, we can reasonably conclude that monoterpenes amide derivatives should be a kind of compound with high antibacterial activities, and they can be used in clinical practice as antimicrobial drugs in the future. In order to search for novel safe anti-bacterial agents with high activity, a series of novel monoterpene type amide derivatives were designed and synthesized (Scheme 1).

Experimental section

Chemistry

General methods

The NMR spectra were carried out on an AV-500 spectrometer (Bruker, Switzerland) with DMSO-d_6 as solvent and TMS as the internal reference. The FT-IR spectra were recorded on a Nicolte IS10 spectrometer (Thermo, USA) connected to an OMNIC operating system. The ESI^+-MS were carried out on a TSQ



Scheme 1 Synthetic route for the amide derivatives of *p*-menth-3-en-1-amine

Quanturn ultra AM mass spectrometer (Finnigan, USA). Melting points were recorded on a WRS-1B digital melting point apparatus. GC analysis was recorded on an Agilent 6890N/5973N spectrometer and used to confirm the purity of the compounds. Column chromatography was carried out on silicagel (200–300 mesh). Mouse embryo BALB/c 3T3 fibroblasts and human umbilical vein endothelial cell line HUVEC-C were purchased from Jiangsu Keygen Biotech Co., Ltd. DMEM (Dulbecco's modified Eagle's medium) and Kaighn's Modification of Ham's F-12 were purchased from Gibco Invitrogen. Fetal bovine serum (FBS) was purchased from Gibco Invitrogen. MTT was purchased from Biosharp. Turpentine (73% α-pinene and 20% β-pinene) were obtained commercially from Zhuzhou Sonbon Forest Chemical Co., Ltd. and distilled before use. DMSO (>99%) and other reagents were obtained commercially from Shanghai Jingchun Biochemical Technology Co., Ltd. and used without further purification.

General procedures for the synthesis of *N*,*N*′-diacetyl-*p*-menthane-1,8-diamines (2)

First, 60 mL 60% H_2SO_4 was stirred at 30 °C in a flask with a thermometer, a constant pressure funnel and a reflux condenser. To the flask was added 50 g turpentine. 60 mL acetonitrile was added drop-wise, via the constant pressure funnel, into the reaction mixture. Then the temperature of the reaction was increased slowly to 75 °C. The reaction was maintained at this temperature for 8 h and then cooled to room temperature. The reaction mixture was poured into 200 mL ice water, and the pH of the solution was adjusted to 7 with 20% NaOH. The organic phase was concentrated to obtain a yellow viscous solid using a vacuum rotatory evaporator at 50 °C. The viscous solid was recrystallized in ethyl acetate to give 46.69 g *N*, *N*′-diacetyl-*p*-menthane-1,8-diamines **2** ($n_{\text{trans}-}$:$n_{\text{ciss}-}$ = 4:1) in 50.0%

yield. The pure *trans-N,N′*-diacetyl-*p*-menthane-1,8-diamines (**2a**) and *cis-N,N′*-diacetyl-*p*-menthane-1,8-diamines (**2b**) were separated by fractional crystallization of the mixtures from ethyl acetate.

*Trans-N-(2-(4-acetamido-4-methylcyclohexyl)propan-2-yl)acetamide (**2a**)*

White solid. Yield, 39.20%. Purity, 99.51%. m.p.: 196.8–197.6 °C. FT-IR (cm^{-1}): 3304, 3258 (m, ν_{N-H}); 2939 (s, ν_{C-H}); 1638 (s, $\nu_{C=O}$); 1554 (s, δ_{N-H}); 1437, 1367 (m, δ_{C-H}); 1298 (s, ν_{C-N}). ESI^+-MS (45 eV, *m/z*): 255.05 [M + H]. HRMS (ESI) for $C_{14}H_{27}N_2O_2$, calcd 255.2067, found 255.2065 $[M + H]^+$, $\Delta = 0.77$ ppm, DBE = 3. ^1H NMR (DMSO-d_6, 500 MHz), δ_H 7.21 (1H, s, 1-N<u>H</u>COCH$_3$), 7.07 (1H, s, 8-N<u>H</u>COCH$_3$), 2.25 (2H, d, $J = 12.5$ Hz, 2-Ha, 6-Ha), 1.88–1.94 (1H, m, 4-H), 1.82 (3H, s, 1-NHCOC<u>H</u>$_3$), 1.78 (3H, s, 8-NHCOC<u>H</u>$_3$), 1.39 (2H, d, $J = 11.4$ Hz, 3-Ha, 5-Ha), 1.22 (3H, s, 7-H), 1.19–1.20 (2H, m, 2-He, 6-He), 1.17 (6H, s, 9-H, 10-H), 1.02–1.08 (2H, m, 3-He, 5-He). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 169.38 (1-NH<u>C</u>OCH$_3$), 169.00 (8-NH<u>C</u>OCH$_3$), 55.66 (8-C), 52.18 (1-C), 43.54 (4-C), 36.51 (2-C, 6-C), 28.07 (7-C), 24.36 (9-C, 10-C), 24.13 (1-NHCO<u>C</u>H$_3$, 8-NHCO<u>C</u>H$_3$), 22.62 (3-C, 5-C).

*Trans-N-(2-(4-acetamido-4-methylcyclohexyl)propan-2-yl)acetamide (**2b**)*

White solid. Yield, 10.80%. Purity, 98.72%. m.p.: 245.3–246.3 °C. FT-IR (cm^{-1}): 3324 (m, ν_{N-H}); 2940 (s, ν_{C-H}); 1647 (s, $\nu_{C=O}$); 1537 (s, δ_{N-H}); 1441, 1365 (m, δ_{C-H}); 1295 (s, ν_{C-N}). ESI^+-MS (45 eV, *m/z*): 255.14 $[M + H]^+$. HRMS (ESI) for $C_{14}H_{27}N_2O_2$, calcd 255.2067, found 255.2064 $[M + H]^+$, $\Delta = 1.02$ ppm, DBE = 3. ^1H NMR (DMSO-d_6, 500 MHz), δ_H 7.29 (1H, s, 1-N<u>H</u>COCH$_3$), 7.19 (1H, s, 8-N<u>H</u>COCH$_3$), 1.83–1.93 (3H, m, 2-Ha, 4-H, 6-Ha), 1.79 (3H, s, 1-NHCOC<u>H</u>$_3$), 1.77 (3H, s, 8-NHCOC<u>H</u>$_3$), 1.51–1.57 (4H, m, 2-He, 3-Ha, 5-Ha, 6-He), 1.27 (3H, s, 7-H), 1.19 (6H, s, 9-H, 10-H), 1.06–1.17 (2H, m, 3-He, 5-He). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 169.06 (1-NH<u>C</u>OCH$_3$, 8-NH<u>C</u>OCH$_3$), 55.54 (8-C), 52.86 (1-C), 43.99 (4-C), 36.77 (2-C, 6-C), 24.39 (9-C, 10-C), 24.29 (1-NHCO<u>C</u>H3), 24.15 (8-NHCO<u>C</u>H$_3$), 23.21 (3-C, 5-C), 22.03 (7-C).

General procedures for the synthesis of *p*-menth-3-en-1-amine (4)

p-Menth-3-en-1-amine was synthesized from **2** according to the method cited in the literature [17]. First, 398 mL 10% HCl was stirred in a flask at room temperature (25 °C). To the flask was added 101.6 g (0.40 mol) *N,N′*-diacetyl-*p*-menthane-1,8-diamine (**2**). The reaction mixture was refluxed for 8 h. Then, the reaction mixture was cooled to room temperature. The solution of the upper layer was concentrated by vacuum rotatory evaporator to give 43.9 g yellow viscosity material (crude product of **3**). The experiment was repeated three times. The residues were combined. Next, 64.9 g crude product of **3** was added to another flask with a thermometer and a condenser-west tube. To the flask was added 300 mL ethylene glycol and 20 g NaOH. To remove the low-boiling-point substances, the reaction mixture was refluxed until the temperature reached 170 °C. Then, the reaction

mixture was cooled to room temperature, and the condenser-west tube was replaced with a condenser-Allihn type. Then the reaction mixture was refluxed for 13 h and then cooled to room temperature. The reaction mixture was poured into 100 mL water and extracted four times with 100 mL ethyl acetate. Then, 23.3 g (0.15 mol) *p*-menth-3-en-1-amine was obtained after the distillation of the organic phase.

*4-isopropyl-1-methylcyclohex-3-enamine (**4**)*

Colourless liquid. Yield, 37.50%. Purity, 95.03%. b.p.: 184.0 °C. FT-IR (cm^{-1}): 3345, 3272 (m, ν_{N-H}); 3051 (w, $\nu_{= C-H}$); 2955 (s, ν_{C-H}); 1594 (s, δ_{N-H}); 1464, 1375 (m, δ_{C-H}); 1295 (s, ν_{C-N}); 812 (m, $\delta_{=C-H}$). ESI^+-MS (45 eV, *m*/*z*): 154.16 $[M + H]^+$. HRMS (ESI) for $C_{10}H_{20}N$, calcd 154.1590, found 154.1590 $[M + H]^+$, $\Delta = 0.01$ ppm, DBE = 2. ^1H NMR (DMSO-d_6, 300 MHz), δ_H 5.25 (1H, t, 3-C), 2.10–2.18 (1H, m, 8-Ha), 1.81–2.02 (4H, m, 2-Ha, 2-He, 5-Ha, 6-Ha), 1.39 (2H, t, $J = 6.5$ Hz, 5-He, 6-He), 0.97 (6H, d, $J = 0.6$ Hz, 9-H, 10-H), 0.94 (3H, s, 7-H). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 143.61 (4-C), 114.00 (3-C), 52.69 (1-C), 35.24 (2-C), 34.10 (6-C), 31.76 (8-C), 23.21 (7-C), 22.32 (5-C), 20.76 (10-C), 20.67 (9-C).

General procedures for the synthesis of compounds (5a–d)

First, 4 mmol *p*-menth-3-en-1-amine and 6 mmol triethylamine were dissolved in 15 mL dry dichloromethane. Then, 6 mmol acyl chloride was dissolved in 5 mL dry dichloromethane and added dropwise slowly from a pressure-equalising funnel, and the reaction mixture was refluxed for 3–8 h. After cooling to room temperature, the reaction mixture was added to 30 mL H_2O, and the pH was adjusted to 9–11 with a sodium hydroxide solution. The solvent of the organic phase was removed by rotary evaporation, and the crude product was purified by chromatography on silica gel to afford compounds **5a–d**.

*N-(4-isopropyl-1-methylcyclohex-3-en-1-yl)pentanamide (**5a**)*

Colourless viscous liquid. Yield, 97.71%. Purity, 97.82%. b FT-IR (cm^{-1}): 3308 (m, ν_{N-H}); 3073 (w, $\nu_{=C-H}$); 2957 (s, ν_{C-H}); 1641 (s, $\nu_{C=O}$); 1545 (s, δ_{N-H}); 1447, 1369 (m, δ_{C-H}); 1273 (m, ν_{C-N}); 813 (m, $\delta_{=C-H}$). ESI^+-MS (45 eV, *m*/*z*): 238.19 $[M + H]^+$. HRMS (ESI) for $C_{15}H_{28}NO$, calcd 238.2165, found 238.2177 $[M + H]^+$, $\Delta = 4.88$ ppm, DBE = 3. ^1H NMR (DMSO-d_6, 500 MHz), δ_H 7.12 (1H, s, 1-NHCO$(CH_2)_3CH_3$), 5.25 (1H, t, 3-C), 2.32 (1H, d, $J = 17.8$, 2-Ha), 2.15–2.22 (2H, m, 5-Ha, 8-H), 1.97–2.07 (4H, m, 2-He, 6-Ha, 1-NHCOCH_2CH_2-CH_2CH_3), 1.87–1.90 (1H, m, 5-He), 1.43–1.50 (3H, m, 6-He, 1-NHCOCH_2CH_2-CH_2CH_3), 1.28–1.33 (2H, m, 1-NHCOCH_2CH_2CH_2CH_3), 1.26 (3H, s, 7-H), 0.98 (6H, d, $J = 6.9$ Hz, 9-H, 10-H), 0.87 (3H, t, $J = 7.4$ Hz, 1-NHCOCH_2CH_2CH_2-CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 172.45 (1-NHCOCH_2CH_2CH_2CH_3), 142.24 (4-C), 116.27 (3-C), 50.95 (1-C), 37.35 (2-C), 36.35 (1-NHCOCH_2CH_2-CH_2CH_3), 34.67 (6-C), 32.11 (8-C), 28.25 (1-NHCOCH_2CH_2CH_2CH_3), 25.18 (1-NHCOCH_2CH_2CH_2CH_3), 23.27 (7-C), 22.18 (5-C), 21.75 (10-C), 21.61 (9-C), 14.14 (1-NHCOCH_2CH_2CH_2CH_3).

*N-(4-isopropyl-1-methylcyclohex-3-en-1-yl)benzamide (**5b**)*

White solid. Yield, 86.70%. Purity, 98.75%. m.p.: 111.1–112.0 °C. FT-IR (cm^{-1}): 3319 (m, $\nu_{N–H}$); 3061 (w, $\nu_{= C–H}$); 2961 (s, $\nu_{C–H}$); 1634 (s, $\nu_{C=O}$); 1601, 1578, 1491 (w, $\nu_{C=C}$); 1535 (s, $\delta_{N–H}$); 1434, 1370 (m, $\delta_{C–H}$); 1290 (m, $\nu_{C–N}$); 816 (m, $\delta_{=C–H}$). ESI^+-MS (45 eV, *m/z*): 258.08 $[M + H]^+$. HRMS (ESI) for $C_{17}H_{24}NO$, calcd 258.1852, found 258.1857 $[M + H]^+$, $\Delta = 1.66$ ppm, DBE = 7. ^1H NMR (DMSO-d_6, 500 MHz), δ_H 7.77 (2H, d, $J = 7.5$ Hz, H-Ph), 7.49–7.56 (2H, m, H-Ph), 7.42–7.45 (2H, m, 1-NHCO, H-Ph), 5.32 (1H, t, 3-C), 2.55 (1H, d, $J = 20.7$, 2-Ha), 2.32–2.37 (1H, m, 8-H), 2.19–2.23 (1H, m, 5-Ha), 2.15 (1H, d, $J = 17.5$, 2-He), 1.94–2.06 (2H, m, 5-He, 6-Ha), 1.62–1.67 (1H, m, 6-He), 1.41 (3H, s, 7-H), 1.00 (6H, d, $J = 6.9$ Hz, 9-H, 10-H). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 167.05 (1-NHCO), 142.38 (4-C), 136.55 (C-Ph), 131.13 (C-Ph), 128.46 (C-Ph), 127.71 (C-Ph), 116.44 (3-C), 51.98 (1-C), 37.32 (2-C), 34.69 (6-C), 32.11 (8-C), 25.07 (7-C), 23.42 (5-C), 21.87 (10-C), 21.76 (9-C).

*4-Chloro-N-(4-isopropyl-1-methylcyclohex-3-en-1-yl)benzamide (**5c**)*

White solid. Yield, 98.28%. Purity, 96.87%. m.p.: 110.7–111.5 °C. FT-IR (cm^{-1}): 3324 (m, $\nu_{N–H}$); 3054 (w, $\nu_{= C–H}$); 2957 (s, $\nu_{C–H}$); 1633 (s, $\nu_{C=O}$); 1594, 1571, 1485 (w, $\nu_{C=C}$); 1535 (s, $\delta_{N–H}$); 1437, 1369 (m, $\delta_{C–H}$); 1279 (m, $\nu_{C–N}$); 816 (m, $\delta_{=C–H}$). ESI^+-MS (45 eV, *m/z*): 292.11 $[M + H]^+$. HRMS (ESI) for $C_{17}H_{23}ClNO$, calcd 292.1463, found 292.1469 $[M + H]^+$, $\Delta = 2.2$ ppm, DBE = 7. ^1H NMR (DMSO-d_6, 500 MHz), δ_H 7.79 (2H, d, $J = 8.6$ Hz, H-Ar), 7.63 (1H, s, 1-NHCO), 7.51 (2H, d, $J = 8.6$ Hz, H-Ar), 5.31 (1H, t, 3-C), 2.54 (1H, d, $J = 14.2$, 2-Ha), 2.29–2.34 (1H, m, 8-H), 2.18–2.22 (1H, m, 5-Ha), 2.14 (1H, d, $J = 18.7$, 2-He), 1.91–2.04 (2H, m, 5-He, 6-Ha), 1.62–1.67 (1H, m, 6-He), 1.39 (3H, s, 7-H), 0.99 (6H, d, $J = 6.9$ Hz, 9-H, 10-H). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 155.77 (1-NHCO), 142.66 (4-C), 137.40 (C-Ar), 130.60 (C-Ar), 128.89 (C-Ar), 128.10 (C-Ar), 116.75 (3-C), 57.95 (1-C), 37.12 (2-C), 34.82 (6-C), 34.71 (8-C), 26.82 (7-C), 23.77 (5-C), 21.76 (10-C), 21.75 (9-C).

*4-Bromo-N-(4-isopropyl-1-methylcyclohex-3-en-1-yl)benzamide (**5d**)*

White solid. Yield, 93.40%. Purity, 97.85%. m.p.: 92.2–93.5 °C. FT-IR (cm^{-1}): 3329 (m, $\nu_{N–H}$); 3060 (w, $\nu_{= C–H}$); 2955 (s, $\nu_{C–H}$); 1634 (s, $\nu_{C=O}$); 1590, 1567, 1482 (w, $\nu_{C=C}$); 1538 (s, $_{N–H}$); 1445, 1365 (m, $\delta_{C–H}$); 1290 (m, $\delta\nu_{C–N}$); 821 (m, $\delta_{=C–H}$). ESI^+-MS (45 eV, *m/z*): 335.94 $[M + H]^+$. HRMS (ESI) for $C_{17}H_{23}BrNO$, calcd 336.0958, found 336.0956 $[M + H]^+$, $\Delta = 0.77$ ppm, DBE = 7. ^1H NMR (DMSO-d_6, 500 MHz), δ_H 7.73 (2H, d, $J = 8.4$ Hz, H-Ar), 7.63–7.65 (3H, m, 1-NHCO, H-Ar), 5.31 (1H, t, 3-C), 2.54 (1H, d, $J = 14.2$, 2-Ha), 2.29–2.34 (1H, m, 8-H), 2.18–2.21 (1H, m, 5-Ha), 2.14 (1H, d, $J = 19.4$, 2-He), 1.93–2.03 (2H, m, 5-He, 6-Ha), 1.62–1.67 (1H, m, 6-He), 1.39 (3H, s, 7-H), 0.99 (6H, d, $J = 6.9$ Hz, 9-H, 10-H). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 166.00 (1-NHCO), 142.34 (4-C), 135.52 (C-Ar), 131.45 (C-Ar), 129.90 (C-Ar), 124.84 (C-Ar), 116.39 (3-C), 52.16

(1-C), 37.26 (2-C), 34.67 (6-C), 32.07 (8-C), 24.93 (7-C), 23.41 (5-C), 21.84 (10-C), 21.75 (9-C).

General procedures for the synthesis of compounds (5e–m)

First, 9 mmol thionyl chloride was dissolved in 5 mL dry dichloromethane and two DMF drops were added dropwise into a stirred solution of 6 mmol carboxylic acid in 15 mL dry dichloromethane. On completion, the mixture was refluxed for 3–8 h. After this, 4 mmol *p*-menth-3-en-1-amine and 6 mmol triethylamine dissolved in 10 mL dry dichloromethane were added to the above reaction mixture. The mixture was refluxed for 3–8 h. After cooling to room temperature, the reaction mixture was added to 30 mL H_2O, and the pH was adjusted to 9–11 with a sodium hydroxide solution. The solvent of the organic phase was removed by rotary evaporation and the crude product was purified by chromatography on silica gel to afford compounds **5e–m**.

*N-(4-isopropyl-1-methylcyclohex-3-en-1-yl)furan-2-carboxamide (**5e**)*

White solid. Yield, 98.53%. Purity, 99.50%. m.p.: 78.6–80.7 °C. FT-IR (cm^{-1}): 3277 (m, $\nu_{N–H}$); 3054 (w, $\nu_{= C–H}$); 2959 (s, $\nu_{C–H}$); 1636 (s, $\nu_{C=O}$); 1571 (s, $\nu_{C=C}$); 1537 (s, $\delta_{N–H}$); 1445, 1371 (m, $\delta_{C–H}$); 1271 (m, $\nu_{C–N}$); 1190 (s, $\nu_{=C–O–C}$); 813 (m, $\delta_{=C–H}$). ESI^+-MS (45 eV, *m/z*): 248.11 $[M + H]^+$. HRMS (ESI) for $C_{15}H_{22}NO_2$, calcd 248.1645, found 248.1655 $[M + H]^+$, $\Delta = 0.80$ ppm, DBE = 6. ^1H NMR (DMSO-d_6, 500 MHz), δ_H 7.78 (1H, s, H-Furan), 7.21 (1H, s, 1-NHCO), 7.08 (1H, s, H-Furan), 6.60 (1H, s, H-Furan), 5.30 (1H, t, 3-C), 2.50 (1H, d, $J = 24.6$, 2-Ha), 2.23–2.28 (1H, m, 8-H), 2.18–2.22 (1H, m, 5-Ha), 2.14 (1H, d, $J = 17.9$, 2-He), 1.92–2.02 (2H, m, 5-He, 6-Ha), 1.62–1.67 (1H, m, 6-He), 1.38 (3H, s, 7-H), 0.98 (6H, d, $J = 6.8$ Hz, 9-H, 10-H). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 158.05 (1-NHCO), 148.89 (C-Furan), 144.99 (C-Furan), 142.53 (4-C), 116.18 (3-C), 113.31 (C-Furan), 112.11 (C-Furan), 52.03 (1-C), 37.36 (2-C), 34.60 (6-C), 32.18 (8-C), 24.94 (7-C), 23.39 (5-C), 21.79 (10-C), 21.75 (9-C).

*N-(4-isopropyl-1-methylcyclohex-3-en-1-yl)thiophene-2-carboxamide (**5f**)*

White solid. Yield, 97.27%. Purity, 99.50%. m.p.: 110.4–111.9 °C. FT-IR (cm^{-1}): 3328 (m, $\nu_{N–H}$); 3060 (w, $\nu_{= C–H}$); 2955 (s, $\nu_{C–H}$); 1634 (s, $\nu_{C=O}$); 1590 (s, $\nu_{C=C}$); 1538 (s, $\delta_{N–H}$); 1445, 1365 (m, $\delta_{C–H}$); 1270 (m, $\nu_{C–N}$); 822 (m, $\delta_{=C–H}$). ESI^+-MS (45 eV, *m/z*): 264.11 $[M + H]^+$. HRMS (ESI) for $C_{15}H_{22}NOS$, calcd 264.1417, found 264.1426 $[M + H]^+$, $\Delta = 3.65$ ppm, DBE = 6. ^1H NMR (DMSO-d_6, 500 MHz), δ_H 7.77 (1H, d, $J = 3.7$ Hz, H-thiophene), 7.70 (1H, d, $J = 5.1$ Hz, H-thiophene), 7.52 (1H, s, 1-NHCO), 7.12 (1H, dd, $J = 4.9$, 3.8 Hz, H-thiophene), 5.31 (1H, t, 3-C), 2.52 (1H, d, $J = 8.3$, 2-Ha), 2.24–2.29 (1H, m, 8-H), 2.18–2.22 (1H, m, 5-Ha), 2.15 (1H, d, $J = 18.0$, 2-He), 1.94–2.05 (2H, m, 5-He, 6-Ha), 1.63–1.68 (1H, m, 6-He), 1.38 (3H, s, 7-H), 0.99 (6H, d, $J = 6.9$ Hz, 9-H, 10-H). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 161.54 (1-NHCO), 142.38 (4-C), 141.84 (C-thiophene), 130.63 (C-thiophene), 128.39 (C-thiophene), 128.02 (C-thiophene),

116.33 (3-C), 52.31 (1-C), 37.28 (2-C), 34.64 (6-C), 32.27 (8-C), 25.00 (7-C), 23.43 (5-C), 21.82 (10-C), 21.78 (9-C).

*N-(4-isopropyl-1-methylcyclohex-3-en-1-yl)-5-nitrothiophene-2-carboxamide (**5g**)*

Yellow solid. Yield, 96.39%. Purity, 98.79%. m.p.: 115.9–117.2 °C. FT-IR (cm^{-1}): 3299 (m, ν_{N-H}); 3101 (w, $\nu_{= C-H}$); 2962 (s, ν_{C-H}); 1625 (s, $\nu_{C=O}$); 1550 (s, δ_{N-H});1502 (s, $\nu_{C=C}$); 1448, 1352 (m, δ_{C-H}); 1248 (m, ν_{C-N}); 815 (m, $\delta_{=C-H}$). ESI^+-MS (45 eV, *m/z*): 309.08 $[M + H]^+$. HRMS (ESI) for $C_{15}H_{21}N_2O_3S$, calcd 309.1267, found 309.1277 $[M + H]^+$, $\Delta = 3.27$ ppm, DBE = 7. ^1H NMR (DMSO-d_6, 500 MHz), δ_H 8.10 (1H, d, $J = 4.4$ Hz, H-thiophene), 8.06 (1H, s, 1-NHCO), 7.88 (1H, d, $J = 4.4$ Hz, H-thiophene), 5.30 (1H, t, 3-C), 2.51 (1H, d, $J = 15.2$, 2-Ha), 2.23–2.28 (1H, m, 8-H), 2.18–2.20 (1H, m, 5-Ha), 2.15 (1H, d, $J = 13.6$, 2-He), 1.94–2.03(2H, m, 5-He, 6-Ha), 1.61–1.69 (1H, m, 6-He), 1.38 (3H, s, 7-H), 0.97 (6H, d, $J = 6.9$ Hz, 9-H, 10-H). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 159.72 (C-thiophene), 153.04 (1-NHCO), 148.42 (C-thiophene), 142.36 (4-C), 130.30 (C-thiophene), 127.80 (C-thiophene), 116.13 (3-C), 53.07 (1-C), 37.02 (2-C), 34.61 (6-C), 32.03 (8-C), 24.62 (7-C), 23.35 (5-C), 21.72 (10-C), 21.70 (9-C).

*5-chloro-N-(4-isopropyl-1-methylcyclohex-3-en-1-yl)thiophene-2-carboxamide (**5h**)*

Yellow solid. Yield, 98.87%. Purity, 98.95%. m.p.: 117.1–118.6 °C. FT-IR (cm^{-1}): 3310 (m, ν_{N-H}); 3076 (w, $\nu_{= C-H}$); 2956 (s, ν_{C-H}); 1615 (s, $\nu_{C=O}$); 1548 (s, δ_{N-H}); 1521 (s, $\nu_{C=C}$); 1446, 1369 (m, δ_{C-H}); 1270 (m, ν_{C-N}); 809 (s, $\delta_{=C-H}$). ESI^+-MS (45 eV, *m/z*): 298.04 $[M + H]^+$. HRMS (ESI) for $C_{15}H_{21}ClNOS$, calcd 298.1027, found 298.1042 $[M + H]^+$, $\Delta = 5.07$ ppm, DBE = 6. ^1H NMR (DMSO-d_6, 500 MHz), δ_H 7.68 (1H, d, $J = 4.1$ Hz, H-thiophene), 7.64 (1H, s, 1-NHCO), 7.12 (1H, d, $J = 4.0$ Hz, H-thiophene), 5.29 (1H, t, 3-C), 2.50 (1H, d, $J = 18.8$, 2-Ha), 2.22–2.27 (1H, m, 8-H), 2.16–2.20 (1H, m, 5-Ha), 2.13 (1H, d, $J = 16.4$, 2-He), 1.92–2.02 (2H, m, 5-He, 6-Ha), 1.61–1.67 (1H, m, 6-He), 1.36 (3H, s, 7-H), 0.97 (6H, d, $J = 6.9$ Hz, 9-H, 10-H). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 160.48 (1-NHCO), 142.32 (4-C), 141.06 (C-thiophene), 132.91 (C-thiophene), 128.30 (C-thiophene), 128.11 (C-thiophene), 116.25 (3-C), 52.52 (1-C), 37.21 (2-C), 34.63 (6-C), 32.22 (8-C), 24.85 (7-C), 23.40 (5-C), 21.75 (10-C), 21.73 (9-C).

*N-(4-isopropyl-1-methylcyclohex-3-en-1-yl)picolinamide (**5i**)*

Yellow viscous liquid. Yield, 97.88%. Purity, 99.25%. FT-IR (cm^{-1}): 3368 (m, ν_{N-H}); 3054 (w, $\nu_{= C-H}$); 2958 (s, ν_{C-H}); 1676 (s, $\nu_{C=O}$); 1590 (w, $\nu_{C=C}$); 1515 (s, δ_{N-H}); 1432, 1370 (m, δ_{C-H}); 1270 (m, ν_{C-N}); 817 (m, $\delta_{=C-H}$). ESI^+-MS (45 eV, *m/z*): 259.16 $[M + H]^+$. HRMS (ESI) for $C_{16}H_{23}N_2O$, calcd 259.1805, found 259.1808 $[M + H]^+$, $\Delta = 1.23$ ppm, DBE = 7. ^1H NMR (DMSO-d_6, 500 MHz), δ_H 8.60 (1H, d, $J = 4.2$ Hz, H-Pyridine), 8.06 (1H, d, $J = 7.8$ Hz, H-Pyridine), 7.98–8.00 (2H, m, 1-NHCO, H-Pyridine), 7.57–7.60 (1H, m, H-Pyridine), 5.33 (1H, t, 3-C), 2.43 (1H, d, $J = 18.8$, 2-Ha), 2.29–2.34 (1H, m, 8-H), 2.14–2.21 (2H, m, 2-He, 5-Ha), 1.92–1.97 (2H, m, 5-He, 6-Ha), 1.60–1.66 (1H, m, 6-He), 1.45 (3H, s,

7-H), 0.94 (6H, d, $J = 6.9$ Hz, 9-H, 10-H). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 163.33 (1-NHCO), 150.67 (C-Pyridine), 148.60 (C-Pyridine), 143.12 (4-C), 138.33 (C-Pyridine), 126.83 (C-Pyridine), 121.62 (C-Pyridine), 115.81 (3-C), 51.37 (1-C), 37.75 (2-C), 34.63 (6-C), 31.89 (8-C), 25.04 (7-C), 23.31 (5-C), 21.77 (10-C), 21.63 (9-C).

*N-(4-isopropyl-1-methylcyclohex-3-en-1-yl)nicotinamide (**5j**)*

Yellow viscous solid. Yield, 70.95%. Purity, 99.60%. FT-IR (cm^{-1}): 3278 (m, ν_{N-H}); 3054 (w, $\nu_{=C-H}$); 2958 (s, ν_{C-H}); 1641 (s, $\nu_{C=O}$); 1591 (w, $\nu_{C=C}$); 1537 (s, δ_{N-H}); 1446, 1371 (m, δ_{C-H}); 1272 (m, ν_{C-N}); 816 (m, $\delta_{=C-H}$). ESI$^+$-MS (45 eV, *m/z*): 259.14 $[M + H]^+$. HRMS (ESI) for $C_{16}H_{23}N_2O$, calcd 259.1805, found 259.1818 $[M + H]^+$, $\Delta = 5.07$ ppm, DBE = 7. ^{1}H NMR (DMSO-d_6, 500 MHz), δ_H 8.93 (1H, s, H-Pyridine), 8.68 (1H, d, $J = 4.8$ Hz, H-Pyridine), 8.11 (1H, d, $J = 8.0$ Hz, H-Pyridine), 7.82 (1H, s, 1-NHCO), 7.46 (1H, dd, $J = 7.9$, 4.8 Hz, H-Pyridine), 5.31 (1H, t, 3-C), 2.55 (1H, d, $J = 16.1$, 2-Ha), 2.31–2.35 (1H, m, 8-H), 2.18–2.22 (1H, m, 5-Ha), 2.15 (1H, d, $J = 16.5$, 2-He), 1.92–2.06 (2H, m, 5-He, 6-Ha), 1.62–1.68 (1H, m, 6-He), 1.41 (3H, s, 7-H), 0.98 (6H, d, $J = 6.9$ Hz, 9-H, 10-H). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 165.52 (1-NHCO), 151.79 (C-Pyridine), 148.85 (C-Pyridine), 142.34 (4-C), 135.42 (C-Pyridine), 131.88 (C-Pyridine), 123.60 (C-Pyridine), 116.35 (3-C), 52.27 (1-C), 37.23 (2-C), 34.67 (6-C), 32.06 (8-C), 24.95 (7-C), 23.40 (5-C), 21.80 (10-C), 21.71 (9-C).

*N-(4-isopropyl-1-methylcyclohex-3-en-1-yl)pyrazine-2-carboxamide (**5k**)*

White solid. Yield, 94.28%. Purity, 99.80%. m.p.: 67.2–67.5 °C. FT-IR (cm^{-1}): 3346 (m, ν_{N-H}); 3051 (w, $\nu_{=C-H}$); 2959 (s, ν_{C-H}); 1660 (s, $\nu_{C=O}$); 1578 (w, $\nu_{C=C}$); 1522 (s, δ_{N-H}); 1468, 1374 (m, δ_{C-H}); 1290 (m, ν_{C-N}); 817 (m, $\delta_{=C-H}$). ESI$^+$-MS (45 eV, *m/z*): 260.13 $[M + H]^+$. HRMS (ESI) for $C_{15}H_{22}N_3O$, calcd 260.1757, found 260.1778 $[M + H]^+$, $\Delta = 8.05$ ppm, DBE = 7. ^{1}H NMR (DMSO-d_6, 500 MHz), δ_H 9.19 (1H, s, H-Pyrazine), 8.88 (1H, d, $J = 2.5$ Hz, H-Pyrazine), 8.70 (1H, d, $J = 3.9$ Hz, H-Pyrazine), 7.85 (1H, s, 1-NHCO), 5.33 (1H, t, 3-C), 2.47 (1H, d, $J = 15.5$, 2-Ha), 2.29–2.34 (1H, m, 8-H), 2.15–2.23 (2H, m, 2-He, 5-Ha), 1.92–2.01 (2H, m, 5-He, 6-Ha), 1.63–1.69 (1H, m, 6-He), 1.45 (3H, s, 7-H), 0.95 (6H, d, $J = 6.9$ Hz, 9-H, 10-H). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 162.51 (1-NHCO), 147.97 (C-pyrazine), 147.94 (C-pyrazine), 145.35 (C-pyrazine), 143.51 (C-pyrazine), 143.09 (4-C), 115.78 (3-C), 51.90 (1-C), 37.56 (2-C), 34.61 (6-C), 31.83 (8-C), 24.90 (7-C), 23.30 (5-C), 21.78 (10-C), 21.65 (9-C).

*3-chloro-N-(4-isopropyl-1-methylcyclohex-3-en-1-yl)picolinamide (**5l**)*

Yellow viscous liquid. Yield, 96.74%. Purity, 98.68%. FT-IR (cm^{-1}): 3308 (m, ν_{N-H}); 2959 (s, ν_{C-H}); 1678 (s, $\nu_{C=O}$); 1583 (w, $\nu_{C=C}$); 1517 (s, δ_{N-H}); 1427, 1371 (m, δ_{C-H}); 1268 (m, ν_{C-N}); 820 (m, $\delta_{=C-H}$). ESI$^+$-MS (45 eV, *m/z*): 293.09 $[M + H]^+$. HRMS (ESI) for $C_{16}H_{22}ClN_2O$, calcd 293.1415, found 293.1431 $[M + H]^+$, $\Delta = 5.42$ ppm, DBE = 7. ^{1}H NMR (DMSO-d_6, 500 MHz), δ_H

8.01–8.07 (2H, m, H-Pyridine), 7.68–7.70 (2H, m, 1-NHCO, H-Pyridine), 5.31 (1H, t, 3-C), 2.42 (1H, d, $J = 19.2$, 2-Ha), 2.25–2.29 (1H, m, 8-H), 2.13–2.20 (2H, m, 2-He, 5-Ha), 1.88–1.99 (2H, m, 5-He, 6-Ha), 1.59–1.65 (1H, m, 6-He), 1.44 (3H, s, 7-H), 0.94 (6H, d, $J = 6.9$ Hz, 9-H, 10-H). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 162.05 (1-NHCO), 151.53 (C-Pyridine), 149.35 (C-Pyridine), 143.21 (4-C), 141.81 (C-Pyridine), 127.43 (C-Pyridine), 120.98 (C-Pyridine), 115.70 (3-C), 51.68 (1-C), 37.61 (2-C), 34.65 (6-C), 31.81 (8-C), 24.83 (7-C), 23.31 (5-C), 21.75 (10-C), 21.62 (9-C).

*3-chloro-N-(4-isopropyl-1-methylcyclohex-3-en-1-yl)isonicotinamide (**5m**)*

Yellow solid. Yield, 96.47%. Purity, 99.28%. m.p.: 103.7–104.9 °C. FT-IR (cm^{-1}): 3241 (m, ν_{N-H}); 3076 (w, $\nu_{=C-H}$); 2962 (s, ν_{C-H}); 1640 (s, $\nu_{C=O}$); 1590 (w, $\nu_{C=C}$); 1555 (s, δ_{N-H}); 1435, 1373 (m, δ_{C-H}); 1270 (m, ν_{C-N}); 816 (m, $\delta_{=C-H}$). ESI$^+$-MS (45 eV, *m/z*): 292.99 $[M + H]^+$. HRMS (ESI) for $C_{16}H_{22}ClN_2O$, calcd 293.1415, found 293.1415 $[M + H]^+$, $\Delta = 0.04$ ppm, DBE = 7. ^{1}H NMR (DMSO-d_6, 500 MHz), δ_H 8.68 (1H, s, H-Pyridine), 8.58 (1H, d, $J = 4.8$ Hz, H-Pyridine), 8.09 (2H, s, 1-NHCO), 7.36 (1H, d, $J = 4.8$ Hz, H-Pyridine), 5.29 (1H, t, 3-C), 2.44 (1H, d, $J = 18.2$, 2-Ha), 2.31–2.36 (1H, m, 8-H), 2.10–2.24 (3H, m, 2-He, 5-Ha, 6-Ha), 1.95–2.02 (2H, m, 5-He), 1.58–1.67 (1H, m, 6-He), 1.41 (3H, s, 7-H), 1.02 (6H, d, $J = 6.9$ Hz, 9-H, 10-H). ^{13}C NMR (DMSO-d_6, 125 MHz), δ_C 164.58 (1-NHCO), 149.63 (C-Pyridine), 148.57 (C-Pyridine), 144.86 (C-Pyridine), 142.44 (4-C), 127.81 (C-Pyridine), 123.12 (C-Pyridine), 116.07 (3-C), 52.57 (1-C), 37.18 (2-C), 34.74 (6-C), 32.08 (8-C), 25.21 (7-C), 23.28 (5-C), 21.87 (10-C), 21.68 (9-C).

Antimicrobial assay

The antimicrobial activity of the compounds was evaluated by the broth dilution method for the determination of MIC values. The MIC of the test compound was determined by the micro-broth dilution method [18]. Tested pathogenic microorganisms are as follows: gram-positive *Staphylococcus aureus* (CMCC-26069), gram-negative *Klebsiella pneumoniae* (GIM-1.279) and *Candida albicans* (ATCC-10231). LB broth and potato dextrose broth (PDB) medium were as the culture medium of bacteria and fungi, respectively.

In detail, the test compounds were first dissolved in dimethyl sulfoxide (DMSO) and diluted by sterile medium at 900 μg/mL concentration (containing 3% DMSO). Duplicate two fold serial dilutions of each sample were added into medium (containing 3% DMSO) for final concentrations of 900, 450, 225, 112.5, 56.25, 28.125, 14.0625, 7.0313, 3.5156, 1.7578 and 0.8789 μg/mL. The test microorganisms were diluted by sterile water to obtain a microorganism suspension of which the OD600 value equalled 1. After this, it was diluted 5000 times with test medium as the test microbe dilution. Then, 100 μL solution of the test compound and 100 μL test microorganism dilution were added to a separate well of the 96-well plates. In addition, 100 μL medium (containing 3% DMSO) and 100 μL test microorganism dilution were added into the 96-well plates as control. After inoculation, the bacteria and fungi were incubated at 37 °C and 30 °C for 24 h, respectively.

Cell cultures and sample preparation

First, the test compounds were dissolved in DMSO and diluted by the corresponding complete medium (DMEM containing 10% FBS and 4 mM L-glutamine for BALB/c 3T3 cells, and Kaighn's Modification of Ham's F-12 Medium containing 15% FBS for HUVEC-C cells) at 10 μmol/L concentration (DMSO concentration is <0.1%). Next, an ampoule containing the mouse embryo BALB/c 3T3 fibroblasts or human umbilical vein endothelial cell line HUVEC-C was removed from liquid nitrogen and fast unfrozen in a water bath at 37 °C. The cellular suspension was transferred to a culture flask containing the corresponding complete medium. To eliminate residues from the freezing medium, the medium was changed when the cells adhered to the culture flask. The cultures were incubated in a humidified atmosphere of 5% CO_2, 95% air at 37 °C.

Cytotoxicity assays (MTT)

After 1–2 generations of reproduction, the cells were cultivated in a 96-well plate at a density of 3.5×10^3 cells in 100 μL of the corresponding complete medium in each well for 24 h (culture condition: 5% CO_2, 95% air at 37 °C). Then, 100 μL solution of the test compound and 100 μL medium (as negative control) were added to a separate well of the 96-well plates, respectively. Then, the cultures were incubated at 37 °C. After 48 h of incubation, 20 μl MTT solution (5 mg/mL) was added to each well and allowed to incubate for a further 4 h. Then, the supernatant was discarded and 0.15 mL DMSO was added to each well to dissolve the formed crystals. Later, the absorbance at a wavelength of 490 nm (Microplate Reader, EL-x800; BioTek Instruments, lnc., Winooski, USA) was recorded.

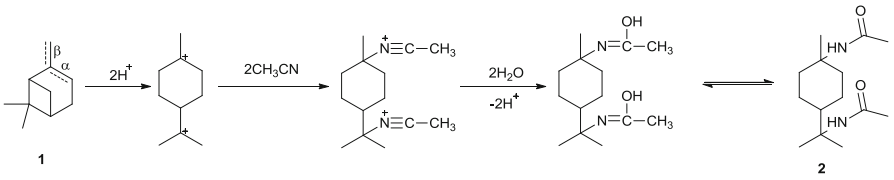
$$y = \frac{x_2 - x_1}{x_2}$$

where: y is the inhibition rate of cell growth; x_2 is the OD value of the blank control group; and x_1 is the OD value of experimental group.

Results and discussion

Synthesis of *N*,*N*′-diacetyl-p-menthane-1,8-diamines (2)

In the course of our investigations of the synthesis of *N*,*N*′-diacetyl-*p*-menthane-1,8-diamines, our work group found that turpentine is a cheap and commercially available alternative to terpine hydrate. *N*,*N*′-diacetyl-*p*-menthane-1,8-diamines (**2**) was obtained in 50.0% yield when turpentine was treated with acetonitrile in the presence of 60% H_2SO_4 aqueous solution. The reaction mechanism is shown in Scheme 2. Firstly, turpentine was attacked by protons to form a carbonium ion. After this, the carbonium ion was attacked by CH_3CN to form the compound **2**. Conclusions concerning the spatial structure of the isomeric *N*,*N*′-diacetyl-*p*-menthane-1,8-diamines (**2a** and **2b**) isolated were drawn on the basis of the results



Scheme 2 The reaction mechanism of turpentine to **N,N′**-diacetyl-p-menthane-1,8-diamines

of ^{13}C NMR spectroscopy. According to the literature, the signal of the CH_3-7 methyl group with the equatorial orientation is shifted upfield by approximately 6 ppm compared with an axially oriented group [19]. In our case, the CH_3-7 methyl group for compound (**2b**) was 22.03 ppm, and for compound (**2a**) it was 28.07 ppm. Therefore, compound (**2b**) was the cis-isomer having the axial orientation of the amide group and the equatorial orientation of the methyl group, and compound (**2a**) was the structure of the trans-isomer.

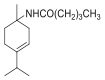
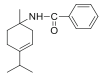
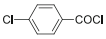
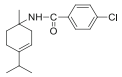
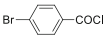
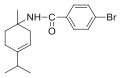
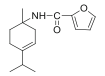
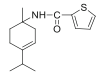
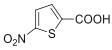
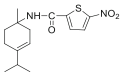
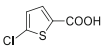
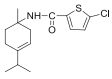
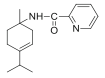
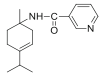
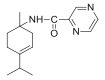
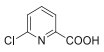
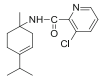
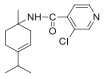
Synthesis of *p*-menth-3-en-1-amine amide derivatives (5a–m)

The *p*-menth-3-en-1-amine amide derivatives were synthesized in high yield (70.95–98.53%, Table 1) according to the synthetic sequences illustrated in Scheme 1. Compounds **(5a–d)** were prepared through nucleophilic additional-elimination reaction of compound **4** with acyl chloride in the presence of triethylamine and methylene chloride. However, compounds **(5e–m)** were synthesized from compound **4** and carboxylic acid due to the high cost of the corresponding acyl chlorides. In the FT-IR spectra of compounds (**5a–m**), the appearance of characteristic bands at 1700–1600 cm^{-1} due to the unsaturated carbonyl group, suggested the condensation of compound **4** with methylene chloride or carboxylic acid. Furthermore, these compounds were further confirmed by ESI^+-MS, HRMS, ^1H NMR and ^{13}C NMR.

Evaluation of antimicrobial activity in vitro

All of these compounds were screened in vitro for antimicrobial activity against gram-positive *Staphylococcus aureus*, gram-negative *Klebsiella pneumonia* and *Candida albicans*. The commercial antimicrobial agents (kanamycin sulfate and rifampicin) were used as controls. The results are listed in Table 2. The compound **4** exhibited remarkable antimicrobial activity against gram-positive bacterium *Staphylococcus aureus*. The possible antimicrobial mechanism was that the amino group could easily react with *N*-acetylmuramic acid and inhibit the synthesis of peptidoglycan (the main component of the cell walls of gram-positive bacteria); however, there was only a little peptidoglycan in the cell walls of gram-negative bacterium and the cell walls of fungi had no peptidoglycan [20–22]. Therefore, compound **4** showed much worse antimicrobial activity against gram-negative bacterium *Klebsiella pneumonia and fungi Candida albicans* than gram-positive bacterium *Staphylococcus aureus*.

Table 1 The preparation of *p*-menth-3-en-1-amine amide derivatives

Product	Acyl chloride/carboxylic acid	Structure of Product	Yield/%	Time/h
5a	$CH_3(CH_2)_3COCl$		97.71	5
5b			86.70	6
5c			98.28	5
5d			93.40	5
5e			98.53	9
5f			97.27	13.5
5g			96.39	13
5h			98.87	13
5i			97.88	13
5j			70.95	13.5
5k			94.28	12
5l			96.74	13
5m			96.47	13

Five synthesized compounds had antimicrobial activity against *Klebsiella pneumoniae*. Compound **5g** displayed the same activity with kanamycin sulfate and rifampicin. MIC (the minimum inhibition concentration) value of compound **5g**

Table 2 Antimicrobial activity of the title compounds

Compound	MIC (μg/mL)		
	Staphylococcus aureus	*Klebsiella pneumoniae*	*Candida albicans*
2a	>450	>450	>450
2b	>450	>450	>450
4	56.25	450	112.5
5a	>450	>450	450
5b	>450	>450	450
5c	>450	>450	450
5d	>450	>450	450
5e	>450	450	450
5f	>450	>450	>450
5g	112.5	0.44	450
5h	>450	>450	112.5
5i	>450	450	450
5j	450	225	225
5k	>450	450	225
5l	450	>450	>450
5m	>450	>450	450
Kanamycin sulfate	0.44	0.44	112.5
Rifampicin	3.52	0.44	450

only was 0.44 μg/mL. Furthermore, most of the synthesized compounds had antimicrobial activity against fungi *Candida albicans*.

The antimicrobial activity against *Staphylococcus aureus* and *Klebsiella pneumonia* had not been changed obviously when a chlorine or bromine atom was introduced into the *p*-menth-3-en-1-amine amide derivatives containing benzene, a thiophene ring or a pyridine ring. However, the antimicrobial activity against *Candida albicans* was increased when a chlorine atom was introduced into the thiophene amide derivative of compound **4** (**5e** → **5h**). Moreover, introduction of a nitro group into the thiophene amide derivative of compound **4** was favorable to antimicrobial activity against *Klebsiella pneumonia* (**5f** → **5g**).

Evaluation of Cytotoxicity

The cytotoxicity of the synthesized compounds against BALB/c 3T3 cells and HUVEC-C cells was determined, and the results are listed in Table 3. The growth of these cells had not been clearly affected when they were treated with a 10 μmol/L concentration of the title compounds. The inhibition rates of these synthesized compounds against the growth of BALB/c 3T3 cells and HUVEC-C cells were 2.17–10.11 and 0.23–10.89%, respectively (Table 3).

Table 3 Cytotoxicity of the title compounds

Compound	Inhibition rate (%) of cell growth at 10 μmol/L	
	BALB/c 3T3 cells	HUVEC-C cells
2a	4.51	10.89
2b	8.03	0.99
4	10.11	3.96
5a	3.25	0.74
5b	4.30	0.19
5c	3.25	9.90
5d	3.43	0.99
5e	2.34	2.87
5f	2.17	0.50
5g	6.08	8.76
5h	8.66	3.92
5i	6.81	4.15
5j	8.66	5.07
5k	2.39	0.23
5l	7.00	2.07
5m	6.81	4.84
Kanamycin sulfate	0.41	0.93
Rifampicin	7.48	7.84

Conclusion

In summary, a series of 1-amino-*p*-menth-3-ene amide derivatives were synthesized using turpentine as starting material. Their structures were identified by FT-IR, ESI^+-MS, ^1H NMR and ^{13}C NMR. At the same time, antimicrobial activities of these compounds were evaluated. Some compounds showed antimicrobial activities against gram-positive *Staphylococcus aureus* and gram-negative *Klebsiella pneumonia*, and most of these compounds displayed antimicrobial activities against *Candida albicans*. Compound **5g** exhibited the same activity against *Klebsiella pneumoniae* with kanamycin sulfate and rifampicin, and the MIC was 0.44 μg/mL. In addition, these compounds including compound **5g** had almost had no toxicity to BALB/c 3T3 cells and HUVEC-C cells. Therefore, compound **5g** is a promising antibacterial agent against *Klebsiella pneumoniae*.

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