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RESEARCH ARTICLE

Synthesis and anticancer activity of (+)-nopinone-based 2-amino-3-cyanopyridines

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Abstract Twelve (+)-nopinone-based 2-amino-3-cyanopyridines 4a–l were synthesized from (–)-β-pinene. The structures of these compounds were characterized by FT-IR, ^{1}H NMR, and ESI-MS. All the compounds were tested for their anticancer activity against lung cancer cell line A549, gastric cancer cell line MKN45 and breast cancer cell line MCF7 by MTT method, respectively. The results showed that compounds 4f, 4j and 4k had promising anticancer activity against these cancer cell lines, in particular, compound 4f exhibited broad-spectrum and highly efficient anticancer activity against cell lines A549, MKN45 and MCF7 with IC_{50} of 23.78, 67.61 and 53.87 μmol·L^{-1}, respectively. The preliminary analysis of the structure activity relationship implied that the Br or Cl substituted group of the benzene ring in these derivatives significantly contributed to the anticancer activity.

Keywords β-pinene, nopinone, synthesis, 2-amino-3-cyanopyridine, anticancer

1 Introduction

Natural products are important source for drug development in all major disease areas due to their structure diversity and low side effects[1]. β-Pinene is a naturally occurring monoterpene and commonly occurs in many natural essential oils, including turpentine, tarragon and tea tree oils[2–4]. Research has shown that β-pinene and its derivatives displayed broad-spectrum biological activity, such as anticancer, antibacterial, antifungal, antidiabetic and antiallergic activity[5–10]. Hence, exploiting β-pinene and its derivatives for their biological activities has significant potential.

2-amino-3-cyanopyridines are known to have a range of biological activity[11–14]. The anticancer activity of these compounds has especially attracted great interest because of their different modes of action[15]. With both β-pinene and 2-amino-3-cyanopyridine having potential anticancer activity, there is a possibility that synthesis of the β-pinene derivatives with a 2-amino-3-cyanopyridine core may lead to more active anticancer agents.

In this study, a series of (+)-nopinone-based 2-amino-3-cyanopyridines were prepared (Fig. 1), and their anticancer activity against human cancer cell lines A549, MKN45 and MCF7 were evaluated.

2 Material and methods

(–)-β-Pinene was purchased from the domestic spice company Jiangxi Jishui Hongda Natural Perfume Co., Limited, China, and other compounds were of reagent grade. Melting points were determined in a WRS-2 melting point apparatus (Shanghai Precision and Scientific Instrument Co. Ltd., China) and were uncorrected. IR spectra were recorded on a Nicolet IS10 FT-IR spectrometer (Nicolet, Madison, USA). ^{1}H NMR spectra were recorded on a Bruker DKX500 NMR spectrometer (Bruker, Karlsruhe, Germany) using $CDCl_3$ or DMSO-*d*6 as solvent, and TMS as internal standard. Mass spectra were recorded on an Agilent-5973 spectrometer (ESI source). Purity of compounds was detected by Agilent 1260 high performance liquid chromatography (Agilent, Santa Clara, USA) and Fuli GC-9750 gas chromatography (Zhejiang Fuli analysis instrument Co. Ltd., China). All reactions were followed by thin layer chromatography

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(TLC). Cancer cell lines (human lung cancer cell A549, human gastric cancer cell MKN45, human breast cancer cell MCF7) were supplied by the Institute of Biochemistry and Cell Biology, Shanghai Institute of Biological Science, Chinese Academy of Sciences, China. Neurobasal medium (RPMI 1640) and fetal calf serum were supplied by Gibco™, USA and (4,5-dimetylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) by AMRESCO, USA.

2.1 Synthesis of (+)-nopinone (2)

(+)-Nopinone (2) was prepared by gradually adding potassium permanganate (23.705 g, 0.15 mol) into the mixture of (–)-β-pinene (1) (6.951 g, 0.05 mol) and catalytic amount of sulfuric acid in solvent acetone, reaction was stirred at room temperature for 8 h. Then, the resulting mixture was filtered, extracted by ethyl acetate, washed with water and concentrated under reduced pressure.

2.2 General procedure for the synthesis of (+)-nopinone-based 2-amino-3-cyanopyridine derivatives 4a–l

Each of the aromatic aldehydes 3a–l (4 mmol) was added to a solution of (+)-nopinone (2) (4 mmol) in absolute ethanol (5 mL) containing ammonium acetate (6 mmol), malononitrile (4 mmol), and catalyst ytterbium triflate (0.2 mmol). The reaction mixture, in each case, was heated under reflux for 12 h, then cooled and concentrated by rotary evaporation, and compounds 4a–l were obtained after further purification by silica gel chromatography.

2.3 Anticancer activity evaluation

The cells were thawed and grown in RPMI 1640 medium with 10% fetal bovine serum and 0.25% trypsin at 37°C in a humidified (95%) incubator with CO_2 (5%). Then, the cells in logarithmic phase was collected, trypsinized with 0.25% trypsin/0.02% EDTA and diluted into cell suspensions. The cell suspensions were aliquoted (90 μL per well) into 96-well plates, incubated for 24 h, and then treated with 10 μL testing compounds at different concentrations (3.13, 6.25, 12.5, 25, 50 and 100 μmol·L^{-1}). The treated 96-well plates were incubated for 48 h at 37°C in a humidified 5% CO_2/95% air mixture. Ten microliter of 5 mg·mL^{-1} MTT was added to the cell cultures and incubated for 4 h. Formed formazan crystals were dissolved in 150 μL dimethyl sulfoxide, and the plates were analyzed using a microplate reader at 570 nm wavelength. The experiment was repeated three times. Cell inhibition rate was calculated by:

$$\text{Inhibition}(\%) = \left(1 - \frac{\text{average test absorption}}{\text{average control absorption}}\right) \times 100$$

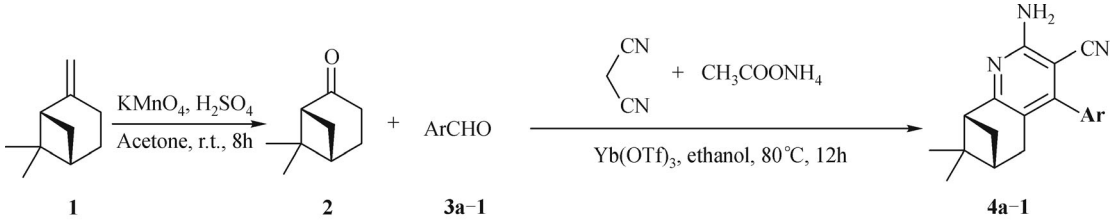
Half maximal inhibitory concentration (IC_{50}) was calculated according to the improved Karber method[16].

3 Results

3.1 Synthesis and characterization

According to the literature, the optimum reaction conditions to synthesize compound 2 were to add 1 equivalent (–)-β-pinene, 3 equivalent $KMnO_4$, 0.162 equivalent H_2SO_4 in acetone, then stirring at 15–25°C for 5 h[17]. In the present research, the reaction time was extended to 8 h in order to deplete the (–)-β-pinene, and the obtained (+)-nopinone was directly used in the following reaction without further purification. The structure of (+)-nopinone was confirmed by FT-IR, ^{1}H NMR and ESI-MS, and values were in good agreement with those reported in the literature[18].

Multicomponent reaction has been widely used in the synthesis of 2-amino-3-cyanopyridine[19,20]. Compared with the common reaction method[21], multicomponent reaction is more facile, effective and economical. Therefore, a series of 2-amino-3-cyanopyridine compounds 4a–l derived from (–)-nopinone were synthesized through the



Ar = a, phenyl; b, 2-Cl; c, 2-OCH_3; d, 3-OH; e, 3-OCH_3; f, 3-Br; g, 3, 4-diF; h, 3, 4-diOCH_3; i, 4-F; j, 4-Cl; k, 4-Br; l, 4-CH_3

Fig. 1 Synthestic route of (+)-nopinone-based 2-amino-3-cyanopyridine derivatives 4a–l. r.t., room temperature.

one-pot multicomponent reaction. Furthermore, a diversity of substitutions including methyl-, methoxy-, fluoro-, chloro- and bromo- groups was introduced into the derivatives for high throughput biological screening. The characterization data of these synthesized compounds are given in Table 1.

3.2 Anticancer activity evaluation

Anticancer activity evaluation were carried out for all compounds. The inhibition rates at different concentrations and the IC_{50} values of the compounds against cell lines A549, MKN45 and MCF7 are given in Table 2. Compound 4f exhibited good activity against A549 cells, its IC_{50} was 23.78 μmol·L^{-1}. Compounds 4j and 4k exhibited moderate activity against A549 cells, their IC_{50} were 91.29 and 37.35 μmol·L^{-1}, respectively. Additionally, compounds 4f, 4j and 4k exhibited moderate activity against MKN45, their IC_{50} were 67.61, 79.61 and 91.66 μmol·L^{-1}, respectively. Compound 4f showed good activity against

Table 1 Characterization data of (+)-nopinone and (+)-nopinone–based 2-amino-3-cyanopyridines

Compound	Characterization data
2	Yield 82%, GC purity 90.35%, $[\alpha]_D^{18}$ + 33.9° (c 1.0 $CHCl_3$), FT-IR ν (cm^{-1}): 2948, 2874 (C–H stretching vibration), 1705 (C = O stretching vibration), 1459, 1386 (C–H bending vibration). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.56 (pd, J = 12.4, 5.0 Hz, 3H, –CH– and–CH_2–), 2.41–2.19 (m, 2H, –CH_2–), 2.12–1.88 (m, 2H, –CH_2–), 1.59 (d, J = 9.9 Hz, 1H, –CH–), 1.33 (s, 3H, –CH_3), 0.86 (s, 3H, –CH_3). ESI-MS: m/z 138.1 $[M]^+$
4a	Yield 75%, HPLC purity = 91.46%, (XDB-C-18 column; MeOH:H_2O/80:20), t_r = 12 min; pale yellow crystals, m.p. 209–210°C; FT-IR ν (cm^{-1}): 3409, 3305, 3150 (N–H), 2949, 2925, 2842(C–H), 2203(C≡N), 1641, 1557, 1497, 1466 (phenyl), 1376 (C–H); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.60–7.43 (m, 3H, phenyl), 7.34 (dd, J = 7.7, 1.7 Hz, 2H, phenyl), 5.40 (s, 2H, –NH_2), 2.92 (t, J = 5.5 Hz, 1H, –CH–), 2.69 (dt, J = 9.9, 5.7 Hz, 1H, –CH_2–), 2.58 (d, J = 2.9 Hz, 2H, –CH_2–), 2.34–2.18 (m, 1H, –CH–), 1.41 (s, 3H, –CH_3), 1.32 (d, J = 9.8 Hz, 1H, –CH_2–), 0.76 (s, 3H, –CH_3); ESI-MS: m/z 290.4 $[M + 1]^+$; 312.4 $[M + 23]^+$
4b	Yield 60%, HPLC purity = 98.23%, (XDB-C-18 column; MeOH:H_2O/80:20), t_r = 12 min; pale yellow crystals, m.p. 205–206°C; FT-IR ν (cm^{-1}): 3382, 3306, 3184 (N–H), 2950, 2917, 2840 (C–H), 2211 (C≡N), 1640, 1598, 1561, 1483, 1463 (phenyl), 1378 (C–H); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.63 (dd, J = 6.3, 2.9 Hz, 1H, phenyl), 7.58–7.45 (m, 2H, phenyl), 7.457.27 (m, 1H, phenyl), 6.66 (s, 2H, –NH_2), 3.28 (s, 1H, –CH–), 2.73 (t, J = 5.5 Hz, 1H, –CH_2–), 2.68–2.58 (m, 1H, –CH_2–), 2.35–2.22 (m, 2H, –CH_2–), 2.19 (d, J = 2.8 Hz, 1H, –CH–), 1.35 (s, 3H, –CH_3), 1.24 (d, J = 9.6 Hz, 1H, –CH_2–), 0.69 (d, J = 5.1 Hz, 3H, –CH_3); ESI-MS: m/z 324.8 $[M + 1]^+$; 346.8 $[M + 23]^+$
4c	Yield 77%, HPLC purity = 99.12%, (XDB-C-18 column; MeOH:H_2O/80:20), t_r = 12 min; pale yellow crystals, m.p. 207–208°C; FT-IR ν (cm^{-1}): 3388, 3304, 3173 (N–H), 2935, 2839 (C–H), 2208 (C≡N), 1640, 1605, 1561, 1498, 1459 (phenyl), 1379 (C–H); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.54–7.36 (m, 1H, phenyl), 7.24–7.12 (m, 2H, phenyl), 7.06 (t, *J* = 7.4 Hz, 1H, phenyl), 6.46 (s, 2H, –NH_2), 3.93–3.56 (m, 3H, –CH_3), 3.26 (d, *J* = 11.9 Hz, 1H, –CH–), 2.70 (t, *J* = 5.5 Hz, 1H, –CH_2–), 2.60 (dt, *J* = 11.3, 5.5 Hz, 1H, –CH–), 2.40–2.24 (m, 1H, –CH_2–), 2.17 (d, *J* = 2.8 Hz, 1H, –CH–), 1.34 (s, 3H, –CH_3), 1.18 (dd, *J* = 17.5, 9.5 Hz, 1H, –CH_2–), 0.67 (d, *J* = 14.0 Hz, 3H, –CH_3); ESI-MS: m/z 320.4 $[M + 1]^+$; 342.4 $[M + 23]^+$
4d	Yield 55%, HPLC purity = 99.58%, (XDB-C-18 column; MeOH:H_2O/80:20), t_r = 12 min; pale yellow crystals, m.p. 264–265°C; FT-IR ν (cm^{-1}): 3459(O–H), 3442, 3232 (N–H), 2922 (C–H), 2217 (C≡N), 1631, 1560, 1499, 1439 (phenyl), 1370 (C–H); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.60 (s, 1H, –OH), 7.29 (t, *J* = 7.8 Hz, 1H, phenyl), 6.84 (dd, *J* = 8.1, 1.8 Hz, 1H, phenyl), 6.72 (d, *J* = 7.4 Hz, 1H, phenyl), 6.68 (s, 1H, phenyl), 6.53 (s, 2H, –NH_2), 3.28 (s, 1H, –CH–), 2.71 (t, *J* = 5.5 Hz, 1H, –CH_2–), 2.65–2.56 (m, 1H, –CH–), 2.42 (dd, *J* = 29.5, 8.6 Hz, 2H, –CH_2–), 2.20 (s, 1H–CH–), 1.34 (s, 3H, –CH_3), 1.21 (d, *J* = 9.5 Hz, 1H, –CH–), 0.66 (s, 3H, –CH_3); ESI-MS: m/z 306.4 $[M + 1]^+$; 328.4 $[M + 23]^+$
4e	Yield 87%, HPLC purity = 94.58%, (XDB-C-18 column; MeOH:H_2O/80:20), t_r = 12 min; pale yellow crystals, m.p. 145–146°C; FT-IR ν (cm^{-1}): 3390, 3306, 3171 (N–H), 2933 (C–H), 2207 (C≡N), 1641, 1610, 1560, 1492, 1463 (phenyl), 1370 (C–H); ^{1}H NMR (300 MHz, DMSO-d6) δ: 7.42 (t, J = 8.0 Hz, 1H, phenyl), 7.08–6.98 (m, 1H, phenyl), 6.89 (s, 2H, phenyl), 6.55 (s, 2H, –NH_2), 3.80 (s, 3H, –CH_3), 3.30 (s, 1H, –CH–), 2.72 (t, J = 5.5 Hz, 1H, –CH_2–), 2.67–2.56 (m, 1H, –CH–), 2.37 (dd, J = 16.5, 1.9 Hz, 1H, –CH_2–), 2.26–2.15 (m, 1H, –CH–), 1.34 (s, 3H, –CH_3), 1.23 (d, J = 9.5 Hz, 1H, –CH_2–), 0.67 (s, 3H, –CH_3); ESI-MS: m/z 320.4 $[M + 1]^+$; 342.4 $[M + 23]^+$
4f	Yield 79%, HPLC purity = 98.93%, (XDB-C-18 column; MeOH:H_2O/80:20), t_r = 12 min; pale yellow crystals, m.p. 242–243°C; FT-IR ν (cm^{-1}): 3432, 3330 (N–H), 2978, 2929 (C–H), 2211 (C≡N), 1628, 1553, 1478, 1450 (phenyl), 1368 (C–H); ^{1}H NMR (300 MHz, DMSO-d6) δ: 7.67 (d, *J* = 8.0 Hz, 1H, phenyl), 7.60 (s, 1H, phenyl), 7.48 (t, *J* = 7.8 Hz, 1H, phenyl), 7.37 (d, *J* = 7.6 Hz, 1H, phenyl), 6.62 (s, 2H, –NH_2), 3.28 (s, 1H, –CH–), 2.72 (t, *J* = 5.5 Hz, 1H, –CH_2–), 2.66–2.57 (m, 1H, –CH–), 2.34 (d, *J* = 16.3 Hz, 1H, –CH_2–), 2.20 (s, 1H, –CH–), 1.34 (s, 3H, –CH_3), 1.24 (d, *J* = 9.4 Hz, 1H, –CH_2–), 0.67 (s, 3H, –CH_3); ESI-MS: m/z 369.3 $[M + 1]^+$; 390.3, 392.3 $[M + 23]^+$
4g	Yield 52%, HPLC purity = 94.94%, (XDB-C-18 column; MeOH:H_2O/80:20), t_r = 12 min; pale yellow crystals, m.p. 174–175°C; FT-IR ν (cm^{-1}): 3406, 3300, 3161 (N–H), 2924, 2837 (C–H), 2207 (C≡N), 1638, 1589, 1562, 1480, 1465 (phenyl), 1385 (C–H). ^{1}H NMR (300 MHz, DMSO-d6) δ: 7.67–7.49 (m, 2H, phenyl), 7.24 (d, *J* = 4.3 Hz, 1H, phenyl), 6.63 (s, 2H, –NH_2), 3.27 (s, 1H, –CH–), 2.72 (t, *J* = 5.5 Hz, 1H, –CH_2–), 2.68–2.57 (m, 1H, –CH–), 2.36 (d, *J* = 16.5 Hz, 1H, –CH_2–), 2.28–2.11 (m, 1H, –CH–), 1.34 (s, 3H, –CH_3), 1.23 (d, *J* = 9.5 Hz, 1H, –CH_2–), 0.67 (s, 3H, –CH_3); ESI-MS: m/z 326.4 $[M + 1]^+$; 348.1 $[M + 23]^+$
4h	Yield 73%, HPLC purity = 99.47%, (XDB-C-18 column; MeOH:H_2O/80:20), t_r = 12 min; pale yellow crystals, m.p. 195–196°C; FT-IR ν (cm^{-1}): 3416, 3305, 3180 (N–H), 2952, 2935, 2835 (C–H), 2203 (C≡N), 1637, 1603, 1556, 1514, 1461 (phenyl), 1370 (C–H); ^{1}H NMR (300 MHz, DMSO-d6) δ: 7.06 (d, *J* = 8.2 Hz, 1H, phenyl), 6.94 (s, 1H, phenyl), 6.88 (d, *J* = 8.1 Hz, 1H, phenyl), 6.49 (s, 2H, –NH_2), 3.78 (t, *J* = 17.4 Hz, 6H,, –CH_3), 3.27 (s, 1H, –CH–), 2.71 (t, *J* = 5.5 Hz, 1H, –CH_2–), 2.66–2.57 (m, 1H, –CH–), 2.41 (d, *J* = 16.5 Hz, 1H, –CH_2–), 2.21 (s, 1H, –CH–), 1.34 (s, 3H, –CH_3), 1.24 (d, *J* = 9.5 Hz, 1H, –CH_2–), 0.66 (s, 3H, –CH_3); ESI-MS: m/z 350.2 $[M + 1]^+$; 372.2 $[M + 23]^+$

(*Continued*)

Compound	Characterization data
4i	Yield 71%, HPLC purity = 99.30%, (XDB-C-18 column; MeOH:H_2O/80:20), t_r = 12 min; pale yellow crystals, m.p. 212–213°C; FT-IR v (cm^{-1}): 3461, 3292, 3161 (N–H), 2963 (C–H), 2202 (C≡N), 1625, 1607, 1564, 1511, 1456 (phenyl), 1379 (C–H); ^{1}H NMR (300 MHz, DMSO-d6) δ: 7.42 (dd, J = 8.5, 5.6 Hz, 2H, phenyl), 7.33 (t, J = 8.9 Hz, 2H, phenyl), 6.58 (s, 2H, –NH_2), 3.27 (s, 1H, –CH–), 2.72 (t, J = 5.5 Hz, 1H, –CH_2–), 2.65–2.56 (m, 1H, –CH–), 2.36 (dd, J = 16.4, 2.2 Hz, 1H, –CH_2–), 2.24–2.16 (m, 1H, –CH–), 1.34 (s, 3H, –CH_3), 1.22 (d, J = 9.5 Hz, 1H, –CH_2–), 0.67 (s, 3H, –CH_3); ESI-MS: m/z 308.2 $[M + 1]^+$; 330.2 $[M + 23]^+$
4j	Yield 78%, HPLC purity = 96.37%, (XDB-C-18 column; MeOH:H_2O/80:20), t_r = 12 min; pale yellow crystals, m.p. 167–168°C; FT-IR v (cm^{-1}): 3379, 3311, 3189 (N–H), 2954, 2933 (C–H), 2205 (C≡N), 1639, 1603, 1574, 1556, 1494, 1462 (phenyl), 1369 (C–H); ^{1}H NMR (300 MHz, DMSO-d6) δ: 7.57 (d, J = 8.4 Hz, 2H, phenyl), 7.40 (d, J = 8.3 Hz, 2H, phenyl), 6.61 (s, 2H, –NH_2), 3.29 (d, J = 19.9 Hz, 1H, –CH–), 2.72 (t, J = 5.5 Hz, 1H, –CH_2–), 2.36 (d, J = 16.7 Hz, 2H, –CH_2–), 2.20 (s, 1H, –CH–), 1.35 (d, J = 3.8 Hz, 3H, –CH_3), 1.22 (d, J = 9.5 Hz, 1H, –CH_2–), 0.67 (s, 3H, –CH_3); ESI-MS: m/z 325.1 $[M + 1]^+$; 346.1, 348.1 $[M + 23]^+$
4k	Yield 74%, HPLC purity = 97.76%, (XDB-C-18 column; MeOH:H_2O/80:20), t_r = 12 min; pale yellow crystals, m.p. 209–210°C; FT-IR v (cm^{-1}): 3453, 3299, 3149 (N–H), 2954, 2925 (C–H), 2211 (C≡N), 1636, 1592, 1555, 1491, 1458 (phenyl), 1370 (C–H); ^{1}H NMR (300 MHz, DMSO-d6) δ: 7.71 (d, J = 8.4 Hz, 2H, phenyl), 7.34 (d, J = 8.3 Hz, 2H, phenyl), 6.61 (s, 2H, –NH_2), 3.29 (d, J = 19.9 Hz, 1H, –CH–), 2.74 (t, J = 5.5 Hz, 1H, –CH_2–), 2.61 (dt, J = 22.3, 8.6 Hz, 1H, –CH_2–), 2.36 (d, J = 16.6 Hz, 1H–CH_2–), 2.20 (s, 1H, –CH–), 1.34 (s, 3H, –CH_3), 1.23 (d, J = 9.6 Hz, 1H, –CH_2–), 0.67 (s, 3H, –CH_3); ESI-MS: m/z 369.1 $[M + 1]^+$; 390.1, 392.1, 393.1 $[M + 23]^+$
4l	Yield 73%, HPLC purity = 94.72%, (XDB-C-18 column; MeOH:H_2O/80:20), t_r = 12 min; pale yellow crystals, m.p. 182–183°C; FT-IR v (cm^{-1}): 3405, 3311, 3189 (N–H), 2931(C–H), 2204 (C≡N), 1687, 1637, 1599, 1556, 1515, 1461 (phenyl), 1371 (C–H); ^{1}H NMR (300 MHz, DMSO-d6) δ: 7.31 (d, J = 7.9 Hz, 2H, phenyl), 7.23 (d, J = 7.9 Hz, 2H, phenyl), 6.52 (s, 2H, –NH_2), 3.27 (s, 1H, –CH–), 2.71 (t, J = 5.5 Hz, 1H, –CH_2–), 2.41–2.33 (m, 5H, –CH_3 and–CH_2–), 2.19 (s, 1H, –CH–), 1.34 (d, J = 3.4 Hz, 3H, –CH_3), 1.21 (d, J = 9.5 Hz, 1H, –CH_2–), 0.66 (s, 3H, –CH_3); ESI-MS: m/z 304.2 $[M + 1]^+$; 326.2 $[M + 23]^+$

MCF7. Its IC_{50} was 53.87 μmol·L^{-1}, which was better than that of dasatinib (with IC_{50} of 55.07 μmol·L^{-1}). Generally, among these (+)-nopinone-based 2-amino-3-cyanopyridine derivatives, compounds 4f, 4j and 4k showed promising anticancer activity against the selected cancer cells. Compound 4f, in particular, was effective against all of the selected cancer cells.

The concentrations of test compounds had great impact on their inhibition rates. Figure 2 shows the inhibition rates of compounds 4f, 4j, 4k and dasatinib against cell lines A549 and MKN45 at different test concentrations. The inhibition rates of these compounds against cell lines A549 and MKN45 were observed to increase with the increasing test concentration. For cell line A549 at a test concentration of 100 μmol·L^{-1}, the inhibition rates of compounds 4f and 4k were comparable to that of dasatinib.

4 Discussion

4.1 Synthesis

For the four component reaction between an aldehyde, a ketone, malononitrile and ammonium acetate, earlier

Table 2 IC_{50} values of (+)-nopinone-based 2-amino-3-cyanopyridines against three human cancer cell lines

Compound	A549	MKN45	MCF7
4a	> 100	> 100	> 100
4b	> 100	> 100	> 100
4c	> 100	> 100	> 100
4d	> 100	> 100	> 100
4e	> 100	> 100	> 100
4f	23.78	67.61	53.87
4g	> 100	> 100	> 100
4h	> 100	> 100	> 100
4i	> 100	> 100	> 100
4j	91.29	79.61	> 100
4k	37.35	91.66	> 100
4l	> 100	> 100	> 100
1	> 100	> 100	> 100
2	> 100	> 100	> 100
Dasatinib	15.71	24.49	55.07

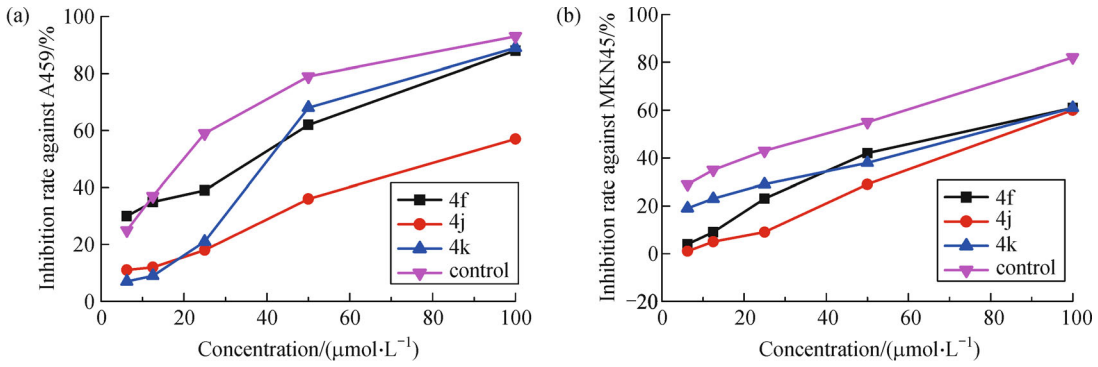


Fig. 2 Inhibition rate of three derivatives against A549 (a) and MKN45 (b) at different concentrations

studies have found that *para*-substituted and *meta*-substituted aromatic aldehydes tolerated the reaction and yielded considerable products, but *ortho*-substituted aromatic aldehydes only gave trace products[19]. However, in this study, we successfully obtained relevant products using *ortho*-substituted aromatic aldehydes by the one-pot four-component reactions (compounds 4b and 4c). One possible reason for this result was that the steric hindrance effect of the pinane ring was smaller than that of two neighboring substituents in the pyridine ring.

4.2 Structure activity relationship

A preliminary analysis of the structure activity relationship of the (+)-nopinone-based 2-amino-3-cyanopyridine derivatives showed that the substituted groups in the benzene ring influenced the anticancer activity. A derivative with a Br or Cl substituted group at the *meta*-position or *para*-position of the benzene ring normally had anticancer activity, such as compounds 4f, 4j and 4k. Furthermore, compound 4f with Br substituted group at the *meta*-position of the benzene ring showed better anticancer activity than the derivatives with other substituted groups at the same position (such as compounds 4d and 4e).

5 Conclusions

A series of (+)-nopinone-based 2-amino-3-cyanopyridine derivatives were synthesized and evaluated for their anticancer activity against A549, MKN45 and MCF7. The result showed that compounds 4f, 4j and 4k showed promising anticancer activity against the selected cancer cells. Compound 4f exhibited broad spectrum and highly efficient anticancer activity against cancer cell lines A549, MKN45 and MCF7 with IC_{50} of 23.78, 67.61 and 53.87 μmol·L^{-1}, respectively. Compound 4j showed moderate anticancer activity against cell lines A549 and MKN45 with IC_{50} of 91.29 and 79.61 μmol·L^{-1}, respectively. Compound 4k exhibited moderate anticancer activity against cell lines A549 and MKN45 with IC_{50} of 37.35 and 91.66 μmol·L^{-1}, respectively. Compared with dasatinib, compound 4f obtained comparable anticancer activity against A549 and better anticancer activity against MCF7. The preliminary analysis of the structure activity relationship implied that the substituted groups in the benzene ring exert an influence on the anticancer activity. The findings of this study could aid in the further design and development of new anticancer analogs of monoterpene derivatives with enhanced bioactivity.

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Compliance with ethics guidelines Shengliang Liao, Shibin Shang, Minggui Shen, Xiaoping Rao, Hongyan Si, Jie Song, and Zhanqian Song declare that they have no conflict of interest or financial conflicts to disclose.

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