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RESEARCH ARTICLE

Asymmetric synthesis and evaluation of α-quaternary chiral lactam derivatives as novel anticancer agents

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Abstract Asymmetric synthesis of α-quaternary chiral lactam derivatives as novel anticancer agents and evaluation of their cytotoxic potentials and spectrums are reported. Among the developed lactam derivatives, the most active new compounds (*S*)-**4m** and (*S*)-**4n** synthesized via asymmetric phase-transfer catalytic alkylation in very high optical yields (98 % ee) show promising in vitro anticancer activities with low micromolar IC_{50} values against colon, uterus, lung, and breast human cancer cells.

Keywords α-Quaternary chiral lactam · Phase-transfer catalytic alkylation · Colon · Lung · Breast cancers · Anticancer activity

Introduction

Cancer is one of the fatal and universal diseases (Jemal et al. 2008) that are still demanded novel therapeutic agents which belong to new structural class for high efficacies and low side effects. Among the abundant anticancer small molecules, one of the famous structural classes is oxindole, a bicyclic aromatic heterocycle with benzene ring fused to γ-lactam. As a natural product which has oxindole structure, spirotryprostatin A has been well known for its anticancer activities by inhibition of the G2/M progression in mouse cdc2 mutant tsFT210 cells (Cui et al. 1996; Galliford et al. 2007). Also, sunitinib was developed as an oxindole multitargeted receptor tyrosine kinase inhibitor and has been widely used in the treatment of renal cell carcinoma (Demetri et al. 2006; Chow and Eckhardt 2007; Motzer et al. 2007). In addition, diverse derivatives based on oxindole have been reported for development of new type anticancer candidates (Natarajan et al. 2004; Silva et al. 2010; Kamal et al. 2011).

Compared with oxindole structures, however, there have been few studies of lactam core without fused benzene ring for anticancer activities. Particularly, unlike β-lactams (Banik et al. 2004; Ruf et al. 2008; Banik and Becker 2010), anticancer activities of γ- and δ-lactams have been rarely studied (Kim et al. 2007; Choi et al. 2012). Recently, we have reported the synthetic methods for α-quaternary chiral α-alkyl-*tert*-butoxycarbonyllactams by using chiral phase-transfer catalysts (PTCs) (Park et al. 2011, 2012). The phase-transfer catalytic reaction has been widely accepted as one of the most efficient synthetic methods at the aspect of economical and environmental concerns (Maruoka and Ooi, 2003; Lygo and Andrews 2004; ODonnell 2004; Hashimoto and Maruoka 2007; Jew and Park 2009). As a progressive application of our developed methods, α-quaternary chiral γ- and δ-lactam derivatives **4** were synthesized to investigate their possibilities as a new structural class for anticancer activities (Fig. 1).

In this paper, we reported asymmetric synthesis of novel α-quaternary chiral lactam derivatives **4** by modification of functional groups (X, Y, R), ring sizes (*n*), and configurations

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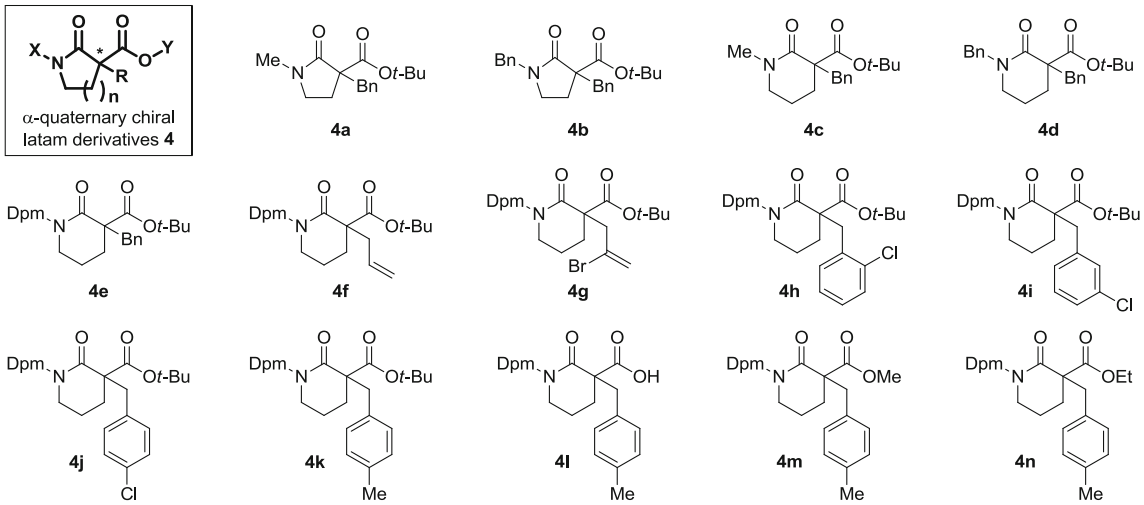


Fig. 1 α-Quaternary chiral lactam derivatives **4**

of chiral center (*). Also, limited structure–activity relationships (SARs) of lactams **4** were determined by in vitro MTT assay. Furthermore, the most active compounds were investigated against four different cancer cell lines to verify the potent scopes of their anticancer activities.

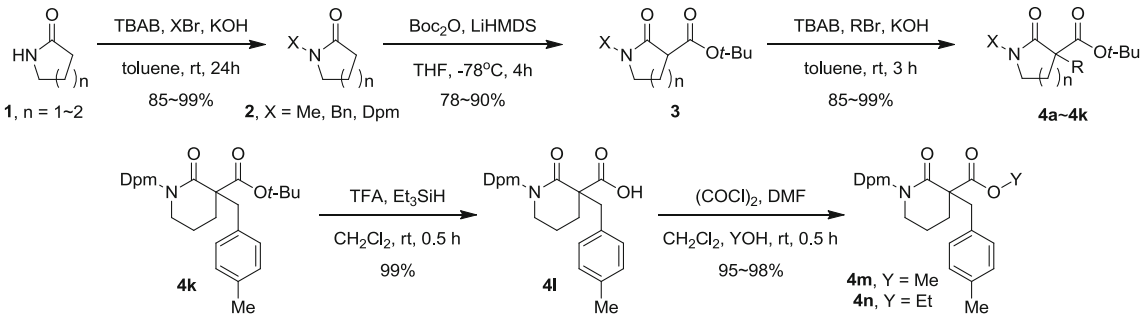
Materials and methods

Chemistry

All reagents bought from commercial sources were used as sold. Organic solvents were concentrated under reduced pressure using a Büchi rotary evaporator. Syringes, needles and cannulae were oven dried at 100 °C. As the commercially available KOH was a pellet type, KOH should be grinded to the powder form for successful reaction and high enantiopurity. EYELA PSL-1400 was used for low temperature stirring and the rate was 7. Phase-transfer catalyst (*S*,*S*)-**5** and (*R*,*R*)-**5** (Ooi et al. 2003) was purchased from the commercial source (Wako). TLC analyses were performed using Merck precoated TLC plate (silica gel 60 GF254, 0.25 mm). Flash column chromatography was carried out using E. Merck Kieselgel 60 (230–400 mesh). Instrument (Hitachi, L-2130) and software (Hitachi, Version LaChrom 8908800-07) were used as HPLC. The enantiomeric excess (ee) of the products was determined by HPLC using 4.6 mm × 250 mm Daicel Chiralcel OD-H and Daicel Chiralpak AD-H. Infrared (IR) spectra were recorded on a JASCO FT/IR-300E and Perkin-Elmer 1710 FT spectrometer. Nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectra were measured on VNMRS500 [500 MHz (^{1}H), 125 MHz (^{13}C)], JEOL JNM-LA 300 [300 MHz (^{1}H), 75 MHz (^{13}C)] spectrometer, and JEOL JNM-GSX 400 [400 MHz (^{1}H), 100 MHz (^{13}C)] spectrometer, using $CHCl_3$-*d* as solvents, and were reported in ppm relative to $CHCl_3$ (δ 7.24) for ^{1}H-NMR and relative to the central $CDCl_3$ (δ 77.00) resonance for ^{13}C-NMR. Coupling constants (*J*) in ^{1}H-NMR are in Hz. High-resolution mass spectra (HRMS) were measured on a JEOL JMS-AX 505wA, JEOL JMS-HX/HX 110A spectrometer. Melting points were measured on a Büchi B-560 melting point apparatus and were not corrected. Optical rotations were measured on a JASCO polarimeter P-2000 series.

General procedure for racemic lactams

As substrates for phase-transfer catalytic alkylation, compounds **3** [n = 1–2, X = Me, Bn, diphenylmethyl (Dpm)] were synthesized from corresponding compounds **2** which were purchased from commercial sources or synthesized from lactams **1**, following our previous methods (Park et al. 2011, 2012). As a representative method for racemic **4a–4k**, α-Bromo-*p*-xylene (517.2 mg, 2.74 mmol) was added to a solution of compound **3e** (200 mg, 0.548 mmol) and tetra-*n*-butylammonium bromide (17.7 mg, 0.055 mmol) in toluene (2.5 mL). At room temperature, solid KOH (153.6 mg, 2.74 mmol) was quickly added to the reaction mixtures after weighing and stirred for 3 h. The reaction mixtures was diluted with ethyl acetate (40 mL), washed with brine (3 × 20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. After purification of the residue by column chromatography (silica gel, hexane:EtOAc = 20:1), racemic **4k** was obtained in Scheme 1. Spectroscopic characterizations of known compounds **4a–4g** were in the previous reports (Park et al. 2011, 2012).



Scheme 1 Synthesis of racemic lactam derivatives **4a–n**

General procedure for modification of Y group

Trifluoroacetic acid (2.5 mL) was added to the reaction mixture of compound **4k** (260 mg, 0.554 mmol) and triethylsilane (0.22 mL 1.38 mmol) in dichloromethane (2.5 mL) (Mehta et al. 1992). The reaction mixture was stirred for 1 h at room temperature and concentrated in vacuo. Purification of the by column chromatography (silica gel, from hexane:EtOAc = 10:1 to only EtOAc) gave carboxylic acid **4l**. Oxalyl chloride (0.042 mL, 0.484 mmol) and DMF (1 drop) were added to compound **4l** (50.0 mg, 0.121 mmol) in dichloromethane (1 mL) at room temperature and stirred for 1 h at room temperature (Wissner et al. 1978). Then, MeOH (1 mL) or EtOH (1 mL) was added to the reaction mixture, stirred for another 1 h at room temperature, and concentrated in vacuo. After purification of the residue by column chromatography (silica gel, hexane:EtOAc = 10:1), corresponding methyl ester **4m** and ethyl ester **4n** were obtained in Scheme 1.

General procedure asymmetric phase-transfer catalytic alkylation

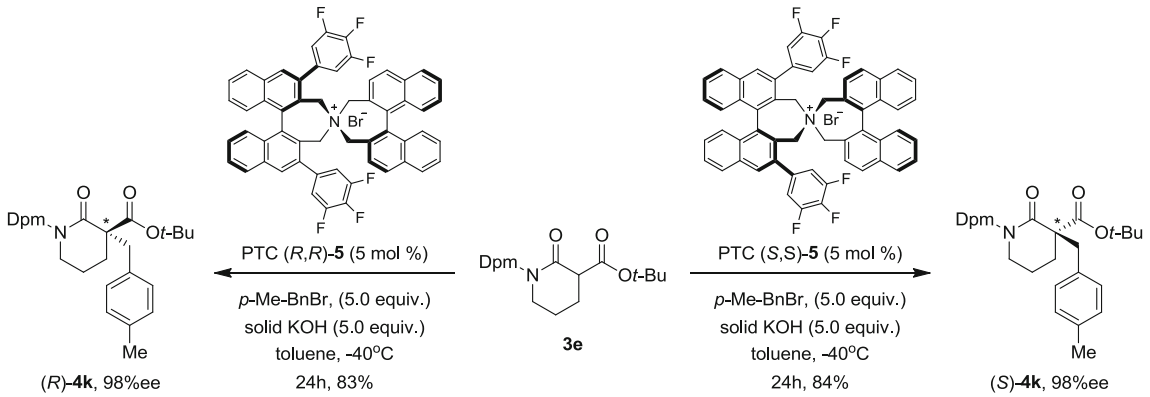
As a representative method for chiral lactams, α-bromo-*p*-xylene (129.3 g, 0.685 mmol) was added to a solution of compound **3e** (50 mg, 0.137 mmol) and (*S*,*S*)-3,4,5-trifluorophenyl-NAS bromide [(*S*,*S*)-**5**, 6.2 mg, 0.0068 mmol] in toluene (0.5 mL). At −40 °C, solid KOH (38.5 mg, 0.685 mmol) was added to the reaction mixtures and stirred for 24 h. After weighing of the powdered KOH, it was quickly added to the reaction mixture. EYELA PSL-1400 was used for low temperature stirring and the stirring rate was 7. The reaction mixtures was diluted with ethyl acetate (10 mL), washed with brine (2 × 2 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. After purification of the residue by column chromatography (silica gel, hexanes:EtOAc = 20:1), (*S*)-**4k** was obtained in Scheme 2. Absolute configuration of (*S*)-**4k** was determined based on X-ray data of (*S*)-*tert*-butyl 1-benzhydryl-3-(4-bromobenzyl)-2-oxopiperidine-3-carboxylate (Park et al. 2011) that was synthesized via reaction mechanism same with (*S*)-**4k** (Maruoka and Ooi 2003; Lygo and Andrews 2004; ODonnell 2004; Hashimoto and Maruoka 2007; Jew and Park 2009). Another absolute configurations of (*R*)-**4k**, (*S*)-**4m**, (*R*)-**4m**, (*S*)-**4n**, and (*R*)-**4n** were assigned by comparison with (*S*)-**4k.**

Tert-Butyl 1-benzhydryl-3-(2-chlorobenzyl)-2-oxopiperidine-3-carboxylate (4 h)

Pale yellow solid; 87 % yield; m.p. 120 °C; IR (KBr) 3062, 3030, 2976, 2933, 2877, 2349, 2308, 1733, 1636, 1475, 1446, 1367, 1295, 1252, 1200, 1148, 1118, 1052, 1033, 772, 756, 725, 699 cm^{-1}; HRMS (FAB) : calcd. for $[C_{30}H_{33}ClNO_3]^+$: 490.2149, found: 490.2155; ^{1}H-NMR (300 MHz, CDCl3) δ 7.30–7.17 (m, 11H), 7.08–7.02 (m, 3H), 6.94–6.88 (m, 1H), 3.62–3.51 (dd, J_1 = 19.41 Hz, J_2 = 13.92 Hz, 2H), 2.98–2.91 (m, 1H), 2.56–2.47 (m, 1H), 2.07–2.01 (m, 1H), 1.93–1.66(m, 3H), 1.45 (s, 9H) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.19, 168.77, 138.47, 138.23, 135.49, 135.32, 132.29, 129.35, 129.29, 128.26, 128.21, 128.19, 127.77, 127.44, 127.15, 126.72, 81.95, 60.26, 56.79, 43.89, 36.67, 29.22, 27.91, 20.04 ppm.

Tert-Butyl 1-benzhydryl-3-(3-chlorobenzyl)-2-oxopiperidine-3-carboxylate (4i)

Pale yellow viscous oil; 89 % yield; IR (KBr) 3061, 3029, 3005, 2932, 1731, 1638, 1598, 1572, 1495, 1481, 1455, 1431, 1393, 1368, 1356, 1291, 1253, 1201, 1149, 1079, 842, 786, 765, 739, 701 cm^{-1}; HRMS (FAB): calcd. for $[C_{30}H_{33}ClNO_3]^+$: 490.2149, found: 490.2161; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.32–7.17 (m, 11H), 7.09–7.01 (m, 4H), 3.29 (dd, J_1 = 404.5 Hz, J_2 = 13.5 Hz, 2H), 2.98–2.95 (m, 1H), 2.53–2.51 (m, 1H), 2.03–1.97 (m, 2H), 1.93–1.88 (m, 1H), 1.80–1.74 (m, 1H), 1.47 (s, 9H) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.28, 168.24, 139.26, 138.41, 138.35, 133.84, 130.89, 129.36, 129.35, 129.15, 128.36, 128.31, 128.30,



Scheme 2 Enantioselective synthesis of (*S*)-**4k** and (*R*)-**4k** via phase-transfer catalytic alkylation

127.43, 127.16, 126.79, 82.13, 60.20, 55.91, 43.91, 40.87, 29.54, 27.98, 20.10 ppm.

Tert-Butyl 1-benzhydryl-3-(4-chlorobenzyl)-2-oxopiperidine-3-carboxylate (**4j**)

White solid; 85 % yield; m.p. 141 °C; IR (KBr) 3061, 3030, 2956, 2918, 2870, 2849, 2349, 2308, 1733, 1637, 1490, 1456, 1368, 1253, 1199, 1149, 1114, 851, 770, 739, 720, 700 cm^{-1}; HRMS (FAB): calcd. for $[C_{30}H_{33}ClNO_3]^+$: 490.2149, found: 490.2161; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.31–7.19 (m, 9H), 7.11 (s, 4H), 6.98–6.96 (m, 2H), 3.27 (dd, $J_1 = 408.5$ Hz, $J_2 = 13.3$ Hz, 2H), 3.71–3.65 (m, 1H), 2.95–2.92 (m, 1H), 2.53–2.48 (m, 1H), 1.99–1.79 (m, 3H), 1.47 (s, 9H) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.41, 168.49, 138.49, 138.18, 135.56, 132.46, 132.29, 129.24, 128.39, 128.29, 128.28, 128.24, 127.55, 127.24, 82.04, 60.22, 55.88, 42.90, 40.65, 29.69, 27.97, 20.25 ppm.

(*S*)-*Tert*-Butyl 1-benzhydryl-3-(4-methylbenzyl)-2-oxopiperidine-3-carboxylate (**4k**)

White solid; 84 % yield; m.p. 92 °C; $[\alpha]_D^{23}$ +26.86 (*c* 1.0, $CHCl_3$); HPLC analysis (DIACEL Chiralcel OD-H, hexane:2-propanol = 95:5, flow rate = 1.0 mL/min, 23 °C, $\lambda = 254$ nm) retention time = *S* (major) 5.7 min, *R* (minor) 9.1 min, 98 % ee; IR (KBr) 2930, 1734, 1638, 1495, 1455, 1367, 1291, 1254, 1149, 1117, 1032, 854, 804, 703 cm^{-1}; HRMS (FAB): calcd. for $[C_{31}H_{36}NO_3]^+$: 470.2695, found: 470.2691; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.25–7.15 (m, 8H), 7.03–6.91 (m, 7H), 3.21 (dd, $J_1 = 243.24$ Hz, $J_2 = 13.47$ Hz, 2H), 2.83–2.79 (m, 1H), 2.43 (m, 1H), 2.25 (s, 3H), 1.92–1.78 (m, 4H), 1.42 (s, 9H) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.71, 168.83, 138.72, 138.44, 135.96, 133.98, 130.84, 129.45, 128.83, 128.45, 128.25, 128.14, 127.34, 127.17, 81.77, 60.18, 56.01, 43.93, 40.96, 29.70, 28.00, 21.06, 20.33, 1.02 ppm.

(*R*)-*Tert*-Butyl 1-benzhydryl-3-(4-methylbenzyl)-2-oxopiperidine-3-carboxylate (**4k**)

White solid; 83 % yield; m.p. 92 °C; $[\alpha]_D^{23}$ −51.01 (*c* 1.0, $CHCl_3$); HPLC analysis (DIACEL Chiralcel OD-H, hexane:2-propanol = 95:5, flow rate = 1.0 mL/min, 23 °C, $\lambda = 254$ nm) retention time = *R* (major) 5.24 min, *S* (minor) 6.69 min, 98 % ee.

1-Benzhydryl-3-(4-methylbenzyl)-2-oxopiperidine-3-carboxylic acid (**4l**)

White solid; 99 % yield; m.p. 110 °C; IR (KBr) 3087, 3060, 3028, 3006, 2938, 2888, 2598, 1955, 1905, 1732, 1636, 1600, 1578, 1514, 1496, 1487, 1446, 1357, 1308, 1291, 1233, 1199, 1182, 1159, 1119, 812, 755, 741, 718, 704 cm^{-1}; HRMS (FAB): calcd. for $[C_{29}H_{31}NO_3]^+$: 442.2382, found: 442.2376; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.41 (br s, 1H), 7.25–7.15 (m, 6H), 7.04–6.93 (m, 9H), 3.21 (dd, $J_1 = 144.9$ Hz, $J_2 = 13.5$ Hz, 2H), 2.90–2.86 (m, 1H), 2.67–2.59 (m, 1H), 2.32–2.18 (m, 1H), 2.25 (s, 3H), 1.80–1.73 (m, 1H), 1.60–1.58 (m, 2H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.05, 172.33, 137.47, 137.31, 136.88, 132.14, 130.24, 129.09, 128.92, 128.64, 128.41, 128.19, 127.76, 127.73, 61.42, 55.12, 45.13, 44.15, 27.05, 21.03, 19.92 ppm.

(*S*)-Methyl 1-benzhydryl-3-(4-methylbenzyl)-2-oxopiperidine-3-carboxylate (**4m**)

White solid; 95 % two step yields; m.p. 83 °C; $[\alpha]_D^{23}$ +40.86 (*c* 1.0, $CHCl_3$); HPLC analysis (DIACEL Chiralpak AD-H, hexane:2-propanol = 80:20, flow rate = 1.0 mL/min, 23 °C, $\lambda = 254$ nm) retention time = *R* (minor) 10.25 min, *S* (major) 22.07 min, 98 % ee; IR (KBr) 3028, 2950, 2927, 2877, 2852, 2349, 2310, 1741, 1639, 1514, 1496, 1433, 1356, 1290, 1244, 1199, 1158, 1118, 770, 720, 704 cm^{-1}; HRMS (FAB): calcd. for $[C_{28}H_{29}NO_3]^+$: 428.2226, found: 428.2226; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.34–7.20 (m, 9H), 7.09–7.00 (m, 6H),

3.71 (s, 3H), 3.29 (dd, $J_1 = 227.9$ Hz, $J_2 = 13.5$ Hz, 2H), 2.84–2.77 (m, 1H), 2.47–2.44 (m, 1H), 2.25 (s, 3H), 2.04–1.76 (m, 4H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.10, 168.49, 138.51, 138.29, 136.11, 133.63, 130.72, 129.38, 128.85, 128.30, 128.23, 128.17, 127.38, 127.16, 60.37, 55.74, 52.58, 44.05, 40.64, 29.54, 21.03, 20.02 ppm.

(*R*)-Methyl 1-benzhydryl-3-(4-methylbenzyl)-2-oxopiperidine-3-carboxylate (**4m**)

White solid; 94 % two step yields; m.p. 83 °C; $[\alpha]_D^{23}$ −36.22 (*c* 1.0, $CHCl_3$); HPLC analysis (DIACEL Chiralpak AD-H, hexane:2-propanol = 80:20, flow rate = 1.0 mL/min, 23 °C, $\lambda = 254$ nm) retention time = *R* (major) 10.47 min, *S* (minor) 27.00 min, 98 % ee.

(*S*)-Ethyl 1-benzhydryl-3-(4-methylbenzyl)-2-oxopiperidine-3-carboxylate (**4n**)

White solid; 97 % two step yields; m.p. 110 °C; $[\alpha]_D^{23}$ +37.83 (*c* 1.0, $CHCl_3$); HPLC analysis (DIACEL Chiralpak AD-H, hexane:2-propanol = 80:20, flow rate = 1.0 mL/min, 23 °C, $\lambda = 254$ nm) retention time = *R* (minor) 11.15 min, *S* (major) 13.08 min, 98 % ee; IR (KBr) 3060, 3028, 2929, 2870, 2349, 2309, 1894, 1737, 1638, 1514, 1496, 1483, 1455, 1356, 1306, 1291, 1242, 1197, 1183, 1158, 1115, 1095, 1030, 814, 739, 720, 704 cm^{-1}; HRMS (FAB): calcd. for $[C_{27}H_{27}NO_3]^+$: 414.2069, found: 414.2067; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.32–7.21 (m, 9H), 7.09–6.99 (m, 6H), 4.26–4.19 (m, 2H), 3.33 (dd, $J_1 = 392.0$ Hz, $J_2 = 13.5$ Hz, 2H), 2.91–2.90 (m, 1H) 2.52–2.47 (m, 1H), 2.31 (s, 3H), 1.99–1.41 (m, 4H), 1.31–1.29 (m, 3H), ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.69, 163.54, 138.51, 138.25, 136.06, 133.71, 130.75, 129.45, 128.83, 128.25, 128.23, 128.14, 127.39, 127.15, 61.54, 60.25, 55.58, 44.02, 40.61, 29.60, 21.05, 20.02, 14.10 ppm.

(*R*)-Ethyl 1-benzhydryl-3-(4-methylbenzyl)-2-oxopiperidine-3-carboxylate (**4n**)

White solid; 97 % two step yields; m.p. 110 °C; $[\alpha]_D^{23}$ −46.40 (*c* 1.0, $CHCl_3$); HPLC analysis (DIACEL Chiralpak AD-H, hexane:2-propanol = 80:20, flow rate = 1.0 mL/min, 23 °C, $\lambda = 254$ nm) retention time = *R* (major) 11.06 min, *S* (minor) 13.34 min, 98 % ee.

Biological assay

The cancer cell lines used in this study include human cervical carcinoma (HeLa), human lung carcinoma (A549), human colon carcinoma (HCT-116), and human breast carcinoma (MDA-MB-231). All cells were purchased from American Type Culture Collection (ATCC, USA) and grown in DMEM (Dulbecco's modified Eagles Medium). To ensure growth and viability of the cells, the mediums were supplemented with 10 % FBS (Gibco) and incubated in a humidified atmosphere with 5 % CO_2 at 37 °C. Cells were also maintained in the exponential growth phase, and routinely certified as mycoplasma free. Cell cytotoxicity was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. 2×10^3 cells were seeded in 96-well plates and then treated with either 0.1 % dimethyl sulfoxide (DMSO) as a negative control or the indicated concentration of derivatives in medium with 0.1 % FBS for 3 days. MTT solution was added for 4 h. After the used media containing MTT solution was removed, the formed formazan crystals were dissolved in DMSO. The plates were read at 540 nm in a microplate reader (Tecan GmbH, Gröding, Austria).

Results and discussions

Based on the MTT assay in HeLa cell lines (concentration of lactam derivatives = 10 μM in DMSO), fourteen racemic lactam derivatives **4a–n** were synthesized (Scheme 1) and compared with their anticancer activities and 0.1 % DMSO as a negative control (Fig. 2). Following Scheme 1, racemic lactam derivatives **4a–k** were synthesized from commercially available lactams **1**, butyrolactam ($n = 1$) and δ-valerolactam ($n = 2$), or *N*-alkylated lactam **2** (X = Me) in 2 or 3 steps that already had been reported (Park et al., 2011, 2012). Tetra-*n*-butylammonium bromide (TBAB) was used as PTC for *N*-alkylation of **1** and synthesis of racemic mixtures **4a–k** from corresponding compounds **3**. Compounds **3** were successively synthesized from compounds **2** with di-*tert*-butyl dicarbonate (Boc_2O) by using lithium bis(trimethylsilyl) amide (LiHMDS) as a base.

First, for determination of limited SAR in *N*-alkyl groups (X) and ring sizes (*n*), we fixed that Y is *tert*-Bu and R is Bn to

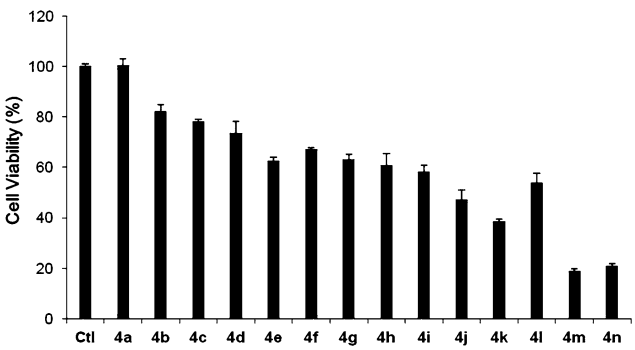


Fig. 2 The cell viability of HeLa cells with racemic lactam derivatives **4a–n** using a typical MTT assay

observe anticancer activities of compounds **4a–e**. The results revealed that δ-valerolactam structure ($n = 2$) was better than butyrolactam structure ($n = 1$) for high activity and bulky *N*-alkyl group, Dpm, was the best for anticancer activities among *N*-alkyl groups (X = Me, Bn, Dpm). Then, for SAR of R groups, ester part (Y = *tert*-Bu), ring size ($n = 2$), and *N*-alkyl group (X = Dpm) were fixed and R groups were changed from Bn to allyl groups in compounds **4f** and **4g**. Compound **4f** (R = allyl) showed lower activity than that of **4e** (R = Bn), but compound **4g** (R = 2-bromoallyl) showed similar activities compared with **4e**.

Since compound **4e** was still the highest active compound, compounds **4h–k** were synthesized following Scheme 1 to investigate SAR about locations and electronic effects of functional groups on a benzene ring of R group in **4e**. Cl was introduced as an electron withdrawing group (EWG) and Me group was chosen as an electron donating group (EDG). Anticancer activity comparison with compounds **4h–k** by MTT assay revealed that whether EWG or EDG were on benzene ring, locations of functional groups, especially *para*-position on benzene ring, were important for higher anticancer activity. As a result of anticancer activity relationships between EWG and EDW, compound **4k** (R = *p*-Me-Bn; EDG) showed higher activity than that of compound **4j** (R = *p*-Cl-Bn; EWG) and compound **4k** (X = Dpm, $n = 2$, R = *p*-Me-Bn) was the most active compound among the lactam derivatives **4a–k** which have same Y (*tert*-Bu).

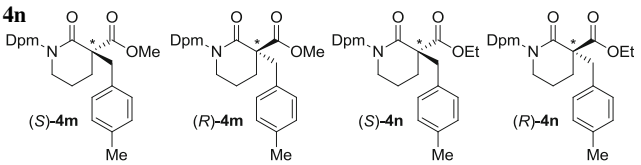
Next, the lactam derivative **4k** was chosen for further SAR to know the effects of ester part, Y groups (Scheme 1). From compound **4k** (Y = *tert*-Bu), carboxylic acid **4l** (Y = H) was synthesized with trifluoroacetic acid (TFA) and triethylsilane in 99 % yield (Mehta et al. 1992). In the presence of oxalyl chloride and dimethylformamide (DMF), addition of alcohols (MeOH and EtOH) to carboxylic acid **4l** (Y = H) gave corresponding ester **4m** (Y = Me, 94 % 2 step yields) and **4n** (Y = Et, 97 % 2 step yields) successively (Wissner et al. 1978). As shown in Fig. 2, relatively low anticancer activity of carboxylic acid **4l** (Y = H) demonstrated that ester moieties were essential structures for high activities of lactam derivatives. Also, the anticancer activities of compounds **4m** and **4n** which had smaller size Y groups were higher than anticancer activity of **4k** which had bulky *tert*-Bu in Y group.

Lactam derivatives **4m** (X = Dpm, Y = Me, R = *p*-Me-Bn) and **4n** (X = Dpm, Y = Et, R = *p*-Me-Bn) which showed the highest anticancer activities among fourteen racemic lactam derivatives **4a–n** were selected to investigate the effects of chiralities (*) for SAR and the scopes of anticancer activities. Following Scheme 2, α-quaternary chiral lactam (*S*)-**4k** (84 % yield, 98 % ee) and (*R*)-**4k** (83 % yield, 98 % ee) were synthesized successfully in very high enantioselectivities with PTCs, (*S*,*S*)-**5** and (*R*,*R*)-**5** (Ooi et al. 2003), respectively. As described method for modification of Y groups in Scheme 1, (*S*)-**4k** were changed to (*S*)-**4m** (Y = Me, 95 % 2 step yields, 98 % ee) and (*S*)-**4n** (Y = Et, 97 % 2 step yields, 98 % ee) while (*R*)-**4k** were turned to (*R*)-**4m** (Y = Me, 94 % 2 step yields, 98 % ee) and (*R*)-**4n** (Y = Et, 97 % 2 step yields, 98 % ee). In Table 1, the IC_{50} values of (*S*)-**4m**, (*R*)-**4m**, (*S*)-**4n**, (*R*)-**4n** and a positive control, 5-fluorouracil (5-FU), were determined in four different human cancer cells (colon, HCT-116; uterus, HeLa; lung, A549; breast, MDA-MB-231). Interestingly, (*S*)-enantiomers of **4m** and **4n** showed significantly greater anticancer activities than those of (*R*)-enantiomers in all four cancer cells. Especially, (*S*)-**4m** had good anticancer activities as well as a well-known chemotherapy agent, 5-FU (Cohen et al. 1958; Longley et al. 2003), in all four human cancer cell lines.

In conclusion, we developed novel anticancer agents, α-quaternary chiral lactam derivatives, (*S*)-methyl 1-benzhydryl-3-(4-methylbenzyl)-2-oxopiperidine-3-carboxylate [(*S*)-**4m**] and (*S*)-ethyl 1-benzhydryl-3-(4-methylbenzyl)-2-oxopiperidine-3-carboxylate [(*S*)-**4n**], synthesized them by using asymmetric phase-transfer catalytic alkylation as a key step in very high optical yields (98 % ee), and evaluated their anticancer activities and scopes in different human cancer cells. The facile synthetic routs, low micromolar IC_{50} values and broad spectrums against four human cancer cells make (*S*)-**4m** and (*S*)-**4n** practical candidates for further exploitations of biological activities as anticancer therapeutic agents. Further investigations of detailed SARs and mechanistic studies for anticancer activities are now in progress.

Table 1 In vitro broad spectrum antiproliferative activity of α-quaternary chiral lactam derivatives **4m** and

4n



Compd.	μM			
	HCT-116 (colon)	HeLa (uterus)	A549 (lung)	MDA-MB-231 (breast)
(*S*)-**4m**	2.84 ± 0.16	3.25 ± 0.14	4.35 ± 0.50	4.16 ± 0.15
(*R*)-**4m**	6.43 ± 0.31	7.21 ± 0.27	5.59 ± 0.40	8.16 ± 0.64
(*S*)-**4n**	4.24 ± 0.46	5.86 ± 0.46	3.72 ± 0.85	4.57 ± 0.36
(*R*)-**4n**	5.77 ± 0.58	6.82 ± 0.83	7.72 ± 0.86	5.13 ± 0.37
5-FU	4.80 ± 0.40	4.37 ± 0.23	6.09 ± 0.34	5.40 ± 0.62

Average IC_{50} values are shown. Each compound was tested at six different concentrations, and each drug dilution was repeated three times. Cells treated with DMSO (equivalent volume) were used as a vehicle control

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