HETEROCYCLES, Vol. 97, No. 1, 2018, pp. 560 - 568. © 2018 The Japan Institute of Heterocyclic Chemistry
Received, 26th Janary, 2018, Accepted, 16th February, 2018, Published online, 1st March, 2018
DOI: 10.3987/COM-18-S(T)25

SYNTHESIS OF *N*-ω-PHENYLALKYL-4-(*p*-CHLOROPHENYL)-PIPERIDIN-4-OL ANALOGUES WITH POTENT ANTIPROLIFERATIVE ACTIVITY AGAINST HCT-116 CELLS

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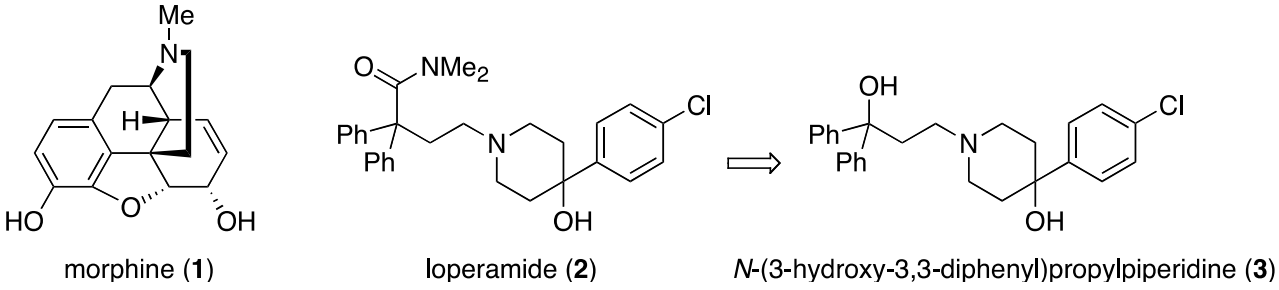
This paper is dedicated to Professor Kiyoshi Tomioka, Doshisha Women's College, on the celebration of his 70th birthday.

Abstract – Some opioid analogues, such as morphine and loperamide, were reported to exhibit weak antiproliferative activity against tumor cells. In a study of loperamide analogues, we found that adding an *N*-ω-phenylalkyl group onto the 4-arylpiperidin-4-ol unit can have important effects on the antiproliferative activity of such compounds against HCT-116 cells. We optimized the distance between the phenyl group and 4-arylpiperidin unit to promote such activity.

In 2014, around 14.1 million people were expected to develop cancer annually, according to the World Cancer Report 2014 compiled by the World Health Organization's International Agency for Research on Cancer (IRAC).[1] In 2015, cancer accounted for 8.8 million deaths, which was nearly 1/6th of all deaths. Lung cancer was the most common cause of cancer death, followed by liver and colorectal cancer. Chemotherapy, surgery, and radiotherapy, are used to treat many types of cancer.[2] In addition, opioid analgesics, such as morphine (**1**), are used to relieve cancer pain in terminal-stage cancer. Recently, it was reported that high-dosage morphine treatment unexpectedly extended the life spans of advanced

cancer patients;[3] threfore, opioid alkaloids might inhibit cancer progression. Indeed, opioid analogues have been reported to attenuate the proliferation of some tumor cells, e.g., such effects have been reported for morphine against PC-9 human lung cancer cells, human neuroblastoma cells, and MCF-7 and MDA-MB231 human breast cancer cells;[3-5] for DADLE, DSLET, ethylketocyclazocine, and etorphine against T47D human breast cancer cells;[6] for buprenorphine and loperamide (**2**) against A549 human lung carcinoma cells;[7] and for endomorphins against HL-60 human leukemia cells.[8] Although some opioid alkaloids have been demonstrated to inhibit tumor cell growth, the molecular mechanisms underlying effects are still unclear because high doses of opioids are required to induce such inhibitory activity. development of opioid analogues with higher antiproliferative activity could help to elucidate these molecular mechanisms in more detail.

Recently, we synthesized loperamide analogues and assessed their antiproliferative activity against HCT-116 human colon tumor cells and HL-60 cells.[9] The 4-phenylpiperidin-4-ol unit in loperamide an important role in its antiproliferative activity, and the substituted to the *N*-diphenylcarbinol unit on the nitrogen atom produces a molecule with stronger antiproliferative activity than loperamide. In the loperamide derivatives examined in our previous study, the *N*-diphenylpropanol analogue (**3** in Figure 1) exhibited the most potent antiproliferative activity (its activity against HCT-116 cells was 2.46-fold than that of loperamide). Interestingly, we also found that the *N*-diphenylethanol analogue displayed weaker antiproliferative activity than the corresponding propanol analogue; therefore, we analyzed the distance between the 4-phenylpiperidin-4-ol unit and the aryl and/or hydroxyl unit in the current study.



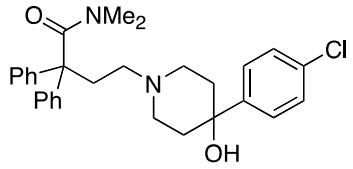
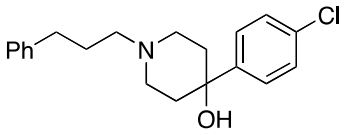
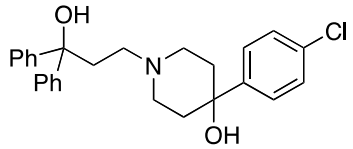
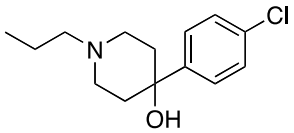
morphine (**1**) loperamide (**2**) *N*-(3-hydroxy-3,3-diphenyl)propylpiperidine (**3**)

Figure 1. Structures of morphine (**1**), loperamide (**2**), and the *N*-diphenylpropanol analogue **3**

The antiproliferative activity of the examined molecules against HCT-116 cells was shown in Table 1. The *N*-ω-monophenylpiperidine analogue **7c**, whose synthesis was shown in Scheme 1, displayed weaker antiproliferative activity than loperamide (**2**) and the *N*-ω-diphenylpropanol analogue **3**. The IC_{50} value of **7c** was 49.86 μM. In addition, the propyl analogue **8**, in which the hydroxyl and diphenyl groups found at the ω position in analogue **3** had been removed, did not demonstrate any antiproliferative activity.

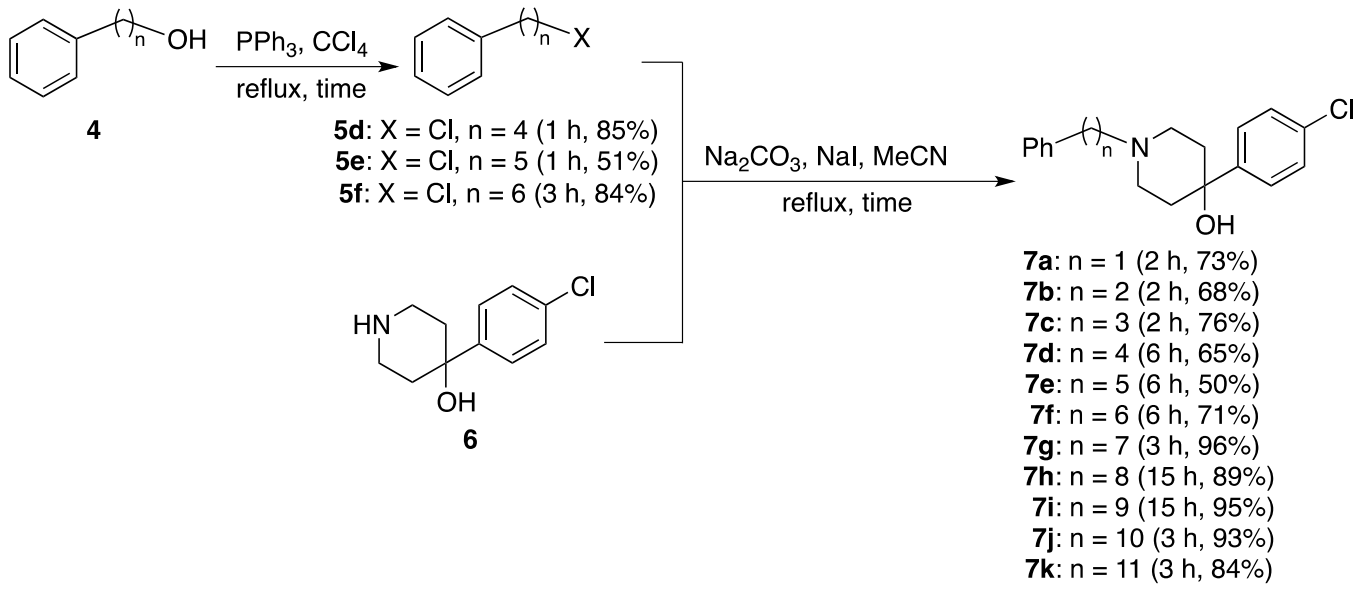
These finding convinced us that the presence of at least one phenyl group at the ω-position in the analogue **7c** could be essential for antiproliferative activity, and so we synthesized *N*-ω-monophenylpiperidine analogue with alkyl chains of different lengths and analyzed their antiproliferative activity.

Table 1. Antiproliferative activity of *N*-ω-substituted-propyl analogues against HCT-116 cells

compoumd	IC_{50} value (μM)[a]	compoumd	IC_{50} value (μM)[a]
 loperamide (**2**)	23.72 ± 0.71	 **7c**	49.86 ± 2.17
 **3**	9.66 ± 0.11	 **8**	> 100

[a] Data are shown as mean ± SEM values.

The syntheses of the *N*-ω-phenylalkylpiperidine analogues **7a-k** according to the previously reported procedure was shown in Scheme 1.[9] The alkylating reagents **5a-c** (X = Br, n = 1-3), **5g**, **5j**, **5k** (X = Br,

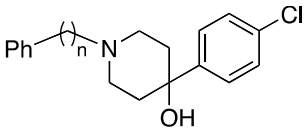


Scheme 1. Syntheses of the *N*-ω-phenylalkylpiperidine analogues **7a-k**

n = 7, 10, 11) and **5h**, **5i** (X = Cl, n = 8, 9), were commercially available, and 4-phenylbutyl chloride (**5d**), 5-phenylpentyl chloride (**5e**) and 6-phenylhexyl chloride (**5f**) were synthesized from the corresponding alcohols in 85%, 51%, and 84% yield, respectively. Subsequently, the *N*-ω-phenylalkylpiperidine analogues **7a-k** were afforded in yields greater than 50% using the ω-phenylalkyl halide **5**. The chemical structures of the desired products were confirmed by spectroscopic analysis.

The antiproliferative activity of the synthesized *N*-ω-phenylalkylpiperidine analogues **7a-k**, which had alkyl chains of different lengths, against HCT-116 cells was assessed (Table 2). The benzyl and analogues **7a** and **7b** exhibited IC_{50} values of >100 μM. These findings are similar to our previous data, which indicated that a diphenylethanol analogue displayed weaker antiproliferative activity than the corresponding diphenylpropanol analogue.[9] The elongation of the alkyl chain into a nonyl led to an increase in antiproliferative activity. The activity of the analogues with hexyl or longer alkyl chains was greater than that of the *N*-diphenylpropanol analogue **3**, and the IC_{50} value of the 9-phenylnonyl analogue which was the most potent of the tested analogues, was 1.79 μM. Further elongation of the alkyl chain resulted in reduced antiproliferative activity; therefore, the IC_{50} value for the nonyl analogue **7i** was identified as the local (or global) minimum value among the *N*-ω-phenylalkylpiperidine analogues **7a-k**.

Table 2. Antiproliferative activity of *N*-ω-phenylalkyl analogues against HCT-116 cells



compoumd		IC_{50} value (μM)[a]	compoumd		IC_{50} value (μM)[a]
7a	n = 1	> 100	**7g**	n = 7	3.70 ± 0.20
7b	n = 2	> 100	**7h**	n = 8	2.21 ± 0.08
7c	n = 3	49.86 ± 2.17	**7i**	n = 9	1.79 ± 0.07
7d	n = 4	31.59 ± 0.76	**7j**	n = 10	1.96 ± 0.06
7e	n = 5	15.39 ± 0.62	**7k**	n = 11	3.73 ± 0.06
7f	n = 6	6.08 ± 0.20	*N*-diphenylpropanol **3**		9.66 ± 0.11

[a] Data are shown as mean ± SEM values.

Some opioid analogues, such as morphine and loperamide, exhibit antiproliferative activity against tumor cells, but this activity is quite weak. Therefore, the development of opioid analogues with more potent antiproliferative activity is necessary. In the current study, the activity of 4-arylpiperidin-4-ol analogues against HCT-116 cells was examined, and an *N*-ω-phenylalkyl group was identified as being necessary for such activity. The *N*-phenylnonyl analogue **7i** was the most potent of the synthesized compounds.

EXPERIMENTAL

General method

All new compounds were fully identified. Melting points were obtained with a Yanagimoto micro-melting-point apparatus and are uncorrected. IR spectra of solids (KBr) and liquids (NaCl) were recorded on a JASCO FT/IR-4100 spectrophotometer and are expressed in reciprocal centimeter (cm^{-1}). ^1H NMR spectra were recorded on a JEOL AL-300 (300 MHz) spectrometer or Brucker AVANCE500 (500 MHz) spectrometer, with tetramethylsilane used as the internal standard. ^{13}C NMR spectra were obtained on a JEOL AL-300 spectrometer (75 MHz) or Brucker AVANCE500 (125 MHz) spectrometer, with $CDCl_3$ used as the internal standard (δ = 77.0). EI-MS was recorded on a JEOL JMS-GC mate II spectrometer. All column chromatography-based chromatographic isolation procedures were accomplished with silica gel (60N, spherical neutral, 63-210 μm) (Kanto Chemical).

General Procedures for synthesis 1-chloro-ω-phenylalkane. Triphenylphosphine (2.72 g, 10.4 was added to the 4-phenyl-1-butanol (1.2 g, 8.0 mmol) in CCl_4 (10 mL) at room temperature under atmosphere of argon, and the mixture was refluxed for 1 h. The reaction quenched with water and extracted with hexane. The extracts were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography with hexane/AcOEt (100:1) to give (4-chlorobutyl)benzene (**5d**) as a colorless oil in 85% yield. The analytical data were identical with those of a literature compound.[10] IR (neat): ν = 2940 (CH_2), 699 (Alkyl-Cl); ^1H NMR (500 MHz, $CDCl_3$): δ = 1.72-1.86 (4H, m), 2.64 (2H, t, J = 7.2 Hz), 3.54 (2H, t, J = 6.3 Hz), 7.15-7.21 (3H, m), 7.25-7.31 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.5, 32.1, 35.1, 44.9, 125.9, 128.4, 141.8; MS (EI) *m/z* 168 ($[M]^+$).

(5-Chloropentyl)benzene (5e). Yield 51%, colorless oil. The analytical data were identical with those of a literature compound.[11] IR (neat): ν = 2933 (CH_2), 697 (Alkyl-Cl); ^1H NMR (500 MHz, $CDCl_3$): δ = 1.44-1.53 (2H, m), 1.61-1.69 (2H, m), 1.76-1.84 (2H, m), 2.62 (2H, t, J = 7.7 Hz), 3.52 (2H, t, J = 6.8 Hz), 7.15-7.21 (3H, m), 7.25-7.31 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 26.6, 30.7, 32.5, 35.8, 45.0, 125.8, 128.3, 128.4, 142.3; MS (EI) *m/z* 182 ($[M]^+$).

(6-Chlorohexyl)benzene (5f). Yield 84%, colorless oil. The analytical data were identical with those of a literature compound.[12] IR (neat): ν = 2932 (CH_2), 699 (Alkyl-Cl); ^1H NMR (500 MHz, $CDCl_3$): δ = 1.32-1.41 (2H, m), 1.42-1.51 (2H, m), 1.60-1.68 (2H, m), 1.72-1.82 (2H, m), 2.61 (2H, t, J = 7.7 Hz), 3.52 (2H, t, J = 6.8 Hz), 7.14-7.21 (3H, m), 7.25-7.31 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 26.7, 28.5, 31.2, 32.5, 35.8, 45.1, 125.7, 128.3, 128.4, 142.6; MS (EI) *m/z* 196 ($[M]^+$).

General Procedures for synthesis *N*-ω-phenylalkyl-4-(*p*-chlorophenyl)piperidin-4-ol. Sodium carbonate (372 mg, 3.51 mmol) was added to the solution of benzyl bromide (**5a**) (300 mg, 1.75 mmol),

(*p*-chlorophenyl)piperidin-4-ol (**6**) (447 mg, 2.11 mmol) and sodium iodide (316 mg, 2.11 mmol) in dry MeCN (2.0 mL) at room temperature under atmosphere of argon, and the mixture was refluxed for indicated hours. The reaction quenched with water and extracted with $CHCl_3$. The extracts were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography with $CHCl_3$/MeOH (50:1) to give *N*-benzyl-4-(*p*-chlorophenyl)piperidin-4-ol (**7a**) as a yellow oil in 73% yield. The analytical data were identical with those of a literature compound.[13] IR (neat): ν = 3390 (OH), 2942 (CH_2), 1095 (Ar-Cl); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.62-1.80 (3H, m), 2.12 (2H, dt, *J* = 4.4, 13.0 Hz), 2.45 (2H, dt, *J* = 2.5, 12.0 Hz), 2.78 (2H, dd, *J* = 2.4, 8.9 Hz), 3.57 (2H, s), 7.23-7.37 (7H, m), 7.41-7.47 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 38.5, 49.4, 63.2, 71.1, 126.2, 127.1, 128.2, 128.4, 129.2, 132.7, 138.4, 147.0; MS (EI) *m/z* 301 ([M]$^+$); HR-MS (EI) Calcd for $C_{18}H_{20}ClNO$: 301.1233. Found: 301.1225.

4-(*p*-Chlorophenyl)-1-phenethylpiperidin-4-ol (7b). Yield 68%, white solid. The analytical data were identical with those of a literature compound.[13] Mp 124-128 °C; IR (KBr): ν = 3150 (OH), 2938 (CH_2), 1089 (Ar-Cl); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.68 (1H, brs), 1.72-1.79 (2H, m), 2.15 (2H, dt, *J* = 4.3, 13.1 Hz), 2.52 (2H, dt, *J* = 2.3, 12.1 Hz), 2.64-2.71 (2H, m), 2.82-2.94 (4H, m), 7.18-7.24 (3H, m), 7.27-7.34 (4H, m), 7.43-7.48 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 33.8, 38.5, 49.4, 60.7, 71.1, 126.1, 126.1, 128.4, 128.4, 128.7, 132.8, 140.4, 146.9; MS (EI) *m/z* 315 ([M]$^+$); HR-MS (EI) Calcd for $C_{19}H_{22}ClNO$: 315.1390. Found: 315.1406.

4-(*p*-Chlorophenyl)-1-(3-phenylpropyl)piperidin-4-ol (7c). Yield 76%, white solid. The analytical data were identical with those of a literature compound.[14] Mp 93-95 °C (lit. 96-97 °C); IR (KBr): ν = 3134 (OH), 2944 (CH_2), 1096 (Ar-Cl); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.62 (1H, brs), 1.69-1.75 (2H, m), 1.83-1.91 (2H, m), 2.13 (2H, dt, *J* = 4.2, 13.1 Hz), 2.37-2.48 (4H, m), 2.67 (2H, t, *J* = 7.7 Hz), 2.78-2.87 (2H, m), 7.16-7.22 (3H, m), 7.25-7.33 (4H, m), 7.41-7.47 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.6, 33.8, 38.4, 49.4, 58.1, 71.0, 125.8, 126.1, 128.3, 128.4, 128.4, 132.8, 142.0, 146.8; MS (EI) *m/z* 329 ([M]$^+$); HR-MS (EI) Calcd for $C_{20}H_{24}ClNO$: 329.1546. Found: 329.1538.

4-(*p*-Chlorophenyl)-1-(4-phenylbutyl)piperidin-4-ol (7d). Yield 65%, white solid. The analytical data were identical with those of a literature compound.[15] Mp 108-110 °C (*lit.* 113-114 °C); IR (KBr): ν = 3177 (OH), 2922 (CH_2), 1085 (Ar-Cl); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.53-1.61 (2H, m), 1.61-1.74 (5H, m), 2.10 (2H, dt, *J* = 4.3, 13.1 Hz), 2.33-2.45 (4H, m), 2.64 (2H, t, *J* = 7.5 Hz), 2.74-2.83 (2H, m), 7.14-7.20 (3H, m), 7.24-7.33 (4H, m), 7.41-7.46 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 26.6, 29.4, 35.8, 38.4, 49.4, 58.6, 71.0, 125.7, 126.1, 128.3, 128.4, 128.4, 132.8, 142.4, 146.8; MS (EI) *m/z* 343 ([M]$^+$); HR-MS (EI) Calcd for $C_{21}H_{26}ClNO$: 343.1703. Found: 343.1697.

4-(*p*-Chlorophenyl)-1-(5-phenylpentyl)piperidin-4-ol (7e). Yield 50%, pale yellow solid. The

analytical data were identical with those of a literature compound.[14] Mp 98-102 °C (*lit.* 103-105 °C); IR (KBr): ν = 3057 (OH), 2933 (CH_2), 1094 (Ar-Cl); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.37 (2H, quint, J = 7.6 Hz), 1.51-1.77 (7H, m), 2.13 (2H, dt, J = 3.7, 13.0 Hz), 2.33-2.46 (4H, m), 2.62 (2H, t, J = 7.7 Hz), 2.77-2.88 (2H, m), 7.15-7.20 (3H, m), 7.24-7.33 (4H, m), 7.41-7.47 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 26.9, 27.3, 31.4, 35.9, 38.5, 49.5, 58.8, 71.2, 125.6, 126.1, 128.3, 128.4, 128.4, 132.8, 142.7, 146.9; MS (EI) *m/z* 357 ([M]$^+$); HR-MS (EI) Calcd for $C_{22}H_{28}ClNO$: 357.1859. Found: 357.1865.

4-(*p*-Chlorophenyl)-1-(6-phenylhexyl)piperidin-4-ol (7f). Yield 71%, pale yellow solid. Mp 103-105 °C; IR (KBr): ν = 3171 (OH), 2931 (CH_2), 1096 (Ar-Cl); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.30-1.41 (4H, m), 1.47-1.77 (7H, m), 2.12 (2H, dt, J = 4.2, 13.1 Hz), 2.33-2.45 (4H, m), 2.61 (2H, t, J = 7.7 Hz), 2.76-2.87 (2H, m), 7.15-7.20 (3H, m), 7.24-7.33 (4H, m), 7.42-7.47 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 26.9, 27.5, 29.2, 31.4, 35.9, 38.4, 49.5, 58.9, 71.1, 125.6, 126.1, 128.2, 128.4, 128.4, 132.8, 142.8, 146.9; MS (EI) *m/z* 371 ([M]$^+$); HR-MS (EI) Calcd for $C_{23}H_{30}ClNO$: 371.2016. Found: 371.2021.

4-(*p*-Chlorophenyl)-1-(7-phenylheptyl)piperidin-4-ol (7g). Yield 96%, pale yellow solid. Mp 86-88 °C; IR (KBr): ν = 3055 (OH), 2922 (CH_2), 1093 (Ar-Cl); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.23-1.40 (6H, m), 1.46-1.77 (7H, m), 2.14 (2H, dt, J = 3.6, 13.0 Hz), 2.32-2.48 (4H, m), 2.60 (2H, t, J = 7.7 Hz), 2.76-2.89 (2H, m), 7.14-7.20 (3H, m), 7.24-7.33 (4H, m), 7.41-7.48 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 27.0, 27.6, 29.2, 29.4, 31.4, 36.0, 38.5, 49.5, 58.9, 71.1, 125.6, 126.1, 128.2, 128.4, 128.4, 132.8, 142.8, 146.9; MS (EI) *m/z* 385 ([M]$^+$); HR-MS (EI) Calcd for $C_{24}H_{32}ClNO$: 385.2172. Found: 385.2172.

4-(*p*-Chlorophenyl)-1-(8-phenyloctyl)piperidin-4-ol (7h). Yield 89%, pale yellow solid. Mp 88-89 °C; IR (KBr): ν = 3173 (OH), 2919 (CH_2), 1095 (Ar-Cl); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.24-1.38 (8H, m), 1.47-1.76 (7H, m), 2.13 (2H, dt, J = 4.3, 13.1 Hz), 2.33-2.46 (4H, m), 2.60 (2H, t, J = 7.8 Hz), 2.77-2.87 (2H, m), 7.14-7.20 (3H, m), 7.24-7.33 (4H, m), 7.41-7.48 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 26.9, 27.6, 29.2, 29.4, 29.5, 31.4, 35.9, 38.3, 49.4, 58.9, 71.0, 125.5, 126.1, 128.2, 128.4, 128.4, 132.7, 142.8, 146.9; MS (EI) *m/z* 399 ([M]$^+$); HR-MS (EI) Calcd for $C_{25}H_{34}ClNO$:399.2329. Found: 399.2327.

4-(*p*-Chlorophenyl)-1-(9-phenylnonyl)piperidin-4-ol (7i). Yield 96%, pale yellow solid. Mp 72-74 °C; IR (KBr): ν = 3163 (OH), 2922 (CH_2), 1097 (Ar-Cl); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.23-1.38 (10H, m), 1.46-1.76 (7H, m), 2.14 (2H, dt, J = 4.1, 13.1 Hz), 2.33-2.46 (4H, m), 2.60 (2H, t, J = 7.8 Hz), 2.77-2.89 (2H, m), 7.14-7.20 (3H, m), 7.24-7.33 (4H, m), 7.41-7.47 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 27.1, 27.7, 29.3, 29.5, 29.5, 29.6, 31.5, 36.0, 38.5, 49.5, 59.0, 71.2, 125.6, 126.1, 128.2, 128.4, 128.4, 132.8, 142.9, 147.0; MS (EI) *m/z* 413 ([M]$^+$); HR-MS (EI) Calcd for $C_{26}H_{36}ClNO$: 413.2485.

Found: 413.2482.

4-(*p*-Chlorophenyl)-1-(10-phenyldecyl)piperidin-4-ol (7j). Yield 93%, white solid. Mp 86-88 °C; IR (KBr): ν = 3178 (OH), 2924 (CH_2), 1095 (Ar-Cl); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.23-1.37 (12H, m), 1.46-1.65 (5H, m), 1.68-1.76 (2H, m), 2.13 (2H, dt, J = 4.0, 13.0 Hz), 2.33-2.46 (4H, m), 2.60 (2H, t, J = 7.8 Hz), 2.77-2.89 (2H, m), 7.14-7.20 (3H, m), 7.24-7.33 (4H, m), 7.42-7.47 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 27.1, 27.7, 29.3, 29.5, 29.5, 29.6, 31.5, 36.0, 38.5, 49.5, 59.0, 71.2, 125.5, 126.1, 128.2, 128.4, 128.4, 132.7, 142.9, 146.9; MS (EI) *m/z* 427 ([M]$^+$); HR-MS (EI) Calcd for $C_{27}H_{38}ClNO$: 427.2642. Found: 427.2643.

4-(*p*-Chlorophenyl)-1-(11-phenylundecyl)piperidin-4-ol (7k). Yield 84%, pale yellow solid. Mp 53-55 °C; IR (KBr): ν = 3168 (OH), 2922 (CH_2), 1098 (Ar-Cl); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.21-1.37 (14H, m), 1.47-1.76 (7H, m), 2.14 (2H, dt, J = 3.3, 12.8 Hz), 2.32-2.47 (4H, m), 2.60 (2H, t, J = 7.8 Hz), 2.77-2.89 (2H, m), 7.14-7.20 (3H, m), 7.24-7.33 (4H, m), 7.42-7.47 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 26.8, 27.6, 29.3, 29.5, 29.5, 29.6, 31.5, 36.0, 38.3, 49.4, 58.9, 71.0, 125.5, 126.1, 128.2, 128.4, 128.4, 132.8, 142.9, 146.8; MS (EI) *m/z* 441 ([M]$^+$); HR-MS (EI) Calcd for $C_{28}H_{40}ClNO$: 441.2798. Found:441.2800.

Cell lines and cell cultures

To evaluate the antiproliferative activity of the compounds, HCT-116 cells (derived from human colon cancer), which were purchased from the American Type Culture Collection (VA, USA), were used in this study. The HCT-116 cells were maintained in McCOY 5A medium supplemented with L-glutamine and 10% heat inactivated (55 °C for 30 min) fetal bovine serum at 37 °C in an atmosphere of 5% CO_2.

Cell viability assays

The HCT-116 cell viability assay was carried out using the MTT method, which was described by Mosmann.[16] The cells were placed in 96-well flat-bottomed tissue culture plates, with 2.0 x 10^3 cells in 100 μL culture medium in each well. The cells were incubated at 37 °C in an atmosphere of 5% CO_2 for 24 h to allow them to attach to the wells. Then, they were treated with the indicated concentrations of the test agents in culture medium. Following a further 48 h incubation, 10 μL of MTT (5 mg/mL in phosphate-buffered saline) were added to each well, and the plate was incubated for 4 h to allow the metabolism of MTT by cellular mitochondrial dehydrogenases. The excess MTT was aspirated, and the resultant formazan crystals were dissolved by adding 100 μL of DMSO. The absorbance of purple formazan was read at 570 nm using a microplate reader. The results shown as percentages relative to the values for the untreated controls.

Statistical calculations

Concentration-cell viability relationships were fitted to a four-parameter logistic equation using a nonlinear curve-fitting program and were used to obtain IC_{50} values (Kaleida-Graph; Synergy Software, Reading, PA). Where appropriate, the results were expressed as mean ± standard error of the mean values for one of at least three similar experiments ($n \geq 3$).

ACKNOWLEDGEMENTS

This study was supported in part by a Grant-in Aid for Scientific Research from the Japan Society for promotion of Science (JSPS); for analyzed the antiproliferative activity (No. 17K08369).

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