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Total synthesis and anticancer activity studies of the stereoisomers of asperphenamate and patriscabratine

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Abstract

All stereoisomers of asperphenamate **1a** and patriscabratine **2a** were achieved with a high yield, and total synthesis of **2a** is firstly described here. The absolute configuration of patriscabratine was determined as (*S*,*S*). The compounds **1a–d** and **2a–d** have been tested by MTT assay in T47D, MDA-MB231, HL60, Hela and SGC-7901 cell lines *in vitro*. Among them, the (*R*,*S*) stereoisomer shows the strongest anticancer effects, while the (*S*,*R*) shows the weakest one.

Keywords: *N*,*N*′-substituted phenylalanine-phenylalaninol ester; Asperphenamate; Patriscabratine; Total synthesis; Anticancer activity

Asperphenamate **1a**, which has a *N*,*N*′-substituted phenylalanine-phenylalaninol ester framework (Fig. 1), was first isolated by Clark from *Aspergillus flavus*. The stereochemistry of **1a** was established as (*S*,*S*) configuration at its chiral centers [1]. Recently, **1a** was isolated by our group from raw malt [2], a traditional medicine for the treatment of mammary hyperplasia. A preliminary bioassay proved that compound **1a** displayed potent antiproliferative activity in the MCF-7 cell line [3].

Patriscabratine **2a** (Fig. 1), which is the analog of asperphenamate, was isolated from ethanol extracts of *Patrinia scabra* bunge [4], a traditional medicine for the treatment of acute leukemia. The ethanol extracts displayed significant cytotoxic activity against acute leukemia as well as inhibitory activity on ehrlich ascites carcinoma [5]. And because of its similar natural structure to **1a**, the compound **2a** is expected to show anticancer activity.

The further biological tests are impeded by the low natural abundance of asperphenamate and patriscabratine. The absolute configuration of patriscabratine was undetermined and its total synthesis was also not developed. In order to compare the activities among stereoisomers of **1** and **2** and obtain the potent compound, total synthesis of their stereoisomers and biological evaluation were studied.

The total synthesis of asperphenamate was documented [6,7]. Because of the unsatisfying yields of these methods, we developed a more efficient method for all stereoisomers of asperphenamate and patriscabratine.

Additionally, all stereoisomers of patriscabratine and asperphenamate have been tested for their antiproliferative activities in T47D, MDA-MB231, HL60, Hela, and SGC-7901 cell lines *in vitro* using the MTT method [8].

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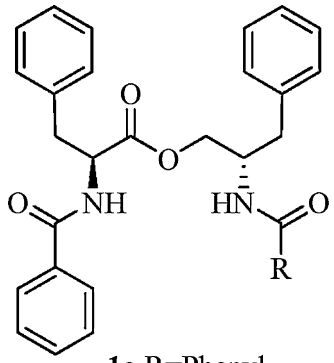
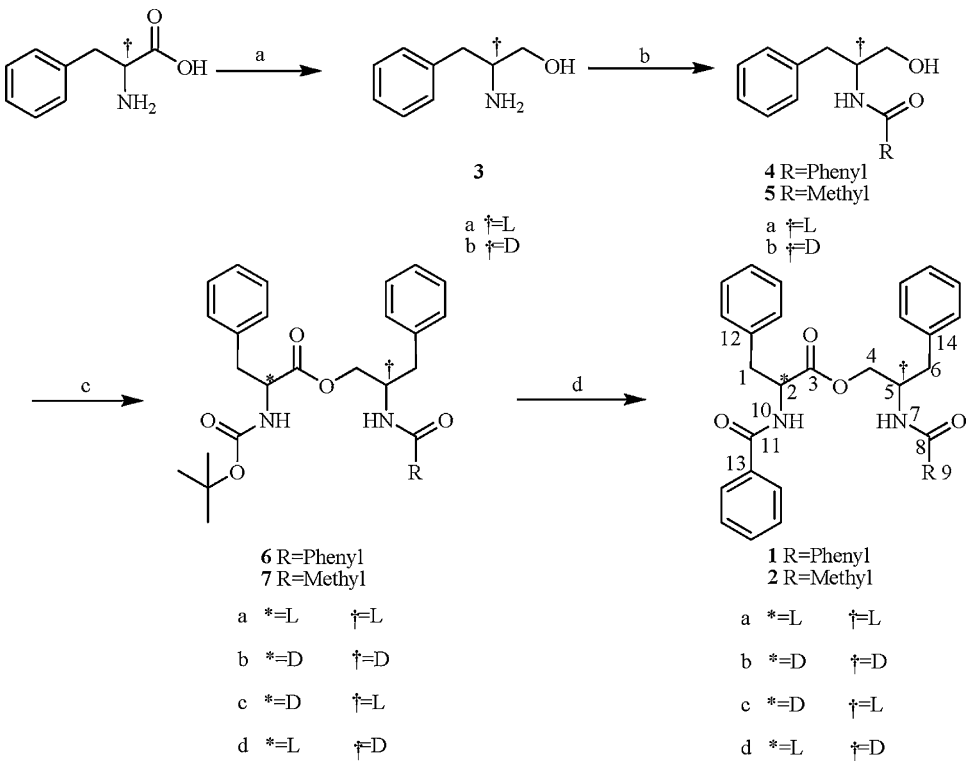


Fig. 1. The structure of aperphenamate **1a** and patriscabratine **2a**.

As shown in Scheme 1, starting from optically pure (L or D)-phenylalanine, (L or D)-phenylalaninol **3** was prepared according to the method developed by McKennon [9]. N-benzoyl-(L or D)-phenylalaninol **4** was synthesized by Lewanowicz [10]. N-acetyl-(L or D)-phenylalaninol **5** was readily obtained from acetic anhydride by the same reaction as the synthesis of **4**. Condensation of commercially available N-*tert*-butoxycarbonyl-(L or D)-phenylalanine with **4** or **5** was promoted by 1,3-dicyclohexylcarbodiimide (DCC) to afford key intermediates N-benzoyl-*O*-(N′-*tert*-butoxycarbonyl-(L or D)-phenylalanyl)-(L or D)-phenylalanin-ol **6** or N-acetyl-*O*-(N′-*tert*-butoxycarbonyl-(L or D)-phenylalanyl)-(L or D)-phenylalaninol **7**. Removal of the N-*tert*-butoxycarbonyl protecting groups of **6** or **7** was achieved by treatment with 3 equivalents of 1.5 mol/L dry hydrochloride in ethyl acetate, followed by the reaction of



Scheme 1. The synthetic route of patriscabratine and asperphenamate. Condition and reagent: (a) $H_2SO_4/NaBH_4$, THF, r.t., 98%; (b) **4**: benzoyl chloride, K_2CO_3, MeOH, r.t., 95%; **5**: $(CH_3CO)_2O$, K_2CO_3, MeOH, r.t., 92%; (c) N-*tert*-butoxycarbonyl-(L or D)-phenylalanine, DCC, $CHCl_3$, r.t., 65%; (d) (i) 1.5 mol/L HCl/AcOEt, r.t., 76%; (ii) benzoyl chloride, pyridine, r.t., 90%.

Table 1
The cytotoxic activities *in vitro* (IC_{50} at μmol/L) in five human cancer cell lines.

Compounds	IC_{50} (μmol/L)				
	T47D	MDA-MB231	HL60	Hela	SGC-7901
1a[a]	92.3	96.5	97.9	nt[b]	nt[b]
1b	18.7	23.1	86.7	30.7	32.7
1c	8.2	11.9	67.2	25.7	20.8
1d	35.8	47.6	94.1	46.6	40.9
2a	>100	>100	>100	nt[b]	nt[b]
2b	88.4	90.9	92.4	50.9	46.7
2c	83.6	84.7	87.3	32.9	34.4
2d	99.7	98.5	97.2	67.8	58.9

[a] **1a** exibited strong activity against MCF-7 and A549 cell lines. The EC_{50} (mg/mL) value was both 2.5 [3].
[b] Not tested.

the deprotected products with benzoyl chloride in pyridine gave the stereoisomers **1a–d** and **2a–d** the structures were confirmed by IR, ^{1}H NMR data, ^{13}C NMR, ESIMS and HR-MS [11,12].

The *in vitro* anticancer activities of **1a–d** and **2a–d** were investigated by the standard MTT method in T47D, MDA-MB231, HL60, Hela, and SGC-7901 cell lines. The results are shown in Table 1.

Asperphenamate **1a** showed weak activity against T47D, MDA-MB231 and HL60. Patriscabratine **2a** was ineffective against cell lines tested. **1c** exhibited the most potent activities against all of cell lines, especially breast cancer cell lines T47D and MDA-MB231 (IC_{50} = 8.2 and 11.9 μmol/L, respectively). All stereoisomers showed more potent activity than reference drug **1a** except **2a**.

In the stereoisomers of asperphenamate, **1c** expressed the strongest anticancer effects, while the **1d** displayed the weakest. On the other hand, **2c** showed the broadest activity against all of cell lines and **2d** was the weakest in patriscabratine class of compounds.

It is noteworthy that the benzoyl-substituted compounds (**1a–d**) are more potent than the acetyl-substituted compounds (**2a–d**).

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[11] Compound **1a**: m.p. 200–202 °C; $[\alpha]_D^{20}$ −98.6 (*c* 0.76, pyridine); ESIMS *m/z*: 507.2 $[M+1]^+$, 529.2 $[M+Na]^+$; HR-MS: 529.2098 $[M+Na]^+$ (Calcd. for $C_{32}H_{30}N_2O_4Na$, 529.2103); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.08–7.59(m, 20H, ArH), 6.60(s, 1H, 10-NH), 6.51(d, 1H, 7-NH, *J* = 6.3), 4.77–4.79(m, 1H, 2-H), 4.45(m, 1H, 5-H), 4.40–4.45(dd, 1H, *J* = 11.4, 3.0 Hz, 4-Ha), 3.86–3.90(dd, 1H, *J* = 11.4, 4.2 Hz, 4-Hb), 3.07–3.16(m, 2H, 1-H), 2.84–2.89(dd, 1H, *J* = 13.2, 7.2 Hz, 6-Ha), 2.75–2.80(dd, 1H, *J* = 13.2, 8.1 Hz, 6-Hb); ^{13}C NMR (75 MHz, DMSO-*d6*): δ 171.6, 166.8, 166.3, 138.4, 137.9, 134.6, 133.7, 131.6, 131.3, 129.2 × 3, 129.1 × 3, 128.3 × 3, 127.5 × 3, 127.3 × 3, 126.6 × 2, 126.3, 65.6, 54.6, 50.0, 36.4, 36.1; IR(KBr) (cm^{-1}): 697($\delta_{ar.}$, mono-suvst.), 1218(ν_{C-O}, ester), 1533(δ_{N-H}), 1639($\nu_{C=O}$, amide), 1751($\nu_{C=O}$, ester), 3312(ν_{N-H}); **1b**: m.p. 209–211 °C; $[\alpha]_D^{20}$ 98.6 (*c* 0.76, pyridine); IR, ESIMS, HR-MS, ^{1}H NMR and ^{13}C NMR spectra were identical with **1a**; **1c**: m.p. 210–213 °C; $[\alpha]_D^{20}$ −35.8 (*c* 0.76, pyridine); ESIMS *m/z*: 507.2 $[M+1]^+$; HR-MS:529.2098 $[M+Na]^+$ (Calcd. for $C_{32}H_{30}N_2O_4Na$, 529.2103); ^{1}H NMR (300 MHz, DMSO-*d6*): δ 8.85(d, 1H, 10-NH, *J* = 7.5), 8.32(d, 1H, 7-NH, *J* = 8.4), 7.16–7.81(m, 20H, ArH), 4.62–4.68(m, 1H, 2-H), 4.35–4.43(m, 1H, 5-H), 4.09–4.21(m, 2H, 4-H), 3.11–3.18(m, 2H, 1-H), 2.84(m, 2H, 6-H); ^{13}C NMR (75 MHz, DMSO-*d6*): δ 171.3, 166.6, 166.3, 138.4, 136.9, 134.6, 133.7, 131.6, 131.3, 129.2 × 3, 129.1 × 3, 128.3 × 3, 127.5 × 3, 127.3 × 3, 126.6 × 2, 126.3, 65.6, 54.6, 50.0, 36.4, 36.1; IR(KBr) (cm^{-1}): 697($\delta_{ar.}$, mono-suvst.), 1218(ν_{C-O}, ester), 1533(δ_{N-H}), 1639($\nu_{C=O}$, amide), 1751($\nu_{C=O}$, ester), 3312(ν_{N-H}); **1d**: m.p. 212–214 °C; $[\alpha]_D^{20}$ 35.8 (*c* 0.76, pyridine); IR, ESIMS, HR-MS, ^{1}H NMR and ^{13}C NMR spectra were identical with **1c**.

[12] **2a**: m.p. 186–188 °C; $[\alpha]_D^{20}$ −32.6 (*c* 1.0, methanol); ESIMS *m/z*: 467.9 $[M+Na]^+$; HR-MS: 467.1944 $[M+Na]^+$ (Calcd. for $C_{27}H_{28}N_2O_4Na$, 467.1947); ^1H NMR (300 MHz, $CDCl_3$): δ 7.14–7.73(m, 15H, ArH), 6.61(s, 1H, 10-NH), 6.10(s, 1H, 7-NH), 4.87–4.89(m, 1H, 2-H), 4.41(m, 1H, 5-H), 4.45–4.48(m, 1H, 4-Ha), 3.84–3.87(dd, 1H, *J* = 11.1, 3.2 Hz, 4-Hb), 3.22–3.29(dd, 1H, *J* = 6.6 Hz, 13.8 Hz, 1-Ha), 3.18–3.21(dd, 1H, *J* = 7.1 Hz, 11.9 Hz, 1-Hb), 2.83–2.87(dd, 1H, *J* = 13.4, 8.4 Hz, 6-Hb), 2.72–2.79(dd, 1H, *J* = 13.4, 7.2 Hz, 6-Hb), 1.88(s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.4, 169.7, 167.2, 136.9, 135.5, 133.0, 131.8, 128.9 × 3, 128.5 × 3, 128.4 × 2, 128.3 × 2, 127.1, 126.7 × 2, 126.4,64.8, 54.2, 49.1, 37.2, 37.0, 22.9; IR (KBr) (cm^{-1}): 699 and 747(δ_{ar}, mono-suvst.), 1213(ν_{C-O}, ester), 1448(δ_{CH_2}), 1539(δ_{N-H}), 1646($\nu_{C=O}$, amide), 1746($\nu_{C=O}$, ester), 3300(ν_{N-H}); **2b**: m.p. 188–189 °C; $[\alpha]_D^{20}$ 33.0 (*c* 1.0, methanol); IR, ESIMS, HR-MS, ^1H NMR and ^{13}C NMR spectra were identical with **2a**; **2c**: m.p. 178–181 °C; $[\alpha]_D^{20}$ −18.5 (*c* 1.0, methanol); ESIMS *m/z*: 467.9 $[M+Na]^+$; HR-MS:467.1944 $[M+Na]^+$ (Calcd. for $C_{27}H_{28}N_2O_4Na$, 467.1947); ^1H NMR (300 MHz, $CDCl_3$): δ 7.06–7.75(m, 15H, ArH), 6.61–6.64(d, 1H, *J* = 6.8 Hz, 10-NH), 5.78–5.81(d, 1H, *J* = 8.4 Hz, 7-NH), 4.96–4.98(m, 1H, 2-H), 4.35-4.37(m, 1H, 5-H), 4.30–4.34(dd, 1H, *J* = 10.8, 3.4 Hz, 4H-a), 3.93–3.98(dd, 1H, *J* = 10.8, 3.6 Hz, 4H-b), 3.25–3.27(m, 2H, 1-H), 2.71–2.76(dd, 1H, *J* = 13.5, 6.6 Hz, 6H-a), 2.61–2.66(dd, 1H, *J* = 13.5, 8.1 Hz, 6H-b), 1.92(s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.1, 169.4, 167.2, 136.9, 135.5, 133.0, 132.7, 128.9 × 3, 128.5 × 3, 128.4 × 2, 128.3 × 2, 127.1, 126.7 × 2, 126.4,64.8, 54.2, 49.1, 37.2, 37.0, 22.9; IR (KBr) (cm^{-1}): 699 and 747(δ_{ar}, mono-suvst.), 1213(ν_{C-O}, ester), 1452(δ_{CH_2}), 1545(δ_{N-H}), 1646($\nu_{C=O}$, amide), 1746($\nu_{C=O}$, ester), 3300(ν_{N-H}); **2d**: m.p. 180–184 °C; $[\alpha]_D^{20}$ 18.5 (*c* 1.0, methanol); IR, ESIMS, HR-MS, ^1H NMR and ^{13}C NMR spectra were identical with **2c**.