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치오우레이도 MAPP 유도체의 합성과 세포독성

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Synthesis and Cytotoxicity of Thioureido MAPP Derivatives

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Abstract — The 2-amino-1-phenylpropanols **4~7** were reacted with isothiocyanates to afford the 20 thioureido MAPP derivatives **8a~11e**, which were tested their cytotoxic activity by MTT assay. The cytotoxicity of alkylthioureido compounds were increased and decreased by alkyl chain length from C_8 to C_{14} and of phenylthioureido compounds showed poor activity. The compounds (**8b, 8c, 9b, 9c, 10b, 10c, 11b, 11c**) with C_{10} and C_{12} alkyl chains gave stronger activity than reference compound B13. The IC_{50} of compound **8c** was 1.05 μM and 3 times stronger activity than B13. The stereochemistry of synthesized compounds affected very little to cytotoxic activity.

Keywords □ MAPP, cytotoxicity

Ceramide는 세포사멸, 세포분화, 세포성장 억제, Parkinson병 및 Alzheimer병 등에 관여하는 중요한 이차전달 물질이다.[1-4] 암세포에서 ceramide의 농도는 정상 세포에서보다 매우 낮고, 천연 ceramide와 유사한 ceramide 유도체 또는 ceramide를 분해하는 ceramidase의 억제제 등을 암세포에 처리하여 암세포내의 ceramide 농도가 증가하면, cytochrome가를 유리되고 caspase cascade를 활성화시켜 암세포의 성장을 억제하지만, 정상세포에 대한 독성은 낮다고 보고되었다.[5,6] 그리고, ceramidase를 억제하는 D-e-MAPP와 B13 등의 *N*-alkyl-phenylamino alcohol류[7-9]와 C_2-ceramide, C_6-ceramide, C_8-ceramide 등의 ceramide 유도체가 암세포의 성장억제를 보여주었다.[10] 따라서, ceramide 유도체나 ceramidase의 억제제와 같이 세포내 ceramide의 농도를 높여줄 수 있는 화합물들은 암세포에 대해서만 선택적인 독성을 나타내는 새로운 항암치료제가 될 수 있다.

본 연구에서는 ceramidase를 억제하는 D-e-MAPP와 B13 화합물의 구조를 바탕으로 다양한 stereoisomer 구조와 alkyl기 길이를 갖으며, CH_2CO 부위를 동등체인 NHCS로 치환한 20종의 D-e-MAPP 유도체를 합성하고 그 세포독성효과를 측정하였다.

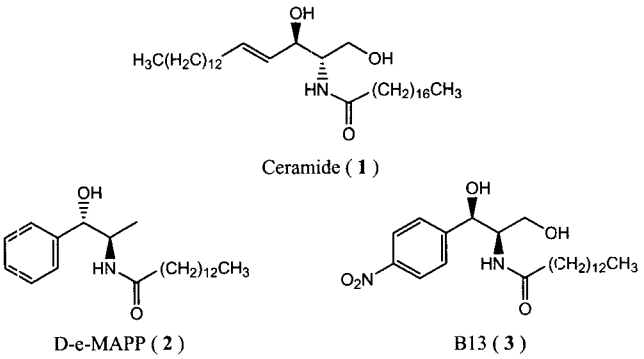

Fig. 1 – Structure of ceramide (**1**) and ceramidase inhibitors (**2** and **3**).

실험방법

시약 및 기기

본 실험에서 사용된 시약들은 Aldrich사와 Fluka사의 것을 사용하였고, 각종 용매는 특급시약을 사용하였다. ^{1}H-NMR spectra는 Varian Gemini 2000(300 MHz)을 사용하여 얻었고, $CDCl_3$의 경우 TMS(tetramethylsilane)를 내부 표준물질로 사용하였다. TLC는 Merck silica gel 60 F_{254}를 사용하였고, UV Lamp로 spot을 확인하였다. 흡광도는 JASCO DIP-370을 사용하였고, 용점측정은 Buchi Melting point B-540을 사용하였으며

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IR spectra는 Jasco FT/IR 300E을 사용하여 얻었다. Column chromatography는 silica gel(Merck type 9355, 230-400 mesh)를 사용하였다.

일반적인 Thioureido MAPP 화합물의 합성방법

Amine 화합물 **4~7**(1.2 mmol)을 ethanol(25 m*l*)와 $CHCl_3$ (25 m*l*)의 혼합 용매에 녹인 후, 실온에서 isothiocyanate(1.4 mmol)를 천천히 적가하여 4시간 동안 반응시킨다. 반응액에 ethylacetate(200 m*l*)를 가하고 5% citric acid(400 m*l*×4)로 세척한 후, 유기층을 무수 $MgSO_4$로 건조하고 감압 농축하여 crude 화합물을 얻는다. 이 crude 화합물을 Silicagel column chromatography로 정제하여 목적 화합물 **8a~11e**를 얻었다.

(1S, 2R) 1-(2-Hydroxy-1-methyl-2-phenyl-ethyl)-3-octyl-thiourea의 합성(8a) – (1R, 2S) 2-Amino-1-phenyl-1-propanol **4** (182 mg, 1.2 mmol)과 octylisothiocyanate(240 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 33.5%; R_f=0.33(ethylacetate : hexane=1 : 4); $[\alpha]_D^{20}$= -28.50(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1140, 1620, 2960, 3110, 3440; ^{1}H-NMR($CDCl_3$) δ : 0.89(t, 3H, *J*=6.6 Hz, CH_3), 1.06(d, 3H, *J*=6.9 Hz C_1-CH_3), 1.23-1.43(m, 10H, $(CH_2)_5$), 1.545-1.69(m, 2H, NH-CH_2<u>CH_2</u>), 3.23-3.38(m, 2H, NH-<u>CH_2</u>), 4.99-5.00(m, 1H, C_1-H), 5.55(d, 1H, *J*=7.5 Hz, C_2-H), 7.21-7.41(m, 5H, phenyl).

(1S, 2R) 1-Decyl-3-(2-hydroxy-1-methyl-2-phenyl-ethyl)-thiourea의 합성(8b) – (1R, 2S) 2-Amino-1-phenyl-1-propanol **4** (182 mg, 1.2 mmol)과 decylisothiocyanate(280 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 33.6%; R_f=0.33(ethylacetate : hexane=1 : 3); $[\alpha]_D^{20}$= -27.50(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1020, 1625, 2965, 3160, 3460; ^{1}H-NMR($CDCl_3$) δ : 0.81(t, 3H, *J*=6.6 Hz, CH_3), 0.99(d, 3H, *J*=6.9 Hz, C_1-CH_3), 1.01-1.39(m, 14H, $(CH_2)_7$), 1.39-1.61(m, 2H, NH-CH_2<u>CH_2</u>), 3.10-3.39(m, 2H, NH-<u>CH_2</u>), 4.81-4.99(m, 1H, C_1-H), 5.53(d, 1H, *J*=7.8 Hz, C_2-H), 7.19-7.40(m, 5H, phenyl).

(1S, 2R) 1-Dodecyl-3-(2-hydroxy-1-methyl-2-phenyl-ethyl)-thiourea의 합성(8c) – (1R, 2S) 2-Amino-1-phenyl-1-propanol **4** (182 mg, 1.2 mmol)과 dodecylisothiocyanate(318 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 18.6%; R_f=0.33(ethylacetate : hexane=1 : 3); $[\alpha]_D^{20}$= -28.50(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1100, 1640, 2960, 3135, 3390; ^{1}H-NMR($CDCl_3$) δ : 0.88(t, 3H, *J*=6.6 Hz, CH_3), 1.05(d, 3H, *J*=6.9 Hz C_1-CH_3), 1.20-1.40(m, 18H, $(CH_2)_9$), 1.38-1.49(m, 2H, NH-CH_2<u>CH_2</u>), 3.22-3.40(m, 2H, NH-<u>CH_2</u>), 4.99-5.01(m, 1H, C_1-H), 5.69(d, 1H, *J*=7.5 Hz, C_2-H), 7.20-7.40(m, 5H, phenyl).

(1S, 2R) 1-(2-Hydroxy-1-methyl-2-phenyl-ethyl)-3-tetra-decyl-thiourea의 합성(8d) – (1R, 2S) 2-Amino-1-phenyl-1-propanol **4**(182 mg, 1.2 mmol)과 tetradecylisothiocyanate(358 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 27.6%; R_f=0.25(ethylacetate : hexane=1 : 3); $[\alpha]_D^{20}$= -28.50(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1140, 1620, 2960, 3140, 3400; ^{1}H-NMR($CDCl_3$) δ : 0.88(t, 3H, *J*=6.6 Hz, CH_3), 1.05(d, 3H, *J*=6.9 Hz C_1-CH_3), 1.19-1.40(m, 22H, $(CH_2)_{11}$), 1.55-1.69(m, 2H, NH-CH_2<u>CH_2</u>), 3.21-3.40(m, 2H, NH-<u>CH_2</u>), 4.96-5.02(m, 1H, C_1-H), 5.62(d, 1H, *J*=7.5 Hz, C_2-H), 7.21-7.40(m, 5H, phenyl).

(1S, 2R) 1-(4-Chloro-phenyl)-3-(2-hydroxy-1-methyl-2-phenyl-ethyl)-thiourea의 합성(8e) – (1R, 2S) 2-Amino-1-phenyl-1-propanol **4**(182 mg, 1.2 mmol)과 4-chlorophenylisothiocyanate(235 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 87.2%; R_f=0.33(ethylacetate : hexane=1 : 3); $[\alpha]_D^{20}$= -19.40(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1090, 1560, 3160; ^{1}H-NMR($CDCl_3$) δ : 1.03(d, 3H, *J*=6.9 Hz, C_1-CH_3), 5.04-5.11(m, 1H, C--H), 6.04-6.16(m, 1H, C--H), 7.10-7.40(m, 9H, phenyl).

(1R, 2S) 1-(2-Hydroxy-1-methyl-2-phenyl-ethyl)-3-octyl-thiourea의 합성(9a) – (1S, 2R) 2-Amino-1-phenyl-1-propanol **5** (182 mg, 1.2 mmol)과 octylisothiocyanate(240 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 33.0%; R_f=0.25(ethylacetate : hexane=1 : 2); $[\alpha]_D^{20}$= +10.60(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1140, 1590, 2920, 3140, 3445; ^{1}H-NMR($CDCl_3$) δ : 0.88(t, 3H, *J*=6.6 Hz, CH_3), 1.06(d, 3H, *J*=6.9 Hz C_1-CH_3), 1.19-1.40(m, 10H, $(CH_2)_5$), 1.49-1.69(m, 2H, NH-CH_2<u>CH_2</u>), 3.22-3.39(m, 2H, NH-<u>CH_2</u>), 4.98-5.00(m, 1H, C_1-H), 5.52(d, 1H, *J*=8.1 Hz, C_2-H), 7.21-7.41(m, 5H, phenyl).

(1R, 2S) 1-Decyl-3-(2-hydroxy-1-methyl-2-phenyl-ethyl)-thiourea의 합성(9b) – (1S, 2R) 2-Amino-1-phenyl-1-propanol **5** (182 mg, 1.2 mmol)과 decylisothiocyanate(280 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 59.0%; R_f=0.33(ethylacetate : hexane=1 : 4); $[\alpha]_D^{20}$= +10.60; ^{1}H-NMR($CDCl_3$) δ : 0.88(t, 3H, *J*=6.6 Hz, CH_3), 1.05(d, 3H, *J*=6.9 Hz C_1-CH_3), 1.29-1.40(m, 14H, $(CH_2)_7$), 1.50-1.69(m, 2H, NH-CH_2<u>CH_2</u>), 3.20-3.39(m, 2H, NH-<u>CH_2</u>), 4.95-5.01(m, 1H, C_1-H), 5.56(d, 1H, *J*=7.5 Hz, C_2-H), 7.19-7.40(m, 5H, phenyl).

(1R, 2S) 1-Dodecyl-3-(2-hydroxy-1-methyl-2-phenyl-ethyl)-thiourea의 합성(9c) – (1S, 2R) 2-Amino-1-phenyl-1-propanol **5**

(182 mg, 1.2 mmol)과 dodecylisothiocyanate(318 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 21.5%; R_f=0.33(ethylacetate : hexane=1 : 4); $[\alpha]_D^{20}$= +5.40(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1240, 1610, 2935, 3090, 3460; ^{1}H-NMR($CDCl_3$) δ : 0.88(t, 3H, *J*=6.6 Hz, CH_3), 1.04(d, 3H, *J*=6.9 Hz C_1-CH_3), 1.190-1.40(m, 18H, $(CH_2)_9$), 1.50-1.70(m, 2H, NH-$CH_2\underline{CH_2}$), 3.29-3.40(m, 2H, NH-$\underline{CH_2}$), 4.94-5.04(m, 1H, C_1-H), 5.66(d, 1H, *J*=7.5 Hz, C_2-H), 7.21-7.40(m, 5H, phenyl).

(1R, 2S) 1-(2-Hydroxy-1-methyl-2-phenyl-ethyl)-3-tetradecyl-thiourea의 합성(9d) – (1S, 2R) 2-Amino-1-phenyl-1-propanol **5**(182 mg, 1.2 mmol)과 tetraisothiocyanate(358 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 19.8%; R_f=0.29(ethylacetate : hexane=1 : 3); $[\alpha]_D^{20}$= +7.20(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1210, 1620, 2950, 3110, 3446; ^{1}H-NMR($CDCl_3$) δ : 0.88(t, 3H, *J*=6.6 Hz, CH_3), 1.05(d, 3H, *J*=6.9 Hz C_1-CH_3), 1.19-1.41(m, 22H, $(CH_2)_{11}$), 1.57-1.69(m, 2H, NH-$CH_2\underline{CH_2}$), 3.22-3.39(m, 2H, NH-$\underline{CH_2}$), 4.93-5.01(m, 1H, C_1-H), 5.60(d, 1H, *J*=9.0 Hz, C_2-H), 7.20-7.40(m, 5H, phenyl).

(1R, 2S) 1-(4-Chloro-phenyl)-3-(2-hydroxy-1-methyl-2-phenyl-ethyl)-thiourea의 합성(9e) – (1S, 2R) 2-Amino-1-phenyl-1-propanol **5**(182 mg, 1.2 mmol)과 4-chlorophenylisothiocyanate (235 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 70.2%; R_f=0.33(ethylacetate : hexane=1 : 3); $[\alpha]_D^{20}$= +58.90(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1100, 1540, 3120; ^{1}H-NMR($CDCl_3$) δ : 1.02(d, 3H, *J*=6.6 Hz C_1-CH_3), 5.02-5.11(m, 1H, C_1-H), 6.09-6.19(m, 1H, C_2-H), 7.10-7.40(m, 9H, phenyl).

(R) 1-(1-Hydroxymethyl-2-phenyl-ethyl)-3-octyl-thiourea의 합성(10a) – (R)-2-Amino-1-phenyl-3-propanol **6**(182 mg, 1.2 mmol)과 octylisothiocyanate(240 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 38.9%; R_f=0.20(ethylacetate : hexane=1 : 2); $[\alpha]_D^{20}$= +41.60(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1240, 1550, 2910, 3170, 3370; ^{1}H-NMR($CDCl_3$) δ : 0.88(t, 3H, *J*=6.6 Hz, CH_3), 1.00-1.39(m, 10H, $(CH_2)_5$), 1.40-1.59(m, 2H, NH-$CH_2\underline{CH_2}$), 2.86(dd, 1H, J_1=8.1 Hz, J_2=7.8 Hz, C_2-H) 2.98(dd, 1H, J_1=6.3 Hz, J_2=6.3 Hz, C_2-H) 3.19-3.39(m, 2H, NH-$\underline{CH_2}$), 3.58-3.81(m, 2H, $\underline{CH_2}$OH), 4.42-4.60(m, 1H, C_1-H), 7.19-7.39(m, 5H, phenyl).

(R) 1-Decyl-3-(1-hydroxymethyl-2-phenyl-ethyl)-thiourea의 합성(10b) – (R)-2-Amino-1-phenyl-3-propanol **6**(182 mg, 1.2 mmol)과 decylisothiocyanate(280 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 32.0%; R_f=0.12(ethylacetate : hexane=1 : 3); $[\alpha]_D^{20}$= +48.20(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1140, 1560, 2970, 3060, 3450; ^{1}H-NMR($CDCl_3$) δ : 0.88(t, 3H, *J*=6.6 Hz, CH_3), 1.09-1.37(m, 14H, $(CH_2)_7$), 1.42-1.59(m, 2H, NH-$CH_2\underline{CH_2}$), 2.87(dd, 1H, J_1=7.8 Hz, J_2=7.8 Hz, C_2-H) 2.99(dd, 1H, J_1=6.3 Hz, J_2=6.3 Hz, C_2-H) 3.20-3.39(m, 2H, NH-$\underline{CH_2}$), 3.59-3.80(m, 2H, $\underline{CH_2}$OH), 4.42-4.62(m, 1H, C_1-H), 7.19-7.39(m, 5H, phenyl).

(R) 1-Dodecyl-3-(1-hydroxymethyl-2-phenyl-ethyl)-thiourea의 합성(10c) – (R)-2-Amino-1-phenyl-3-propanol **6**(182 mg, 1.2 mmol)과 dodecylisothiocyanate(318 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 24.8%; R_f=0.29(ethylacetate : hexane=1 : 3); $[\alpha]_D^{20}$= +45.80(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1110, 1520, 2890, 3090, 3340; ^{1}H-NMR($CDCl_3$) δ : 0.88(t, 3H, *J*=6.6 Hz, CH_3), 1.19-1.39(m, 18H, $(CH_2)_9$), 1.40-1.59(m, 2H, NH-$CH_2\underline{CH_2}$), 2.88(dd, 1H, J_1=7.8 Hz, J_2=7.5 Hz, C_2-H) 3.00(dd, 1H, J_1=6.0 Hz, J_2=6.3 Hz, C_2-H) 3.12-3.37(m, 2H, NH-$\underline{CH_2}$), 3.59-3.80(m, 2H, $\underline{CH_2}$OH), 4.49-4.64(m, 1H, C_1-H), 7.19-7.39(m, 5H, phenyl).

(R) 1-(1-Hydroxymethyl-2-phenyl-ethyl)-3-tetradecyl-thiourea의 합성(10d) – (R)-2-Amino-1-phenyl-3-propanol **6** (182 mg, 1.2 mmol)과 tetradecylisothiocyanate(358 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 20.8%; R_f=0.13(ethylacetate : hexane=1 : 3); $[\alpha]_D^{20}$= +43.00(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1260, 1510, 2910, 3160, 3470; ^{1}H-NMR($CDCl_3$) δ : 0.88(t, 3H, *J*=6.6 Hz, CH_3), 1.19-1.39(m, 22H, $(CH_2)_{11}$), 1.42-1.58(m, 2H, NH-$CH_2\underline{CH_2}$), 2.89(dd, 1H, J_1=7.8 Hz, J_2=8.1 Hz, C_2-H) 3.01(dd, 1H, J_1=6.3 Hz, J_2=6.3 Hz, C_2-H) 3.19-3.29(m, 2H, NH-$\underline{CH_2}$), 3.59-3.80(m, 2H, $\underline{CH_2}$OH), 4.58-4.62(m, 1H, C1-H), 7.19-7.39(m, 5H, phenyl).

(R) 1-(4-Chloro-phenyl)-3-(1-hydroxymethyl-2-phenyl-ethyl)-thiourea의 합성(10e) – (R)-2-Amino-1-phenyl-3-propanol **6**(182 mg, 1.2 mmol)과 4-chlorophenylisothiocyanate(235 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 98.1%; R_f=0.33(ethylacetate : hexane=1 : 3); $[\alpha]_D^{20}$= +108.40(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1120, 1550, 3120; ^{1}H-NMR($CDCl_3$) δ : 2.89-3.00(m, 2H, C_2-H), 3.59-3.82(m, 2H, $\underline{CH_2}$OH), 4.79-4.90(m, 1H, C_1-H), 7.10-7.39(m, 9H, pheny).

(S) 1-(1-Hydroxymethyl-2-phenyl-ethyl)-3-octyl-thiourea의 합성(11a) – (S)-2-Amino-1-phenyl-3-propanol **7**(182 mg, 1.2

mmol)과 octylisothiocyanate(240 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 25.0%; R_f=0.33(ethylacetate : hexane=1 : 2); $[\alpha]_D^{20}$= -47.40(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1170, 1490, 2900, 3100, 3370; ^{1}H-NMR($CDCl_3$) δ : 0.88(t, 3H, *J*=6.6 Hz, CH_3), 1.19-1.39(m, 10H, $(CH_2)_5$), 1.40-1.59(m, 2H, NH-$CH_2\underline{CH_2}$), 2.89(dd, 1H, J_1=8.1 Hz, J_2=7.5 Hz, C_2-H) 3.01(dd, 1H, J_1=6.3 Hz, J_2=6.3 Hz, C_2-H) 3.19-3.31(m, 2H, NH-$\underline{CH_2}$), 3.59-3.80(m, 2H, $\underline{CH_2}$), 4.50-4.69(m, 1H, C_1-H), 7.20-7.39(m, 5H, phenyl).

(S) 1-Decyl-3-(1-hydroxymethyl-2-phenyl-ethyl)-thiourea의 합성(11b) – (S)-2-Amino-1-phenyl-3-propanol **7**(182 mg, 1.2 mmol)과 decylisothiocyanate(280 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 58.8%; R_f=0.14(ethylacetate : hexane=1 : 3); $[\alpha]_D^{20}$= -47.20(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1120, 1560, 2910, 3160, 3460; ^{1}H-NMR($CDCl_3$) δ : 0.88(t, 3H, *J*=6.6 Hz, CH_3), 1.19-1.39(m, 14H, $(CH_2)_7$), 1.40-1.59(m, 2H, NH-$CH_2\underline{CH_2}$), 2.88(dd, 1H, J_1=7.8 Hz, J_2=7.8 Hz, C_2-H) 2.99(dd, 1H, J_1=5.7 Hz, J_2=6.3 Hz, C_2-H) 3.19-3.37(m, 2H, NH-$\underline{CH_2}$), 3.59-3.80(m, 2H, $\underline{CH_2}$OH), 4.49-4.65(m, 1H, C_1-H), 7.19-7.39(m, 5H, phenyl).

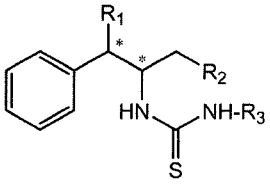
(S) 1-Dodecyl-3-(1-hydroxymethyl-2-phenyl-ethyl)-thiourea의 합성(11c) – (S)-2-Amino-1-phenyl-3-propanol **7**(182 mg, 1.2 mmol)과 dodecylisothiocyanate(318 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 21.9%; R_f=0.17(ethylacetate : hexane=1 : 3); $[\alpha]_D^{20}$= -39.00(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1210, 1530, 2970, 3150, 3410; ^{1}H-NMR($CDCl_3$) δ : 0.88(t, 3H, *J*=6.6 Hz, CH_3), 1.19-1.39(m, 18H, $(CH_2)_9$), 1.41-1.59(m, 2H, NH-$CH_2\underline{CH_2}$), 2.88(dd, 1H, J_1=7.8 Hz, J_2=7.5 Hz, C_2-H) 3.00(dd, 1H, J_1=6.3 Hz, J_2=6.3 Hz, C_2-H) 3.19-3.37(m, 2H, NH-$\underline{CH_2}$), 3.49-3.80(m, 2H, $\underline{CH_2}$OH), 4.49-4.69(m, 1H, C_1-H), 7.19-7.39(m, 5H, phenyl).

(S) 1-(1-Hydroxymethyl-2-phenyl-ethyl)-3-tetradecyl-thiourea의 합성(11d) – (S)-2-Amino-1-phenyl-3-propanol **7** (182 mg, 1.2 mmol)과 tetradecylisothiocyanate(358 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 23.7%; R_f=0.20(ethylacetate : hexane=1 : 3); $[\alpha]_D^{20}$= -36.60(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1200, 1500, 2910, 3170, 3370; ^{1}H-NMR($CDCl_3$) δ : 0.88(t, 3H, *J*=6.6 Hz, CH_3), 1.19-1.39(m, 22H, $(CH_2)_{11}$), 1.41-1.59(m, 2H, NH-$CH_2\underline{CH_2}$), 2.886dd, 1H, J_1=7.8 Hz, J_2=8.1 Hz, C_2-H) 3.00(dd, 1H, J_1= 6.3 Hz, J_2=6.6 Hz, C_2-H) 3.12-3.31(m, 2H, NH-$\underline{CH_2}$), 3.57-3.80(m, 2H, $\underline{CH_2}$OH), 4.49-4.69(m, 1H, C1-H), 7.19-7.39(m, 5H, phenyl).

Table I – Cytotoxicity of thioureido MAPP derivatives in HL-60 cells



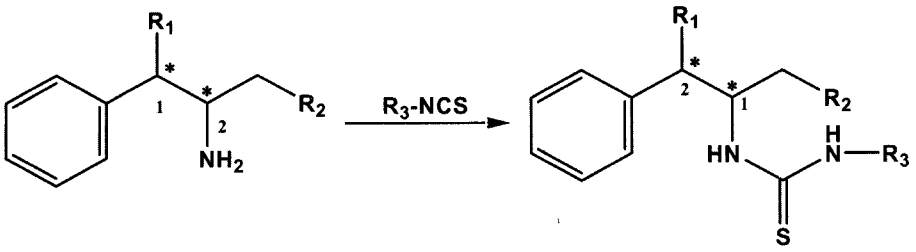
Compd.	R_1	R_2	R_3	Config.	IC_{50} (μM)
8a	OH	H	C_8H_{17}	1S, 2R	12.59
8b	OH	H	$C_{10}H_{21}$	1S, 2R	1.53
8c	OH	H	$C_{12}H_{25}$	1S, 2R	1.05
8d	OH	H	$C_{14}H_{29}$	1S, 2R	6.75
8e	OH	H	C_6H_4-4-Cl	1S, 2R	12.94
9a	OH	H	C_8H_{17}	1R, 2S	10.12
9b	OH	H	$C_{10}H_{21}$	1R, 2S	1.73
9c	OH	H	$C_{12}H_{25}$	1R, 2S	1.52
9d	OH	H	$C_{14}H_{29}$	1R, 2S	3.96
9e	OH	H	C_6H_4-4-Cl	1R, 2S	7.85
10a	H	OH	C_8H_{17}	R	3.10
10b	H	OH	$C_{10}H_{21}$	R	2.01
10c	H	OH	$C_{12}H_{25}$	R	2.20
10d	H	OH	$C_{14}H_{29}$	R	2.88
10e	H	OH	C_6H_4-4-Cl	R	9.04
11a	H	OH	C_8H_{17}	S	5.02
11b	H	OH	$C_{10}H_{21}$	S	1.78
11c	H	OH	$C_{12}H_{25}$	S	1.50
11d	H	OH	$C_{14}H_{29}$	S	3.74
11e	H	OH	C_6H_4-4-Cl	S	61.58
B13					2.99

(s) 1-(4-Chloro-phenyl)-3-(1-hydroxymethyl-2-phenyl-ethyl)-thiourea의 합성(11e) – (S)-2-Amino-1-phenyl-3-propanol **7**(182 mg, 1.2 mmol)과 4-chlorophenylisothiocyanate(235 mg, 1.4 mmol)을 사용하여 합성하였다.

Yield : 83.7%; R_f=0.33(ethylacetate : hexane=1 : 3); $[\alpha]_D^{20}$= -105.60(*c*, 0.5 in CH_3OH); IR(KBr) cm^{-1}; 1080, 1560, 3160; ^{1}H-NMR($CDCl_3$) δ : 2.89-3.04(m, 2H, C_2-H) 3.59-3.89(m, 2H, CH_2OH) 4.79-4.90(m, 1H, C_1-H), 7.10-7.39(m, 9H, phenyl).

세포독성효과를 측정(MTT assay)[11]

합성 화합물은 PBS에 녹여서 농도별로 20 μl씩 각 well에 첨가하고, 암세포(HL-60 cells)와 화합물이 접종된 plate를 37°C, 5% CO_2 하에서 4일 간 배양한다. 각 well에 0.1 mg의 MTT를 가하고 다시 37°C, 5% CO_2 하에서 4시간 배양한다. plate를 원심 분리하여 생성된 formazan 결정을 가라앉히고, 배지는 30 μl 정도만 남기고 제거하였다. 생성된 formazan 결정을 용해하려고 DMSO를 150 μl씩 가한 후, 96 well-plate용 광도계(ELISA



Compd.	R_1	R_2	Config.
4	OH	H	1R, 2S
5	OH	H	1S, 2R
6	H	OH	2R
7	H	OH	2S

Compd.	R_1	R_2	R_3	Config.
8a	OH	H	C_8H_{17}	1S, 2R
8b	OH	H	$C_{10}H_{21}$	1S, 2R
8c	OH	H	$C_{12}H_{25}$	1S, 2R
8d	OH	H	$C_{14}H_{29}$	1S, 2R
8e	OH	H	C_6H_4-4-Cl	1S, 2R
9a	OH	H	C_8H_{17}	1R, 2S
9b	OH	H	$C_{10}H_{21}$	1R, 2S
9c	OH	H	$C_{12}H_{25}$	1R, 2S
9d	OH	H	$C_{14}H_{29}$	1R, 2S
9e	OH	H	C_6H_4-4-Cl	1R, 2S
10a	H	OH	C_8H_{17}	R
10b	H	OH	$C_{10}H_{21}$	R
10c	H	OH	$C_{12}H_{25}$	R
10d	H	OH	$C_{14}H_{29}$	R
10e	H	OH	C_6H_4-4-Cl	R
11a	H	OH	C_8H_{17}	S
11b	H	OH	$C_{10}H_{21}$	S
11c	H	OH	$C_{12}H_{25}$	S
11d	H	OH	$C_{14}H_{29}$	S
11e	H	OH	C_6H_4-4-Cl	S

Scheme 1 – Synthesis of thioureido MAPP derivatives.

reader)로 540 nm에서의 흡광도를 측정하였다. 50% 억제농도(IC_{50})는 대조군에 대해 생존율이 50%가 되도록 하는 약물의 농도이며 이 IC_{50} 값을 항암 효과의 지표로 삼았다.

결과 및 고찰

합성한 화합물의 선광도($[\alpha]_D^{20}$)는 모핵이(1S, 2R)인 화합물(**8a~8e**)에서 -19.40~-28.50, 모핵이(1R, 2S)인 화합물(**9a~9e**)에서 5.40~58.86, 모핵이(R)인 화합물(**10a~10e**)에서 41.60~108.40, 모핵이(S)인 화합물(**11a~11e**)에서 -36.60~-105.60이 나왔다. 화합물(**8a~9e**)의 ^{1}H-NMR에서 C_2-H가 5.53~6.20 ppm, C_1-H가 4.81~5.12 ppm, C_1-CH_3는 0.99~1.06 ppm에서 흡수대가 나타났고, 화합물(**10a~11e**)의 ^{1}H-NMR에서 $^1C_2$-H가 2.87~3.05 ppm, C_1-H가 4.43~4.91 ppm, CH_2OH는 3.59~3.90 ppm에서 흡수대가 나타났다. IR에서 NH는 3340~3470 cm^{-1}, -OH는 3060~3170 cm^{-1}, -C=S는 1020~1260 cm^{-1}에서 흡수대가 나타났다. 지방족체인 화합물의 합성수득률은 20~40% 정도로 낮았지만, 방향족 화합물은 70% 이상 높았다.

합성한 화합물 20종에 대하여 MTT assay를 사용하여 IC_{50}값을 측정한 결과, 지방족 체인의 탄소 수가 8개에서 14개 및 phenyl로 갈수록 세포독성 효과가 증가하다가 다시 감소하였고, 탄소의 수가 10개와 12개인 화합물이 가장 우수한 세포독성 효과를 나타내었다. 대조화합물로 사용한 B13보다 우수한 세포독성효과를 나타낸 화합물은 지방족 체인의 탄소 수가 10개와 12개인 **8b**, **8c**, **9b**, **9c**, **10b**, **10c**, **11b**, **11c**이며, 이중에서 가장 우수한 효과를 나타낸 화합물은 **8c**이고, IC_{50}값은 1.05 μM이며 B13보다 3배 우수한 세포독성 효과를 나타내었다. 한편, 각 화합물의 stereoisomer에 대한 세포독성 효과에는 큰 차이가 없었다.

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