Thiazoline Analogues of Epiderstatin, New Inhibitors of Cell Cycle of tsFT-210 Cells

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In the course of our study on an alternative synthesis of (±)-epiderstatin, we found some interesting observations, which helped us for preparing epiderstatin analogues. We developed a new methodology for the syntheses of epiderstatin analogues by using an unusual reaction to study their structure-activity relationship. During study, we found that epiderstatin is not a real inhibitor of signal transduction of EGF, but the inhibition arose from acetoxycycloheximide contaminating the preparation of the natural epiderstatin.[1] However, thiazoline analogues of epiderstatin showed unexpected biological activities. In this paper, we wish to report the syntheses of analogues, **1a**, **1b**, **2a**, **2b**, **3a** and **3b**, shown in Fig. 1 and their biological activities.

The syntheses of the racemic epiderstatin analogues, **4**, **5**, **10** and **11**, are outlined in Fig. 2. Treatment of *cis*, *trans* mixture of 3,5-dimethyl-2-thioglutarimide[2] (**1**) with (carbethoxymethylene)triphenylphosphorane (toluene, reflux for 22 hours) gave an ethyl ester (**2**) in 42% yield. Hydrolysis of **2** (LiOH, H_2O-MeOH-dimethoxyethane (1:2:2), r.t., 12 hours) afforded a carboxylic acid (**3**) with isomerization of the double bond at C_5 position in 51% yield. Condensation of **3** with 2-mercaptothiazoline (dicyclohexylcarbodiimide, dimethylaminopyridine, 0°C→r.t., 5 hours)[3] afforded stereoselectively the desired thioester (3S*/5S* isomer (**4**): 3S*/5R* isomer (**5**), ratio of 2:98) in 47% yield. Isomerization of the double bond into *Z*-isomer at C_6 position during the reaction may be caused by the intramolecular hydrogen-bonding stabilization between C_8 carbonyl and the amide proton. 3-Hydroxymethylglutarimide (**9**) was synthesized as indicated in Fig. 3. Treatment of 3-Phenylglutaric acid[4] (**6**) with urea at 150°C gave **7** in 97% yield. Oxidative degradation of **7** ($NaIO_4$, Ru(III)Cl_3 r.t., 36 hours) afforded the desired product (**8**) in 85% yield. Chemoselective reduction[5] of **8** using N,N-dimethylchloromethyleneiminium chloride and sodium borohydride gave 3-Hydroxymethylglutarimide (**9**) in 60% yield. Condensation of **3** with **9** afforded ester (3S*/5S* isomer (**10**): 3S*/5R* isomer (**11**), ratio of 9:91) in 19% yield. Optical resolution of each analogue was carried out by chiral HPLC to give optically pure samples shown in Fig. 1.

The absolute configurations of synthetic epiderstatin and nine analogues, **1a**, **1b**, **2a**, **2b**, **3a**, **3b**, were determined by NMR and CD analyses. The absolute configurations of C_3/C_5 *trans* isomers, **2a** and **2b**, were determined by the comparison of their Cotton effects, $\lambda_{332}+3.9$ for **2a** and $\lambda_{332}-3.0$ for **2b**, with the data ($\lambda_{287}+1.78$) of synthetic epiderstatin. The absolute configurations of *cis* isomers, **1a**, **1b**, **3a**, **3b** were determined by the application of allylic axial chirality approach[1] at C_5 axial protons which were deducible from the large coupling constant value (12.7 Hz) between 4-H and 5-H for each analogue.

After separation and characterization of each isomer, we examined the flat reversion activities on *src*^{ts}-NRK cells, the cell cycle inhibitory activities on tsFT-210 cells, effects on [^{3}H]thymidine uptake into EGF-stimulated Balb/MK cells and the antifungal activities. Any an-

Fig. 1. Structures of epiderstatin analogues.

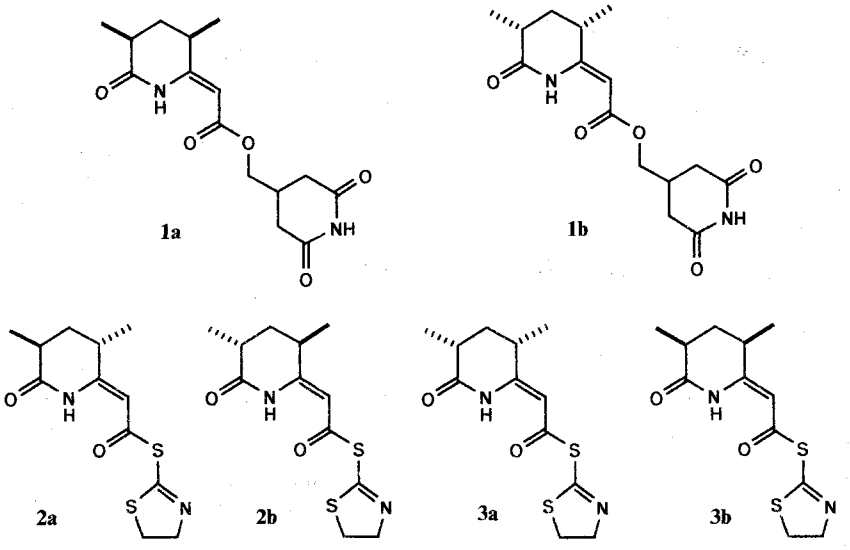


Fig. 2. Syntheses of racemic forms of epiderstatin analogues **4**, **5**, **10** and **11**.

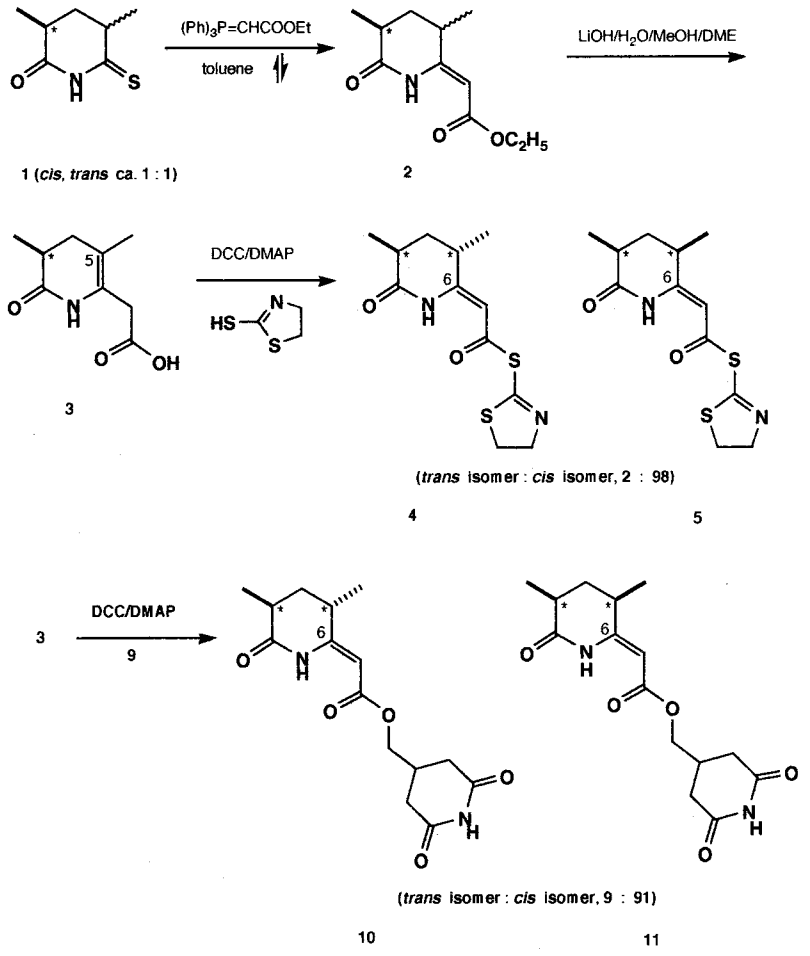


Fig. 3. Synthesis of 3-hydroxymethylglutarimide.

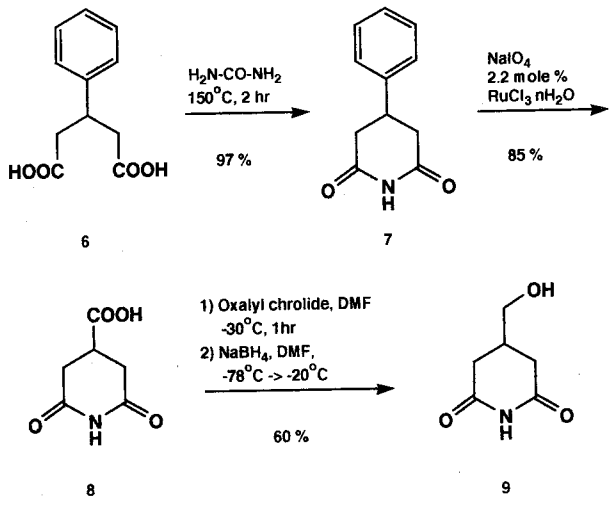
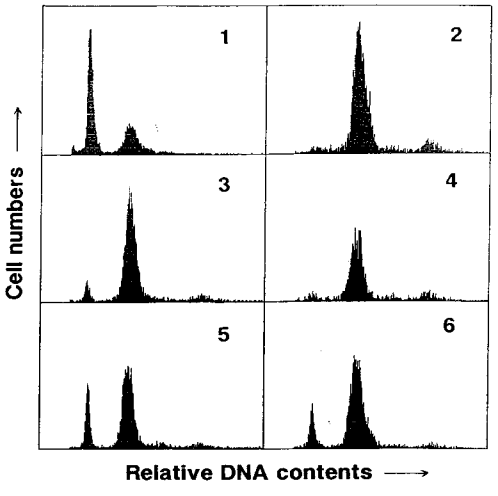


Fig. 4. Effects of thiazoline analogues, **2a**, **2b**, **3a** and **3b**, on the cell cycle of tsFT-210 cells.



Exponentially growing cells were synchronized at the G_2 phase by incubation at 39°C (non-permissive temperature) for 16 hours and the cells were released to 32°C for 4 hours after treatment with the samples. 1: no drug control; 2: staurosporine (0.43 μM); 3, 4, 5, 6: thiazoline analogues **2a**, **2b**, **3a** and **3b**, respectively (each 88 μM).

alogue as well as synthetic epiderstatin[1] did not induce the flat reversion of the *src*^{ts}-NRK cells at the dose of 50 μg/ml. However the thiazoline analogues, **2a**, **2b**, **3a** and **3b**, showed different biological activities. The analogues showed antifungal activities against *Piricularia oryzae* (**2a**: 11 mm, **2b**: 15 mm, **3a**: negative, **3b**: negative, each at 350 μg/disc, conventional paper disc method) and inhibited the cell cycle progression of tsFT-210 cells at G_2/M phase as shown in Fig. 4. The activities of C_3/C_5 *trans* isomers, **2a** and **2b**, were rather stronger than those of *cis* isomers, **3a** and **3b**. Although staurosporine, which is a broad spectrum inhibitor of protein kinases, inhibited the cell cycle progression of tsFT-210 cells under the random culture condition as well as the synchronized culture condition, thiazoline analogues, **2a**, **2b**, **3a**, **3b**, arrested the cell cycle only under the synchronized culture condition. Thus these thiazoline analogues may be useful as selective inhibitors of cell cycle progression of tsFT-210 cells synchronized at G_2 phase.

Experimental

General Methods

Melting points were obtained using a Yanagimoto micro-melting point apparatus and were uncorrected. Optical rotations were taken on a JASCO DIP-370 polarimeter. CD spectra were measured on a JASCO J-270 spectropolarimeter. ^{1}H and ^{13}C NMR were measured on JEOL EX-270, α-400 and GSX-500 instruments. MS were measured on Hitachi M-80 and JEOL HX-110 mass spectrometers. Spectral properties of key intermediates (**2**, **3**, **9**) and synthetic analogues (**1a**, **1b**, **2a**, **2b**, **3a**, **3b**) are as follows. Compound **2**: EI-MS *m/z* 211 (M^+); ^{1}H NMR ($CDCl_3$) δ 1.23, 1.26, 1.27, 1.28 (total 6H, each d, J=7 Hz), 1.267 (3H, t, J=7 Hz), 1.5 (1/2H, dt, J=13, 12.5 Hz, *cis* isomer), 1.8 (1H, m, *trans* isomer), 1.94 (1/2H, ddd, J=13, 5.0, 4.5 Hz; *cis* isomer), 2.54, 2.65 (total 2H, each m, *trans* and *cis* isomers), 4.16, 4.17 (total 2H, each m), 4.87 (1/2H, s; *trans* isomer), 4.91 (1/2H, d, J=2 Hz; *cis* isomer), 10.6, 10.8 (total 1H, each br s). Compound **3**: The acid was highly unstable and immediately used for the next reaction; ^{1}H NMR ($CDCl_3$) δ 1.12 (3H, d, J=6.9 Hz), 1.69 (3H, s), 2.0 (1H, dd, J=16.5, 10.8 Hz), 2.2 (1H, dd, J=16.5, 6.6 Hz), 2.4 (1H, m), 3.1 (2H, br s), 7.27 (1H, br s), 8.6 (1H, br s). Compound **9**: EI-MS *m/z* 143 (M^+); ^{1}H NMR (CD_3OD) δ 2.28 (1H, m), 2.43 (2H, dd, J=17.5, 11.0 Hz), 2.62 (2H, dd, J=17.5, 4.2 Hz), 3.52 (2H, d, J=5.4 Hz). Compounds **1a** and **1b**: Optical resolution of *cis* isomer (**11**) was carried out by chiral HPLC (CHIRALPAK AS, 10×250 mm; EtOH, Flow rate 1.2 ml/minute; detector wave length, 295 nm; temperature, 25°C); retention time (Rt) for **1a**, 29.8 minutes; Rt for **1b**, 37.3 minutes. **1a**: $[\alpha]_D^{24}$ +6.9° (*c* 0.22, CH_2Cl_2); CD $\Delta\varepsilon^{24}$ (*c* 0.005, MeOH) λ_{267} +1.35; EI-MS *m/z* 308 (M^+); ^{1}H NMR (CD_2Cl_2) δ 1.22 (6H, d, J=6.8 Hz), 1.49 (1H, dt, J=13.2, 12.7 Hz), 1.94 (1H, ddd, J=13.2, 4.9, 4.4 Hz), 2.42 (2H, ddd, J=17, 11.7, 1.5 Hz), 2.54 (2H, m), 2.66 (1H, m), 2.72 (2H, dd, J=17, 4.2 Hz), 4.10 (1H, dd, J=5.9, 1.5 Hz), 4.92 (1H, d, J=1.5 Hz), 7.93 (1H, br s), 10.62 (1H, br s). **1b**: $[\alpha]_D^{24}$ −10.3° (*c* 0.18, CH_2Cl_2); CD $\Delta\varepsilon^{24}$ (*c* 0.005, MeOH) λ_{267} −1.39. Compounds **2a** and **2b**: Optical resolution of *trans* isomer (**4**) was carried out by chiral HPLC (CHIRALPAK AS, 10×250 mm; EtOH, Flow rate 2.2 ml/minute; detector wave length, 295 nm; temperature, 60°C); Rt for **2a**, 14 minutes; Rt for **2b**, 24.4 minutes. Compound **2a**: MP 108.5~110°C; $[\alpha]_D^{24}$ +26.3° (*c* 1.33, CH_2Cl_2); CD $\Delta\varepsilon^{24}$ (*c* 0.005, MeOH) λ_{332} +3.9; EI-MS *m/z* 284 (M^+); ^{1}H NMR (CD_2Cl_2) δ 1.31 (3H, d, J=7.3 Hz), 1.25 (3H, d, J=6.8 Hz), 1.82 (2H, m), 2.68 (1H, dquint, J=9.3, 6.8 Hz), 2.8 (1H, m), 3.28 (2H, t, J=7.6 Hz), 4.52 (2H, t, J=7.6 Hz), 6.67 (1H, s), 10.9 (1H, br s). Compound **2b**: MP 104.5~106°C; $[\alpha]_D^{24}$ −21.8° (*c* 1.42, CH_2Cl_2); CD $\Delta\varepsilon^{24}$ (*c* 0.005, MeOH) λ_{332} −3.0. Compounds **3a** and **3b**: Optical resolution of *cis* isomer (**5**) was carried out by chiral HPLC (CHIRALPAK AS, 10×250 mm; EtOH, Flow rate 2.2 ml/minute; detector wave length, 295 nm; temperature, 60°C); Rt for **3a**, 15 minutes; Rt for **3b**, 20.5 minutes. Compound **3a**: MP 153~154°C; $[\alpha]_D^{24}$ −33.2° (*c* 1.0, CH_2Cl_2); CD $\Delta\varepsilon^{24}$ (*c* 0.005, MeOH) λ_{329} −2.4; , EI-MS *m/z* 284 (M^+); ^{1}H NMR (CD_2Cl_2) δ 1.23 (3H, d J=6.8 Hz), 1.25 (3H, d, J=6.8 Hz), 1.53 (1H, dt, J=13.2, 12.7 Hz), 1.97 (1H, ddd, J=13.2, 4.9, 4.4 Hz), 2.56 (1H, dqd, J=12.7, 6.7, 4.9 Hz), 2.75 (1H, dqdd, J=12.7, 6.8, 4.4, 2.0 Hz), 3.29 (2H, t, J=7.3 Hz), 4.54 (2H, t, J=7.3 Hz), 6.83 (1H, d, J=2 Hz), 11.1 (1H, br s). Compound **3b**: MP 156~157°C; $[\alpha]_D^{24}$ +33.5° (*c* 1.0, CH_2Cl_2); CD $\Delta\varepsilon^{24}$ (*c* 0.005, MeOH) λ_{329} +3.1.

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