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SEMISYNTHESIS OF TRIPTOLIDE ANALOGUES PART IV: EFFECTS OF C-14 CARBAMOTHIOATE SUBSTITUENTS ON CYTOTOXIC ACTIVITIES

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Abstract – Four C-14 carbamothioate triptolide analogues were prepared and their cytotoxic activities on A549 human lung tumor cells and HT29 human colon tumor cells were evaluated. The activities of the prepared compounds were weaker than those of the parent compound, triptolide (**1**). However, some differences were noted in the activities of prepared compounds. The leaving ability and bulkiness of the amine components of their carbamothioate groups appeared to affect their cytotoxic activities.

Triptolide (**1**) and its related compounds **2** and **3**, which are isolated from *Tripterygium wilfordii* (Celastaceae) (Figure 1),[1-3] comprising significant antileukemic activities,[1,2] have a unique continuous triepoxide system on the B/C ring system. Because another analogue **4** from the same plant without 12α,13α-oriented epoxide group shows rather weak cytotoxic activity, the 12α,13α-oriented epoxide group is considered

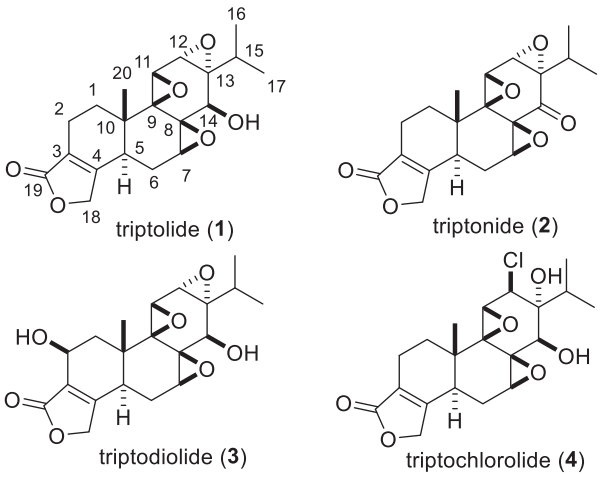


Figure 1. Structures of Triptolide (**1**) and Its Reated Compounds (**2**−**4**)

essential for cytotoxicity in triptolide analogues.[1,4] Previously, we reported the preparation and cytotoxic activities of a series of triptolide derivatives, *i.e.*, three triptolide epoxide-transposition analogues and the reaction intermediates,[5] a series of reaction products derived from the treatment of triptolides with fluorinating agents,[6] and γ-lactone-modified and/or C-14 substituent-modified products.[7] The results suggested that an electron-negative substituent at C-14, particularly of β-orientation, would increase the cytotoxic activity (Figure 2). The active analogue among the previously examined analogues was a triptolide imidazole-carbothioate,[5] whose cytotoxic activity was relatively strong. Thus, in the present study, we prepared three more carbamothioate analogues of triptolide and examined their cytotoxic activities.

Imidazole-carbothioate analogue **5**,[5] prepared by heating a mixture of triptolide, 4-dimethylaminopyridine (DMAP), and *N,N′*-thiocarbonyldiimidazole (TCDI) in CH_2Cl_2 at 60 °C in a sealed tube under an Ar atmosphere (yield 98%; structure elucidated by 1D ^{1}H- and ^{13}C-NMR and 2D-NMR spectroscopy; and assignment of ^{1}H-and ^{13}C-NMR as shown in Table 2), was used for carbamothioate analogue preparation.

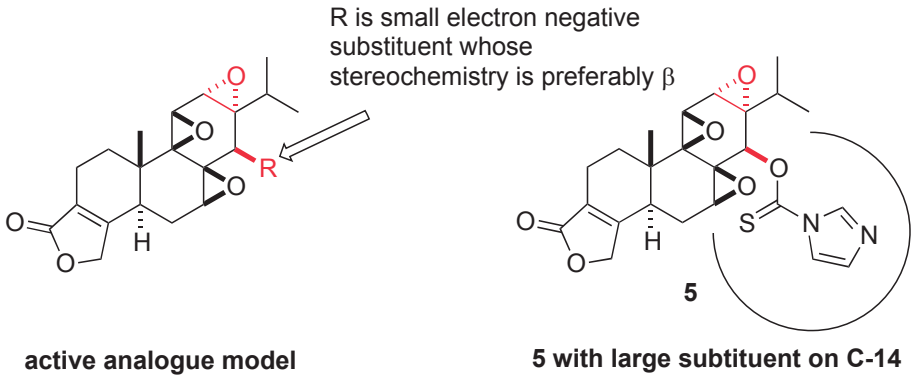
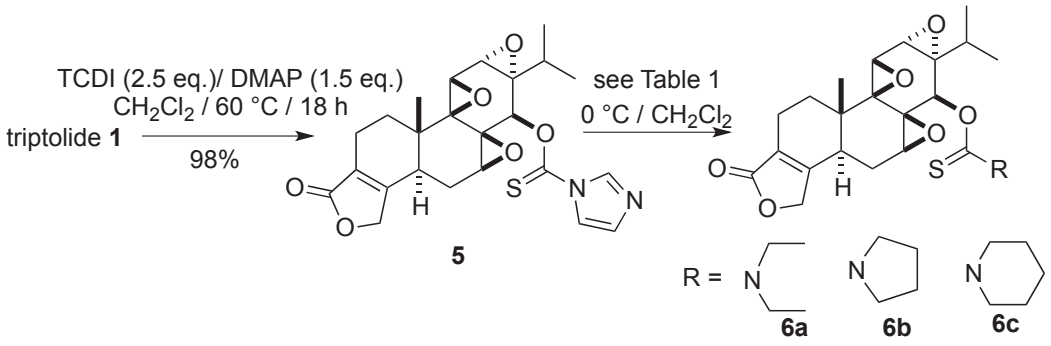


Figure 2. Biologically Active Triptolide Analogue Model and Imidazole Carbothioate Analogue **5**

Transthioamidation of **5** with secondary amines, performed by treating **5** with diethylamine or pyrrolidine or piperidine under the reaction conditions shown in Scheme 1 and Table 1, produced the corresponding



Scheme 1. Synthesis of Analogues **5** and **6a-c**

amine-carbothioate analogues **6a-c** in fair to excellent yields. The structure of **6a** was identified by using 1D ^{1}H- and ^{13}C-NMR and 2D-NMR spectroscopy (Table 2).

Table 1. Reactions of Imidazole-Carbothioate Analogue (**5**) with Secondary Amines

entry	amine (eq.)	reaction time (h)	product (yield %)
1	diethylamine (75)	1.5	---[a)]
2	diethylamine (57)	1	**6a** (49)
3	diethylamine (42)	1	**6a** (65)
4	pyrrolidine (46)	1	**6b** (50)
5	pyrrolidine (40)	1	**6b** (50)
6	piperidine (39)	1	**6c** (100)

[a] Complex mixture.

The cytotoxic activities of **5**, the prepared **6a-c** and the mother triptolide (**1**) were evaluated on A549 human lung tumor cells (A549) and HT29 human colon-tumor cells (HT29). The results are shown in Figure 3. Although the cytotoxic activities of the synthesized analogues (**5** and **6a-c**) were generally weaker than those of the mother triptolide (**1**), analogue **5** might still be considered cytotoxic on both A549 human lung tumor cells and HT29 human colon tumor cells. The difference in the activity between **5** and **6a-c** is mostly due to the difference in the leaving ability of the amine component. The imidazole group in **5**, which has good leaving ability, only slightly affects the cytotoxic activity. During assay, **5** may produce some triptolide (**1**). The amine components in **6a-c** with smaller leaving activity than **5**

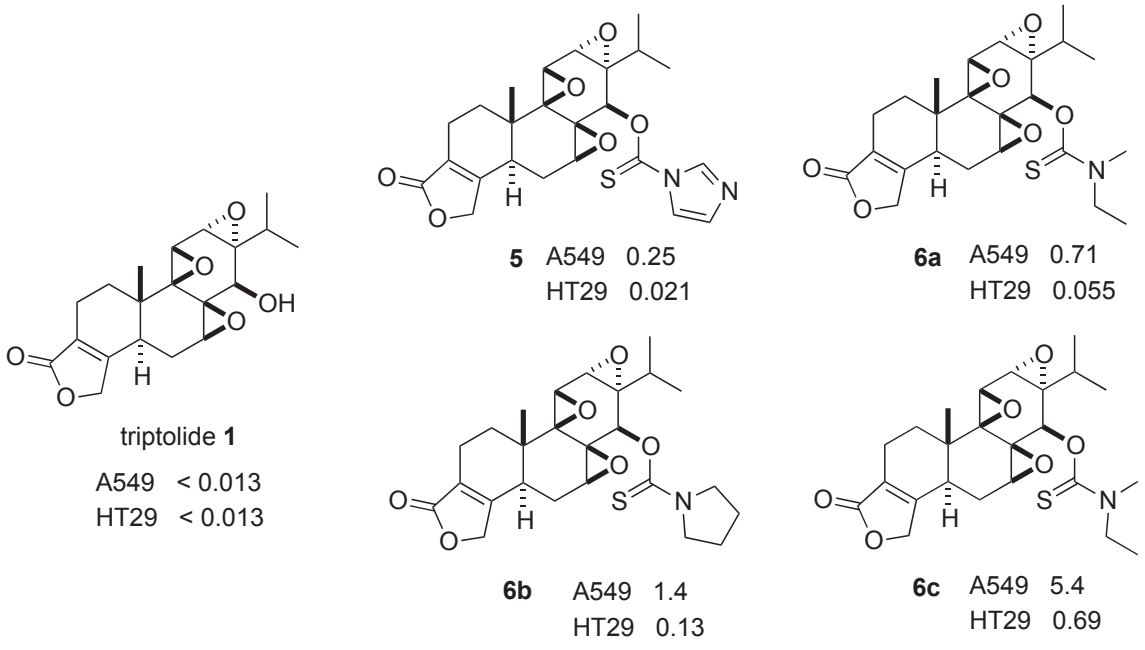


Figure 3. Cytotoxic Activities (IC_{50} values, μM) on A549 and HT29 Cells of Analogues **5** and **6a-c**

affect the activity more obviously (**6a** > **6b** > **6c**). The differences in activity among **6a-c** might be due to differences in the bulkiness of the amine component.

Overall, these results indicate that the leaving ability and bulkiness of the amine component of the carbamothioate analogues (**5** and **6a-c**) are related to their cytotoxic activities. These findings will be useful for designing prodrug analogues, including antibody-drug conjugates, of triptolide (**1**).

Table 2. ^{1}H-NMR ($CDCl_3$, 500 MHz, δ in ppm and *J* in Hz) and ^{13}C-NMR ($CDCl_3$, 125 MHz, δ in ppm) data of compounds **5**[a] and **6a**[a]

position	**5**			**6a**		
	δ_C	δ_H	HMBC[b]	δ_C	δ_H	HMBC[b]
1	29.8 (t)	1.57 (1H, dd, *J* = 12.0, 5.2)	2, 3, 5, 6, 9, 10, 19, 20	29.9 (t)	1.56 (1H, ddd, *J* = 12.5, 5.4, 1.1)	2, 3, 5, 9, 10, 19, 20
		1.23 (1H, ddd, *J* = 12.0, 12.0, 5.8)	2, 3, 5, 9, 10, 20		1.22 (1H, dd, *J* = 12.5, 5.9)	2, 3, 5, 9, 10, 20
2	17.0 (t)	2.31 (1H, br d)	4, 10	17.1 (t)	2.32 (1H, m)	3, 4, 10
		2.12 (1H, m)			2.17-2.08 (1H, m)	3, 4, 10
3	125.6 (s)			125.5 (s)		
4	159.6 (s)			160.1 (s)		
5	40.2 (d)	2.71 (1H, m)	3, 4, 6, 10, 20	40.4 (d)	2.71 (1H, m)	6, 10, 20
6	23.2 (t)	2.19 (1H, ddd, *J* = 14.9, 5.6, 5.6)	4, 5, 7, 10	23.4 (t)	2.16 (1H, ddd, *J* = 14.8, 5.9, 5.9)	4, 5, 7, 10
		1.895 (1H, m)			1.90 (1H, dd, *J* = 14.8, 13.5)	4, 5, 7, 8, 10, 14
7	61.0 (d)	3.54 (1H, d, *J* = 5.6)	5, 6, 8, 9, 14	60.7 (d)	3.54 (1H, d, *J* = 5.6)	4, 5, 6, 8
8	60.3 (s)			60.5 (s)		
9	63.6 (s)			63.8 (s)		
10	35.6 (s)			35.7 (s)		
11	55.5 (d)	3.90 (1H, d, *J* = 3.1)	8, 10, 12, 13	55.7 (d)	3.84 (1H, d, *J* = 3.1)	8, 10, 13
12	55.3 (d)	3.60 (1H, d, *J* = 3.1)	9, 11, 15,	55.2 (d)	3.53 (1H, dd, *J* = 3.1, 0.8)	9, 11
13	63.1 (s)			63.9 (s)		
14	80.5 (d)	5.77 (1H, s)	8, 10, 12, 13, 15	77.4 (d)	5.89 (1H, d, *J* = 0.6)	C=S, 7, 8, 10, 12, 13, 15
15	28.4 (d)	1.903 (1H, m)		28.3 (d)	1.97 (1H, sep, *J* = 6.9)	12, 13, 14, 16, 17
16	17.7 (q)	1.00 (3H, d, *J* = 6.8)	15, 20	17.8 (q)	1.03 (3H, d, *J* = 6.9)	13, 15, 17
17	16.9 (q)	0.87 (3H, d, *J* = 6.8)	12, 15, 20	17.3 (q)	0.89 (3H, d, *J* = 6.9)	12, 13, 15, 16
18	69.9 (t)	4.68 (1H, m)	3, 4, 19	70.0 (t)	4.69 (1H, m)	3, 4
		4.64 (1H, m)	3, 4, 19		4.65 (1H, m)	3, 4
19	173.0 (s)			173.2 (s)		
20	13.6 (q)	1.01 (3H, s)	1, 9, 10, 5, 11	13.6 (q)	1.04 (3H, s)	1, 5, 9, 10
C=S	184.5 (s)			187.5 (s)		
imidazole-2	137.3 (d)	8.45 (1H, br s)	imidazole-4, 5			
imidazole-4	131.0 (d)	7.05 (1H, s)	imidazole-2, 5			
imidazole-5	118.5 (d)	7.73 (1H, d, *J* = 1.1)	imidazole-2, 4			
Me<u>C</u>H$_2$N				48.2 (t)	3.98 (1H, dq, *J* = 14.1, 7.1)	C=S, Me<u>C</u>H$_2$-, MeC<u>H</u>$_2$-
					3.73 (1H, dq, *J* = 14.1, 7.1)	C=S, Me<u>C</u>H$_2$-, MeC<u>H</u>$_2$-
Me<u>C</u>H$_2$N				43.9 (t)	3.77 (1H, dq, *J* = 14.1, 7.1)	C=S, Me<u>C</u>H$_2$-, MeC<u>H</u>$_2$-
					3.49 (1H, dq, *J* = 14.1, 7.1)	C=S, Me<u>C</u>H$_2$-, MeC<u>H</u>$_2$-
<u>Me</u>CH$_2$N				13.3 (q)	1.27 (3H, t, *J* = 7.1)	<u>Me</u>CH$_2$-
<u>Me</u>CH$_2$N				11.9 (q)	1.27 (3H, t, *J* = 7.1)	<u>Me</u>CH$_2$-

[a] Assignments were confirmed by COSY, HMQC or HSQC, and NOESY experiments. [b] HMBC correlations are from proton(s) started to the indicate carbon(s).

EXPERIMENTAL

General Experimental Procedures

Melting points were determined on a Yanaco MP-J3 apparatus and recorded uncorrected. Optical rotations were measured using either a JASCO P-1020 or P-1030 digital polarimeter, with IR spectra recorded using a JASCO FT/IR 620 or a 4100 spectrophotometer. NMR spectra were recorded on a JEOL JMN-ECA 500 and a Bruker DRX500 spectrometer at 300K. The chemical shifts (δ) are reported in ppm relative to the residual $CDCl_3$ resonance at 7.26 ppm for ^{1}H-NMR and to the $CDCl_3$ resonance at 77.0 ppm for ^{13}C-NMR. Mass spectra were obtained with a Micromass LCT spectrometer with a time-of-flight analyzer. Cytotoxic activities were assayed on A549 human lung cells and HT29 human colon-tumor cells and the results are shown as IC_{50} (μM).

Cells and Culture

Human lung and colorectal cancer cell lines, A549 and HT29, were obtained from ATCC (Lockville, MD, USA). A549 and HT29 cells were maintained in Dulbecco's modified Eagle's medium (D-MEM) (D6046, D6046) or D-MEM/F-12 medium (D8062, Sigma) with 10% heat-inactivated fetal bovine serum or 5 mg/mL of gentamicin, respectively, at 37 °C in a humidified atmosphere containing 5% CO_2.

Growth–Inhibition Assay

A 190 μL volume of an exponentially growing cell suspension (1×10^4 cells/1.9 mL) was seeded in a 96-well microtiter plate. After 24 h from seeding of the tumor cells, 10 μL of each drug at various concentrations was added. After incubation for 96 h at 37 °C, 10 μL of TetraColor ONE (Seikagaku Biobusiness Corporation, Tokyo, Japan) was added to each well and the plates were incubated for a further 1 h at 37 °C. After incubation, the optical density was measured at 450 nm using a microplate reader (SPECTRA max PLUS, Molecular Devices, CA, USA). The concentration causing 50% inhibition of cell proliferation (IC_{50}) was then calculated from a linear regression analysis of the linear portion of the growth curves.

Preparation of Triptolide Imidazole-Carbamothioate Analogue (**5**)

A mixture of **1** (20 mg, 0.056 mmol), TCDI (24.8 mg, 0.14 mmol), and DMAP (10 mg, 0.084 mmol) in dry CH_2Cl_2 (3.0 mL) was heated at 50 °C in a sealed tube for 18 h under an Ar atmosphere. Then, the solvent was evaporated *in vacuo* to give an oily residue, which was purified by SiO_2-MPLC (hexane/Me_2CO = 2:1) to give **5** as an amorphous solid (26 mg) in 98% yield.

Compound **5**: Colorless amorphous solid (EtOAc), mp 143-145 °C (dec.); $[\alpha]_D$ –49.6 (*c* 0.59, $CHCl_3$); ^{1}H-NMR and ^{13}C-NMR data are shown in Table 2; IR (neat) 1752 (C=O) cm^{-1}; HRMS (ESI) calcd for $C_{24}H_{27}N_2O_6S$ $[M+H]^+$: 471.1590, found: 471.1556.

Preparationof Amine-Carbamothioate Analogues (**6a**–**c**)

Amine (diethylamine, pyrrolidine, or piperidine; 39-57 eq.) was added to a CH_2Cl_2 (1 mL) solution of **5** (13 mg, 0.027 mmol, 1.0 eq.) at 0 °C. After stirring at the same temperature for 1 h under an Ar atmosphere, the reaction mixture was diluted with $CHCl_3$ (30 mL). The organic layer was then separated and the aqueous layer was extracted with $CHCl_3$ (20 mL × 2). The combined extracts were sequentially washed with 5% HCl (20 mL × 3), sat.$NaHCO_3$ (20 mL × 3), and sat. NaCl (20 mL ×3) solutions. The organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo* to give a viscous oil, which was purified by SiO_2-MPLC (hexane/Me_2CO = 4:1) to give the products, **6a-c**. The yields are given in Table 1.

6a: Colorless amorphous solid (EtOAc), mp 101 °C; $[\alpha]_D$ –68.3 (*c* 0.12, $CHCl_3$); ^{1}H-NMR and ^{13}C-NMR data are shown in Table 2; IR (neat) 1752 (C=O) cm^{-1}; HRMS (ESI) calcd for $C_{25}H_{34}NO_6S$ $[M+H]^+$: 476.2107, found: 476.2100.

6b: Colorless amorphous solid (EtOAc), mp 165-171 °C $[\alpha]_D$ –0.3 (*c* 0.2, $CHCl_3$); ^{1}H-NMR (500 MHz, 300K, $CDCl_3$) δ 5.81 (1H, s), 4.71-4.64 (2H, m), 3.84-3.71 (4H, br m), 3.64-3.59 (1H, br m), 3.56 (1H, d, *J* = 5.6 Hz), 3.53 (1H, d, *J* = 1.3 Hz), 2.72-2.69 (1H, br m), 2.33-2.30 (1H, br m), 2.22-2.09 (2H, br m), 2.02-1.87 (6H, br m), 1.60-1.53 (3H, br m), 1.25 (9H, s), 1.05-1.03 (6H, m), 0.88 (4H, d, *J* = 6.9 Hz); ^{13}C-NMR (125 MHz, 300K, $CDCl_3$) δ 185.2, 173.2, 160.1, 125.5, 77.3, 77.21, 77.17, 76.8, 70.0, 63.8, 63.7, 60.1, 60.0, 55.6, 55.0, 48.5, 40.4, 35.6, 29.9, 29.7, 28.1, 25.5, 24.5, 23.4, 17.8, 17.2, 17.0, 13.6; IR (neat) 1752 (C=O) cm^{-1}; HRMS (ESI) calcd for $C_{25}H_{32}NO_6S$ $[M+H]^+$: 474.1950, found: 474.1949.

6c: Colorless amorphous solid (EtOAc), mp 114 °C; $[\alpha]_D$ –60.5 (*c* 0.44, $CHCl_3$); ^{1}H-NMR (500 MHz, 300K, $CDCl_3$) δ 5.84 (1H, s), 4.71-4.64 (2H, m), 4.22-4.18 (1H, br m), 4.08−4.04 (1H, br m), 3.94-3.89 (1H, br m), 3.84-3.838 (1H, m), 3.78-3.73 (1H, br m), 3,53-3.52 (2H, m), 2.71-2,69 (1H, br m), 2.33-2.30 (1H, m), 2.18-2.09 (2H, br m), 2.04-1.97 (1H, m), 1.92-1.87 (1H, m), 1.74-1.53 (8H, br m), 1.25 (5H, s), 1.04 (6H, d, *J* = 5.5 Hz), 0.89 (4H, d, *J* = 6.9 Hz); ^{13}C-NMR (125 MHz, 300K, $CDCl_3$) δ 187.0, 178.5, 173.2, 160.1, 125.5, 77.9, 77.25, 76.74, 76.64, 70.0, 63.78, 63.75, 60.7, 60.5, 55.7, 55.1, 51.8, 47.3, 40.3,

35.6, 29.8, 29.6, 28.3, 26.0, 25.2, 24.4, 23.4, 17.8, 17.2, 17.0, 13.6; IR (neat) 1754 (C=O) cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{34}NO_6S$ $[M+H]^+$: 488.2107, found: 488.2107.

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Conflict of Interest. The authors declare no conflict of interest.

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