Supporting Information

(*R*)- and (*S*)-3-Fluorothalidomides: Isosteric Analogs of Thalidomide

Yoshio Takeuchi,* Tomoki Shiragami, Kenichi Kimura, Emiko Suzuki, and Norio Shibata

Faculty of Pharmaceutical Sciences, Toyama Medical & Pharmaceutical University, Sugitani 2630, Toyama, 930-0194 Japan.

takeuchi@ms.toyama-mpu.ac.jp

General Information. Melting points were determined on a Yanagimoto micro-melting point apparatus and uncollected. IR spectra (cm^{-1}) were recorded on a Perkin-Elmer 1600 spectrometer. ^{1}H-NMR spectra were measured as solutions in $CDCl_3$, and chemical shifts are expressed in ppm relative to internal Me_4Si (0.00 ppm) and were recorded on a JEOL GX-270 (270 MHz) or a Varian Gemini 300 (300 MHz) spectrometer. ^{19}F-NMR spectra were measured with $CFCl_3$ as an internal standard and were taken with a JEOL GX-270 (254 MHz) spectrometer. Upfield shifts are quoted as negative δ values. EI mass spectra were taken with a JEOL JMS-D300 spectrometer. Column chromatography and preparative TLC were performed on BW-200 (Fuji Silysia) and Kieselgel 60 (Merck, art. 7748), respectively. All reactions involving oxygen- or moisture-sensitive compounds were carried out under a dry N_2 atmosphere. Unless otherwise noted, reagents were added by syringe. THF was distilled from sodium/benzophenone immediately prior to use.

3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-2-oxo-piperidin-1-carboxylic acid *tert*-butyl ester (5)

To a stirred solution of **4** (1.00 g, 4.10 mmol) in acetonitrile (30ml) was added DMAP (0.60 g, 4.91 mmol) and di-*t*-butyl dicarbonate (1.00 g, 4.58 mmol) at room temperature. After the mixture was stirred for overnight, 3% citric acid was added and the resulting mixture was extracted with AcOEt (300 ml). The organic layer was washed with brine (30 ml), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was recrystallized from ethanol to yield **5** (910mg, 92%) as colorless crystals. mp 204—207 ℃; IR (KBr) 1778, 1726, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.79 (m, 4H), 4.89 (dd, *J* = 7.0, 12.0 Hz, 1H), 3.89 (ddt, *J*= 2.0, 4.5, 13.0 Hz, 1H), 3.75 (ddt, *J* = 4.0, 11.0, 13.0 Hz, 1H), 2.33 (m, 1H), 2.06 (m, 3H), 1.51 (s, 9H); MS *m/z* 288 (M^+–*t*-Bu); HRMS calcd for $C_{14}H_{12}O_5N_2$ 288.0718, found 288.0746.

3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-3-fluoro-2-oxo-piperidin-1-carboxylic acid *tert*-butyl ester (6)

To a stirred solution of **5** (1.00 g, 2.91 mmol) in THF (20 ml) was added a 1.0 M solution of LiHMDS in THF (3.5 ml, 3.5 mmol) at –78 ℃ under nitrogen. After the mixture was allowed to warm to –40 ℃ over 20 min, gaseous $FClO_3$ [freshly generated from KF (2.00 g) and $FHSO_4$ (8.0 ml) under a stream of nitrogen at 80 ℃] was introduced for 2h. Saturated aqueous NH_4Cl (20 ml) was added to the reaction mixture. The mixture was extracted with AcOEt (200 ml). The organic layer was washed brine (50 ml), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was recrystallized from ethanol to yield **6** (750 mg, 71%) as colorless crystals. mp 203—205 ℃; IR (KBr) 1784, 1747, 1732, 1714 cm^{-1}; ^{19}F NMR ($CDCl_3$) δ –127 (ddd, *J* = 1.9, 11.1, 15.7 Hz); ^1H NMR ($CDCl_3$) δ 7.84 (m, 4H), 3.99 (dt, *J* = 6.5, 13.5 Hz, 1H), 3.58 (ddd, *J* = 6.0, 8.0, 13.5 Hz, 1H), 3.27 (ddt, *J* = 6.5, 15.0, 16.0 Hz, 1H), 2.40 (dddd, *J* = 6.0, 9.0, 11.0, 15.0 Hz, 1H), 2.10 (m, 1H), 1.87 (m, 1H), 1.58 (s, 9H); MS m/z 306 (M^+–*t*-Bu), 262 (M^+–Boc); HRMS calcd for $C_{13}H_{11}O_3N_2F$ ($C_{18}H_{19}O_5N_2F$–$C_5H_8O_2$) 262.0758, found 262.0754.

2-(3-Fluoro-2-oxo-piperidin-3-yl)-isoindol-1,3-dione (7)

A solution of **6** (700 mg, 1.93 mmol) in CH_2Cl_2 (100 ml) was stirred with TFA (3.0 ml) for 1h. The solvent was then evaporated *in vacuo* and the remaining acid was thoroughly removed by co-evaporations with toluene. The residue was recrystallized from ethanol to yield **7** (424mg, 84%) of colorless crystal. mp 156—159 ℃; IR (KBr) 3224, 1799, 1735, 1688 cm^{-1}; ^{19}F NMR ($CDCl_3$) δ –136 (dd, 12.0, 19.4 Hz); ^1H NMR ($CDCl_3$) δ 7.82 (m, 4H), 6.29 (br, 1H), 3.48 (m, 2H), 2.87 (dddd, J = 3.5, 9.0, 14.0, 19.5 Hz, 1H), 2.51 (dddd, J= 3.5, 9.0, 12.5, 14.0 Hz, 1H), 2.10 (m, 1H), 1.89 (m, 1H); MS m/z 262 (M^+); HRMS calcd for $C_{13}H_{11}O_3N_2F$ 262.0756, found 262.0753.

2-(3-Fluoro-2,6-dioxo-piperidin-3-yl)-isoindol-1,3-dione (3, 3-fluorothalidmide)

A solution of **7** (350 mg, 1.34 mmol) in AcOEt (20 ml) / CH_2Cl_2 (5 ml) was dropped into a 10% aqueous solution of $NaIO_4$ (30 ml) in the presence of RuO_2 (90.0 mg, 0.676 mmol). The reaction mixture was stirred for overnight at 40 ℃, then a small amount of 2-propanol was added. Insoluble materials were removed by filtration. The filtrate was poured into water (30 ml), and extracted with AcOEt (200 ml). The organic layer was washed with 10% $Na_2S_2O_3$ (30 ml), brine (30 ml), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was recrystallized from ethanol to furnish **3** (330 mg, 90%) as colorless crystals. mp 229—232 ℃; IR (KBr) 3297, 1794, 1742, 1726, 1702 cm^{-1}; ^{19}F NMR ($CDCl_3$) δ –132 (m); ^1H NMR ($CDCl_3$) δ 8.05 (br, 1H), 7.89 (m, 4H), 3.59 (m, 1H), 2.90 (m, 1H), 2.55 (m, 2H); MS m/z 276 (M^+); HRMS calcd for $C_{13}H_9O_4N_2F$ 276.0533, found 276.0520. Anal. Calcd for $C_{13}H_9O_4N_2F$: C, 56.53; H, 3.28; N, 10.14. Found: C, 56.42; H, 3.19; N, 9.91.

[(3*S*)-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-3-fluoro-2,6-dioxo-piperidin-1-yl]-acetic acid *tert*-butyl ester (9)

To a stirred solution of (*S*)-**3** (less polar isomer, 56.8 mg, 0.206 mmol) with K_2CO_3 (41.0 mg, 0.30 mmol) in DMF (1.0 ml) was added *tert*-butyl bromoacetate (0.044 ml, 0.30 mmol)

at room temperature. After the reaction mixture was stirred for 4 h, 1N HCl (1.0 ml) was added and the mixture was extracted with AcOEt (50 ml). The organic layer was washed water (20 ml) , brine (20 ml), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica-gel eluting with 30% AcOEt in hexane to give **9** (84.8 mg). Although this compound **9** contained some impurities, it was used for next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 7.78—7.91 (m, 4H), 4.56 (d, J = 16.5 Hz, 1H), 4.41 (d, J = 16.5 Hz, 1H), 3.56—3.66 (m, 1H), 2.92—3.03 (m, 1H), 2.62—2.69 (m, 1H), 2.44—2.57 (m, 1H), 1.46 (s, 9H).

[(3*S*)-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-3-fluoro-2,6-dioxo-piperidin-1-yl]-acetic acid (10)

TFA (1.0 ml) was added to a stirred solution of **9** (84.8 mg) in CH_2Cl_2 (1.0 ml) at room temperature. After the reaction mixture was stirred for 2 h, the solvent was evaporated *in vacuo* to furnish **10** (76.4 mg) as a crude oil, which was used for next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 7.79—7.91 (m, 4H), 4.73 (d, J = 17.5 Hz, 1H), 4.57 (d, J = 17.5 Hz, 1H), 3.59—3.68 (m, 1H), 2.94—3.05 (m, 1H), 2.64—2.71 (m, 1H), 2.44—2.60 (m, 1H).

[(3*S*)-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-3-fluoro-2,6-dioxo-piperidin-1-yl]-acetic acid 1,6-dibromonaphthalen-2-yl ester (8)

To a stirred solution of **10** (76.4 mg) in CH_2Cl_2 (3.0 ml) was added 1,6-dibromonaphthol (91.0 mg, 0.30 mmol), DMAP (5.0 mg, 0.040 mmol) and DCC (62.0 mg, 0.30 mmol) at room temperature. After the reaction mixture was stirred for 12 h, acetonitrile (5.0 ml) was added and then insoluble materials were removed by filtration through celite pad. After the solvent was evaporated off *in vacuo*, the residue was purified by chromatography on silica-gel eluting with 25% AcOEt in hexane to give a solid, which was recrystallized from AcOEt / hexane / EtOH to yield **8** as colorless crystals. mp 183—185 °C; $[\alpha]_{26}^{D}$ = –148.6° (*c* 0.35,

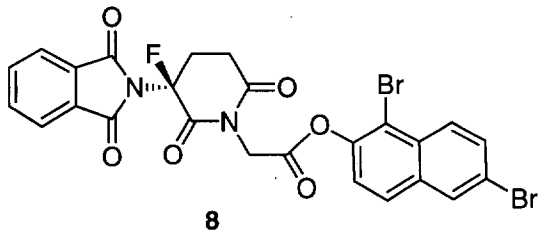
CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 8.14 (d, *J* = 8.0 Hz, 1H), 8.00 (d, *J* = 9.5 Hz, 1H), 7.80—7.93 (m, 4H), 7.66 (dd, *J* = 2.0, 9.5 Hz, 1H), 7.33 (d, *J* = 9.0 Hz, 1H), 5.08 (d, *J*= 17.0 Hz, 1H), 4.89 (d, *J* = 17.0 Hz, 1H), 3.61—3.72 (m, 1H), 2.97—3.09 (m, 1H), 2.68—2.75 (m, 1H), 2.46—2.64 (m, 1H).

Assay of tumor necrosis factor-α (TNF-α) produced from human peripheral blood lymphocytes (PBL) cultivated *in vitro*.

The PBL from four healthy male volunteer were separated from heparinized whole blood (20 ml) by a density centrifugation method using a lymphocyte separation medium (H-SMF, d=1.077,JIMRO Inc., Takasaki, Japan). PBL were cultured at 37 ℃ for 18 hours in 5% CO_2 in air at a concentration of 1 x 10^6 cells per milliliter of RPMI1640 medium (Gibco BRL, Grand Island, NY) supplemented with 10% (v/v) fetal bovine serum (ICN Biomedicals, Aurora, OH), 100 U/ml penicillin (Gibco BRL), and 100 μg/ml lipopolysaccharide (LPS, *Escherichia coli* O111:B4, Difco Laboratories, Detroit, MI) for further 6 hours in the presence or absence of Thalidomide (Tocris, Ballwin, MO), (*R*)-**3**, (*S*)-**3**, or dexamethasone (DMS, Sigma Chemicals, St. Louis, MO). The conditioned medium thus prepared were collected for assay of TNF-α level by using a commercially available ELISA kit (DuoSe T, Gemzyme, Cambridge, MA).

X-Ray analysis of (*S*)-8

The crystal used for X-ray study had dimentions of approximately 0.2 x 0.08 x 0.03 mm. Crystal data: C25H15Br2FN2O6, Mr=618.21, triclinic, space group P1, a=10.0383(15)Å, b=11.7550(12)Å, c=5.2155(19)Å, α=96.067(16)°, β=95.938(21)°, γ=75.485(10)°, *V*=590.55(21)Å3, *Z*=1, *R* [I>3.00σ(I)] =0.031, *R* w, 0.044. Data were collected at room temperature.



8

Table 1	Data Collection condition
Crystal size	0.2 x 0.08 x 0.03 mm
Radiation	Graphite - monochromated CuK α
2 θ range	4 - 120.1_
Scan method	ω - 2 θ
Scan speed	8_ / min
Temperature	23°C

Table 2	Crystal data
Formula	$C_{25}H_{15}Br_2FN_2O_6$
Formula weight	618.21
Crystal system	Triclinic
Space group	*P*1
Lattice parameters	*a* = 10.0383 (15) Å
	b = 11.7550 (12) Å
	c = 5.2155 (19) Å
	α = 96.067 (16) °
	β = 95.938 (21) °
	γ = 75.485 (10) °
	V = 590.55 (21) Å^3
Z value	1

Table 3a Result of absolute configuration analysis for model 1

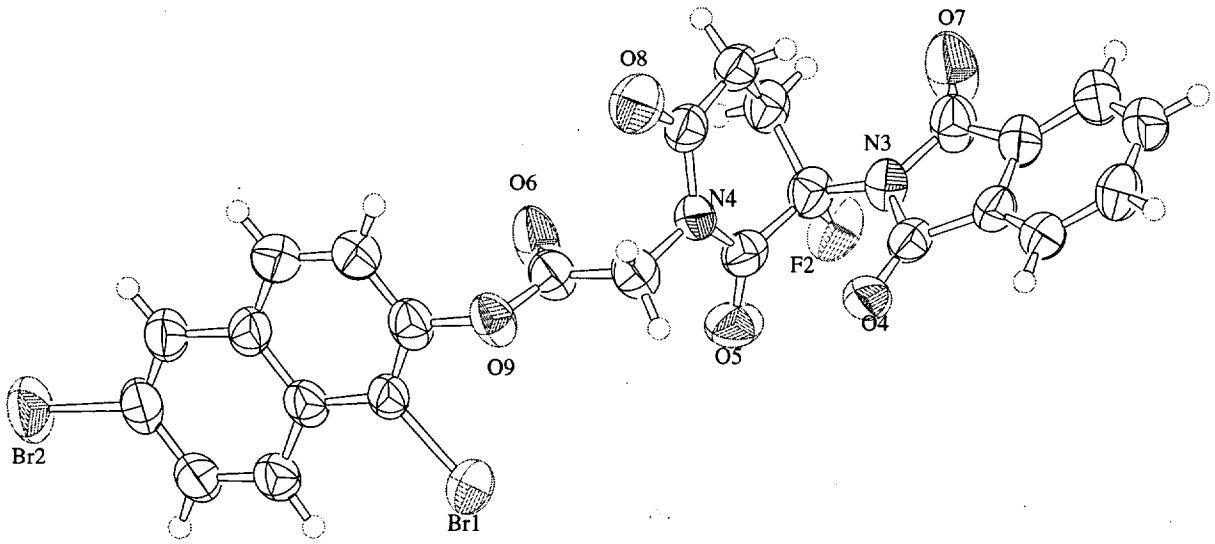
Summary	# of refls.
Reflections processed	529
Fc difference agrees with Fo:	433
Fc difference disagrees with Fo:	96

Table 3b Result of absolute configuration analysis for model 2

Summary	# of refls.
Reflections processed	525
Fc diference agrees with Fo:	99
Fc difference disagrees with Fo:	426

Table 4 Refinement Statistics

No. Observations (I > 3.00 σ (I))	71
No. Variables	322
Reflection / Parameter Ratio	9.2
R factor	0.031
weighted *R* factor	0.044
Goodness of Fit Indicator	1.17
Max. Shift / Error in Final Cycle	0.00
Max. peak in Final Diff. Map	0.34 e^- / $Å^3$
Min. peak in Final Diff. Map	-0.23 e^- / $Å^3$


O8
O7
N3
O6
N4
F2
O4
O5
O9
Br2
Br1