Tumor Necrosis Factor-Alpha Production Enhancing Activity of Substituted 3′-Methylthalidomide: Influence of Substituents at the Phthaloyl Moiety on the Activity and Stereoselectivity

Hiroyuki MIYACHI,[a] Yukiko KOISO,[a] Ryuichi SHIRAI,[a] Satomi NIWAYAMA,[b] Jun O. LIU,[b] and Yuichi HASHIMOTO*,[a]

Institute of Molecular and Cellular Biosciences, The University of Tokyo,[a] 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–0032, Japan, and Departments of Biology and Chemistry, Center for Cancer Research, Massachusetts Institute of Technology,[b] Cambridge, Massachusetts 02139, U.S.A. Received February 18, 1998; accepted March 28, 1998

The synthesis and tumor necrosis factor (TNF)-α production enhancing activity of substituted 3′-methylthalidomides on human leukemia cell line HL-60 stimulated with 12-*O*-tetradecanoyl-phorbol 13-acetate (TPA) are described. Though the introduction of an electron-donating amino group at the phthaloyl moiety of α-methylthalidomides enhanced the activity, substituted α-methylthalidomides showed decreased stereoselectivity as compared to that of non-substituted α-methylthalidomide. The data indicates that the TNF-α production enhancing activity of thalidomide derivatives depends on both the electronic-state of substituents at the fused benzene ring and the stereochemistry of the glutarimide moiety.

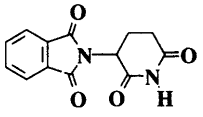
Key words tumor necrosis factor-α; 3′-methylthalidomide; human leukemia cell line; 12-*O*-tetradecanoyl-phorbol 13-acetate

Thalidomide [2-(2,6-dioxo-3-piperidinyl)-1*H*-isoindole-1,3(2*H*)-dione] (Fig. 1) was used as a hypnotic/sedative agent in the late 1950s and the early 1960s, but was withdrawn from the market because of its teratogenicity.[1] In spite of its teratogenicity, there has been a resurgence of interest in the drug in recent years due to its potential usefulness for the treatment of various diseases, including acquired immunodeficiency syndrome (AIDS),[2] graft-*versus*-host disease (GVHD),[3] leprosy,[4] and other related diseases.[5] The effectiveness of the drug in these diseases has been attributed to its regulatory activity on tumor necrosis factor-alpha (TNF-α) production.[6] TNF-α, an important cytokine produced mainly by activated monocytes/macrophages, plays a critical role in certain physiological immune systems but it causes severe damage to the host when produced in excess. Therefore, TNF-α can be regarded as possessing both favorable and unfavorable effects. These pleiotropic effects of TNF-α indicate that TNF-α production enhancers in some cases and production inhibitors in other cases would be useful as biological response modifiers (BRMs) under various circumstances.[7]

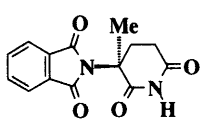
We found recently that the regulation by thalidomide of TNF-α production is bidirectional and specific to cell type and to inducer, *i.e.*, (1) thalidomide enhances 12-*O*-tetradecanoyl-phorbol 13-acetate (TPA)-induced TNF-α production by human leukemia HL-60 cells (TPA/HL-60 system), while it inhibits TPA-induced TNF-α production in another human leukemia cell line, THP-1 (TPA/THP-1 system), and (2) it inhibits TNF-α production by both HL-60 and THP-1 cells when the cells are stimulated with okadaic acid (OA) (OA/HL-60 and OA/THP-1 systems).[8] Based on these findings, we have been engaged in structural modification of thalidomide with the aim of creating superior regulators of TNF-α production. Structure–activity relationship studies of *N*-phenyl phthalimides, structurally simplified analogs of thalidomide, indicated that TNF-α production-regulating activity depends on the steric effect of the substituents introduced at the imide nitrogen moiety and the electronic effect of the substituents introduced at the phthaloyl moiety.[9,10] We also found a clear enantio-dependence of the inducer-specific bidirectional TNF-α roduction-regulating activity. In the case of the optically active thalidomide analogues, (*S*)- and (*R*)-2-(2,6-dioxo-3-methyl-3-piperidinyl)-1*H*-isoindole-1,3(2*H*)-dione (3′-methylthalidomide) (Fig. 1), only the (*S*)-form shows TNF-α production-enhancing activity in the TPA/HL-60 system. In contrast, the (*R*)-form shows much more potent TNF-α production-inhibiting activity than the (S)-form in the OA/HL-60 system.[11]

In this paper we describe the synthesis and TNF-α production-enhancing activity of novel substituted 3′-methylthalidomide derivatives in the TPA/HL-60 system.

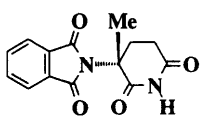
The synthetic route to the optically active 3′-methylthalidomide derivatives is shown in Chart 1. Optically pure 3-amino-3-methylpiperidine-2-ones ((*R*)-**6**, (*S*)-**6**), that were prepared by optical resolution of racemic 3-amino-3-methylpiperidine-2-one with the use of optically active binaphthyl phosphoric acid,[12] were treated with substituted phthalic anhydrides to give (*R*)- and (*S*)-**7**,



Thalidomide



(S)-3′-Methylthalidomide



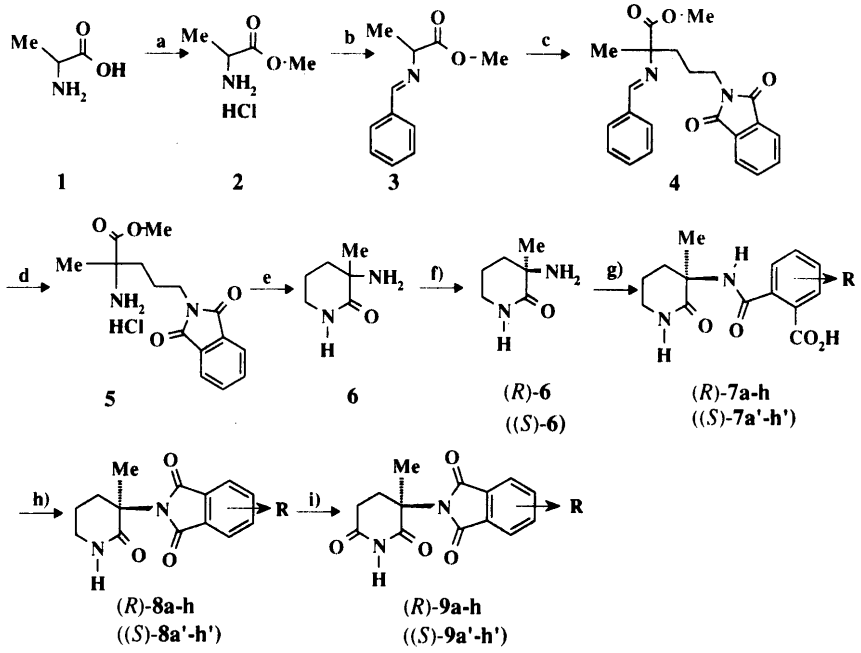
(R)-3′-Methylthalidomide

Fig. 1. Structures of Thalidomide and 3′-Methylthalidomides

* To whom correspondence should be addressed.

© 1998 Pharmaceutical Society of Japan

NII-Electronic Library Service



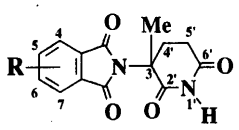
a) $SOCl_2$/MeOH b) PhCHO, TEA, $MgSO_4$/CH_2Cl_2
c) LDA, *N*-(3-Iodopropyl)phthalimide/THF
d) 1 N HCl e) Hydrazine hydrate, MeONa/ MeOH
f) Optical Resolution g) Phthalic anhydrides/1,4-dioxane
h) Heat under vacuum i) *m*-CPBA/CCl_4

Chart 1. Preparation of Substituted-3′-methylthalidomides

respectively. Compounds **7** were heated under vacuum to form the phthalimide ring ((*R*)- and (*S*)-**8**), following 3-chloroperoxybenzoic acid oxidation to give desired 3′-methylthalidomide derivatives ((*R*)- and (*S*)-**9**). The effects on TNF-α production of the synthesized compounds in the TPA/HL-60 system were measured according to the previously described method[7] and the results are summarized in Table 1. As shown in Table 1, the (*R*)-form of non-substituted 3′-methylthalidomide ((*R*)-**9a**) showed no or very weak enhancement on TNF-α production by TPA-stimulated HL-60 cells at the concentration range investigated (1 μM to 30 μM). Introduction of substituents at the phthaloyl moiety of (*R*)-**9a** generally caused the appearance of TNF-α production enhancing activity, and the compounds that have an electron donating amino group at the 4-position ((*R*)-**9c**) showed the most potent activity (552% at 1 μM). The 4-substituted analogs ((*R*)-**9b**, (*R*)-**9c**) show higher activity than the corresponding 5-substituted analogs ((*R*)-**9e**, (*R*)-**9f**).

As already reported,[9] the (*S*)-form of non-substituted 3′-methylthalidomide ((*S*)-**9a**) showed dose dependent enhancement on TNF-α production in the TPA/HL-60 system at the concentration range investigated (1 μM to 30 μM). In the (*S*)-series, introduction of an electron-donating amino group ((*S*)-**9c**, (*S*)-**9f**) also increased the activity considerably. But contrary to the results obtained from the (*R*)-series of compounds, other substituents ((*S*)-**9b**, (*S*)-**9d**, (*S*)-**9e**, (*S*)-**9g**, (*S*)-**9h**) exhibited less potent TNF-α production enhancing activity as compared to that of (*S*)-**9a**. Therefore, in each enantiomer, introduction

Table 1. TNF-α Production Enhancing Activity of 3′-Methylthalidomides



Compd	R	Amount of TNF-a (%)[a]					
		0.1 μM[b]	0.3 μM[b]	1 μM[b]	3 μM[b]	10 μM[b]	30 μM[b]
None		..... 100 (69 pg/ml)					
(*R*)-**9a**	H	—	—	93	93	92	114
(*S*)-**9a**	H	—	—	223	385	619	480
(*R*)-**9b**	4-NO_2	—	—	—	—	169	352
(*S*)-**9b**	4-NO_2	—	—	—	—	312	486
(*R*)-**9c**	4-NH_2	128	208	552	514	—	—
(*S*)-**9c**	4-NH_2	258	695	845	881	—	—
(*R*)-**9d**	4-F	—	—	—	—	120	135
(*S*)-**9d**	4-F	—	—	—	—	276	301
(*R*)-**9e**	5-NO_2	—	—	—	—	151	225
(*S*)-**9e**	5-NO_2	—	—	—	—	310	355
(*R*)-**9f**	5-NH_2	106	103	132	135	—	—
(*S*)-**9f**	5-NH_2	116	253	486	526	—	—
(*R*)-**9g**	5-Me	—	—	—	—	189	321
(*S*)-**9g**	5-Me	—	—	—	—	370	453
(*R*)-**9h**	4,5,6,7-F	—	—	127	409	173[c]	—
(*S*)-**9h**	4,5,6,7-F	—	—	152	358	145[c]	—

a) The amount of TNF-α produced in the presence of 10 nM TPA alone was defined as 100%. *b*) Concentration of test compounds. *c*) This compound showed cytotoxicity at concentrations higher than 10 μM.

NII-Electronic Library Service

of an electron-donating amino group increased TNF-α production enhancing activity. It is interesting to note that in a series of *N*-phenylphthalimide derivatives, introduction of an electron-withdrawing nitro group increased the enhancing activity, and introduction of an electron-donating amino group decreased the activity.[9] These contradictory results, *i.e.*, different structure–activity relationships, might indicate that the target molecule(s) of these two types of compounds are different between *N*-phenylphthalimides and 3′-methylthalidomides, although the elicited biological response is the same. If this is the case, the target molecule of *N*-phenylphthalimides would seem to prefer electron withdrawing groups on the phthaloyl moiety and recognize only the (*S*)-isomer. On the other hand, the target molecule of 3′-methylthalidomides would prefer electron donating groups on the phthaloyl moiety.

As for enantioselectivity, non-substituted α-methylthalidomides ((*R*)-**9a**, (*S*)-**9a**) showed distinct enantioselective TNF-α production enhancing activity. The maximum selectivity ratio was 6.73 at 10 μM. Introduction of substituents at the phthaloyl moiety generally decreased the stereoselectivity, except 5-amino-3′-methylthalidomides ((*R*)-**9f**, (*S*)-**9f**) (maximum selectivity ratio was 3.90 at 3 μM).

In conclusion, we have developed 3′-methylthalidomide analogs, (*S*)- and (*R*)-[2-(2,6-dioxo-3-methyl-3-piperidinyl)-5-amino-1*H*-isoindole-1,3(2*H*)-dione] (5-amino-3′-methylthalidomides (*S*)-**9f** and (*R*)-**9f**, respectively), which possesses more potent TNF-α production enhancing activity while retaining stereoselectivity on TPA-stimulated HL-60 cells. Further pharmacological evaluation is in progress, including inducer- and cell-type-specific bidirectional TNF-α production.

Experimental

General Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H-NMR spectra were measured in $CDCl_3$ with tetramethylsilane and/or the solvent peak as the internal standard, on a JEOL JNM-A500 spectrometer. Mass spectra (MS) were obtained on a JEOL JMS-HX110 spectrometer. Optical rotations were measured on a JASCO DIP-100 digital polarimeter. Column chromatography was carried out on Merck Silica gel 60. Analytical thin-layer chromatography (TLC) was performed on Merck precoated Silica gel $60F_{254}$ plates, and the compounds were visualized by UV illumination (254 nm) or by heating to 150 °C after spraying with phosphomolybdic acid in ethanol. Elemental analysis were performed in the microanalytical laboratory of the Institute of Molecular and Cellular Biosciences, The University of Tokyo.

Cells and Measurement of TNF-α HL-60 cells were maintained as previously described.[7] The exponentially growing cells in RPMI1640 medium supplemented with 10% v/v fetal bovine serum (2.5×10^5 cells in 0.5 ml) were treated or not treated with TPA or OA for 16 h at 37 °C using 24-well multidish plates. For testing the effects of compounds, cells were treated with TPA (10 nM) as an inducer in the presence or absence of a sample compound at the concentrations indicated in Table 1. Then the number of cells was counted, the cellular morphology was checked under a microscope, and the cells were collected by centrifugation (2000 rpm × 10 min). The amount of TNF-α in the supernatant was measured by the use of a human TNF-α ELISA system (Amersham Co.) according to the supplier's protocol. The amount of TNF-α produced in the presence of inducer alone was defined as 100%. The amount of TNF-α produced by stimulated HL-60 cells in the presence of test compounds showed some variation from experiment to experiment. However, the order of efficacy of the test compounds was reproducible. Therefore, typical data obtained in experiments performed at the same time are presented. In each set of experiments, the assay was performed in duplicate or triplicate (the mean value was taken) and at least three different times. A typical set of data obtained at the same time is presented in Table 1.

Chemicals The compounds listed in Table 1 were prepared by condensation of optically pure 3-amino-3-methylpiperidine-2-ones ((*R*)-**6**, (*S*)-**6**) and substituted phthalic anhydrides, followed by imide formation by heating under vacuum and 3-chloroperoxybenzoic acid oxidation (overall yield 20—30%). The products were purified by silica gel column chromatography and recrystallization.

(*R*)-3′-Methyl-4-nitrothalidomide ((*R*)-**9b**): mp 153—155 °C (from ethanol/acetone); High MS (EI+) *m/z* Calcd for $C_{14}H_{11}N_3O_6$ 317.0648, Found 317.0650; $[\alpha]_D^{25}$ −23.3°(*c*=0.064, *N*,*N*-dimethylformamide); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.09 (3H, s), 2.13—2.17 (2H, m), 2.72—2.80 (2H, m), 7.94 (1H, t, *J*=7.81 Hz), 7.96 (1H, br s), 8.11 (2H, dt, *J*=7.81, 0.98 Hz); *Anal.* Calcd for $C_{14}H_{11}N_3O_6$: C, 53.00; H, 3.49; N, 13.25, Found: C, 52.93; H, 3.43; N, 13.21.

(*S*)-3′-Methyl-4-nitrothalidomide ((*S*)-**9b**): mp 153—155 °C (from ethanol/acetone);/High MS (EI+) *m/z* Calcd for $C_{14}H_{11}N_3O_6$ 317.0648, Found 317.0666; $[\alpha]_D^{25}$ 22.2° (*c*=0.08, *N*,*N*-dimethylformamide); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.09 (3H, s), 2.13—2.17 (2H, m), 2.72—2.80 (2H, m), 7.94 (1H, t, *J*=7.81 Hz), 7.96 (1H, br s), 8.11 (2H, dt, *J*=7.81, 0.98 Hz); *Anal.* Calcd for $C_{14}H_{11}N_3O_6$: C, 53.00; H, 3.49; N, 13.25. Found: C, 52.84; H, 3.53; N, 12.99.

(*R*)-4-Amino-3′-methylthalidomide ((*R*)-**9c**): mp 228—230 °C (from ethanol/acetone); High MS (EI+) *m/z* Calcd for $C_{14}H_{13}N_3O_4$ 287.0906, Found 287.0906; $[\alpha]_D^{25}$ −25.2° (*c*=0.08, *N*,*N*-dimethylformamide); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.02 (3H, s), 2.03—2.10 (1H, m), 2.67—2.71 (2H, m), 2.80—2.87 (1H, m), 5.23 (2H, br s), 6.86 (1H, d, *J*=8.30 Hz), 7.11 (1H, d, *J*=6.84 Hz), 7.43 (1H, t, *J*=8.30 Hz), 7.89 (1H, br s); *Anal.* Calcd for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56; N, 14.63. Found: C, 58.38; H, 4.95; N, 13.33.

(*S*)-4-Amino-3′-methylthalidomide ((*S*)-**9c**): mp 228—230 °C (from ethanol/acetone); High MS (EI+) *m/z* Calcd for $C_{14}H_{13}N_3O_4$ 287.0906, Found 287.0937; $[\alpha]_D^{25}$ 26.2° (*c*=0.07, *N*,*N*-dimethylformamide); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.02 (3H, s), 2.03—2.10 (1H, m), 2.67—2.71 (2H, m), 2.80—2.87 (1H, m), 5.23 (2H, br s), 6.86 (1H, d, *J*=8.30 Hz), 7.11 (1H, d, *J*=7.81 Hz), 7.42 (1H, dd, *J*=8.30, 732 Hz), 7.89 (1H, br s); *Anal.* Calcd for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56; N, 14.63. Found: C, 58.28; H, 4.82; N, 13.55.

(*R*)-4-Fluoro-3′-methylthalidomide ((*R*)-**9d**): mp 193—194 °C (from ethyl acetate/acetone); High MS (EI+) *m/z* Calcd for $C_{14}H_{11}FN_2O_4$ 290.0703, Found 290.0711; $[\alpha]_D^{25}$ −27.7°(*c*=0.09, *N*,*N*-dimethylformamide); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.06 (3H, s), 2.09—2.17 (1H, m), 2.70—2.73 (2H, m), 2.75—2.83 (1H, m), 7.41 (1H, t, *J*=8.30 Hz), 7.66 (1H, d, *J*=6.84 Hz), 7.73—7.78 (1H, m), 7.86 (1H, br s); *Anal.* Calcd for $C_{14}H_{11}FN_2O_4 1/4CH_3CO_2C_2H_5$: C, 57.69; H, 4.20; N, 8.97. Found: C, 57.39; H, 4.25; N, 8.80.

(*S*)-4-Fluoro-3′-methylthalidomide ((*S*)-**9d**): mp 193—194 °C (from ethyl acetate/acetone); High MS (EI+) *m/z* Calcd for $C_{14}H_{11}FN_2O_4$ 290.0703, Found 290.0710; $[\alpha]_D^{25}$ 26.0° (*c*=0.06, *N*,*N*-dimethylformamide); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.06 (3H, s), 2.09—2.14 (1H, m), 2.70—2.74 (2H, m), 2.76—2.82 (1H, m), 7.40 (1H, t, *J*=8.30 Hz), 7.65 (1H, d, *J*=6.84 Hz), 7.73—7.78 (1H, m), 8.03 (1H, br s); *Anal.* Calcd for $C_{14}H_{11}FN_2O_4 1/4CH_3CO_2C_2H_5$: C, 57.69; H, 4.20; N, 8.97. Found: C, 57.29; H, 4.20; N, 8.77.

(*R*)-3′-Methyl-5-nitrothalidomide ((*R*)-**9e**): mp 293—295 °C (from ethanol/acetone); High MS (EI+) *m/z* Calcd for $C_{14}H_{11}N_3O_6$ 317.0648, Found 317.0627; $[\alpha]_D^{25}$ −29.2°(*c*=0.166, *N*,*N*-dimethylformamide); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.10 (3H, s), 2.14—2.18 (1H, m), 2.73—2.81 (3H, m), 7.88 (1H, br s), 8.04 (1H, d, *J*=8.79 Hz), 8.63 (1H, dd, *J*=8.30, 1.95 Hz), 8.65 (1H, d, *J*=1.47 Hz); *Anal.* Calcd for $C_{14}H_{11}N_3O_6 1/6H_2O$: C, 52.50; H, 3.57; N, 13.12. Found: C, 52.44; H, 3.46; N, 12.85.

(*S*)-3′-Methyl-5-nitrothalidomide ((*S*)-**9e**): mp 293—295 °C (from ethanol/acetone); High MS (EI+) *m/z* Calcd for $C_{14}H_{11}N_3O_6$ 317.0648, Found 317.0626; $[\alpha]_D^{25}$ 27.9° (*c*=0.146, *N*,*N*-dimethylformamide); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.10 (3H, s), 2.14—2.17 (1H, m), 2.74—2.81 (3H, m), 7.88 (1H, br s), 8.04 (1H, d, *J*=8.79 Hz), 8.63 (1H, dd, *J*=8.30, 1.95 Hz), 8.65 (1H, d, *J*=1.47 Hz); *Anal.* Calcd for $C_{14}H_{11}N_3O_6 1/3H_2O$: C, 52.02; H, 3.64; N, 13.00. Found: C, 52.10; H, 3.43; N, 12.74.

(*R*)-5-Amino-3′-methylthalidomide ((*R*)-**9f**): mp 272—274 °C (from ethanol/acetone); High MS (EI+) *m/z* Calcd for $C_{14}H_{13}N_3O_4$ 287.0906,

NII-Electronic Library Service

Found 287.0923; $[\alpha]_D^{25}$ −30.2° (c=0.08, *N*,*N*-dimethylformamide); ^{1}H-NMR (500 MHz, DMSO-d_6) δ 1.85 (3H, s), 1.97—2.00 (1H, m), 2.45—2.56 (2H, m), 2.67—2.73 (1H, m), 6.52 (2H, s), 6.80 (1H, dd, J=8.30, 1.96 Hz), 6.88 (1H, d, J=1.96 Hz), 7.44 (1H, d, J=8.30 Hz), 10.9 (1H, s); *Anal.* Calcd for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56; N, 14.63. Found: C, 58.04; H, 4.90; N, 13.13.

(*S*)-5-Amino-3′-methylthalidomide ((*S*)-**9f**): mp 272—274 °C (from ethanol/acetone); High MS (EI+) m/z Calcd for $C_{14}H_{13}N_3O_4$ 287.0906, Found 287.0916; $[\alpha]_D^{25}$ 29.0° (c=0.07, *N*,*N*-dimethylformamide); ^{1}H-NMR (500 MHz, DMSO-d_6) δ 1.85 (3H, s), 1.97—2.09 (1H, m), 2.45—2.56 (2H, m), 2.64—2.73 (1H, m), 6.52 (2H, s), 6.80 (1H, dd, J=8.30, 1.96 Hz), 6.88 (1H, d, J=1.96 Hz), 7.45 (1H, d, J=8.30 Hz), 10.9 (1H, s); *Anal.* Calcd for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56; N, 14.63. Found: C, 58.14; H, 4.89; N, 13.30.

(*R*)-3′,5-Dimethylthalidomide ((*R*)-**9g**): mp 221—223 °C (from ethanol/acetone); High MS (EI+) m/z Calcd for $C_{15}H_{14}N_2O_4$ 286.0954, Found 287.0963; $[\alpha]_D^{25}$ −26.0° (c=0.11, *N*,*N*-dimethylformamide); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.04 (3H, s), 2.06—2.12 (1H, m), 2.51 (3H, s), 2.67—2.72 (2H, m), 2.75—2.85 (1H, m), 7.53 (1H, d, J=7.81 Hz), 7.62 (1H, s), 7.70 (1H, d, J=7.81 Hz), 7.88 (1H, br s); *Anal.* Calcd for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.79. Found: C, 62.60; H, 4.87; N, 9.65.

(*S*)-3′,5-Dimethylthalidomide ((*S*)-**9g**): mp 221—223 °C (from ethanol/acetone); High MS (EI+) m/z Calcd for $C_{15}H_{14}N_2O_4$ 286.0954, Found 287.0983; $[\alpha]_D^{25}$ 25.0° (c=0.10, *N*,*N*-dimethylformamide); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.04 (3H, s), 2.06—2.16 (1H, m), 2.51 (3H, s), 2.67—2.72 (2H, m), 2.75—2.85 (1H, m), 7.53 (1H, d, J=7.81 Hz), 7.62 (1H, s), 7.70 (1H, d, J=7.81 Hz), 7.91 (1H, br s); *Anal.* Calcd for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.79. Found: C, 62.56; H, 4.87; N, 9.68.

(*R*)-4,5,6,7-Tetrafluoro-3′-methylthalidomide ((*R*)-**9h**): mp 200—201 °C (from ethanol/acetone); High MS (EI+) m/z Calcd for $C_{14}H_8F_4N_2O_4$ 344.0420. Found 344.0433; $[\alpha]_D^{25}$ −29.8° (c=0.155, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.05 (3H, s), 2.11—2.15 (1H, m), 2.71—2.76 (3H, m), 7.97 (1H, br s); *Anal.* Calcd for $C_{14}H_8F_4N_2O_4$: C, 48.85; H, 2.34; N, 8.14. Found: C, 48.95; H, 2.47; N, 8.30.

(*S*)-4,5,6,7-Tetrafluoro-3′-methylthalidomide ((*S*)-**9h**): mp 200—201 °C (from ethanol/acetone); High MS (EI+) m/z Calcd for $C_{14}H_8F_4N_2O_4$ 344.0420, Found 344.0401; $[\alpha]_D^{25}$ 30.6° (c=0.105, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.05 (3H, s), 2.11—2.15 (1H, m), 2.71—2.76 (3H, m), 7.97 (1H, br s); *Anal.* Calcd for $C_{14}H_8F_4N_2O_4$: C, 48.85; H, 2.34; N, 8.14. Found: C, 49.05; H, 2.47; N, 7.92.

Physicochemical properties of (*R*)-**9a** and (*S*)-**9a** have been described previously.[13]

References

1) Kelsey F. O., *Teratogenicity*, **38**, 221—226 (1988).
2) Makonkawkeyoon S., Limon-Pombre R. N. R., Moreira A. L., Schauf V., Kaplan G., *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 5974—5978 (1993).
3) Perker P. M., Chao N., Nademanee A., O'Donnell M., Schmidt G. M., Snyder D.S., Stein A. S., Smith E. P., Molina A., Stepan D. E., Kashyap A., Planas I., Spielberger R., Somlo G., Margolin K., Zwingenberger K., Wilsman K., Negrin R. S., Long G., Niland J. C., Blume K. G., Forman S. J., *Blood*, **86**, 3604—3609 (1995).
4) Atra E., Sato E. I., *Clin. Exp. Rheumatol.*, **11**, 487—493 (1993).
5) Ochonisky S., Verroust J., Bastuji-Garin S., Gherardi R., Revuz J., *Arch. Dermatol.*, **130**, 66—69 (1994).
6) Sampio E. P., Sarno E.N., Galilly R., Cohn Z. A., Kaplan G., *J. Exp. Med.*, **173**, 699—703 (1991).
7) Shibata Y., Sasaki K., Hashimoto Y., Iwasaki S., *Biochim. Biophys. Res. Commun.*, **205**, 1992—1997 (1994).
8) Miyachi H., Azuma A., Hioki E., Iwasaki S., Kobayashi Y., Hashimoto Y., *Biochem. Biophys. Res. Commun.*, **224**, 426—430 (1996).
9) Miyachi H., Azuma A., Ogasawara A., Uchimura E., Watanabe N., Kobayashi Y., Kato F., Kato M., Hashimoto Y., *J. Med. Chem.*, **40**, 2858—2865 (1997).
10) Miyachi H., Azuma A., Hashimoto Y., *Yakugaku Zasshi*, **117**, 91—107 (1997).
11) Miyachi H., Azuma A., Hioki E., Iwasaki S., Kobayashi Y., Hashimoto Y., *Biochem. Biophys. Res. Commun.*, **224**, 426—430 (1996).
12) Knabe J., Omlor G., *Arzneim. Forsch.*, **40**, 32—37 (1990).
13) Nishimura K., Hashimoto Y., Iwasaki S., *Chem. Pharm. Bull.*, **42**, 1157—1159 (1994).

NII-Electronic Library Service