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ENANTIO-DEPENDENCE OF INDUCER-SPECIFIC BIDIRECTIONAL REGULATION OF TUMOR NECROSIS FACTOR (TNF)-ALPHA PRODUCTION: POTENT TNF-α PRODUCTION INHIBITORS

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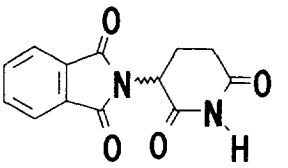
Abstract: In optically active phthalimide analogs of thalidomide, the inducer-specific TNF-α production-enhancing and -inhibiting activities are separated. This implies that the target molecules of the two activities are different. Copyright © 1996 Elsevier Science Ltd

Thalidomide [N(α)-phthalimidoglutarimide, **1**] is a hypnotic/sedative agent which has been withdrawn from the market because of its teratogenicity.[1] In spite of this, there has been a resurgence of interest in the drug in recent years due to its potential for the treatment of acquired immunodeficiency syndrome (AIDS), graft-*versus*-host disease (GVHD), Behcet's disease, leprosy, and other related diseases.[2-6] The effectiveness of the drug in these diseases has been attributed to its inhibitory activity on tumor necrosis factor (TNF)-α production.[2,7,8] TNF-α is a pleiotropic cytokine produced by activated macrophages, and has been regarded as an attractive target of biological response modifiers (BRMs).[9]

Recently, we have reported that the TNF-α production-regulating activity of **1** is inducer-specific and bidirectional, *i.e.*, **1** inhibits TNF-α production by human leukemia HL-60 cells when the cells are stimulated with okadaic acid (OA), while it enhances TNF-α production by the same cell line when the cells are stimulated with 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[10] From the standpoint of medicinal chemistry, it would be of great benefit if the bidirectional TNF-α production-regulating activity could be separated, because inhibition, but not enhancement, of TNF-α production is considered to be the mechanism of the beneficial effects of **1**.[2,6,7]

Though the widely prevailing hypothesis is that only one optical isomer of **1** is biologically active, it is difficult to observe the biological activity of each isomer separately, because racemization of **1** is fast under physiological conditions.[11] To overcome this problem, we previously prepared (S)- and (R)-α-methyl-N(α)-phthalimidoglutarimides [(S)-**2** and (R)-**2**], which do not



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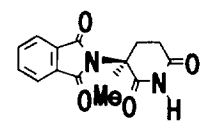
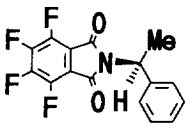
racemize.[11] Analysis of the TNF-α production-regulating activity of (S)-**2** and (R)-**2** using HL-60 cells indicated that only (S)-**2** is active in enhancement of TPA-induced TNF-α production,[11] while both (S)-**2** and (R)-**2** are active in inhibition of OA-induced TNF-α production, with the latter possessing higher activity than the former. Based on these previous results, we anticipated that separation of the bidirectional TNF-α production-regulating activity could be achieved by the use of optically active phthalimide analogs.

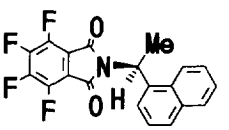
We are interested in structural modification of **1** and have prepared various phthalimide derivatives which possess TNF-α production-regulating activity.[12-17] These previous studies provided some indications for designing new analogs, *i.e.*, (i) introduction of fluorine into the phthalimide moiety enhances the activity and selectivity of the compounds, and (ii) introduction of a methyl group at the α-position enhances the activity. Accordingly, we have designed and prepared optically pure forms of 2-(1-phenylethyl)-4,5,6,7-tetrafluoro-1*H*-isoindole-1,3-dione (FPTP : **3**), 2-(1-naphthylethyl)-4,5,6,7-tetrafluoro-1*H*-isoindole-1,3-dione (FPTN : **4**), and 2-(1-cyclohexylethyl)-4,5,6,7-tetrafluoro-1*H*-isoindole-1,3-dione (FPTH : **5**). In this paper, we report that the inducer-specific TNF-α production-enhancing and -inhibiting activities are separated in the optically pure forms of these tetrafluoro-phthalimide analogs.



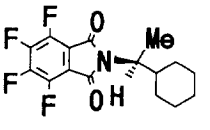
(S)-**2**



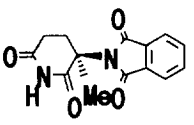
(S)-**3** : (S)-FPTP



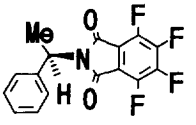
(S)- **4** : (S)-FPTN



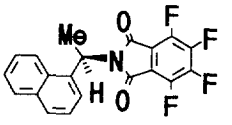
(S)-**5** : (S)-FPTH



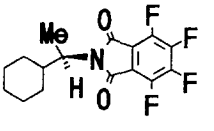
(R)-**2**



(R)-**3** : (R)-FPTP



(R)- **4** : (R)-FPTN



(R)-**5** : (R)-FPTH

The (S)- and (R)-forms of compounds **3**-**5** were prepared by condensation of phthalic anhydride with appropriate optically pure isomers of amines. The chemical and analytical data of the prepared compounds are given in the *Notes*.[18]

TNF-α production-regulating activity of the prepared compounds was assayed as described previously.[12-17] Briefly, exponentially growing HL-60 cells (1 x 10^5 cells/ml RPMI-1640 medium containing 10% v/v fetal bovine serum) were treated with TPA (10 nM) or OA (50 nM) in the absence or presence of various concentrations of test compounds at 37°C for 16

h. The cells were collected by centrifugation (2000 rpm), and the amount of produced TNF-α in the supernatant was measured with an ELISA system (Amersham, Human TNF-α ELISA Kit) according to the supplier's protocol. The amount of TNF-α is presented as a percentage of the amount produced in the presence of stimulator (TPA or OA) alone, taken as 100%. The results are shown in Table I and Fig. 1.

As shown in the Table I, only the (S)-forms of **2**, **3**, and **5** showed enhancing activity in TPA-induced TNF-α production. The (R)-forms were all inactive. In the case of **4**, the (S)- and (R)-forms were both inactive. Though the percentages of TNF-α production enhancement by the (S)-forms of **3** and **5** were not high (less than 300%) compared with that of (S)-**2** (358%), they elicit the activity at much lower concentration (300 nM) than in the case of (S)-**2** (30 μM). (S)-**3** and (S)-**5** showed enhancing activity comparable to that of **1** at one hundredth of the concentration of **1**. The effectiveness of the tetrafluorinated analogs at very low concentrations is in accordance with our previous results on the structure-activity relationships of phthalimide analogs.[13-18]

Table I. Inducer-Specific Bidirectional Regulation of TNF-α Production by HL-60 Cells.

Compound	Concentration	Amount of TPA-induced TNF-α %[a]	Amount of OA-induced TNF-α %[b]
1	30 μM	135	58
(S)-**2**	30 μM	**358**	75
(R)-**2**	30 μM	103	**39**
(S)-FPTP [(S)-**3**]	0.3 μM	**143**	70
(R)-FPTP [(R)-**3**]	0.3 μM	97	**2**
(S)-FPTN [(S)-**4**]	0.3 μM	101	101
(R)-FPTN [(R)-**4**]	0.3 μM	100	**2**
(S)-FPTH [(S)-**5**]	0.3 μM	**124**	65
(R)-FPTH [(R)-**5**]	0.3 μM	98	**10**

a. The amount of TNF-α produced by HL-60 cells in the presence of TPA (10 nM) alone was defined as 100%.

b. The amount of TNF-α produced by HL-60 cells in the presence of OA (50 nM) alone was defined as 100%.

In contrast, the (R)-forms of compounds **2**, **3**, and **5** showed more potent inhibitory activity than the corresponding (S)-isomers on OA-induced TNF-α production, at the concentrations at which the (S)-isomers elicit enhancing activity on TPA-induced TNF-α production In the case of compound **4**, of which the (S)- and (R)-isomers were both inactive in enhancement of TPA-induced TNF-α production, the (S)-isomer was inactive and the (R)-isomer was a very potent inhibitor of OA-induced TNF-α production, like (R)-**3**. That is, addition of 300 nM (R)-**3** or (R)-**4** completely inhibited the TNF-α production (the dose response curves are shown in Fig. 1).

The results indicate that the bidirectional TNF-α production-regulating activities can be separated by the use of optically active phthalimide analogs, **2**-**5**. The separation of these activities implies that the target molecules of these compounds (and also of **1**) for enhancement of TPA-induced TNF-α production and inhibition of OA-induced TNF-α production are different. The target molecule(s) in the former system (denoted as an "enhancing factor" in this paper) should strictly recognize only the (S)-isomers of **2**-**5**, while that in the latter system (denoted as an "inhibiting factor" in this paper) favors the (R)-isomers over the corresponding (S)-isomers.

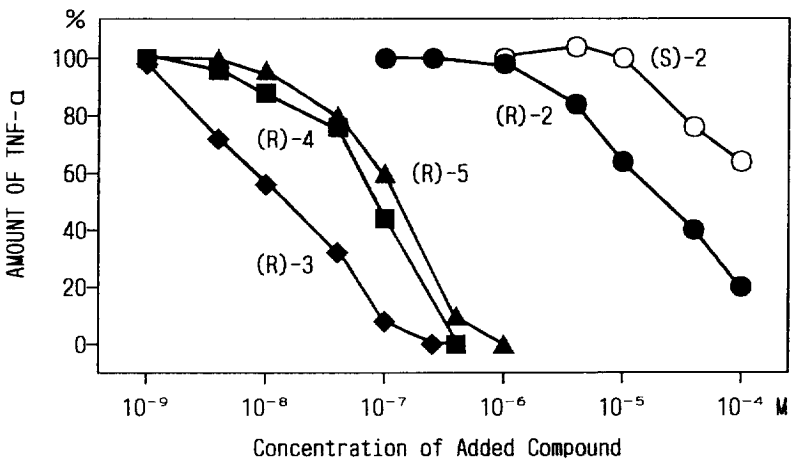


Fig. 1. Inhibition of OA-Induced TNF-α Production

In the case of thalidomide (**1**), a racemic mixture, the "enhancing" and "inhibiting" factors would both be activated. This implies that the "enhancing factor" should be dominant in the TPA-induced TNF-α production system, while the "inhibiting factor" should be

dominant in the OA-induced TNF-α production system. Though other explanations are possible, this is an attractive working hypothesis which can be tested by searching for and characterizing the putative "enhancing" and "inhibiting" factors in HL-60 cells.

As shown in Fig. 1, (R)-**3**, (R)-**4** and (R)-**5** are potent inhibitors of OA-induced TNF-α production, without no enhancing activity on TPA-induced TNF-α production (Table I). Though the (S)-isomers of **3**, **5**, and **2** (and **1**) showed moderate inhibitory activity on OA-induced TNF-α production and enhancing activity on TPA-induced TNF-α production, (S)-**4** showed no activity in either system. These optically active phthalimide analogs with TNF-α production-regulating activity seem to be superior lead compounds in the development of BRMs targeting TNF-α as novel drugs for the treatment of immunodiseases, and should also be useful in investigation of the regulatory mechanisms of TNF-α production.

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18) (S)-2-(1-Phenylethyl)-4,5,6,7-tetrafluoro-1*H*-isoindole-1,3-dione [(S)-FPTP-00, (S)-**3**]: m.p. 95–96°C. $[\alpha]^{20}_D$ = −42.2° (c = 0.386, AcOEt). M^+ = 323. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 1.91 (3H, d, J = 7.32 Hz), 5.53 (1H, q, J = 7.32 Hz), 7.29–7.37 (3H, m), 7.48 (2H, d, J = 7.32 Hz). Anal. calcd for $C_{16}H_9F_4NO_2$: C, 59.45; H, 2.81; N, 4.33. Found: C, 59.50; H, 2.81; N, 4.36.

(R)-2-(1-Phenylethyl)-4,5,6,7-tetrafluoro-1*H*-isoindole-1,3-dione [(R)-FPTP-00, (R)-**3**]: m.p. 95.5–96°C. $[\alpha]^{20}_D$ = 41.5° (c = 0.348, AcOEt). M^+ = 323. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 1.92 (3H, d, J = 7.32 Hz), 5.53 (1H, q, J = 7.32 Hz), 7.29–7.37 (3H, m), 7.48 (2H, d, J = 7.32 Hz). Anal. calcd for $C_{16}H_9F_4NO_2$: C, 59.45; H, 2.81; N, 4.33. Found: C, 59.41; H, 2.88; N, 4.45.

(S)-2-(1-Naphthylethyl)-4,5,6,7-tetrafluoro-1*H*-isoindole-1,3-dione [(S)-FPTN-00, (S)-**4**]: b.p. 240°C (1 mmHg). $[\alpha]^{20}_D$ = −42.1° (c = 0.097, EtOH). M^+ = 373. ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 2.01 (3H, d, J = 6.84 Hz), 6.28 (1H, q, J = 6.84 Hz), 7.46 (1H, t, J =6.84 Hz), 7.50–7.54 (2H, m), 7.84 (2H, t, J = 8.30 Hz), 7.97 (1H, d, J = 7.32.Hz), 8.10 (1H, t, J = 8.30 Hz). Anal. calcd for $C_{20}H_{11}F_4NO_2$: C, 64.35; H, 2.97; N, 3.75. Found: C, 64.35; H, 2.92; N, 3.90.

(R)-2-(1-Naphthylethyl)-4,5,6,7-tetrafluoro-1*H*-isoindole-1,3-dione [(R)-FPTN-00, (R)-**4**]: b.p. 240°C (1 mmHg). $[\alpha]^{20}_D$ = 40.9° (c = 0.089, EtOH). M^+ = 373. ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 2.02 (3H, d, J = 6.84 Hz), 6.29 (1H, q, J = 6.84 Hz), 7.47 (1H, t, J =6.84 Hz), 7.51–7.55 (2H, m), 7.85 (2H, t, J = 8.30 Hz), 7.98 (1H, d, J = 7.32.Hz), 8.10 (1H, t, J = 8.30 Hz). Anal. calcd for $C_{20}H_{11}F_4NO_2$: C, 64.35; H, 2.97; N, 3.75. Found: C, 64.35; H, 2.92; N, 3.82.

(S)-2-(1-Cyclohexylethyl)-4,5,6,7-tetrafluoro-1*H*-isoindole-1,3-dione [(S)-FPTH, (S)-**5**]: m.p. 147–148°C. $[\alpha]^{20}_D$ = 5.26° (c = 0.618, AcOEt). M^+ = 329. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 0.86–1.00 (2H, m), 1.10–1.28 (3H, m), 1.44 (3H, d, J = 6.84 Hz), 1.52–2.00 (5H, m), 3.94–4.00 (1H, m). Anal. calcd for $C_{16}H_{15}F_4NO_2$: C, 58.36; H, 4.59; N, 4.25. Found: C, 58.51; H, 4.69; N, 4.25.

(R)-2-(1-Cyclohexylethyl)-4,5,6,7-tetrafluoro-1*H*-isoindole-1,3-dione [(R)-FPTH, (R)-**5**]: m.p. 147–148°C. $[\alpha]^{20}_D$ = −5.13° (c = 0.658, AcOEt). M^+ = 329. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 0.86–1.00 (2H, m), 1.10–1.28 (3H, m), 1.44 (3H, d, J = 6.84 Hz), 1.52–2.00 (5H, m), 3.94–4.00 (1H, m). Anal. calcd for $C_{16}H_{15}F_4NO_2$: C, 58.36; H, 4.59; N, 4.25. Found: C, 58.32; H, 4.40; N, 4.35.

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