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Tumor Necrosis Factor-alpha Production-inhibiting Activity of Phthalimide Analogues on Human Leukemia THP-1 Cells and a Structure–Activity Relationship Study

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Abstract—*N*-Substituted phthalimides (2-substituted 1*H*-isoindole-1,3-diones) were prepared and their inhibitory effects on tumor necrosis factor-alpha (TNF-α) production by human leukemia cell line THP-1 stimulated with 12-*O*-tetradecanoylphorbol 13-acetate (TPA) or okadaic acid (OA) were examined. A structure–activity relationship study of these phthalimide analogues revealed that their inhibitory effects on TPA- and OA-induced TNF-α production by THP-1 cells are well correlated to each other, i.e. they may involve the same target molecule(s). An analysis by the use of phthalimide analogue-immobilized affinity gels indicated the existence of several phthalimide-binding proteins in THP-1 cell extract. © 1997 Elsevier Science Ltd.

Introduction

The tumor necrosis factor (TNF) family of ligands and receptors is a large family of cell surface and secreted molecules, mediating host defence and immune regulation. Among the members of this family, TNF-α, which is produced mainly by macrophages and T cells in response to various stimuli and is bioactive both as a transmembrane protein and as a homotrimeric secreted molecule, shows the widest range of activities. These extend beyond the well-characterized pleiotropic pro-inflammatory properties to include diverse signals for cellular differentiation, proliferation and death.[1–3] The growing understanding of the pathophysiological role of TNF-α in acute and chronic diseases including AIDS,[4] tumors[5] and diabetes[6] has led to the development of strategies to intercept the deleterious effects of TNF-α.[7] Clinical anti-TNF strategies, for example, therapy of rheumatoid arthritis with anti-TNF antibodies,[8] have been impressively successful. Another approach would be the use of TNF-α production-inhibitors.

Among the known TNF-α production-inhibitors, thalidomide [*N*-(α)-phthalimidoglutarimide (**1**)] is the most widely used because of its remarkable efficiency in various diseases including AIDS,[9] graft-versus-host disease (GVHD)[10] and Behcet's disease,[11] combined with its low toxicity (except for its severe teratogenicity).[12] We have been engaging in structural development of thalidomide, aiming at the production of superior TNF-α production-regulators, and we have found various potent phthalimde-derived regulators.[13–16] We also analyzed the bidirectional TNF-α production-regulatory activities of phthalimide analogues, including thalidomide, and their enantio-dependence.[16–19]

For the development of these compounds as clinical tools, we required system(s) which mimic TNF-α production by normal human peripheral blood cells. Various human cell lines produce TNF-α in response to various stimuli. However, the greatest difference between such human cell lines and normal human peripheral blood cells is that the latter produce TNF-α in response to lipopolysaccharide (LPS), while very few human cell lines respond to LPS. Recently, a human acute monocytic leukemia cell line, THP-1, established by Tsuchiya et al.,[20] has been reported to produce TNF-α in response to LPS (though treatment with 1,25-dihydroxyvitamin D3 is necessary).[21–23] Therefore, an assay system employing THP-1 cells might be suitable for evaluation of TNF-α production-inhibitors.

In this paper, we firstly describe the structure–activity relationships of *N*-phenyl- and *N*-benzylphthalimides, including newly prepared analogues, based on assays of TNF-α production-inhibiting activity on THP-1 cells. As stimulators of the cells, two potent TNF-α production-inducers, i.e. 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and okadaic acid (OA), were employed. Both TPA and OA are potent TNF-α production-inducers,[17] as well as potent tumor promoters,[24] while their target molecules are quite different, i.e. the target molecules of TPA and OA were reported to be protein kinase C and protein phosphatase As, respectively.[24] The correlation of the TNF-α production-inhibiting activities of the compounds in the TPA- and OA-stimulated systems was examined. Secondly, we describe the design and preparation of affinity gels liganded with potent TNF-α production-inhibiting phthalimide analogues, and the

Key words: tumor necrosis factor-alpha, phthalimide, thalidomide, phthalimide-binding protein, biological response modifier.

detection of phthalimide-binding proteins in THP-1 cell extract by the use of the affinity gels.

Materials and Methods

Chemicals

Thalidomide (**1**) was prepared as described previously.[25] Other phthalimide analogues (**2–42**) were prepared by condensation of phthalic anhydride (or its derivatives) with appropriate amines in good yields.[13–16,26,27] All the compounds gave appropriate analytical values, and their structures were supported by the ^{1}H-NMR spectra and MS. Details, including physicochemical properties, of the prepared compounds will be published elsewhere (most of the compounds have already been reported).[13–16,26,27]

Preparation of affinity gels

The *N*-hydroxysuccinimide ester of agarose (Affi-Gel 10) was purchased from Bio-Rad Laboratories. The gel suspension (3 mL/isopropanol) was washed with 3 mL of *N*,*N*-dimethylformamide (DMF) three times and the gel was resuspended in 1 mL of DMF. To this suspension, 40 mM (*R*)- or (*S*)-2-[1-(4-aminophenyl)-ethyl]-4,5,6,7-tetrafluoro-1*H*-isoindole-1,3-dione [(*R*)-FPTP-00A: **39**, (*S*)-FPTP-00A: **40**] in DMF (1.0 mL) was added at room temperature and the mixture was allowed to stand for 20 h. Then 0.1 mL of 1 M ethanolamine was added to block the unreacted *N*-hydroxysuccimide ester. After further standing for 1 h, the mixture was diluted by addition of 6 mL of DMF, and centrifuged (2000 rpm × 1 min), and the gel was recovered. The gel was washed successively with DMF, ethanol, and distilled water, and stocked in distilled water containing 0.06% sodium azide at 4 °C. The UV analysis indicated that the resultant gels [(*R*)- and (*S*)-FPTP-GELs] contain 20–30 μmol of (*R*)- and (*S*)-FPTP moieties, respectively, per 1 mL of gel-bed.

Cells and measurement of TNF-α

THP-1 cells were maintained as previously described.[18] The exponentially growing cells in RPMI1640 medium supplemented with 10% v/v fetal bovine serum (1.25×10^5 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. To test the effects of compounds, cells were treated with TPA (10 nM) or OA (50 nM) in the presence or absence of a test sample at the concentrations indicated in the tables and figures. 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%.

Analysis of phthalimide-binding proteins in THP-1 cell extract

Cultured THP-1 cells were collected and washed as described previously.[18] The cell pellet was homogenized and fractionated (100,000 g × 1 h). (*R*)- or (*S*)-FPTP-GEL (50 μL of the stock suspension) was washed with 1 mL of incubation buffer [0.15 M NaCl–20 mM Tris (pH 8.0)] and incubated with THP-1 cell extract (200–300 μg protein in 1 mL) at 4 °C for 2 h. The gels were collected by centrifugation (3000 rpm × 4 min, 4 °C), and washed three times with the incubation buffer. The washed gels were treated with a usual SDS-containing sample loading buffer for SDS-PAGE and filtered using filter paper, then the filtrate was analyzed by SDS-PAGE with silver-staining. Samples to be compared were run on the same gel plate (Fig. 4).

Results and Discussion

Production of TNF-α by THP-1 cells

The THP-1 cells used produce no detectable amount of TNF-α under the usual culture conditions but treatment with TPA or OA induces the cells to produce TNF-α. This induction of TNF-α production by TPA or OA is dose-dependent, and we chose concentrations of 10 nM for TPA and 50 nM for OA, because the effects of test compounds were clearly apparent at these concentrations.

The amounts of TNF-α produced by TPA-treated and OA-treated THP-1 cells under the experimental conditions (2.5×10^5 cells/mL, incubated in the presence of 10 nM TPA or 50 nM OA for 16 h) were 150–180 pg/mL and 1000–1200 pg/mL, respectively, and the value separately determined in each set of experiments was taken as 100%. The effect of a compound was represented as the amount (%) of TNF-α produced by the cells in the co-presence of the inducer (TPA or OA) and the test compound. The assay was performed at least in duplicate (the mean value was taken) and at least three times. The results were basically reproducible and a typical set of data obtained at the same time is presented in each table. None of the phthalimides described in this paper induced TNF-α production by themselves in the concentration range investigated. Cell numbers were counted at the time when the amount of TNF-α was measured. Almost no difference in the cell numbers between incubation mixtures in the presence and absence of the test compound was observed in the concentration range investigated.

Structure–activity relationships of *N*-phenylphthalimides

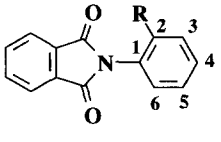
Effects of substituents at the *N*-phenyl group. Unsubstituted *N*-phenylphthalimide (PP-00: **2**), which had been prepared as a lead compound for novel thalidomide substitutes,[13–16] showed moderate TNF-α production-inhibiting activity on both TPA-

treated and OA-treated THP-1 cells (Table 1). The potency of the inhibiting activities is comparable or superior to those of thalidomide (**1**). Mono-alkyl or mono-phenyl substitution at the ortho-position of the *N*-phenyl moiety of *N*-phenylphthalimides (**3–7**)[13] did not cause any appreciable change in the TNF-α production-inhibiting activity of **2** (Table 1).

The effects of *di*-alkyl substitution (**8–13**) are shown in Table 2. Broadly speaking, (i) introduction of two alkyl groups at the ortho-positions of **2** generally caused enhancement of the TNF-α production-inhibiting activity on both TPA- and OA-treated THP-1 cells, and (ii) the steric effect of the two alkyl groups is an important factor, i.e., the inhibitory activity on TPA- and OA-induced TNF-α production by THP-1 cells decreased in the order of diisopropyl analogue (PP-33: **12**) > diethyl analogue (PP-22 :**10**) > dimethyl analogue (PP-11 :**8**) ≥ PP-00 (**2**). PP-33 showed approximately 90% inhibition at 3×10^{-5} M on both TPA- and OA-induced TNF-α production by THP-1 cells, and is much more potent than thalidomide (**1**). The dose–response curves for the inhibitory activities of typical compounds on TNF-α production are shown in Figure 1.

Effects of variation of the phthalimide moiety. First, the effect of modification of the succimide moiety of a potent inhibitor, PP-33 (**12**), was investigated (Table 3). The monothiocarbonyl derivative, PPS-33 (**14**),[26] showed very potent TNF-α production-inhibiting activity on both TPA- and OA-treated THP-1 cells, i.e. it inhibited the production almost completely at 3×10^{-5} M (Table 3 and Fig. 1). Replacement of the imide-carbonyl group of PP-33 (**12**) with a methylimino group (MIP-33: **15**) retained the activity, but elongation of the methyl group to an ethyl group (EIP-33: **16**) considerably decreased the activity.

Table 1. Effects of monosubstituted *N*-phenylphthalimides on TNF-α production



Compd	Code	R	Amount of TNF-alpha (%)[a] TPA/THP1	OA/THP-1
1	Thalidomide		52	56
2	PP-00	H	40	38
3	PP-10	Me	22	36
4	PP-n50	nPen	30	48
5	PP-30	iPr	53	25
6	PP-40	tBu	40	21
7	PP-ph0	Ph	43	46

[a]THP-1 cells were treated with 10 nM TPA or 50 nM OA in the presence of 30 μM test compound. The amount of TNF-α secreted in the culture medium was measured by ELISA. The amount of TNF-α production in the presence of 10 nM TPA alone or 50 nM OA alone was defined as 100%.

Next, the effects of substituents, i.e. an electron-withdrawing nitro group, and electron-donating hydroxy and amino groups, introduced at the condensed benzene ring of the phthalimide moiety (the 4- or 5-position) were investigated (Table 4). All the compounds listed in the table exhibited comparably potent TNF-α production-inhibiting activities on both TPA- and OA-treated THP-1 cells, regardless of the electronic nature and the position of the substituents.

Another structural development of PP-33 (**12**) is tetrahalogenation of the phthalimide moiety (**23–25**).[28] The results are shown in Table 5. It is of great interest that introduction of four fluorine atoms into the phthalimide moiety (FPP-33: **23**) dramatically lowered the concentration of the compound which is necessary to elicit TNF-α production-inhibiting activity. FPP-33 (**23**) inhibited TPA- and OA-induced TNF-α production by THP-1 cells almost completely at 3×10^{-7} M

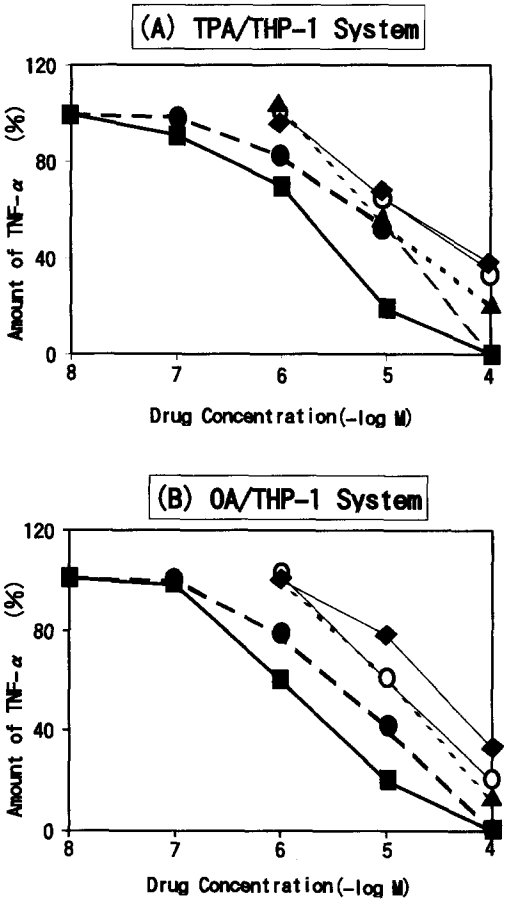
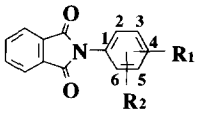


Figure 1. Dose–response curves of typical phthalimide derivatives. Horizontal scale: concentration of added test compound. Vertical scale: amount of TNF-α (%, vide infra). A: TPA-induced TNF-α production-inhibiting activity. THP-1 cells were treated with 10 nM TPA in the presence of a test compound. The amount of TNF-α produced by THP-1 cells stimulated with 10 nM TPA alone was defined as 100%. B: OA-induced TNF-α production-inhibiting activity. THP-1 cells were treated with 50 nM OA in the presence of a test compound. The amount of TNF-α produced by THP-1 cells stimulated with 50 nM OA alone was defined as 100%. ♦: Thalidomide (1), ○: PP-00 (2), ▲: PP-11 (8), ●: PP-33(12), ■: PPS-33(14).

Table 2. Effects of disubstituted *N*-phenylphthalimides on TNF-α production



Compd	Code	R1	R2	Amount of TNF-alpha (%)[a] TPA/THP-1	OA/THP-1
2	PP-00	H	H	40	38
8	PP-11	2-Me	6-Me	31	38
9	PP-0101	3-Me	5-Me	44	42
10	PP-22	2-Et	6-Et	22	20
11	PP-32	2-iPr	6-Et	5	14
12	PP-33	2-iPr	6-iPr	9	12
13	PP-11011[b]			30	31

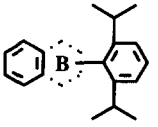
[a]THP-1 cells were treated with 10 nM TPA or 50 nM OA in the presence of 30 μM test compound. The amount of TNF-α secreted in the culture medium was measured by ELISA. The amount of TNF-α production in the presence of 10 nM TPA alone or 50 nM OA alone was defined as 100%.
[b]2-(2,3,5,6-Tetramethylphenyl)-1*H*-isoindole-1,3-dione.

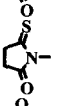
(Table 5 and Fig. 2). On the other hand, the tetrachloro (ClPP-33: **24**) and the tetrabromo (BrPP-33: **25**) analogues are much less active than PP-33 (**12**)/FPP-33 (**23**). These observations suggest that the increase in the activity is specific to tetrafluorination of the phthaloyl group. Similar effects which are unique to tetrafluorination were also reported by us and Niwayama et al.[13,14,16,23,28]

Structure–activity relationships of compounds related to *N*-benzylphthalimides. TNF-α production-inhibiting activities of compounds related to *N*-benzylphthalimide (**26–42**) were investigated (Tables 6 and 7). An apparent enantio-dependence was observed for FPTP (**26**, **27**), FPTN (**28**, **29**) and FP13P (**34**, **35**) in TNF-α production-inhibiting activity on TPA- and OA-treated THP-1 cells (Table 6), i.e., the (*R*)-forms of FPTP, FPTN and FP13P (**26**, **28**, and **34**) are potent TNF-α production-inhibitors, while the corresponding (*S*)-forms (**27**, **29** and **35**) are almost completely inactive.

Hydrogenation of the phenyl group (FPTH: **30**, **31**) and/or elongation of the side-chain methyl group (FPEP: **32**, **33**) of FPTP (**26**, **27**) decreased the enantio-dependence of the activity (Table 6). These structural modifications caused decrease and increase (or appearance) of the activity in (*R*)-isomers and (*S*)-isomers, respectively. We cannot interpret this phenomenon at this stage. Cyclization of the side chain of (*R*)-FPTP (**26**) to give (*R*)-FP13P (**34**) decreased the

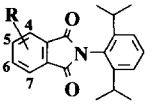
Table 3. Effects of variation of the succinimide moiety of PP-33 on TNF-α production regulation



Compd	B	Code	Amount of TNF-alpha (%)[a] TPA/THP-1	OA/THP-1
12		PP-33	9	12
14		PPS-33	1	5
15		MIP-33	9	18
16		EIP-33	45	33

[a]THP-1 cells were treated with 10 nM TPA or 50 nM OA in the presence of 30 μM test compound. The amount of TNF-α secreted in the culture medium was measured by ELISA. The amount of TNF-α production in the presence of 10 nM TPA alone or 50 nM OA alone was defined as 100%.

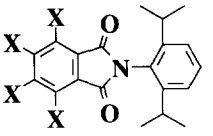
Table 4. Effects of substituents at the phthaloyl moiety of PP-33 on TNF-α production regulation



Compd	Code	R	Amount TNF-alpha (%)[a] TPA/THP-1	OA/THP-1
12	PP-33	H	9	12
17	4NPP-33	$4NO_2$	4	9
18	5NPP-33	$5\text{-}NO_2$	19	6
19	4APP-33	$4\text{-}NH_2$	18	1
20	5APP-33	$5\text{-}NH_2$	9	7
21	4HPP-33	4-OH	18	12
22	5HPP-33	5-OH	1	5

[a]THP-1 cells were treated with 10 nM TPA or 50 nM OA in the presence of 30 μM test compound. The amount of TNF-α secreted in the culture medium was measured by ELISA. The amount of TNF-α production in the presence of 10 nM TPA alone or 50 nM OA alone was defined as 100%.

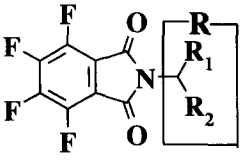
Table 5. Effects of halogenation at the phthaloyl moiety of PP-33 on TNF-α production regulation



Compd	Code	X	Amount of TNF-alpha (%)[a] TPA/THP-1	OA/THP-1	Dose
12	PP-33	H	9	12	30 μM
23	FPP-33	F	8	3	0.3 μM
24	ClPP-33	Cl	20	11	30 μM
25	BrPP-33	Br	19	34	30 μM

[a]THP-1 cells were treated with 10 nM TPA or 50 nM OA in the presence of 30 μM (except FPP-33, 0.3 μM) test compound. The amount of TNF-α secreted in the culture medium was measured by ELISA. The amount of TNF-α production in the presence of 10 nM TPA alone or 50 nM OA alone was defined as 100%.

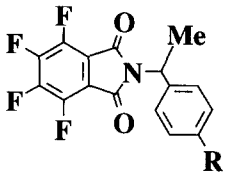
Table 6. Effects of *N*-substituted phthalimides on TNF-α production



Compd	Code	R1	R R2	Amount of TNF-alpha (%)[a] TPA/THP-1	OA/THP-1
26	(R)-FPTP	CH_3		9	16
27	(S)-FPTP	CH_3		95	86
28	(R)-FPTN	CH_3		43	42
29	(S)-FPTN	CH_3		103	101
30	(R)-FPTH	CH_3		66	43
31	(S)-FPTH	CH_3		80	61
32	(R)-FPEP	C_2H_5		38	42
33	(S)-FPEP	C_2H_5		54	76
34	(R)-FP13P	**R =** 		54	39
35	(S)-FP13P	**R =** 		100	101
36	FP22P	**R =** 		54	43

[a]THP-1 cells were treated with 10 nM TPA or 50 nM OA in the presence of 300 nM test compound. The amount of TNF-α secreted in the culture medium was measured by ELISA. The amount of TNF-α production in the presence of 10 nM TPA alone or 50nM OA alone was defined as 100%.

Table 7. Effects of *N*-benzylphthalimides on TNF-α production



Compd	Code	R	Amount of TNF-alpha (%)[a] TPA/THP-1	OA/THP-1
37	(R)-FPTP-00N	NO_2	12	18
38	(S)-FPTP-00N	NO_2	28	19
39	(R)-FPTP-00A	NH_2	32	18
40	(S)-FPTP-00A	NH_2	54	65
41	(R)-FPTP-82	$NHCOC_9H_{19}$	38	28
42	(S)-FPTP-82	$NHCOC_9H_{19}$	44	33

[a]THP-1 cells were treated with 10 nM TPA or 50 nM OA in the presence of 300 nM test compound. The amount of TNF-α secreted in the culture medium was measured by ELISA. The amount of TNF-α production in the presence of 10 nM TPA alone or 50 nM OA alone was defined as 100%.

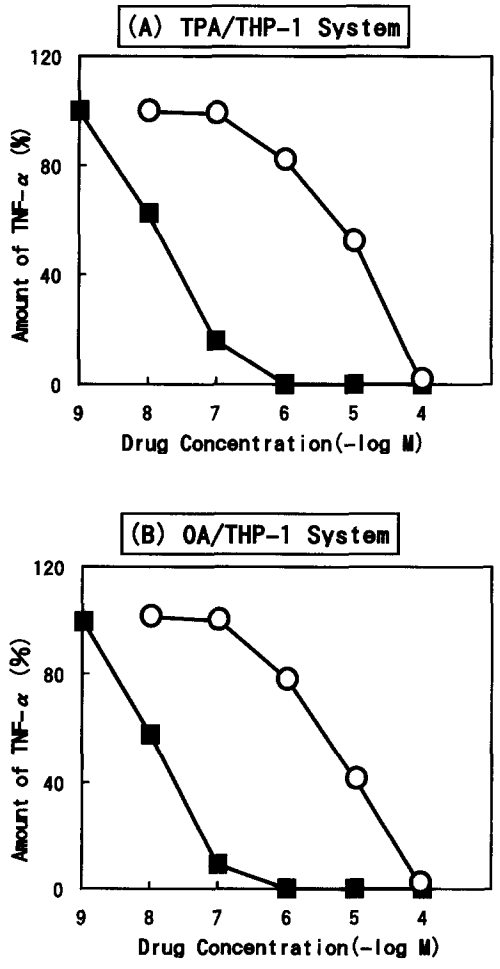


Figure 2. Dose–response curves of halogenated phthalimlde derivatives. Horizontal scale: concentration of added test compound. Vertical scale: amount of TNF-α (%, vide infra). THP-1 cells were treated with 10 nM TPA in the presence of a test compound. The amount of TNF-α produced by THP-1 cells stimulated with 10 nM TPA alone was defined as 100%. ○: PP-33 (12) ■: FPP-33 (23)

activity. FP22P (**36**), a positional isomer of FP13P (**34**, **35**), showed moderate TNF-α production-inhibiting activity which is comparable to that of (*R*)-FP13P (**34**).

Introduction of a substituent (nitro, amino, and acylamino groups) into the para-position of the benzyl moiety of (*R*)-FPTP (**26**) retained or slightly decreased the TNF-α production-inhibiting activity, regardless of the nature of the substituent (**37**, **39** and **41**, in Table 7). On the other hand, introduction of the same groups into (*S*)-FPTP (**35**) dramatically enhanced the activity (**38**, **40**, and **42**). In these series of (*S*)-isomers, introduction of an electron-withdrawing group is much more effective for enhancement of the activity: (*S*)-FPTP-00N (**38**) is a more potent TNF-α production-inhibitor than (*S*)-FPTP-00A (**40**), and (*S*)-FPTP-82 (**42**) showed moderate activity.

Correlation of TNF-α production-inhibiting activities of phthalimides in TPA- and OA-treated THP-1 cells. The studies described above (Tables 1–7) showed that the structure–activity relationships are similar in the assay systems using TPA and OA as TNF-α production-inducers. Analysis of TNF-α production-inhibiting activities of the phthalimides (**1**–**42**) on TPA- and OA-treated THP-1 cells by the method of least squares showed a good linear correlation with an *r*-factor of 0.92 (Fig. 3). This result strongly suggests that the target molecule(s) of the phthalimides is common in TPA- and OA-treated THP-1 cells, even though the mechanisms of TNF-α production-induction by TPA (an activator of protein kinase C) and OA (an inhibitor of protein phosphatase As) should be different.

As a preliminary study, we searched for phthalimide-binding proteins in THP-1 cells using affinity gels liganded with an active phthalimide. As the ligand, we chose para-substituted FPTP (see Materials and Meth-

ods). Treatment of THP-1 whole cell extract with FPTP-GELs and SDS–PAGE of the adsorbed proteins showed the existence of several specific phthalimide-binding proteins with apparent molecular weights ranging from 30 kDa to 115 kDa (Fig. 4, lanes R and S; bands marked with symbols: 33 kDa, 64 kDa, 66 kDa, 90 kDa and 114 kDa). The binding of these proteins to FPTP-GELs was competed out by FPTPs (**26**, **27**). Some specific binding proteins showed enantio-selectivity [Fig. 4, bands marked with an open circle (lane R) and open triangles (lane *S*) are specific to (*R*)- and (*S*)-forms, respectively]. Though only the (*R*)-form (**26**) is active as a TNF-α production-inhibitor in the case of FPTP (**26**, **27**), the (*R*)- and (*S*)-isomers of para-substituted FPTPs, especially (*R*)- and (*S*)-FPTP-82s (**41**, **42**) which are very similar in structure to FPTP-GELs, are comparably active TNF-α production-inhibitors. Therefore, we cannot determine whether an enantio-selective (bands marked with an open circle and open triangles in Fig. 4) or an enantio-non-selective (bands marked with open squares in Fig. 4) binding protein(s) is the true target molecule mediating the activity of phthalimides at this stage. Moreover, we do not know whether active (*R*)- and (*S*)-forms share a common target molecule(s) or not. However, because (*S*)-FPTP (**27**) is inactive or very weakly active (Table 6), the possibility that proteins which bind to only (*S*)-FPTP-GEL and are competed out by (*S*)-FPTP (**27**) (the bands marked with open triangles in lane S of Fig. 4) are target molecules could be excluded. Candidates for the target molecule(s) should bind to only (*R*)-FPTP-GEL or both (*R*)- and (*S*)-FPTP-GELs, and the binding should be efficiently competed out by (*R*)-FPTP (**26**) (the bands marked with an open circle and open squares in Fig. 4). Isolation of such proteins as candidate target molecules of the phthalimides is in progress.

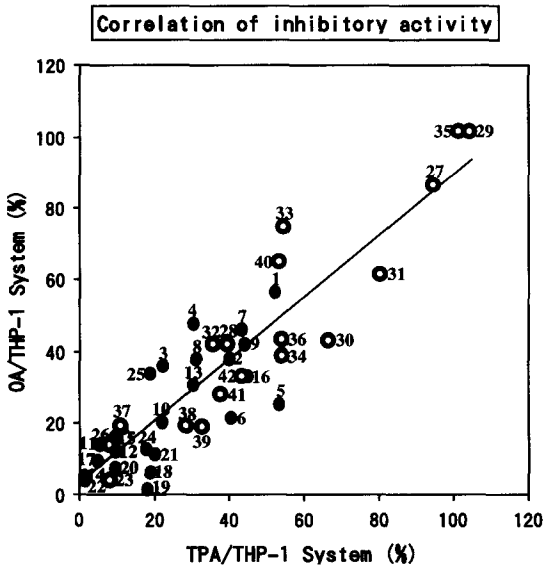


Figure 3. Correlation of the inhibitory activities of phthalimides on TPA- and OA-induced TNF-α production by THP-1 cells. Horizontal scale: TPA-induced TNF-α production-inhibiting activity in THP-1 cells. Vertical scale: OA-induced TNF-α production-inhibiting activity in THP-1 cells. THP-1 cells were treated with 10 nM TPA or 50 nM OA in the presence of a test compound. The amount of TNF-α production by THP-1 cells stimulated with 10 nM TPA or 50 nM OA alone was defined as 100%. ●: non-fluorinated phthalimides, ○: fluorinated phthalimides.

Conclusion

The structure-activity relationships of phenylphthalimides with TNF-α production-inhibiting activity on TPA- and/or OA-treated THP-1 cells was investigated. Several compounds, including PPS-33 (**14**), FPP-33 (**23**), (R)-FPTP (**26**) and (R)-FPTN (**28**), showed potent TNF-α production-inhibiting activity far exceeding that of thalidomide (**1**). These phthalimide analogues seems to have potential for development as novel bioresponse modifiers for a clinical anti-TNF strategy. Pharmacological evaluation of these compounds is in progress.

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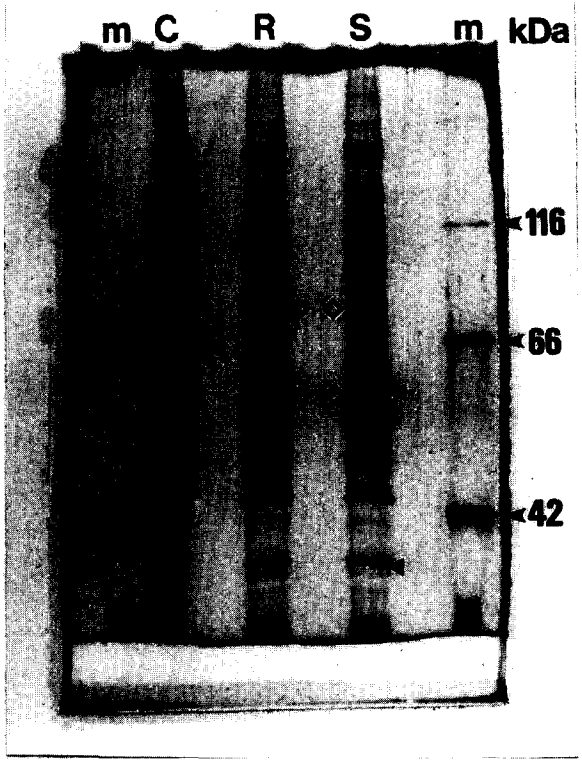

Figure 4. SDS–PAGE analysis of phthalimide-binding proteins. Lane m: molecular weight markers, lane c: control. Whole cell extract was treated with the gel having no phthalimide ligand. Lanes R and S: whole cell extract was treated with (*R*)- and (*S*)-FPTP-Gels, respectively, as described in materials and methods.

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