INDUCER-SPECIFIC REGULATORS OF TUMOR NECROSIS FACTOR ALPHA PRODUCTION

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Novel potent regulators of tumor necrosis factor alpha (TNF-α) production by a human promyelocytic leukemia cell line, HL-60, were prepared. All the compounds showed inducer-specific and bidirectional regulation of TNF-α production, *i.e.*, they enhanced 12-*O*-tetradecanoylphorbol-13-acetate-induced TNF-α production, while they inhibited okadaic acid-induced one.

KEY WORDS tumor necrosis factor; HL-60; phorbol ester; okadaic acid

Tumor necrosis factor alpha (TNF-α) is a cytokine produced by activated monocytes and/or macrophages; it plays a critical role in certain physiological defensive responses, but causes damage to the host when produced in excess.[1] Because TNF-α possesses both favorable and unfavorable effects, regulators of TNF-α production would be useful as biological response modifiers (BRMs).[2] Among such BRMs, N(α)-phthalimidoglutarimide (thalidomide) has been well documented. Though thalidomide was withdrawn from the market because of its teratogenicity,[3] it is potentially useful in the treatment of various diseases including graft-*versus*-host disease and acquired immunodeficiency syndrome.[4-6] We are interested in structural modification of thalidomide and have prepared various phthalimide derivatives which enhances TNF-α production by a human promyelocytic leukemia cell line, HL-60, stimulated with 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[7,8] Recently, we have reported that the TNF-α production-regulating activity of thalidomide is inducer-specific and bidirectional, *i.e.*, thalidomide enhances TPA-induced TNF-α production, while it inhibits okadaic acid (OA)-induced one.[9] Both TPA and OA are tumor promoters which activates protein kinase C and phosphatases 1 and 2A, respectively. In this paper, we describe novel, potent, inducer-specific bidirectional regulators of TNF-α production.

Phenylphthalimide analogs [PP-00, PP-11, PP-33 and FPP-33 (Chart 1)], which we have already reported, are regulators of TNF-α production with wide range of activity.[7-9] We have now developed four classes of compounds based on the phenylphthalimides, by (i) conversion of the phthalimide moiety to a homophthalimide moiety (PIQ-00, -11 and -33), (ii) fusion of an additional benzene ring to the phthalimide moiety (NAP-00, -11 and -33), (iii) decarbonylation of the phthalimide moiety (IP-00, -11 and -33), and (iv) derivatization to monothiocarbonyl analogs (PPS-33 and FPPS-33). All the compounds were synthesized by usual organic chemical methods and gave the expected analytical values (details will be published elsewhere). The TNF-α production-regulating activity of the prepared compounds was measured by ELISA as

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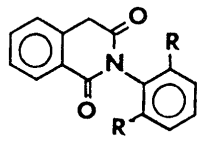
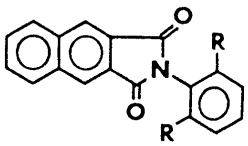
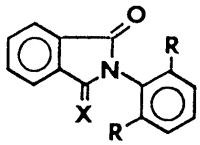
described previously (Table 1).[7,8]



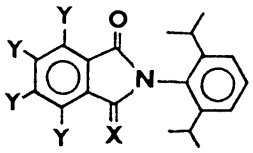
PIQ-00 (R=H)
PIQ-11 (R=Me)
PIQ-33 (R=iPr)



NAP-00 (R=H)
NAP-11 (R=Me)
NAP-33 (R=iPr)



IP-00 (X=H₂,R=H)
IP-11 (X=H₂,R=Me)
PP-00 (X=O, R=H)
PP-11 (X=O, R=Me)



IP-33 (X=H₂, Y=H)
PP-33 (X=O, Y=H)
PPS-33 (X=S, Y=H)
FPP-33 (X=O, Y=F)
FPPS-33 (X=S, Y=F)

Chart 1

Table 1. TNF-α Production-Regulating Activity of the Prepared Compounds

	Amount of TNF-α (%)[a]							
	TPA (3 nM)[b]				Okadaic acid (50 nM)[b]			
	10 nM[c]	100 nM[c]	3 μM[c]	30 μM[c]	10 nM[c]	100 nM[c]	3 μM[c]	30μM[c]
none	-------- 100 (132 pg/ml) --------				-------- 100 (1065 pg/ml) --------			
PP-00	N.A.[d]	N.A.[d]	101	102	N.A.[d]	N.A.[d]	100	58
PP-11	N.A.[d]	99	118	176	N.A.[d]	103	92	40
PP-33	101	147	408	605	100	98	75	18
PIQ-00	N.A.[d]	N.A.[d]	103	113	N.A.[d]	98	90	52
PIQ-11	N.A.[d]	102	115	135	N.A.[d]	100	78	46
PIQ-33	98	100	176	268	102	103	90	50
NAP-00	N.A.[d]	N.A.[d]	97	102	N.A.[d]	99	100	56
NAP-11	N.A.[d]	N.A.[d]	105	125	N.A.[d]	96	81	47
NAP-33	N.A.[d]	100	108	144	N.A.[d]	95	85	48
IP-00	N.A.[d]	98	104	115	N.A.[d]	100	98	60
IP-11	N.A.[d]	101	114	153	N.A.[d]	101	90	45
IP-33	N.A.[d]	103	147	214	N.A.[d]	94	88	40
PPS-33	100	201	610	1120	101	100	46	0
FPP-33	158	345	Toxic	N.A.[d]	73	0	N.A.[d]	N.A.[d]
FPPS-33	200	350	Toxic	N.A.[d]	70	5	0	N.A.[d]

a) The amount of TNF-α produced in the presence of an inducer alone was defined as 100%.
b) Inducer used and its concentration.
c) Concentrations of test compounds.
d) N.A. means not assayed.

As shown in Table 1, PIQ-00, PIQ-11, IP-00, IP-11, NAP-00 and NAP-11 showed no or very weak enhancement on TNF-α production by TPA-stimulated HL-60 cells, while they were moderately active in inhibiting OA-induced TNF-α production. PIQ-33, IP-33 and NAP-33 showed both activities. These results on the structure-activity relationship are consistent with our previous results obtained for phenylphthalimide analogs.[7-9]

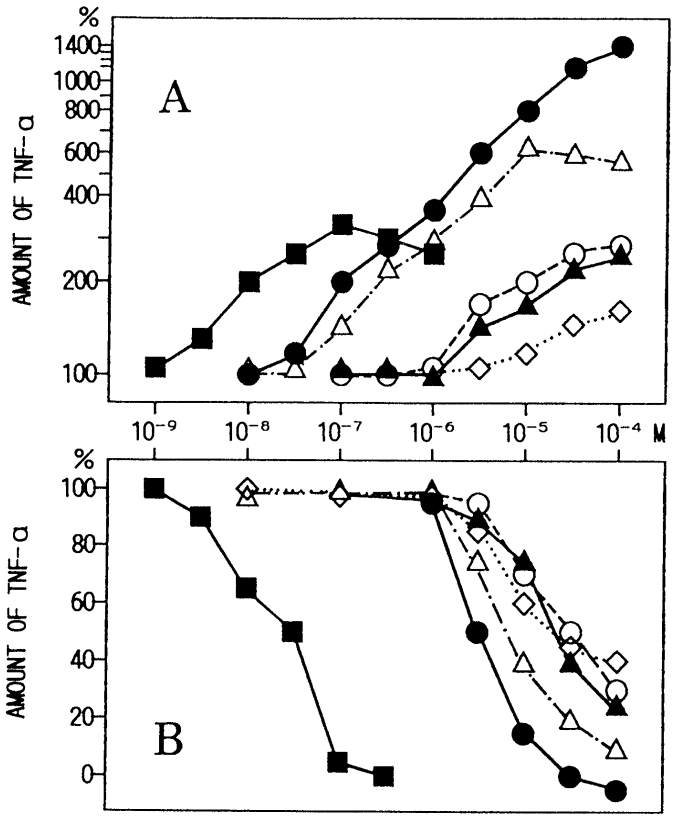


Fig. 1. Dose-Response Curves of Inducer-Specific Bidirectional Regulation of TNF-α Production

HL-60 cells were treated with [A] 3 nM TPA or [B] 50 nM OA in the presence of various concentrations (horizontal scale) of test compounds. The amount of TNF-α secreted in the culture medium (vertical scales) was measured by ELISA.[7-9] The amounts of TNF-α produced in the presence of 3 nM TPA alone and 50 nM OA alone were defined as 100% in panels A and B, respectively.

■: FPPS-33, ●: PPS-33, △: PP-33, ◇: NAP-33, ○: PIQ-33, ▲: IP-33.

Among the compounds newly prepared, PPS-33 showed potent activity in both assay systems, *i.e.*, it enhanced TPA-induced TNF-α production to more than 1400% at 100 μM (Fig. 1), while it completely inhibited OA-induced TNF-α production at 30 μM. Its tetrafluorinated analog, FPPS-33, also showed potent activity in both systems at very low concentrations (Fig. 1). It is noteworthy that the structure-activity relationships for TPA-induced TNF-α production-enhancing activity and OA-induced TNF-α production-inhibiting activity are very similar, but not identical. This means that the target molecules of the compounds in the two assay systems are different but possessing similar ligand-binding affinity.

In conclusion, we have developed phenylthiophthalimide analogs, PPS-33 and FPPS-33, which possess potent, inducer-specific bidirectional TNF-α production-regulating activity.

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