N-ALKYLPHTHALIMIDES: STRUCTURAL REQUIREMENT OF THALIDOMIDAL ACTION ON 12-O-TETRADECANOYLPHORBOL-13-ACETATE-INDUCED TUMOR NECROSIS FACTOR α PRODUCTION BY HUMAN LEUKEMIA HL-60 CELLS

Yoshihiro SHIBATA, Mizue SHICHITA, Keizo SASAKI,* Koji NISHIMURA, Yuichi HASHIMOTO,* Shigeo IWASAKI

Institute of Molecular and Cellular Biosciences, The University of Tokyo,
1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

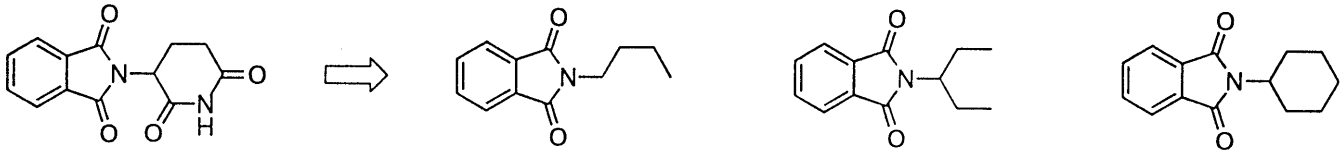
Phthalimide analogs N-substituted with n-butyl, tert-butyl, hexyl and adamantyl groups were designed and prepared as simplified analogs of thalidomide and methylthalidomide. All the compounds prepared except N-n-butylphthalimide showed thalidomidal activity on 12-O-tetradecanoylphorbol-13-acetate-induced tumor necrosis factor (TNF)-α production by human leukemia HL-60 cells. Among the investigated compounds, including thalidomide and methylthalidomide, N-adamantylphthalimide showed the most potent TNF-α production-enhancing activity.

KEYWORDS phthalimide; thalidomide; tumor necrosis factor α; structure-activity relationship

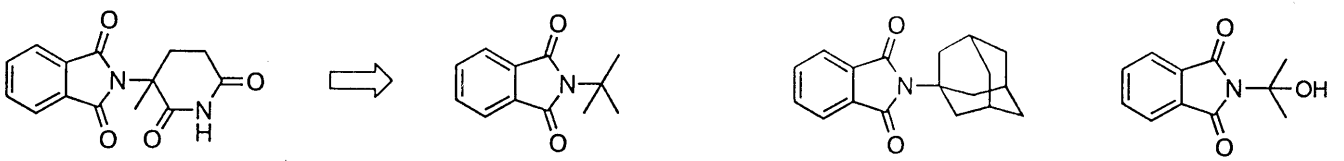
Tumor necrosis factor (TNF)-α is an important cytokine secreted by activated monocyte/macrophages and possesses pleiotropic biological activities including normal B-cell growth stimulation, tumor cell killing activity, angiogenesis stimulation, induction of endotoxic shock, and so on.[1-3] Such biological activities elicited by TNF-α have both favorable and unfavorable effects. Therefore, chemicals which regulate TNF-α production are expected to be useful biological response modifiers (BRM's).

Recently, we reported the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced TNF-α production-enhancing activity of N(α)-phthalimidoglutarimide (1: thalidomide) on human leukemia cell line HL-60.[4] We also reported that N-methylated thalidomide (2: methylthalidomide) and phenethylphthalimide (3c) possess TNF-α production-enhancing activity which is more potent than that of thalidomide.[5,6] To develop superior thalidomidal BRM's, we tried to extract the structural requirements for TNF-α production-enhancing activity from the structures of compounds 1 - 3. In this paper, we report the design, preparation, and effects on TNF-α production of N-alkylphthalimides, 4 - 8, and related compounds, 9 and 10.

First we designed compounds 4 - 6 based on our previous results indicating that (i) unsubstituted phthalimide is inactive and (ii) the glutarimide group of 1 is unnecessary for the activity.[6] Next we designed compounds N-substituted with a tertiary carbon unit, 7 - 8, based on our previous finding that the



1 4 5 6



2 7 8 9

compound bearing a methyl group at the α-position of thalidomide, i.e., 2, possesses much higher activity than 1. Compound 9 was designed as a structural mimic of 7 with enhanced hydrophilicity.

All the compounds, 4 - 9, were prepared by condensation of appropriate amines with phthalic anhydride in good yields. Structure and purity of the prepared compounds were confirmed by NMR, MS and elemental analyses. Analytical values and melting points are listed in Table I.

Effects of the prepared compounds on TPA-induced TNF-α production by HL-60 cells were measured by the previously described method with slight modifications.[4-6] Briefly, exponentially growing HL-60 cells (4 x 10^5 cells/ml) were treated with 3 nM TPA in the presence or absence of the compounds (at the concentration indicated in Table I and Fig. 1) at 37 ℃ for 24 h. Then the concentration of TNF-α in the cell culture supernatant was measured by the use of a human TNF-α ELISA system (Amersham Co.). The results are shown in Table I and Fig. 1.

Though the compound substituted with an n-alkyl group (4) was inactive, secondary alkyl group-bearing compounds (5 and 6) were active. Compound 5 is more potent than the corresponding conformationally restricted compound (6). The compounds substituted with a tertiary carbon unit (7 - 9) were all active. Among them, adamantylphthalimide (8) is the most potent enhancer of TPA-induced TNF-α production by HL-60 cells. The activity is much higher than those of thalidomide (1) and methylthalidomide (2). Substitution of the methyl group of 7 with a hydroxy group, i.e., 9, reduced the activity. The results suggest that the bulkiness of the hydrophobic group at the nitrogen atom of the compound is crucial for potent activity.

Table I. Enhancing Effects of Phthalimide Analogs on TPA-Induced TNF-α Production by HL-60 Cells

Compound	Amount of TNF-α[a] %		mp ℃	Elemental analysis
	10 μM[b]	100 μM[b]		
1	148	155	277-279	Calcd. for $C_{13}H_{10}N_2O_4$: C, 60.47; H, 3.88; N, 10.85. Found: C, 60.75; H, 3.91; N, 11.10.
2	256	325	249	Calcd. for $C_{14}H_{12}N_2O_4$: C, 61.75; H, 4.45; N, 10.29. Found: C, 61.64; H, 4.44; N, 10.31.
3c	176	234	129	Calcd. for $C_{16}H_{13}NO_2$: C, 76.48. H, 5.21; N, 5.57. Found: C, 76.67; H, 5.18; N, 5.52.
4	98	102	34	Calcd. for $C_{12}H_{13}NO_2$: C, 70.92; H, 6.45; N, 6.89. Found: C, 70.86; H, 6.46; N, 6.91.
5	108	225	51	Calcd. for $C_{13}H_{15}NO_2$: C, 71.87; H, 6.96; N, 6.45. Found: C, 71.79; H, 6.88; N, 6.46.
6	104	182	168	Calcd. for $C_{14}H_{15}NO_2$: C, 73.34; H, 6.59; N, 6.11. Found: C, 73.61; H, 6.53; N, 6.24.
7	114	198	233-234	Calcd. for $C_{10}H_{13}NO_2$: C, 70.92; H, 6.45; N, 6.89. Found: C, 71.12; H, 6.67; N, 6.82.
8	383	521	139	Calcd. for $C_{18}H_{19}NO_2$: C, 76.84; H, 6.80; N, 4.98. Found: C, 76.54; H, 6.80; N, 4.90.
9	101	162	67	Calcd. for $C_{11}H_{11}NO_3$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.96; H, 6.01; N, 6.12.
10	292	360	221	Calcd. for $C_{24}H_{23}NO_2$: C, 80.64; H, 6.49; N, 3.92. Found: C, 80.77; H, 6.45; N, 3.90.
11	108	196	177	Calcd. for $C_{24}H_{23}NO_2$: C, 80.77; H, 6.36; N, 4.01. Found: C, 80.64; H, 6.49; N, 3.92.

a) The amount of TNF-α produced in the presence of TPA (3 nM) alone (148 pg/ml) was defined as 100%.
b) Concentration of added compound.

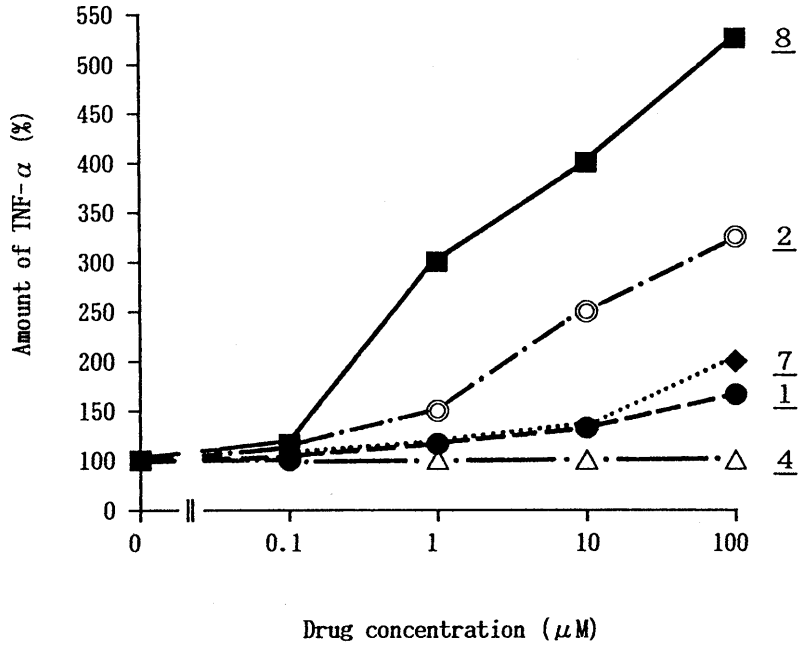
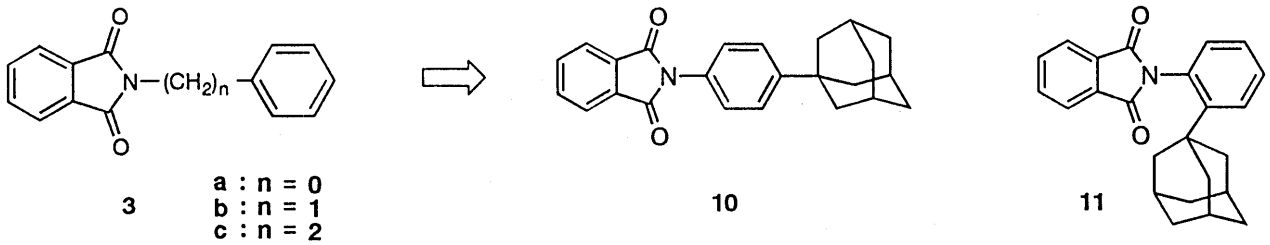


Fig. 1. Enhancing Effects of 1, 2, 4, 7 and 8 on TPA-Induced TNF-α Production by HL-60 Cells

Our previous results indicated that the distance between the phthalimide group and a hydrophobic group is one of the crucial factors for the activity, i.e., 3c is active while molecules, 3a and 3b, are inactive. These results led us to prepare a molecule with a phenyl group as a spacer between a phthalimide group and an adamantyl group, i.e., 10. Compound 10 was rather active, though the activity was lower than that of 8. The ortho-isomer of 10, i.e., 11, showed even weaker activity.



Thus, we found that (i) introduction of a hexan-3-yl group at the nitrogen atom of phthalimide (5) is enough to elicit enhancing activity on TPA-induced TNF-α production by HL-60 cells, and (ii) the bulkiness of the alkyl group at the nitrogen atom is crucial for strong activity. Also, we succeeded in preparing a compound with quite potent activity, i.e., adamantylphthalimide (8). Further investigation on the structure-activity relationships and application of 8 in biological systems are in progress.

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