Benzylphthalimides and Phenethylphthalimides with Thalidomide-Like Activity on the Production of Tumor Necrosis Factor α

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Benzylphthalimide analogs (P1P's) and phenethylphthalimide analogs (P2P's) have been found to exhibit thalidomide-like activity on the production of tumor necrosis factor (TNF)- α by the human leukemia cell line, HL-60, stimulated by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Structure-activity relationships are discussed on the basis of the TNF- α production-enhancing activity. Benzylphthalimide (P1P-00) exhibited activity which is weaker than that of thalidomide, but introduction of a methyl group at the *ortho*-position of the benzyl moiety (P1P-10) resulted an increase to a level comparable with that of thalidomide. Phenethylphthalimide (P2P-00) is more potent than thalidomide, and its fluorinated derivative, 2-phenethyl-4,5,6,7-tetrafluoro-1*H*-isoindole-1,3-dione (FP2P-00), exhibited potent activity at very low concentrations.

Key words thalidomide; phthalimides; tumor necrosis factor α ; structure-activity relationship; biological response modifier

Thalidomide [*N*(α)-phthalimidoglutarimide (**1**)] is a sedative agent with the structure of a substituted phthalimide with a six-membered ring at the isoindolyl nitrogen atom. Although it has been withdrawn from the market because of its teratogenic activity,^{1,2)} the drug is potentially useful in the treatment of graft-versus-host disease (GVHD), leprosy, Behcet's disease, lupus erythematosus, malaria, acquired immunodeficiency syndrome (AIDS), and other related diseases,²⁻⁷⁾ owing to its ability to modulate the host immune system through regulating the production of tumor necrosis factor (TNF)- α by stimulated monocytes and/or macrophages.⁶⁻¹¹⁾ Because TNF- α is an important cytokine with pleiotropic biological effects including normal B-cell growth stimulation,¹²⁾ tumor cell-killing activity,^{8,13)} stimulation of the host immune system,¹⁰⁾ tumor-promoting activity,¹⁴⁾ stimulation of angiogenesis and metastasis,¹⁵⁾ induction of endotoxic shock and tissue inflammation,¹⁶⁾ stimulation of human immunodeficiency virus (HIV) replication,⁶⁾ among others, chemicals which affect TNF- α production are expected to be useful biological response modifiers (BRMs). In this sense, thalidomide seems to be a superior lead compound for the development of potent BRMs, in spite of its teratogenicity and chemical instability.

Recently, we reported that thalidomide acts as a cell-type-specific regulator of TNF- α production, enhancing 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced TNF- α production by human leukemia HL-60 cells.¹⁷⁾ Because optically pure forms of thalidomide readily racemize and undergo spontaneous hydrolysis, we prepared a stable analog of thalidomide, α -methyl-*N*(α)-phthalimidoglutarimide, and found that the (*S*)-form of the compound possesses higher activity than thalidomide.^{18,19)} Through further structural development, we obtained several phthalimide derivatives with potent activity, including adamantylphthalimide (PA-10),²⁰⁾ phenylphthalimide analogs such as 2,6-dimethylphenylphthalimide (PP-11), 2,6-diisopropylphenylphthalimide (PP-33) and 2-(2,6-diisopropylphenyl)-4,5,6,7-tetrafluoro-1*H*-isoindole-1,3-dione (FPP-33),^{21,22)} and phenethylphthal-

imide (P2P-00).²³⁾ In this paper, we describe the synthesis and activities of several benzylphthalimides (P1P's), phenethylphthalimides (P2P's) and related compounds, with emphasis on structure-activity relationships.

RESULTS AND DISCUSSION

Phthalimides Possessing a Non-substituted Phenyl Ring

The structure of thalidomide can be divided into two parts, *i.e.*, the 1*H*-isoindole-1,3-dione moiety and the remaining six-membered ring moiety. Our previous study of the enhancement of TPA-induced TNF- α production in HL-60 cells by alkyl-phthalimides indicated that the structure of the six-membered ring can be simplified, because compounds such as cyclohexylphthalimide (**2**) and *n*-pentanyl-3-phthalimide (**3**) are moderately active.²⁰⁾ Further structural simplification of the six-membered ring moiety led us to design molecules with a 1*H*-isoindole-1,3-dione skeleton connected directly to a phenyl group (PP-00) or spaced by methylene group(s) (PnP-00's, Fig. 1). Thalidomide-like activities elicited by the prepared compounds were assessed by measuring the amount of TNF- α produced by HL-60 cells stimulated with TPA in the presence of the test compounds.¹⁷⁻²³⁾ The investigated compounds themselves, in the absence of TPA, had no effect on TNF- α production by the cells over the concentration ranges examined, under the experimental conditions used. Results are shown in Table 1 as the amount (percentage) of TNF- α , where that produced in the presence of TPA (3 nM) alone is defined as 100%. All the compounds were assayed in duplicate several times. Although considerable variations were observed in the percentages among sets of experiments, the relative efficacy of each compound and its order of potency were reproducible. Therefore, a typical set of data is shown in the Table 1. Similarly, all the results on TNF- α production-enhancing activity in this paper are typical data.

As shown in the Table 1, PP-00 and P3P-00 were inactive, while P2P-00 was more active than thalidomide. P1P-00 also enhanced TPA-induced TNF- α production,

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Fig. 1. Structures of Thalidomide (1), Cyclohexylphthalimide (2), *N*-Neopentyl-3-phthalimide (3), and Phthalimides with a Non-substituted Phenyl Ring

Table 1. Enhancing Effects of Phthalimides Shown in Fig. 1 on TPA-Induced TNF- α Production by HL-60 Cells

Compound	Amount of TNF- α (%)	
	10 μM^b	100 μM^b
None ^{a)}	100	(138 pg/ml)
1	145	178
2	132	194
3	153	212
PP-00	98	94
P1P-00	112	121
P2P-00	162	252
P3P-00	103	96

a) Cells were treated with TPA (3 nM) alone. b) Concentration of the added compound.

Fig. 2. Dose-Dependence Curves of TNF- α Production-Enhancing Activities Elicited by Thalidomide and Phthalimides with a Non-substituted Phenyl Ring

The amount of TNF- α produced in the presence of 3 nM TPA alone was 138 pg/ml, which was taken as 100%.

although its activity was low. The dose-dependence of the activities of these compounds is shown in Fig. 2. The potency of activity can be described as; $n=0 < n=1 < n=2 > n=3$, where n is the number of methylene groups separating the 1*H*-isoindole moiety and the phenyl group (Fig. 1). The results indicate that there are critical structural requirements for phthalimides in terms of their

Table 2. Enhancing Effects of Benzylphthalimide Analogs (at 25 μM) on TPA-Induced TNF- α Production by HL-60 Cells

Compound	R ¹	R ²	R ³	R ⁴	Amount of TNF- α (%; relative value)
P1P-00	H	H	H	H	120 (1.00)
P1P-10	CH ₃	H	H	H	179 (1.49)
P1P-01	H	CH ₃	H	H	152 (1.27)
P1P-001	H	H	CH ₃	H	121 (1.01)
P1P-V0	OCH ₃	H	H	H	133 (1.11)
P1P-0V	H	OCH ₃	H	H	118 (0.98)
P1P-00V	H	H	OCH ₃	H	107 (0.89)
P1P-VV	OCH ₃	H	H	OCH ₃	128 (1.07)
P1P-N0	NO ₂	H	H	H	125 (1.04)
P1P-0N	H	NO ₂	H	H	124 (1.03)
P1P-00N	H	H	NO ₂	H	132 (1.10)
None ^{a)}					100 (165 pg/ml)
Thalidomide (1)					168
P2P-00					209

a) Cells were treated with TPA (3 nM) alone.

TNF- α production-enhancing activity.

P1P's The effects of substituents introduced into the phenyl ring of P1P-00 on TNF- α production-enhancing activity were investigated. As shown in Table 2, introduction of a methyl group at the *ortho*-position (P1P-10) enhanced activity. Substitution of a methyl group at the *meta*-position (P1P-01) also enhanced activity, but similar substitution at the *para*-position (P1P-001) had no effect. Therefore, it appears that the methyl-substituent effect is position-dependent and decreases in the order *ortho* > *meta* > *para*. This order suggests that the substituent effects on TNF- α production-enhancing activity are steric rather than electronic.

Introduction of an electron-donating methoxy group at the *ortho*-position (P1P-V0) also enhanced activity, although the effect was less than that of a methyl group (P1P-10). The order of decreasing activity of the methoxybenzylphthalimides was *o*-methoxy (P1P-V0) > *m*-methoxy (P1P-0V) > *p*-methoxy (P1P-00V), similar to the methylbenzylphthalimides. However, introduction of a methoxy group at the *meta*-position seems to have no effect on activity, because P1P-0V enhanced TNF- α production with almost the same efficacy as non-substituted P1P-00. Moreover, the activity of the *para*-methoxy derivative (P1P-00V) is lower than that of

Fig. 3. Structures of P2P-00 and Related Analogs

P1P-00. These results suggest that introduction of an electron-donating group at the *ortho/para*-positions reduces activity. The higher activity of the *ortho*-methoxy derivative (P1P-V0), compared with P1P-00, can be interpreted in terms of a predominance of steric effects over electronic effects. Introduction of one more methoxy group into P1P-V0 at the other *ortho*-position (P1P-VV) did not enhance the activity of the former (Table 2).

On the other hand, introduction of an electron-withdrawing nitro group at the *ortho*- (P1P-N0) or *meta*-position (P1P-0N) had almost no effect on activity. Introduction at the *para*-position (P1P-00N) caused a slight enhancement of the activity. These results can be interpreted as follows: (1) introduction of an electron-withdrawing group at the *ortho/para*-positions enhances activity, and (2) *ortho*-substituents which enhance activity must be non-polar hydrophobic groups; introduction of a polar group, such as a nitro group, at this position does not enhance activity.

P2P's and Related Compounds Phenethylphthalimide (P2P-00) possesses more potent activity than thalidomide, as shown in Table 1 and Fig. 2, and the distance between the two aromatic moieties (the 1*H*-isoindole-1,3-dione and the phenyl group moieties) seemed to be critical for activity, because P1P-00 and P3P-00 are less active than P2P-00. To investigate the need for two aromatic moieties, hydrogenated derivatives of P2P-00, P2H-00 and H2P-00, and phenethylsuccinimide (S2P-00) were prepared (Fig. 3). Although these compounds all showed TNF- α production-enhancing activity (Table 3), it was lower than that of P2P-00, indicating that the two aromatic components are required for potent activity. To obtain information concerning the active conformation of P2P-

Table 3. Enhancing Effects of P2P's Analogs on TPA-Induced TNF- α Production by HL-60 Cells

Compound	Amount of TNF- α (%)	
	10 μM^b	100 μM^b
None ^{a)}	100	(156 pg/ml)
P2P-00	148	258
PM2P-00	125	214
P2MP-00	113	182
P2P-10	172	281
P2P-V0	192	309
P2H-00	123	192
H2P-00	113	183
S2P-00	106	160
(E)-PDP-00	121	178
(Z)-PDP-00	98	105

a) Cells were treated with TPA (3 nM) alone. b) Concentration of the added compound.

00, cinnamphthalimides [(E)-PDP-00, (Z)-PDP-00] were prepared (Fig. 3). The (Z)-form was inactive and the (E)-form was moderately active (Table 3), suggesting that the active conformation of P2P-00 is *s-trans*-like.

Our previous results suggest that introduction of a methyl group at position α or β to the nitrogen atom increases activity.^{18,20,21} Therefore, we prepared methylated P2P-00 analogs, PM2P-00 and P2MP-00. Although PM2P-00 showed the activity, it was weaker than P2P-00; P2MP-00 was even less active (Table 3). Introduction of a methyl group into P2P-00 at these positions might alter the conformation of the compounds.

As mentioned above, introduction of a hydrophobic group at the *ortho*-position of the phenyl moiety tended

Table 4. Effects of P2P-00 and Its Halogenated Analogs on TPA-Induced TNF- α Production by HL-60 Cells

Compound	X	C_{150}^a (M)	Optimum concentration ^b (M)	Amount of TNF- α at optimum concentration ^b (%)	IC_{50}^c (M)
P2P-00	H	2×10^{-6}	1×10^{-4}	311	$> 1 \times 10^{-4}$
CP2P-00	Cl	1×10^{-6}	2×10^{-6}	305	5×10^{-6}
FP2P-00	F	2×10^{-9}	2×10^{-7}	390	1×10^{-6}
None ^d				100 (146 pg/ml)	
Thalidomide				188	$> 1 \times 10^{-4}$

a) The concentration which is necessary to enhance TNF- α production to 150%.
 b) Optimum concentration: the concentration at which the highest amount of TNF- α is produced under the experimental conditions used. c) Effect of the compound on HL-60 cell growth. d) Cells were treated with TPA (3 nM) alone.

Fig. 4. Dose-Dependence Curves of TNF- α Production-Enhancing Activities Elicited by P2P-00, CP2P-00 and FP2P-00

The amount of TNF- α produced in the presence of 3 nM TPA alone was 156 pg/ml, which was taken as 100%.

to increase activity, and introduction of a methyl or methoxy group at this position, *i.e.*, P2P-10 or P2P-V0 (Fig. 3), enhanced the activity of P2P-00 (Table 3). In this case, a methoxy group was more effective than a methyl group, whereas the reverse was true for the P1P's (*cf.*, P1P-10 and P1P-V0, Table 2).

Previously, we reported that halogenation of the phthalimide moiety has a dramatic effect on the activity,²² which led us to prepare chlorinated and fluorinated analogs of P2P-00, *i.e.*, CP2P-00 and FP2P-00 (Table 4). Both compounds showed potent activity at very low concentrations, although they are also toxic (Fig. 4, Table 4). The effect of FP2P-00 was apparent at a concentration as low as 0.25 nM (Fig. 4). The optimum concentration of FP2P-00 for the enhancement of TPA-in-

duced TNF- α production was 0.25 μ M, which is three orders of magnitude lower than that of P2P-00 (Table 4). At higher concentrations, cell growth was inhibited because of the toxicity of these compounds. The optimum concentration of CP2P-00 was one order higher than that of FP2P-00 and two orders lower than that of P2P-00. The effectiveness of FP2P-00 at concentrations lower than 1 nM suggests the existence of a specific receptor-like target molecule for TNF- α production-enhancing activity. Investigations to identify such target molecule(s), as well as to clarify the molecular mechanism of the TNF- α production-enhancing activity elicited by phthalimide analogs, and to establish further structure-activity relationships, are in progress.

CONCLUSION

P1P's and P2P's, such as P1P-10 and P2P-00, with potent enhancing activity on TPA-induced TNF- α production by HL-60 cells were prepared. The structure-activity relationships of these phthalimide analogs suggest that (1) two phenyl groups and their orientation are critical for activity, and (2) introduction of a hydrophobic group at the *ortho*-position of the 3-phenyl ring enhances activity. The fluorinated analog of P2P-00, FP2P-00, which is structurally very similar to P2P-00, exhibited extremely potent activity at very low concentrations. We suggest that the phthalimide analogs, including thalidomide, elicit their TNF- α production-enhancing activity by interacting with common target molecule(s). These phthalimides should be useful as tools to investigate TNF- α production-regulatory mechanisms, and they also represent lead compounds for the development of superior BRMs targeting TNF- α .

MATERIALS AND METHODS

TNF- α Assay The human leukemia cell line HL-60 was cultured in RPMI1640 medium supplemented with 5% v/v fetal bovine serum (FBS) in a humidified atmosphere of 5% CO₂ in air at 37°C. The exponentially growing cells in RPMI1640 medium supplemented with 10% v/v FBS (5×10^5 cells/ml) were treated with TPA (3 nM) in the presence or absence of sample compounds (at the concentrations indicated in the tables and figures) for 24 h at 37°C. Then the cells were counted and separated by centrifugation (1000 rpm $\times$ 10 min). The amount of TNF- α in the medium (supernatant) was measured using a human TNF- α ELISA system (Amersham Co.) according to the manufacturer's protocol. TNF- α production by HL-60 cells was TPA-dependent, and the amount of TNF- α produced in the presence of 3 nM TPA alone (120–180 pg/ml; presented in the tables) was taken as 100%.

Chemicals (A) P1P's and P2P's were prepared in good yields by condensation of phthalic anhydride and appropriate amines.

PP-00: Colorless needles (EtOH). mp 208°C. ¹H-NMR (500 MHz, CDCl₃) δ : 7.43 (3H, m), 7.52 (2H, m), 7.80 (2H, m), 7.96 (2H, m). Mass (FAB): M+H=224. Anal. Calcd for C₁₄H₉NO₂: C, 75.33; H, 4.06; N, 6.27. Found: C, 75.22; H, 4.16; N, 6.55.

P1P-00: Colorless prisms (benzene). mp 115°C.

¹H-NMR (500 MHz, CDCl₃) δ: 4.85 (2H, s), 7.26 (1H, m), 7.31 (2H, m), 7.40 (2H, m), 7.70 (2H, m), 7.84 (2H, m). Mass: M⁺ = 237. *Anal.* Calcd for C₁₅H₁₁NO₂: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.67; H, 4.70; N, 5.89.

P2P-00: White flakes (benzene-hexane). mp 129 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 2.99 (2H, t, J = 8.0 Hz), 3.93 (2H, t, J = 8.0 Hz), 7.22 (1H, m), 7.27 (2H, m), 7.29 (2H, m), 7.71 (2H, m), 7.83 (2H, m). Mass: M⁺ = 251. *Anal.* Calcd for C₁₆H₁₃NO₂: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.67; H, 5.18; N, 5.52.

P3P-00: White flakes (benzene). mp 48 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 1.93 (2H, m), 2.58 (2H, dd, J = 9.0, 8.0 Hz), 3.64 (2H, t, J = 7.0 Hz), 7.03 (1H, m), 7.09 (2H, m), 7.14 (2H, m), 7.59 (2H, m), 7.72 (2H, m). Mass: M⁺ = 265. *Anal.* Calcd for C₁₇H₁₅NO₂: C, 76.98; H, 5.66; N, 5.28. Found: C, 76.73; H, 5.47; N, 5.26.

P1P-10: Pale brown cubes (benzene-hexane). mp 143 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 2.48 (3H, s), 4.87 (2H, s), 7.13 (1H, m), 7.17–7.27 (3H, m), 7.73 (2H, m), 7.86 (2H, m). Mass: M⁺ = 251. *Anal.* Calcd for C₁₆H₁₃NO₂: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.44; H, 5.32; N, 5.68.

P1P-01: White flakes (benzene). mp 117 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 2.32 (3H, s), 4.81 (2H, s), 7.07 (1H, m), 7.18 (1H, m), 7.21 (1H, m), 7.23 (1H, m), 7.70 (2H, m), 7.85 (2H, m). Mass: M⁺ = 251. *Anal.* Calcd for C₁₆H₁₃NO₂: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.43; H, 5.24; N, 5.62.

P1P-001: Colorless cubes (AcOEt). mp 120 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 2.30 (3H, s), 4.80 (2H, s), 7.12 (2H, d, J = 8.0 Hz), 7.33 (2H, d, J = 8.0 Hz), 7.69 (2H, m), 7.78 (2H, m). Mass: M⁺ = 251. *Anal.* Calcd for C₁₆H₁₃NO₂: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.34; H, 5.14; N, 5.61.

P1P-V0: Colorless prisms (benzene-hexane). mp 135 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 3.85 (3H, s), 4.91 (3H, s), 6.87 (2H, m), 7.17 (1H, m), 7.23 (1H, m), 7.72 (2H, m), 7.86 (2H, m). Mass: M⁺ = 267. *Anal.* Calcd for C₁₆H₁₃NO₃: C, 71.90; H, 4.90; N, 5.24. Found: C, 72.08; H, 4.96; N, 5.34.

P1P-0V: Pale yellow cubes (benzene-hexane). mp 116 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 3.79 (3H, s), 4.82 (2H, s), 6.81 (1H, ddd, J = 8.0, 2.5, 1.0 Hz), 6.97 (1H, dd, J = 2.5, 1.0 Hz), 7.15 (1H, d, J = 8.0 Hz), 7.23 (1H, t, J = 8.0 Hz), 7.71 (2H, m), 7.85 (2H, m). Mass: M⁺ = 267. *Anal.* Calcd for C₁₆H₁₃NO₃: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.85; H, 4.81; N, 5.36.

P1P-00V: White needles (benzene-hexane). mp 189 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 3.77 (3H, s), 4.78 (2H, s), 6.84 (2H, d, J = 8.8 Hz), 7.39 (2H, d, J = 8.8 Hz), 7.70 (2H, m), 7.83 (2H, m). Mass: M⁺ = 267. *Anal.* Calcd for C₁₆H₁₃NO₃: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.69; H, 5.12; N, 5.34.

P1P-VV: White flakes (benzene-hexane). mp 161 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 3.78 (6H, s), 4.93 (2H, s), 6.51 (2H, d, J = 8.0 Hz), 7.17 (1H, t, J = 8.0 Hz), 7.64 (2H, m), 7.77 (2H, m). Mass: M⁺ = 297. *Anal.* Calcd for C₁₇H₁₅NO₄: C, 68.68; H, 5.05; N, 4.71. Found: C, 68.71; H, 5.04; N, 4.74.

P1P-N0: Pale brown cubes (CHCl₃). mp 240 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 5.29 (2H, s), 7.24 (1H, dd, J = 8.0, 1.5 Hz), 7.43 (1H, dt, J = 8.0, 1.5 Hz), 7.55 (1H,

dt, J = 8.0, 1.5 Hz), 7.76 (2H, dd, J = 5.5, 3.0 Hz), 7.89 (2H, dd, J = 5.5, 3.0 Hz), 8.09 (1H, dd, J = 8.0, 1.5 Hz). Mass: M⁺ = 282. *Anal.* Calcd for C₁₅H₁₀N₂O₄: C, 63.83; H, 3.55; N, 9.93. Found: C, 63.77; H, 3.41; N, 9.75.

P1P-0N: Pale yellow needles (benzene-hexane). mp 160 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 4.92 (2H, s), 7.49 (1H, t, J = 8.0 Hz), 7.73 (2H, m), 7.74 (1H, d, J = 8.0 Hz), 7.86 (2H, m), 8.12 (1H, ddd, J = 8.0, 2.5, 1.0 Hz), 8.25 (1H, br t, J = 1.5 Hz). *Anal.* Calcd for C₁₅H₁₀N₂O₄: C, 63.83; H, 3.55; N, 9.93. Found: C, 63.87; H, 3.51; N, 9.73.

P1P-00N: White needles (benzene). mp 175 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 4.94 (2H, s), 7.59 (2H, m), 7.75 (2H, m), 7.88 (2H, m), 8.19 (2H, J = 8.0 Hz). Mass: M⁺ = 282. *Anal.* Calcd for C₁₅H₁₀N₂O₄: C, 63.83; H, 3.55; N, 9.93. Found: C, 63.98; H, 3.58; N, 9.95.

P2P-10: Yellow cubes (benzene-hexane). mp 86 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 2.42 (3H, s), 2.96 (2H, t, J = 7.0 Hz), 3.85 (2H, t, J = 7.0 Hz), 7.10 (2H, m), 7.15 (1H, m), 7.69 (2H, m), 7.82 (2H, m). Mass: M + H = 266. *Anal.* Calcd for C₁₇H₁₅NO₂: C, 76.98; H, 5.66; N, 5.28. Found: C, 77.22; H, 5.67; N, 5.37.

P2P-V0: Yellow cubes (benzene-hexane). mp 117 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 3.01 (2H, t, J = 7.0 Hz), 3.75 (3H, s), 3.95 (2H, t, J = 7.0 Hz), 6.80 (1H, dd, J = 8.0, 2.0 Hz), 6.82 (1H, dt, J = 7.0, 1.0 Hz), 7.10 (1H, dd, J = 7.0, 2.0 Hz), 7.18 (1H, dt, J = 8.0, 2.0 Hz), 7.68 (2H, m), 7.80 (2H, m). Mass: M + H = 282. *Anal.* Calcd for C₁₇H₁₅NO₃: C, 72.60; H, 5.34; N, 4.98. Found: C, 72.76; H, 5.20; N, 5.12.

P2H-00: Colorless flakes (EtOH). mp 60 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 1.39 (2H, m), 1.50 (2H, m), 1.75 (2H, br s), 1.92 (2H, br s), 2.18 (2H, t, J = 7.0 Hz), 3.66 (2H, t, J = 7.0 Hz), 5.12 (1H, br s), 7.60 (2H, m), 7.72 (2H, m). Mass: M + H = 256. *Anal.* Calcd for C₁₆H₁₇NO₂: C, 75.29; H, 6.67; N, 5.49. Found: C, 75.35; H, 6.70; N, 5.38.

PM2P-00: White flakes (AcOEt-hexane). mp 48 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 1.53 (3H, d, J = 6.7 Hz), 3.11 (1H, dd, J = 13.7, 6.7 Hz), 3.32 (1H, dd, J = 13.7, 6.7 Hz), 4.68 (1H, dq, J = 9.4, 6.7 Hz), 7.19 (5H, m), 7.66 (2H, m), 7.76 (2H, m). Mass: M + H = 266. *Anal.* Calcd for C₁₇H₁₅NO₂: C, 76.96; H, 5.70; N, 5.28. Found: C, 76.76; H, 5.64; N, 5.40.

P2MP-00: White flakes (AcOEt-hexane). mp 84.5 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 1.32 (3H, d, J = 8.0 Hz), 3.35 (1H, hex., J = 8.0 Hz), 3.79 (1H, dd, J = 13.5, 8.0 Hz), 3.86 (1H, dd, J = 13.5, 8.0 Hz), 7.20 (1H, m), 7.27 (4H, m), 7.69 (2H, m), 7.80 (2H, m). Mass: M + H = 266. *Anal.* Calcd for C₁₇H₁₅NO₂: C, 76.96; H, 5.70; N, 5.28. Found: C, 76.74; H, 5.60; N, 5.48.

Chemicals (B) Aniline (phenylamine) was condensed with appropriate anhydrides to give the following compounds in good yields.

H2P-00: White flakes (benzene). mp 37 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 1.31 (2H, m), 1.37 (2H, m), 1.57 (2H, m), 1.76 (2H, m), 2.74 (2H, m), 2.90 (2H, dd, J = 8.0, 7.0 Hz), 3.74 (2H, dd, J = 8.0, 7.0 Hz), 7.19 (3H, m), 7.25 (2H, m). Mass: M + H = 258. *Anal.* Calcd for C₁₆H₁₉NO₂: C, 74.71; H, 7.39; N, 5.45. Found: C, 74.49; H, 7.31; N, 5.29.

S2P-00: White flakes (AcOEt). mp 125 °C. ¹H-NMR (500 MHz, CDCl₃) δ: 2.65 (4H, s), 2.88 (2H, dd,

$J=8.0$ Hz), 3.75 (2H, dd, $J=8.0$ Hz), 7.22 (3H, m), 7.29 (2H, m). Mass: $M+H=204$. *Anal.* Calcd for $C_{12}H_{13}NO_2$: C, 70.94; H, 6.40; N, 6.89. Found: C, 70.98; H, 6.42; N, 6.74.

CP2P-00: White flakes (benzene). mp 174°C . $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 2.97 (2H, t, $J=7.0$ Hz), 3.92 (2H, t, $J=7.0$ Hz), 7.22 (3H, m), 7.28 (2H, m). Mass: $M+H=388$, 390. *Anal.* Calcd for $C_{16}H_9Cl_4NO_2$: C, 49.38; H, 2.31; N, 3.60; Cl 36.47. Found: C, 49.16; H, 2.17; N, 3.40; Cl, 36.62.

FP2P-00: Pale yellow needles (benzene). mp 174°C . $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 2.92 (2H, t, $J=7.0$ Hz), 3.81 (2H, t, $J=7.0$ Hz), 7.21 (3H, m), 7.28 (2H, m). Mass: $M+H=324$. *Anal.* Calcd for $C_{16}H_9F_4NO_2$: C, 59.44; H, 2.79; N, 4.33; F, 23.53. Found: C, 59.25; H, 2.97; N, 4.45; F, 23.31.

Chemicals (C) P2P-00 was brominated with NBS (1.1 eq) in the presence of benzoyl peroxide (0.08 eq) to give β -bromophenethylphthalimide, from which elimination of HBr by DBU treatment (1.6 eq) in THF gave (*E*)-PDP-00. Photoisomerization (high-pressure mercury lamp, 400 W, 1 h) of (*E*)-PDP-00 and separation of the isomeric mixture gave (*Z*)-PDP-00 (yield: 18%).

(*E*)-PDP-00: Pale yellow needles (AcOEt). mp 184°C . $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 7.27 (1H, m), 7.35 (2H, m), 7.37 (1H, d, $J=15.2$ Hz), 7.48 (2H, m), 7.66 (1H, d, $J=15.2$ Hz), 7.77 (2H, m), 7.91 (2H, m). Mass: $M^+=249$. *Anal.* Calcd for $C_{16}H_{11}NO_2$: C, 77.10; H, 4.45; N, 5.62. Found: C, 77.18; H, 4.54; N, 5.66.

(*Z*)-PDP-00: Pale yellow needles (AcOEt-hexane). mp 120°C . $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 6.33 (1H, d, $J=9.0$ Hz), 6.70 (1H, d, $J=9.0$ Hz), 7.24 (5H, m), 7.74 (2H, m), 7.86 (2H, m). Mass: $M^+=249$. *Anal.* Calcd for $C_{16}H_{11}NO_2$: C, 77.10; H, 4.45; N, 5.62. Found: C, 77.08; H, 4.54; N, 5.77.

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