Thalidomide Analogs from Diamines: Synthesis and Evaluation as Inhibitors of TNF-α Production

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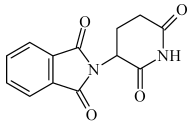
Fourteen thalidomide analogs bearing two phthalimido units were prepared in high yields (83—94%) by condensation of different diamines with phthalic or 3-nitrophthalic anhydride. An *in vitro* investigation of the compounds as inhibitors of the TNF-α production was performed. The inhibition was higher for compounds bearing amino and nitro groups and was modulated by increasing the size of the spacers between the phthalimide groups.

Key words thalidomide; thalidomide analog; TNF-α; diamine; phthalimide

Thalidomide (Fig. 1), a hypnotic/sedative agent, was withdrawn from the market in 1961—1962, because it presented severe teratogenicity.[1—3] However, in recent years, this drug has attracted growing interest due to its potential usefulness for the treatment of various diseases, including leprosy, immunodeficiency syndrome (AIDS), multiple myeloma, various kinds of cancers and other angiogenesis-dependent disorders.[3—9]

In July 1998 thalidomide was approved by the U.S. Food and Drug Administration (FDA) under critical control as a treatment for the erythema nodosum leprosum, an inflammatory complication of Leprosy.[10,11] Thalidomide has been reported to inhibit markedly and selectively TNF-α production,[12—14] but it may also affect to a lesser extent the production of interleukin-1β, IL-2, IL-4, IL-5, IL-6, IL-10 and IFN-γ.[15—17] TNF-α, a highly pleiotropic cytokine produced largely by monocytes and macrophages, is a primary mediator of the inflammatory response and is commonly associated with a broad range of pathological conditions.[18] Polyamines and diamines such as putrescine (1,4-diaminobutane) play key roles in a number of biological processes and possess a variety of pharmacological properties.[19,20] In this context, we chose a series of diamine compounds as analogs of thalidomide containing phthalimide structure. Each compound bears two phthalimido units and was prepared by condensation of these diamines with phthalic or 3-nitrophthalic anhydride (Fig. 2). The effect of these compounds on TNF-α production was evaluated using human peripheral blood mononuclear cell (PBMC) cultures stimulated with LPS.

Our results show that compounds bearing two phthalimido units could easily be obtained in high yield by the synthesis sequence depicted in Fig. 2. The condensation of phthalic or 3-nitrophthalic anhydride with the different diamines commercially available (ethylenediamine, 1,3-propanediamine, 1,4-butanediamine, 1,6-hexanediamine, 1,3-xylylenediamine and a mixture of *cis*, *trans* 1,3-dimethylaminocyclohexane) was carried out in refluxing acetic acid, yielding 83—94% of compounds **2**—**13**. In the case of compounds bearing nitro groups, the reduction of the latter to the corresponding amines was performed by catalytic hydrogenation in dimethylformamide, thus affording compounds **14** and **15** in high yield (90%). Due to the starting material being a mixture of commercially available *cis* and *trans* 1,3-dimethylaminocyclohexane, compounds **12**, **13** and **15** were obtained as a mixture of *cis* and *trans* isomers.



Thalidomide (1)

Fig. 1. The Structure of Thalidomide

The inhibition of TNF-α production by monocytes *in vitro* in the presence of LPS is a good model for evaluation of antiinflammatory drugs.[21,22] To investigate if the prepared compounds were able to inhibit the TNF-α production stimulated by LPS, the PBMC were incubated with different concentrations of the compounds and after 1 h stimulated by LPS. The TNF-α production in the cultures that were not stimulated was less then 300 pg/ml (data not shown). However, the incubation of the cells with LPS (2 μg/ml) was responsible for an increased production of TNF-α (952 ± 65 pg/ml; $n=3$). When the PBMC were stimulated with LPS in the presence of the compounds, with the concentration being between 20 and 880 μM, a remarkable inhibition of TNF-α production was observed (Table 1). Higher TNF-α inhibition by these compounds was not correlated with an effect on cell viability. The Trypan blue test for some compounds showed a slight effect on cell viability, which was not detected by the MTT assay (Table 2). Man and cols described a α-fluoro-4-amino analogue with low cytotoxicity.[7] In our study the amino compounds (**14**, **15**) showed a very slight improvement in viability measured by the Trypan blue in comparison to the nitro compounds (**7**, **13**).

It has been reported that amino or nitro analogues of thalidomide are more potent as TNF-α inhibitors than thalidomide in LPS-stimulated human PBMC.[7,23,24] In agreement, our results show that compounds bearing nitro (**7**—**9**, **11**, **13**) or amino (**14**, **15**) groups exhibited a capacity to inhibit TNF-α production higher than thalidomide (IC_{50} 144 μM, Table 2). Compound **15**, which possess the NH_2 group in the phthalimide ring, was less active than the nitro

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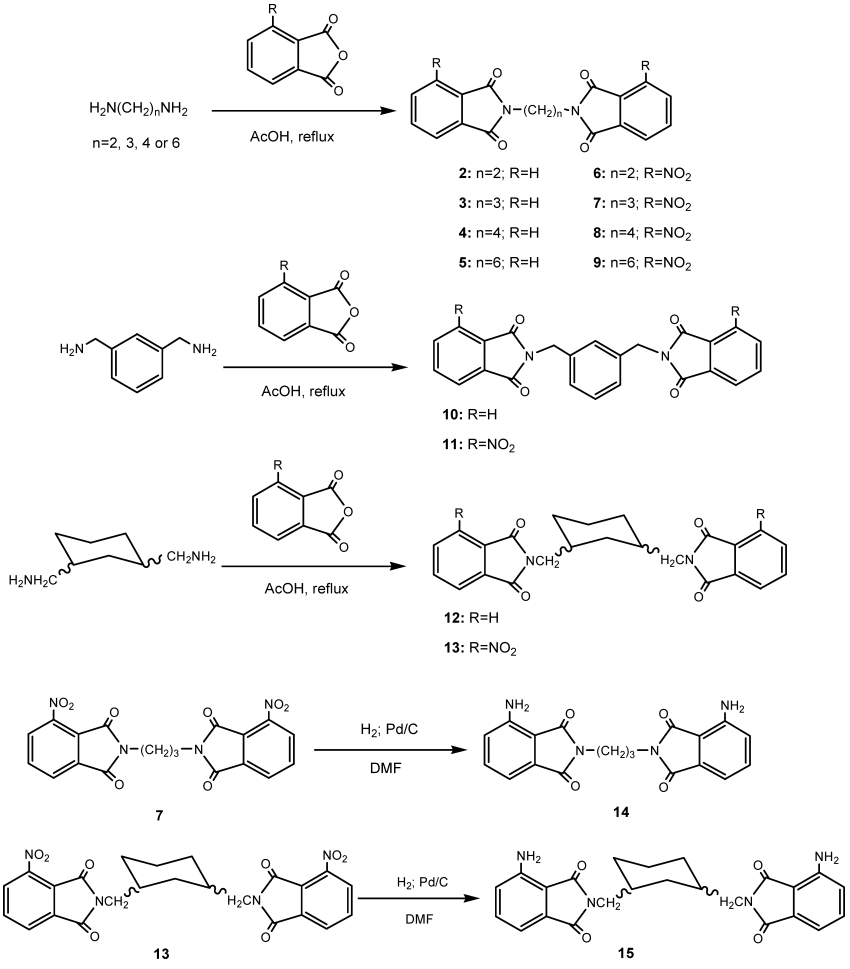

Fig. 2. The Structures and Synthesis Procedures for the Preparation of Compounds **2**—**15**

Table 1. Inhibition of TNF-α Production by Thalidomide Analogs in LPS-Stimulated Peripheral Blood Mononuclear Cells

Concentration[a)]	**2**[b)]	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	Thalidomide
0	952±65[c)] (0%)[d)]	952±65 (0%)	952±65 (0%)	952±65 (0%)	952±65 (0%)	952±65 (0%)	952±65 (0%)	952±65 (0%)	952±65 (0%)	952±65 (0%)	952±65 (0%)	952±65 (0%)	952±65 (0%)	952±65 (0%)	952±65 (0%)
20	0%	0%	0%	0%	0%	0%	593±5.5 (38%)	0%	0%	757±4.9 (20%)	0%	303±26 (68%)	533±2.5 (44%)	634±8.2 (33%)	0%
50	0%	0%	0%	844±9.2 (11%)	0%	647±5.7 (32%)	442±8.0 (53%)	726±35 (24%)	0%	501±4.3 (47%)	0%	245±1.2 (74%)	456±1.0 (52%)	485±8.0 (49%)	697±68 (27%)
110	0%	0%	894±6.7 (6%)	502±9.7 (47%)	907±25 (5%)	401±5.3 (58%)	344±33 (64%)	488±19 (49%)	0%	351±3.9 (63%)	0%	204±1.4 (79%)	399±3.5 (58%)	384±19 (60%)	524±28 (45%)
220	0%	664±35 (30%)	497±5.2 (48%)	318±1.5 (67%)	603±19 (37%)	263±4.0 (72%)	275±9.9 (71%)	345±5.9 (64%)	0%	257±35 (73%)	0%	173±1.3 (82%)	354±37 (63%)	314±53 (67%)	408±9.9 (57%)
440	0%	378±9.6 (60%)	276±4.5 (71%)	201±0.1 (79%)	401±2.2 (58%)	173±4.9 (82%)	220±6.9 (77%)	243±0.9 (74%)	711±8.6 (25%)	188±9.6 (80%)	463±15 (51%)	147±2.6 (84%)	315±22 (67%)	256±22 (73%)	318±20 (67%)
880	693±68 (27%)	215±9.8 (77%)	153±0.1 (84%)	127±2.5 (87%)	266±4.4 (72%)	113±7.8 (88%)	177±4.6 (81%)	172±6.5 (82%)	363±48 (62%)	138±2.7 (85%)	217±3.4 (77%)	125±1.8 (87%)	279±26 (71%)	209±14 (78%)	247±45 (74%)

a) Concentration of the drug (μM). *b*) Thalidomide analog (compounds **2**—**15**). *c*) TNF-α production (pg/ml). Values represent means±S.D. of three separate experiments. *d*) 0% inhibition of TNF-α production means that TNF-α response induced by the compound is greater than or equal to the amount obtained in PBMC cultures stimulated only by LPS. In parenthesis is the inhibition as a percentage of the decline from the LPS-induced 952 pg/ml of TNF-α (0% inhibition level).

derivative (**13**), but this did not occur for compound 14 in comparison with compound **7** (Table 2), which may suggest the influence of the type and size of the spacer between the phthalimido units on the biological activity of the compound. In this context, inhibition of TNF-α production was strongly modulated by the length of the diamine spacer. For example, ethylenediamine (**2**) was much less effective, while butanediamine (**4**) and hexanediamine (**5**), for phthalimide derivatives, were highly effective. As far as we are aware, this work is the first to demonstrate an effect of the size of the diamine spacer of thalidomide analogs bearing two phthalimido units on inhibition of TNF-α. Furthermore, compounds bearing aromatic (**10**) or cyclohexyl (**12**) spacers were less active when compared with compounds containing less rigid structures, such as compounds **3**—**5**. These differences could be due to thalidomide analogs being found in different half-

Table 2. Inhibition of LPS-Induced TNF-α Production in Peripheral Blood Mononuclear Cells (PBMC) by Thalidomide Analogs from Diamines

Compound	IC_{50} (μM)[a]	Cell viability[b] by TB (%)	Cell viability[c] by MTT (%)
2	1220	100	99
3	332	77	99
4	231	97	100
5	119	89	99
6	328	84	99
7	83	95	99
8	40	100	100
9	116	75	100
10	665	79	100
11	56	100	99
12	429	75	99
13	3	93	100
14	39	98	100
15	53	100	100
Thalidomide	144	100	99

a) 5×10^4 PBMC were incubated in the presence of the indicated compounds for 1 h before addition of LPS (2 μg/ml). After 24 h, TNF-α concentration in the culture supernatant was determined by ELISA. IC_{50} for TNF-α inhibition in LPS-stimulated human PBMCs. *b*) Cell viability measured by Trypan blue exclusion. Cytotoxic effects on LPS-treated PBMC after 24 h culture are indicated as percentage of surviving cells. *c*) Cell viability measured by the MTT. The viable cell number was expressed as a percentage relative to control cells, measured as $100\%\times OD_{570}$, treated/OD_{570}, control.

lives, stability, solubility, and potency.[15] Our data are consistent with studies in which modification of thalidomide has enabled the generation of compounds that are more potent TNF-α inhibitors.[7,23—26] The mechanism underlying the action of these compounds remains to be determined. It may include the induction of TNF-α expression, as well as TNF-α synthesis, processing and release.[27] Similar to thalidomide, the compounds may exert their effect by: 1) selective inhibition of TNF-α by enhancing mRNA degradation[27]; 2) binding to α_1-acid glycoprotein (AGP) with high specificity[28]; or, 3) PDE4 inhibition.[29] These deserve further study, as well as other possible biological response mechanisms based on thalidomide which have been described by others.[3]

In conclusion, this work describes the synthesis and characterization of fourteen thalidomide analogs, prepared in good yields using simple methodology. Higher inhibition of TNF-α production was observed for compounds bearing nitro and amino groups and by increasing spacers between the phthalimide groups.

Experimental

General Procedure Melting points were determined with a Thomas–Hoover apparatus and are uncorrected. Infrared spectra were obtained on a Bomem FT IR MB-102 spectrometer in KBr pellets. ^{1}H-NMR (200, 300 MHz) and ^{13}C-NMR (50, 75 MHz) spectra were recorded on Bruker Avance DRX 200 or DRX 300 spectrometers at the Federal University of Minas Gerais and at the Federal University of Juiz de Fora. Elemental analyses were done at the Mycroanalyses Laboratory at ICNS/CNRS, Gif-sur-Yvette, France and at the Central Analítica, USP-Brazil. The progress of all reactions was monitored by thin-layer chromatography, which was performed on 2.0×6.0 cm aluminium sheets precoated with silica gel 60 (HF-254, Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under an ultraviolet light. For column chromatography Merck silica gel (70—230 mesh) was used.

Preparation of Phthalimide Derivatives 2—13 To a solution of phthalic anhydride (10 mmol) or 3-nitrophthalic anhydride (10 mmol) in acetic acid (15 ml) were added the commercially available amines 1,2-ethylenediamine, 1,3-propanediamine, 1,4-butanediamine, 1,6-hexanediamine, 1,3-xylylenediamine or a mixture of *cis* and *trans*-1,3-dimethylaminocyclohexane (5 mmol). The reaction was stirred at reflux for 4—8 h, poured into water, and the resulting precipitate filtered off by suction and recrystallized.

N,*N*′-Diphthaloyl-1,2-ethylenediamine (**2**): Yield: 83%, as white crystals, mp 236—238 °C (from chloroform); lit.[30] mp 236 °C. *Anal.* Calcd for $C_{18}H_{12}N_2O_4$: C, 67.50, H, 3.75, N, 8.75; Found: C, 67.14, H, 3.97, N, 8.95; IR (cm^{-1}) ν: 3464, 2948, 1705; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 4.00 (s, 4H, CH_2), 7.74 (m, 8H, Ph); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 36.8 (CH_2), 123.3—134.0 (Ph), 168.2 (C=O).

N,*N*′-Diphthaloyl-1,3-propanediamine (**3**): Yield: 86%, as white crystals, mp 195—197 °C (from chloroform); lit.[30] mp 198 °C. *Anal.* Calcd for $C_{19}H_{14}N_2O_4$: C, 68.26, H, 4.19, N, 8.38; Found: C, 68.14, H, 3.97, N, 8.45; IR (cm^{-1}) ν: 3457, 2948, 1712; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 2.11 (m, 2H, CH_2), 3.78 (t, 4H, CH_2N), 7.78 (m, 8H, Ph); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 28.3 (CH_2), 35.7 (CH_2N), 123.3—134.2 (Ph), 168.3 (C=O).

N,*N*′-Diphthaloyl-1,4-butanediamine (**4**): Yield: 90%, as white crystals, mp 227—229 °C (from chloroform) lit.[30] mp 227 °C. *Anal.* Calcd for $C_{20}H_{16}N_2O_4$: C, 68.97, H, 4.60, N, 8.05; Found: C, 68.67, H, 4.74, N, 8.24; IR (cm^{-1}) ν: 3458, 2935, 1718; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.70 (m, 4H, CH_2), 3.70 (m, 4H, CH_2N), 7.70 (m, 8H, Ph); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 26.1 (CH_2), 37.5 (CH_2N), 123.3—134.0 (Ph), 168.4 (C=O).

N,*N*-Diphthaloyl-1,6-hexanediamine (**5**): Yield: 93%, as yellow crystals, mp 194—196 °C (from chloroform) lit.[31] mp 196.5—197.5 °C. *Anal.* Calcd for $C_{22}H_{20}N_2O_4$: C, 70.21, H, 5.32, N, 7.45; Found: C, 69.97, H, 5.27, N, 7.42; IR (cm^{-1}) ν: 3462, 2926, 1708; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.32 (m, 4H, CH_2), 1.61 (m, 4H, CH_2), 3.60 (t, 4H, CH_2N), 7.70 (m, 8H, Ph); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 26.5 (CH_2), 28.6 (CH_2), 38.0 (CH_2N), 123.3—133.9 (Ph), 168.5 (C=O).

N,*N*′-Di-(3-nitrophthaloyl)-1,2-ethylenediamine (**6**): Yield: 88%, as a white solid, mp >300 °C; *Anal.* Calcd for $C_{18}H_{10}N_4O_8$: C, 52.68, H, 2.44, N, 13.66; Found: C, 52.78, H, 2.29, N, 13.36; IR (cm^{-1}) ν: 3483, 3084, 1724, 1538; ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 3.87 (s, 4H, CH_2N), 8.06 (t, 2H, H-5, H-5′, $J_{5\text{-}4}=J_{5\text{-}6}=7.8$ Hz), 8.15 (d, 2H, H-6, H-6′, $J_{6\text{-}5}=7.8$ Hz), 8.28 (d, 2H, H-4, H-4′, $J_{4\text{-}5}=7.8$ Hz); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 36.4 (CH_2N), 122.8—144.3 (Ph), 163.3 (C=O), 166.0 (C=O).

N,*N*′-Di-(3-nitrophthaloyl)-1,3-propanediamine (**7**): Yield: 85%, as yellow crystals, mp 198—200 °C (from tetrahydrofuran; *Anal.* Calcd for $C_{19}H_{12}N_4O_8$: C, 53.77, H, 2.83, N, 13.20; Found: C, 53.53, H, 2.95, N, 13.23; IR (cm^{-1}) ν: 3476, 3090, 1712, 1544; ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 2.00 (m, 2H, CH_2, J=7.1 Hz), 3.65 (t, 4H, CH_2N, J=7.1 Hz), 8.04 (t, 2H, H-5, H-5′, $J_{5\text{-}4}=J_{5\text{-}6}=7.5$ Hz), 8.14 (d, 2H, H-6, H-6′, $J_{6\text{-}5}=7.5$ Hz), 8.25 (d, 2H, H-4, H-4′, $J_{4\text{-}5}=7.5$ Hz); ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 26.1 (CH_2), 35.9 (CH_2N), 122.9—144.2 (Ph), 163.2 (C=O), 165.8 (C=O).

N,*N*′-Di-(3-nitrophthaloyl)-1,4-butanediamine (**8**): Yield: 87%, as a yellow solid, mp 249—251 °C (from tetrahydrofuran); *Anal.* Calcd for $C_{20}H_{14}N_4O_8$: C, 54.80, H, 3.20, N, 12.79; Found: C, 54.50, H, 3.47, N, 12.99; IR (cm^{-1}) ν: 3476, 2935, 1718, 1538; ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.63 (m, 4H, CH_2), 3.59 (m, 4H, CH_2N), 8.03 (t, 2H, H-5, H-5′, $J_{5\text{-}4}=J_{5\text{-}6}=7.7$ Hz), 8.14 (d, 2H, H-6, H-6′, $J_{6\text{-}5}=7.7$ Hz), 8.26 (d, 2H, H-4, H-4′, $J_{4\text{-}5}=7.7$ Hz); ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 25.0 (CH_2), 37.5 (CH_2N), 123.0—144.2 (Ph), 163.3 (C=O), 166.0 (C=O).

N,*N*′-Di-(3-nitrophthaloyl)-1,6-hexanediamine (**9**): Yield: 94%, as a yellow solid, mp 198—200 °C (from tetrahydrofuran); *Anal.* Calcd for $C_{22}H_{18}N_4O_8$: C, 56.65, H, 3.86, N, 12.02; Found: C, 56.34, H, 3.97, N, 11.95; IR (cm^{-1}) ν: 3477, 2935, 1717, 1547; ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.30 (s, 4H, CH_2), 1.57 (s, 4H, CH_2), 3.55 (t, 4H, CH_2N, J=6.6 Hz), 8.03 (t, 2H, H-5, H-5′, $J_{5\text{-}4}=J_{5\text{-}6}=7.8$ Hz), 8.13 (d, 2H, H-6, H-6′, $J_{6\text{-}5}=7.8$ Hz), 8.26 (d, 2H, H-4, H-4′, $J_{4\text{-}5}=7.8$ Hz); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 25.8 (CH_2), 27.5 (CH_2), 37.9 (CH_2N), 123.0—144.2 (Ph), 163.4 (C=O), 166.0 (C=O).

N,*N*′-Diphthaloyl-1,3-xylylenediamine (**10**): Yield: 91%, as white crystals, mp 235—237 °C (from chloroform); lit.[32] mp 235—237 °C. *Anal.* Calcd for $C_{24}H_{16}N_2O_4$: C, 72.72, H, 4.04, N, 7.07; Found: C, 72.58, H, 4.25, N, 6.99; IR (cm^{-1}) ν: 3457, 2948, 1705; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 4.81 (s, 4H, CH_2N), 7.26—7.86 (m, 12H, Ph); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 41.4 (CH_2), 123.4—136.8 (Ph), 168.0 (C=O).

N,*N*′-Di-(3-nitrophthaloyl)-1,3-xylylenediamine (**11**): Yield: 92%, as white solid, mp 298—300 °C (from tetrahydrofuran); *Anal.* Calcd for $C_{24}H_{14}N_4O_8$: C, 59.26, H, 2.88, N, 11.52; Found: C, 59.56, H, 2.97, N, 11.36; IR (cm^{-1}) ν: 3470, 2993, 1724, 1531; ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 4.76 (s, 4H, CH_2N), 7.25 (m, 4H, Ph), 8.06 (t, 2H, H-5, H-5′, $J_{5\text{-}4}=J_{5\text{-}6}=7.3$ Hz), 8.17 (d, 2H, H-6, H-6′, $J_{6\text{-}5}=7.3$ Hz), 8.26 (d, 2H, H-4, H-4′, $J_{4\text{-}5}=7.3$ Hz); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 41.2 (CH_2N), 123.1—144.4 (Ph), 163.2 (C=O), 165.9 (C=O).

N,*N*′-Diphthaloyl-*cis*, *trans*-1,3-dimethylaminocyclohexane (**12**): Yield: 90%, as white crystals, mp 173—175 °C (from chloroform); *Anal.* Calcd for

$C_{24}H_{22}N_2O_4$: C, 71.63, H, 5.51, N, 6.96; Found: C, 71.56, H, 5.57, N, 6.76; IR (cm^{-1}) ν: 2929, 1712; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.80—2.08 (m, 10H, CH_2), 3.50—3.56 (2d, 4H, CH_2N), 7.65—7.81 (m, 8H, Ph); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 20.4—44.7 (CH_2), 123.0—133.8 (Ph), 168.5 (C=O).

N,*N*′-Di-(3-nitrophthaloyl)-*cis*, *trans*-1,3-dimethylaminocyclohexane (**13**): Yield: 90%, as a yellow solid, mp 209—212 °C (from tetrahydrofuran); *Anal.* Calcd for $C_{24}H_{20}N_4O_8$: C, 58.54, H, 4.09, N, 11.38; Found: C, 58.63, H, 4.03, N, 11.48; IR (cm^{-1}) ν: 2919, 1712, 1540; ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 0.80—2.09 (m, 10H, CH_2), 3.39—3.50 (2d, 4H, CH_2N), 7.97—8.27 (m, 6H, Ph); ^{13}C-NMR (50 MHz, DMSO-d_6) δ: 20.5—44.0 (CH_2), 122.9—144.2 (Ph), 163.4—166.1 (C=O).

General Procedure for Reduction of Nitro Groups To a solution of compounds **7** or **13** (1 mmol) in dimethylformamide (5 ml) was added 10% palladium on carbon (30 mg). The mixture was stirred under an atmosphere of hydrogen for 4 h, filtered off and concentrated. The residue was purified by column chromatography (CH_2Cl_2/MeOH) yielding the desired amines **14** and **15**, respectively.

N,*N*′-Di-(3-aminophthaloyl)-1,3-propanediamine (**14**): Yield: 90%, as a yellow solid, mp 244—246 °C (from acetone); *Anal.* Calcd for $C_{19}H_{16}N_4O_4$: C, 62.63, H, 4.43, N, 15.38; Found: C, 62.56, H, 4.57, N, 15.36; IR (cm^{-1}) ν: 3458, 3341, 1686; ^{1}H-NMR (300 MHz, acetone-d_6) δ: 1.87 (m, 2H, CH_2, J=7.1 Hz), 3.52 (t, 4H, CH_2N, J=7.1 Hz), 6.41 (s, 4H, NH_2), 6.94 (d, 2H, H-4, J=7.1 Hz), 6.96 (d, 2H, H-6, J=8.4 Hz), 7.41 (dd, 2H, H-5, $J_{5\text{-}4}$=7.1 Hz, $J_{5\text{-}6}$=8.4 Hz); ^{13}C-NMR (50 MHz, acetone-d_6) δ: 27.3 (CH_2), 34.8 (CH_2N), 118.9—146.4 (Ph), 168.0 (C=O), 169.3 (C=O).

N,*N*′-Di-(3-aminophthaloyl)-*cis*, *trans*-1,3-dimethylaminocyclohexane (**15**): Yield: 90%, as a yellow solid, mp 208—210 °C (from acetone); *Anal.* Calcd for $C_{24}H_{24}N_4O_4$: C, 66.65, H, 5.59, N, 12.96; Found: C, 66.56, H, 5.77, N, 13.08; IR (cm^{-1}) ν: 3349, 2929, 1699; ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.80—2.03 (m, 10H, CH_2), 2.99 (s, 4H, NH_2), 3.45 (2d, 4H, CH_2N), 6.98—7.47 (m, 6H, Ph); ^{13}C-NMR (50 MHz, acetone-d_6) δ: 20.2—42.9 (CH_2), 118.8—146.4 (Ph), 168.2 (C=O); 169.6 (C=O).

Determination of TNF-α and Cell Viability Peripheral blood mononuclear cells (PBMC) were isolated from healthy donors by density centrifugation on a Ficoll-Paque Plus (Amersham Bioscience, Uppsala, Sweden). 5×10^4 PBMC were incubated with thalidomide or the analog compounds in RPMI 1640 medium (Gibco, Grand Island, NY, U.S.A.) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 μl/ml non-essential aminoacids and 5% heat-inactivated fetal calf serum, for 1 h before the addition of 2 μg/ml LPS. After 24 h of culture, cell viability was determined by Trypan blue exclusion and by the diphenyltetrazolium assay-MTT.[33] Briefly, MTT (5 mg/ml) was dissolved in RPMI, sterilized through 0.22 μm membranes and added to the plate, 10 μl/well, for 4 h at 37 °C. PBMC were incubated without compounds and used as viability control. Cell viability was directly proportional to OD value. The viable cell number was expressed as a percentage relative to control cells, measured as $100\%\times OD_{570}$, treated/OD_{570}, control. TNF-α levels in culture supernatants were determined by standard ELISA (R&D Systems). Binding of antibodies were detected using the streptavidin-biotinylated horseradish peroxidase complex (Southern Biotechnology Associates, Inc., Birmingham, AL, U.S.A.), TMB and H_2O_2. The reaction was stopped with 1 M sulfuric acid, according to the manufacturer's R&D. The plates were read at 450 nm using an ELISA reader (Spectramax 190-Molecular Devices). Samples were quantified by comparison with standard curves with purified recombinant TNF-α, and values expressed as pg/ml.

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