European Journal of Medicinal Chemistry 44 (2009) 3533–3542

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

# Discovering a new analogue of thalidomide which may be used as a potent modulator of TNF-α production

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ARTICLE INFO

*Article history:*
Received 11 February 2009
Received in revised form
13 March 2009
Accepted 16 March 2009
Available online 26 March 2009

*Keywords:*
Thalidomide
Diphenylmaleimide derivate
TNF-α

ABSTRACT

A new series of imide derivatives related to thalidomide were synthesized and evaluated as modulators of TNF-α production. These derivatives enhance TNF-α production using human leukemia HL-60 cells induced with 12-*O*-tetradecanoylphorbol 13-acetate (TPA), while inhibiting TNF-α production induced with okadaic acid (OA) in the same cell line. The diphenylmaleimide derivative **2f**, was found to be the most active product, producing a strong modulation of the cytokine level.



## 1. Introduction

Thalidomide (Thalomid™, Celgene Corp) (Fig. 1) is a synthetic phthalimido glutamic acid derivative developed as a sedative-hypnotic agent to treat emesis in pregnancy [1]. Despite its early successful clinical results, use was halted because of its teratogenic properties [2]. Nevertheless, there has been a resurgence of interest in the drug in recent years due to its potential usefulness in the treatment of *Erythema Nodosum Leprosum* [3,4] multiple myeloma [5], AIDS, and various cancers [6]. It has been discovered that thalidomide has various biological activities, including, the inhibition of tumor necrosis factor-α (TNF-α) [7,8] production, as well as anti-inflammatory, anti-angiogenic [9], and cycloxygenase inhibitory activities [10]. The TNF-α production inhibitory activity was initially considered to be one of the key thalidomide action mechanisms [11], though the precise molecular mechanism(s) involved remain unclear. An interesting review of thalidomide activity and other less teratogenic homologous has recently been published [12].

Tumor necrosis factor-α (TNF-α), an important cytokine produced by activated monocytes/macrophagues, has been identified as an endotoxin-induced serum factor that causes hemorrhagic necrosis of transplanted solid tumors [13]. TNF-α plays a critical role in several physiological immune systems, and can cause severe damage when it is produced in excess. Therefore, TNF-α can be regarded as possessing both favorable and unfavorable effects. The favorable effects include direct tumor killing action, stimulation of the host's immune system, and acting as a growth factor for normal B-cells. The unfavorable effects include induction of tissue inflammation, a tumor-promoting action, stimulation of human immunodeficiency virus (HIV) replication, and induction of insulin resistance. These pleiotropic effects of TNF-α demonstrate that cytokine production enhancers and inhibitors could be of use as biological response modifiers under various circumstances [14,15].

On this basis, we have synthesized a new series of imide thalidomide derivatives with the aim of creating bidirectional regulators of TNF-α production. As the regulation of this cytokine production was found to be inducer-specific, compounds were tested using the TPA-stimulated HL-60 and OA-stimulated HL-60 assay system [16].

In this paper we describe the synthesis and biological activity of a new series of thalidomide homologues. In order to improve TNF-α production modulator activity, we explored structural modifications of the thalidomide phthaloyl ring (compounds **1a**–**f**). The choice of changes made to the phthalimido moiety was determined

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doi:10.1016/j.ejmech.2009.03.018

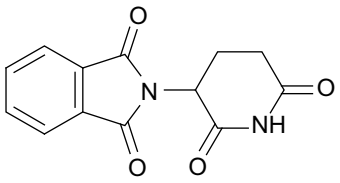


**Fig. 1.** Thalidomide.

by our experience in other areas of cancer research [17,18]. To investigate the contribution of basic residue, a dimethylaminoethyl group was introduced to the glutarimidic nitrogen. This basic residue may also increase water solubility (compounds **2a**–**g**). The open ring intermediate, compounds **3a**–**g**, were also evaluated. Polyamines and diamines play key roles in several of biological processes and possess a variety of pharmacological properties. Thus, we synthesized a new series of bis-glutarimides (compounds **6a**, **d**, **f**–**g**) consisting of two imide units joined by *N*,*N*-bis(2-aminoethyl)-*N*-methylamine. Moreover, two dimeric type thalidomide homologues, **4**–**5**, were obtained (Fig. 2).

## 2. Results and discussion

### 2.1. Chemistry

Thalidomide was prepared following the procedure described by Jönsson [19]. Syntheses of the final compounds are described in Schemes 1–7. Anhydride **7d** was obtained by reaction of 2,3-naphthalenedicarboxylic acid at reflux in acetic anhydride, and **7e** by the Hershberg and Fieser procedure [20]. The other anhydrides are commercially available. Intermediates **7a**–**b**, **d**–**f** were synthesized according the procedure of King and Kidd [21]. These compounds reacted with L-glutamic acid in pyridine at reflux, followed by cyclization in acetic anhydride, to obtain compounds **9a**–**b**, **d**–**f**.

Scheme 2 describes the synthesis of amidoacids **3a**–**f**. The appropriate glutaric anhydrides **9a**, **d**–**f** reacted with gaseous ammonia in dioxane to give a high yield compounds **3a**–**b**, **d**–**f**. The synthesis of compound **3c** was carried out by hydrogenation of **3b** to give 89% yield.

In Scheme 3, we describe the synthesis of glutarimides **1a**–**f**. Glutaric anhydrides **9a**, **d**–**e** were heated in the presence of ammonium carbonate to obtain high yields of **1a**, **d**–**e**. Compound **1f** was obtained by refluxing **3f** with a (1:1) mixture of acetic anhydride:acetyl chloride. Finally, compound **3b** was directly converted into **1b** by heating at 195–250 °C in the absence of solvent and high yields of **1c** was obtained by hydrogenation of **1b**.

*N*-Substituted glutarimides **2a**–**g** were synthesized by treating the corresponding anhydride with *N*,*N*-dimethylethylenediamine followed by cyclization, as depicted in Scheme 4. In this case the amidoacid intermediate was not isolated, and was directly converted into the *N*-substituted glutarimide by reaction with a (1:1) mixture of acetic anhydride:acetyl chloride at reflux temperature. All compounds were isolated as the water-soluble hydrochloride. Finally, compound **2c** was obtained by hydrogenation of the nitro derivative **2b** (Scheme 4).

Compound **11** was obtained by refluxing pyromellitic anhydride **10** with L-glutamic acid in the presence of pyridine. The solid obtained was transformed into the final compound by heating in acetic anhydride. However, all attempts to obtain glutarimides **4** by reaction of **11** with ammonia or ammonium carbonate failed. We also prepared **4**, obtaining good yield (83%) by direct reaction of **10** with of 3-aminopiperidine-2,6-dione hydrochloride 12, following the procedure of Muller et al. [22], as outlined in Scheme 5.

Scheme 6 describes the synthesis of *N*,*N*′ bis-substituted phthalimide **5**. This compound was obtained by direct reaction of **11** with *N*,*N*-dimethylethylenediamine, in a 1:2 ratio, followed by reaction with a (1:1) mixture of acetic anhydride:acetyl chloride at reflux temperature.

Finally, compounds **6a**, **d**, **f**–**g** were synthesized using a similar procedure, starting from glutaric anhydrides **9a**, **d**, **f**–**g** and *N*,*N*-bis(2-aminoethyl)-*N*-methylamine in a 2:1 ratio, as outlined in Scheme 7.

### 2.2. In vitro antiproliferative activity

All compounds and thalidomide were tested for cytotoxicity in a MTT assay [23] against several human cell lines: colon carcinoma DLD-1 and HT-29, Burkitt lymphoma ST-486, leukemia HL-60 and endothelial HUVEC. No antiproliferative effect was detected ($IC_{50} > 50$ μM in all cases).

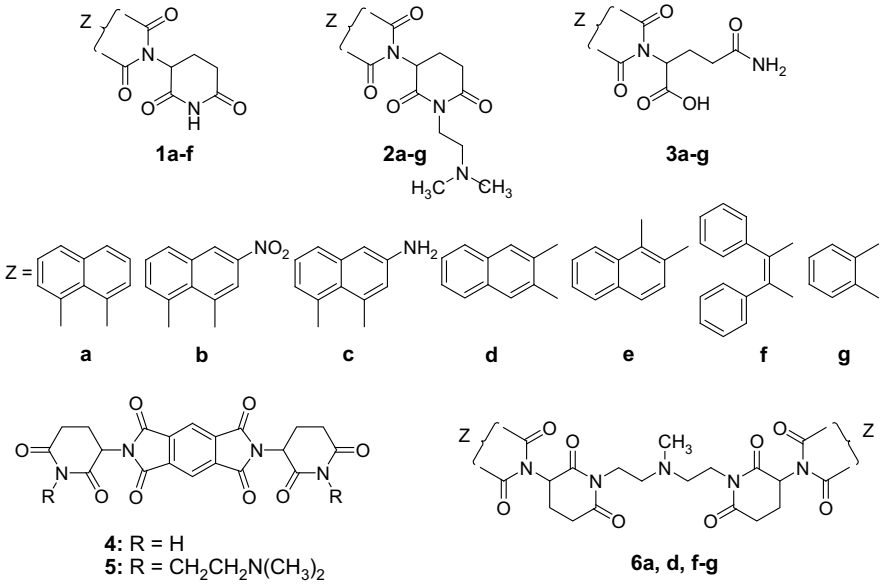
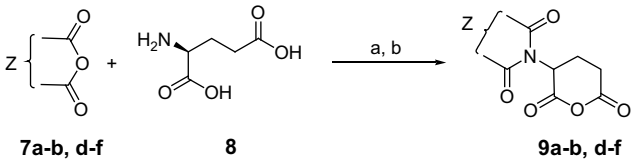



**Fig. 2.** Compounds **1a**–**f**, **2a**–**g**, **3a**–**g**, **4**, **5**, **6a**–**g**.




**Scheme 1.** Reagents: (a) pyridine, Δ; (b) $Ac_2O$, Δ.

### 2.3. TNF-α production-regulatory activity

HL-60 cells do not produce detectable amounts of TNF-α under normal cell culture conditions, but begin to produce it in response to okadaic acid (OA) or 12-*O*-tetradecanoylphorbol 13-acetate (TPA). The cytokine concentration produced by HL-60 cell under our standard experimental conditions ($5 \times 10^5$ cells/mL, incubated with 10 nM TPA or 50 nM OA during 16 h) was defined as 100% of production [24]. The effect of compounds **1**–**6** were represented as the percentage, of TNF-α produced by stimulated HL-60 cells (with TPA or OA) in the presence of each compound. The TNF-α concentration in cell culture medium was quantified using an ELISA. Each assay was performed in triplicate, the means appear in Table 1. None of the tested compounds induced detectable TNF-α production by themselves. Cell number was estimated at the end of the assay. No differences in cell number between treatments with thalidomide and their derivatives were observed.

All treatments show TPA-induced TNF-α production-enhancing activity and OA-induced TNF-α production-inhibiting activity. Therefore, all imide derivates show the same bidirectional modulation of the cytokine production as thalidomide. The dose–response curves for enhancing and inhibiting activities of the most active components on TNF-α production are shown in Fig. 3a,b. The effective concentration ranges of these compounds are roughly the same for the TPA-induced HL-60 and OA-induced HL-60 assay systems.

In view of these results and structural modifications realized, we may summarize that the introduction of the basic chain into the thalidomide glutarimide ring, **2g**, produces a noticeable reduction in ability to regulate cytokine production. Therefore, imidic NH may contribute to this activity. Overproduction of TNF-α is associated with a wide range of pathological conditions, which has led to recent efforts to discover ways to downregulate its production [25]. The thalidomide structural duplication produces an interesting result. The bis-thalidomide joined by a bis-amine **6g**, exhibiting better results than **2g**, but only for the stimulation of cytokine production. However, when **2g** was duplicated using an aromatic bis-imide, **5**, a small improvement in the inhibition of TNF-α production was detected. Similar activity is observed with both, **2g** and **2a** (1,8-naphthalimide). However, when phthalimide is substituted with 2,3-naphthalimide, **2d**, the compound inhibition effect of the TNF-α production is similar to thalidomide, and better than **2g**.

Finally, the diphenylmaleimide group produces a compound with a potent regulatory activity over cytokine production. **2f** has a stimulatory effect over TNF-α production, in cells treated with TPA (672%) and shows inhibitory activity in OA treated cells (6%) (Fig. 4).

Pharmacologic inhibition of TNF-α production or blockade has been a highly effective approach in the treatment of several immunologically mediated diseases, including rheumatoid arthritis [26], Crohn's disease [27], and psoriatic arthritis [28]. Nevertheless, it has recently become clear that blockade of TNF-α action is profoundly immunosuppressive [29,30], and may result in reactivation of tuberculosis [31] and histoplasmosis [32], as well as the emergence of B-cell lymphomas [33]. Due to its modulating capacity, **2f** should be considered for further studies, as an alternative to thalidomide and other regulatory substances (usually blockers) of TNF-α levels.

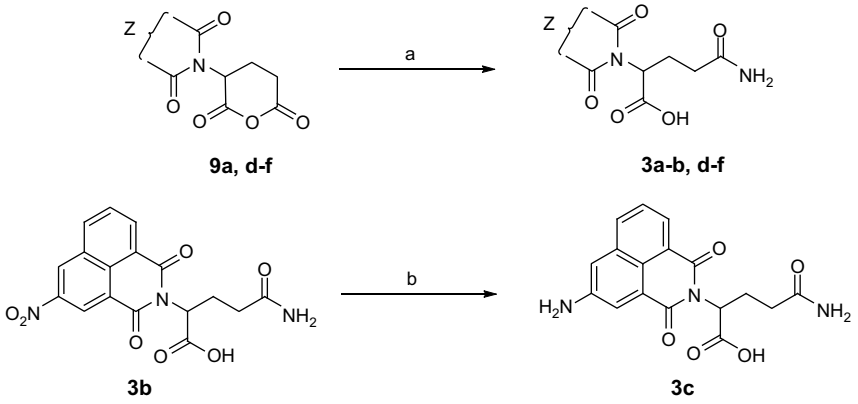
## 3. Conclusions

In conclusion, the phthalimide substitution with diphenylmaleimide moiety appears to be the better option for a TNF-α production modulation of all synthesized compounds. The most important aspect of this compound activity is its strong inhibitory effect. Therefore, the **2f** molecular structure may be considered as a new leader in the design of new TNF-α inhibitors, through modification of the lateral basic chain and the introduction of substituents into one or both phenyl rings. At this point, further experimental work is required to improve TNF-α production modulation.

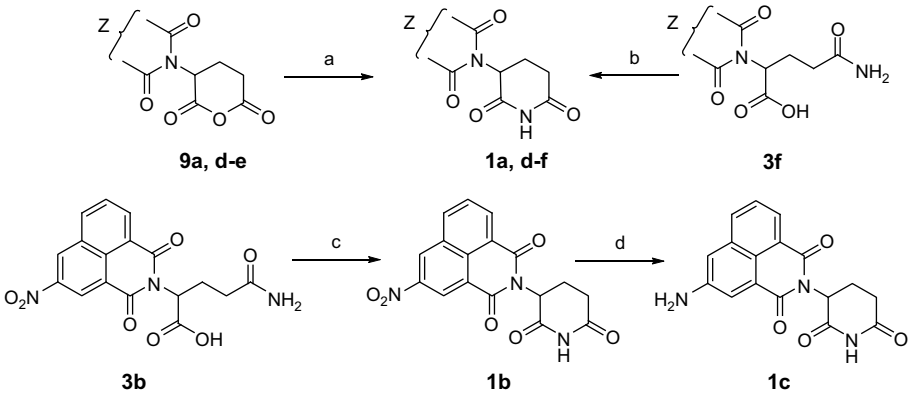
## 4. Experimental protocols

### 4.1. Chemistry

Melting points (uncorrected) were determined on a Stuart Scientific SMP3 apparatus. Infrared (IR) spectra were recorded with a Perkin–Elmer 1330 infrared spectrophotometer. $^1H$ and $^{13}C$ NMR values were recorded on a Bruker 300-AC instrument. Chemical shifts ($\delta$) are expressed in parts per million relative to internal tetramethylsilane; coupling constants ($J$) are in hertz. Mass spectra were run on a HP 5989A spectrometer. Elemental analyses (C, H, N) were performed on a Perkin–Elmer 2400 CHN apparatus at the Microanalyses Service of the University Complutense of Madrid; all the reported values are within ±0.4% of the theoretical




**Scheme 2.** Reagents: (a) (i) $NH_3$ (g), dioxane, RT; (ii) $H_3O^+$; (b) $H_2$, Pd/C 10%, DMF, 50 psi.




**Scheme 3.** Reagents: (a) $(NH_4)_2CO_3$, Δ; (b) $Ac_2O$, AcCl, Δ; (c) Δ, 250 °C; (ii) $H_3O^+$; (d) $H_2$, Pd/C 10%, DMF, 50 psi.

compositions. Thin-layer chromatography (TLC) was run on Merck silica gel 60 F-254 plates. Unless stated otherwise, starting materials used were high-grade commercial products.

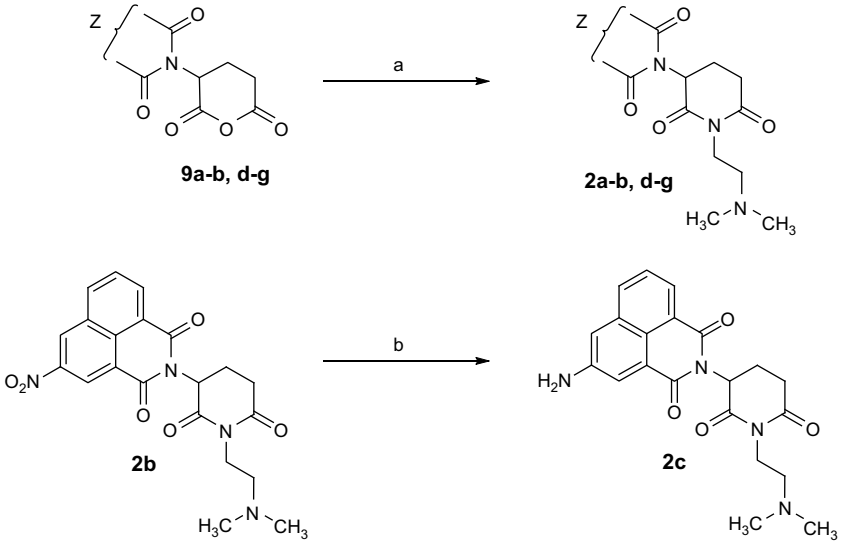
*4.1.1. General procedure for the synthesis of glutaric anhydrides* ***9***

A mixture of the corresponding anhydride **7** (10.0 mmol) and L-glutamic acid (15.1 mmol) was refluxed in dry pyridine (35 mL). Then the mixture was cooled to room temperature and the solvent was evaporated to dryness. Acetic anhydride (12 mL) was added and the mixture was heated under reflux for an additional 0.5 h. After cooling, the precipitate was collected by filtration. The solid was recrystallized from anhydride acetic.

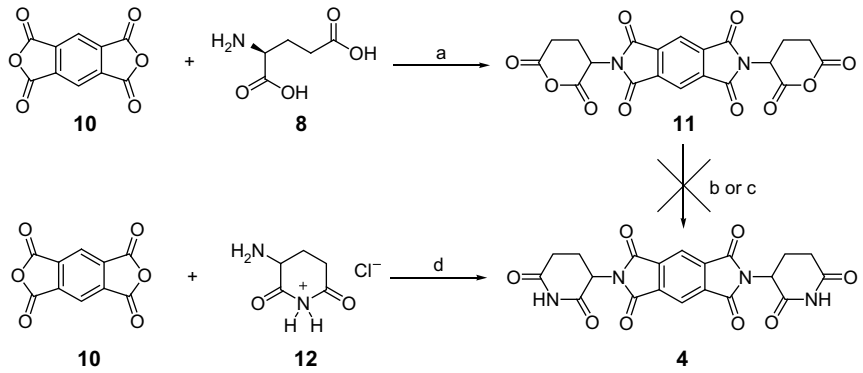
*4.1.1.1. Synthesis of 2-(2,6-dioxotetrahydro-2*H*-pyran-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* ***9a****.* Following the general procedure for the synthesis of glutaric anhydrides, from 1,8-naphthalic anhydride (2.00 g, 10.10 mmol), refluxed for 40 h, 1.43 g (46%) of **9a** were obtained as a yellow solid (mp 276–278 °C). $^1$H NMR (DMSO-$d_6$) $\delta$ 1.90–2.18 (m, 1H, $CH_2$), 2.43–2.57 (m, 1H, $CH_2$), 2.90–2.98 (m, 1H, $CH_2$), 3.12–3.24 (m, 1H, $CH_2$), 6.11 (dd, 1H, CH, $J_1 = 11.6$ Hz, $J_2 = 6.7$ Hz), 7.93 (t, 2H, Ar, $J = 7.9$ Hz), 8.55 (d, 4H, Ar, $J = 7.9$ Hz). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.2, 29.0, 48.9, 121.3, 127.3, 127.4, 131.2, 131.5, 135.1, 165.9, 162.8, 167.0. IR (KBr) 1810, 1765, 1700, 1660.

*4.1.1.2. Synthesis of 2-(2,6-dioxotetrahydro-2*H*-pyran-3-yl)-5-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* ***9b****.* Following the general procedure for the synthesis of glutaric anhydrides, from 3-nitro-1,8-naphthalic anhydride (10.00 g, 50.50 mmol), refluxed for 24 h, 14.00 g (78%) of **9b** were obtained as a pale solid (mp >270 °C dec). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.11–2.17 (m, 1H, $CH_2$), 2.44–2.59 (m, 1H, $CH_2$), 2.91–3.00 (m, 1H, $CH_2$), 3.12–3.24 (m, 1H, $CH_2$), 6.13 (dd, 1H, CH, $J_1 = 11.6$ Hz, $J_2 = 6.1$ Hz), 8.11 (t, 1H, Ar, $J = 7.9$ Hz), 8.74 (d, 1H, Ar, $J = 7.3$ Hz), 8.86 (d, 1H, Ar, $J = 7.3$ Hz), 9.01 (bs, 1H, Ar), 9.57 (s, 1H, Ar). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.1, 29.0, 49.2, 121.9, 123.3, 123.7, 129.4, 129.5, 130.5, 130.9, 134.7, 137.1, 145.9, 161.8, 162.2, 165.6, 166.8. IR (KBr) 1810, 1770, 1715, 1680, 1540, 1350, 1340.

*4.1.1.3. Synthesis of 2-(2,6-dioxotetrahydro-2*H*-pyran-3-yl)-1*H*-benzo[f]isoindole-1,3(2*H*)-dione* ***9d****.* Following the general procedure for the synthesis of glutaric anhydrides, from 2,3-naphthalic anhydride (4.15 g, 20.92 mmol), refluxed for 2 h, 4.84 g (75%) of **9d** were obtained as a white solid (mp >270 °C dec). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.19–2.24 (m, 1H, $CH_2$), 2.64–2.78 (m, 1H, $CH_2$), 3.01–3.26 (m, 2H, $CH_2$), 5.59 (dd, 1H, CH, $J_1 = 12.8$ Hz, $J_2 = 5.5$ Hz), 7.82 (dd, 2H, Ar, $J_1 = 5.8$ Hz, $J_2 = 2.7$ Hz), 8.28 (dd, 2H, Ar, $J_1 = 6.1$ Hz, $J_2 = 3.0$ Hz), 8.58 (s, 2H, Ar). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.4, 29.4, 47.8, 125.2, 126.6, 129.6, 130.4, 135.0, 165.6, 166.3, 166.3. IR (KBr) 1815, 1770, 1710.




**Scheme 4.** Reagents: (a) (i) $H_2NCH_2CH_2N(CH_3)_2$, DMF, RT; (ii) $Ac_2O$, AcCl, Δ; (b) $H_2$, Pd/C 10%, DMF, 50 psi.




**Scheme 5.** Reagents: (a) (i) pyridine, Δ; (ii) $Ac_2O$, Δ; (b) (i) $NH_3$; (ii) $Ac_2O$, AcCl, Δ; (c) $(NH_4)_2CO_3$, Δ; (d) NaAcO, AcOH.

*4.1.1.4. Synthesis of 2-(2,6-dioxotetrahydro-2*H*-pyran-3-yl)-1*H*-benzo[*e*]isoindole-1,3(2*H*)-dione* ***9e***. Following the general procedure for the synthesis of glutaric anhydrides, from 1,2-naphthalic anhydride (2.50 g, 12.61 mmol), refluxed for 2 h, 3.34 g (83%) of **9e** were obtained as a pale solid (mp 234–236 °C). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.13–2.22 (m, 1H, $CH_2$), 2.59–2.74 (m, 1H, $CH_2$), 2.96–3.20 (m, 2H, $CH_2$), 5.52 (dd, 1H, CH, $J_1 = 13.1$ Hz, $J_2 = 5.8$ Hz), 7.79–7.91 (m, 2H, Ar), 7.98 (d, 1H, Ar, $J = 7.9$ Hz), 8.23 (d, 1H, Ar, $J = 7.9$ Hz), 8.49 (d, 1H, Ar, $J = 8.6$ Hz), 8.79 (d, 1H, Ar, $J = 7.9$ Hz). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.6, 29.6, 47.7, 118.5, 123.8, 126.3, 127.1, 129.1, 129.2, 130.1, 130.7, 136.0, 136.2, 165.8, 166.3, 167.1, 167.9. IR (KBr) 1815, 1770, 1710.

*4.1.1.5. Synthesis of 1-(2,6-dioxotetrahydro-2*H*-pyran-3-yl)-3,4-diphenyl-1*H*-pyrrole-2,5-dione* ***9f***. Following the general procedure for the synthesis of glutaric anhydrides, from 2,3-diphenylmaleic anhydride (2.00 g, 7.99 mmol), refluxed for 24 h, 2.10 g (73%) of **9f** were obtained as a yellow solid (mp 219–221 °C). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.10–2.19 (m, 1H, $CH_2$), 2.52–2.67 (m, 1H, $CH_2$), 2.94–3.18 (m, 2H, $CH_2$), 5.45 (dd, 1H, CH, $J_1 = 12.8$ Hz, $J_2 = 5.5$ Hz), 7.42–7.44 (m, 10H, Ar). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.4, 29.4, 47.9, 128.0, 128.5, 129.5, 129.9, 136.2, 165.6, 166.2, 168.9. IR (KBr) 1820, 1770, 1710.

*4.1.2. General procedure for the synthesis of amidoacids* ***3***

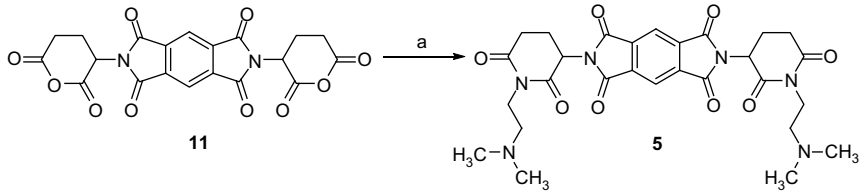
Gaseous $NH_3$ was bubbled through a suspension of anhydride **9** (10.00 mmol) in dioxane (30 ml) for 1.5 h. The precipitate formed was filtered. The solid was dissolved in $H_2O$ (10.0 mL) and acidified to pH 1 with 37% HCl, the precipitate was filtered, washed with $H_2O$ and dried. The solid was recrystallized from glacial acetic acid.

*4.1.2.1. Synthesis of 5-amino-2-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-5-oxopentanoic acid* ***3a***. Following the general procedure for the synthesis of amidoacids, from **9a** (1.00 g, 3.24 mmol), 0.91 g (86%) of **3a** were obtained as a white solid (mp >265 °C dec). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.05–2.19 (m, 2H, $CH_2$), 2.22–2.32 (m, 1H, $CH_2$), 2.40–2.46 (m, 1H, $CH_2$), 5.55 (dd, 1H, CH, $J_1 = 9.5$ Hz, $J_2 = 4.6$ Hz), 6.67 (bs, 1H, $NH_2$), 7.16 (bs, 1H, $NH_2$), 7.92 (dd, 2H, Ar, $J = 7.9$ Hz, $J = 7.3$ Hz), 8.52 (d, 2H, Ar, $J = 7.9$ Hz), 8.54 (d, 2H, Ar, $J = 7.3$ Hz), 12.80 (bs, 1H, OH). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 24.1, 31.7, 52.6, 121.7, 127.4, 127.5, 131.3, 131.4, 134.8, 163.3, 171.0, 173.4. IR (KBr) 3400, 3170, 1700, 1650. MS (ESI), $m/z$ 327 $[M + H]^+$ Anal. ($C_{17}H_{14}N_2O_5 \cdot 0.1H_2O$) C, H, N.

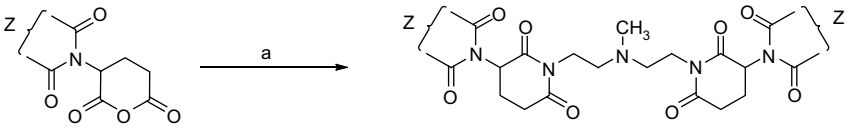
*4.1.2.2. Synthesis of 5-amino-2-(5-nitro-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-5-oxopentanoic acid* ***3b***. Following the general procedure for the synthesis of amidoacids, from **9b** (3.00 g, 8.47 mmol), 2.67 g (85%) of **3b** were obtained as a yellow solid (mp 237–239 °C). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.04–2.46 (m, 4H, 2 × $CH_2$), 5.53 (dd, 1H, CH, $J = 9.8$ Hz, $J = 4.9$ Hz), 6.62 (bs, 1H, $NH_2$), 7.13 (bs, 1H, $NH_2$), 8.09 (t, 1H, Ar, $J = 7.9$ Hz), 8.71 (d, 1H, Ar, $J = 7.3$ Hz), 8.83 (d, 1H, Ar, $J = 7.9$ Hz), 8.99 (d, 1H, Ar, $J = 2.4$ Hz), 9.53 (d, 1H, Ar, $J = 2.5$ Hz), 12.87 (bs, 1H, OH). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 24.1, 31.8, 53.3, 122.1, 123.5, 123.5, 129.4, 129.6, 130.1, 130.9, 134.5, 136.7, 145.9, 162.2, 162.7, 170.8, 173.6. IR (KBr) 3460, 3200, 1710, 1675, 1530, 1340. MS (EI), $m/z$ 353 ($M^+ - H_2O$, 8), 323 (18), 242 (68), 212 (100). Anal. ($C_{17}H_{13}N_3O_7 \cdot 1.1H_2O$) C. H, N.

*4.1.2.3. Synthesis of 5-amino-2-(5-amino-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-5-oxopentanoic acid* ***3c***. A solution of **3b** (0.20 g, 0.54 mmol) in DMF (30 mL) was treated with 10% Pd/C (0.07 g) and hydrogenated for 7 h at 50 psi. The mixture was filtered through a celite pad. The solvent was removed under reduced pressure, the residue was disgregated in AcOEt and filtered to afford **3c** (0,16 g, 89%) as a yellow solid (mp >283 °C dec). $^1$H NMR (DMSO-$d_6$) $\delta$ 1.98–2.27 (m, 3H, 2 × $CH_2$), 2.37–2.44 (m, 1H, $CH_2$), 5.50 (dd, 1H, CH, $J = 10.1$ Hz, $J = 4.6$ Hz), 6.07 (bs, 2H, $NH_2$Ar), 6.67 (bs, 1H, $NH_2$), 7.16 (bs, 1H, $NH_2$), 7.32 (d, 1H, Ar, $J = 2.5$ Hz), 7.64 (t, 1H, Ar, $J = 7.6$ Hz), 7.97 (d, 1H, Ar, $J = 2.5$ Hz), 8.07–8.10 (m, 2H, Ar), 12.73 (bs, 1H, OH). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 24.1, 31.6, 52.5, 112.0, 120.7, 121.4, 122.1, 122.2, 125.9, 127.1, 131.9, 133.6, 148.0, 163.5, 163.7, 171.1, 173.4. IR (KBr) 3420, 3380, 3200, 1695, 1650. MS (ESI), $m/z$ 364 $[M + Na]^+$ Anal. ($C_{17}H_{15}N_3O_5 \cdot 0.65H_2O$) C, H, N.

*4.1.2.4. Synthesis of 5-amino-2-(1,3-dioxo-1*H*-benzo[f]isoindol-2(3*H*)-yl)-5-oxopentanoic acid* ***3d***. Following the general procedure for the synthesis of amidoacids, from **9d** (0.50 g, 1.62 mmol), 0.48 g (91%) of **3d** were obtained as a white solid (mp >223 °C dec).




**Scheme 6.** Reagents: (a) (i) 0.5 $H_2NCH_2CH_2N(CH_3)_2$, DMF, RT; (ii) $H_3O^+$; (iii) $Ac_2O$, AcCl, Δ.




**Scheme 7.** Reagents: (a) (i) 0.5 $H_2NCH_2CH_2N(CH_3)CH_2CH_2NH_2$, DMF, RT; (ii) $H_3O^+$; (iii) $Ac_2O$, AcCl, Δ.

$^1$H NMR (DMSO-$d_6$) δ 2.12 (t, 2H, $CH_2$, $J = 7.3$ Hz), 2.24–2.43 (m, 2H, $CH_2$), 4.83 (dd, 1H, CH, $J = 10.4$ Hz, $J = 4.9$ Hz), 6.74 (bs, 1H, $NH_2$), 7.22 (bs, 1H, $NH_2$), 7.81 (dd, 2H, Ar, $J_1 = 6.1$ Hz, $J_2 = 3.7$ Hz), 8.30 (dd, 2H, Ar, $J_1 = 6.1$ Hz, $J_2 = 3.7$ Hz), 8.59 (s, 2H, Ar), 13.18 (bs, 1H, OH). $^{13}$C NMR (DMSO-$d_6$) δ 24.0, 51.5, 31.4, 124.9, 127.0, 129.5, 130.4, 135.2, 167.1, 170.5, 173.2. IR (KBr) 3480, 3340, 1780, 1710, 1660. MS (ESI), *m/z* 349 $[M + Na]^+$ Anal. ($C_{17}H_{14}N_2O_5$) C, H, N.

*4.1.2.5. Synthesis of 5-amino-2-(1,3-dioxo-1*H*-benzo[*e*]isoindol-2(*3H*)-yl)-5-oxopentanoic acid **3e**.* Following the general procedure for the synthesis of amidoacids, from **9e** (0.50 g, 1.62 mmol), 0.37 g (70%) of **3e** were obtained as a yellow solid (mp 239–241 °C). $^1$H NMR (DMSO-$d_6$) δ 2.15 (t, 2H, $CH_2$, $J = 7.3$ Hz), 2.26–2.47 (m, 2H, $CH_2$), 4.80 (dd, 1H, CH, $J_1 = 10.4$ Hz, $J_2 = 4.9$ Hz), 6.73 (bs, 1H, $NH_2$), 7.21 (bs, 1H, $NH_2$), 7.77–7.89 (m, 2H, Ar), 7.94 (d, 1H, Ar, $J = 8.5$ Hz), 8.21 (d, 1H, Ar, $J = 7.9$ Hz), 8.46 (d, 1H, Ar, $J = 8.5$ Hz), 8.80 (d, 1H, Ar, $J = 8.5$ Hz), 13.10 (bs, 1H, OH). $^{13}$C NMR (DMSO-$d_6$) δ 24.1, 31.4, 51.3, 118.5, 123.9, 126.4, 127.2, 129.1, 129.3, 130.1, 130.8, 135.8, 136.3, 167.9, 168.7, 170.7, 173.2. IR (KBr) 3440, 3200, 1775, 1710. 1660, MS (ESI), *m/z* 349 $[M + Na]^+$ Anal. ($C_{17}H_{14}N_2O_5 \cdot 0.2CH_3COOH$) C, H, N.

*4.1.2.6. Synthesis of 5-amino-2-(2,5-dioxo-3,4-diphenyl-2,5-dihydro-1*H*-pyrrol-1-yl)-5-oxopentanoic acid **3f**.* Following the general procedure for the synthesis of amidoacids, from **9f** (0.80 g, 2.21 mmol), 0.60 g (75%) of **3f** were obtained as a yellow solid (mp 237–239 °C). $^1$H NMR (DMSO-$d_6$) δ 2.14–2.36 (m, 4H, 2 × $CH_2$), 4.71 (dd, 1H, CH, $J_1 = 9.8$ Hz, $J_2 = 5.5$ Hz), 6.78 (bs, 1H, $NH_2$), 7.28 (bs, 1H, $NH_2$), 7.40–7.43 (m, 10H, Ar), 13.24 (bs, 1H, OH). $^{13}$C NMR (DMSO-$d_6$) δ 24.0, 31.5, 51.7, 128.4, 128.6, 129.7, 129.9, 136.0, 169.7, 170.5, 173.2. IR (KBr) 3430, 3200, 1750, 1720, 1690. MS (EI), *m/z* 360 ($M^+ - H_2O$, 26), 249 (63), 178 (100). Anal. ($C_{21}H_{18}N_2O_5 \cdot 0.8H_2O$) C, H, N.

**Table 1**
Effects of thalidomide and imide derivatives (30 μM) in TNF-α production (%) by human leukemia HL-60 cells induced with 10 nM TPA or 50 nM OA. TNF-α concentration in treatments without thalidomide or derivate compounds (only with TPA or OA) was defined as 100%.

| Compound | % TNF-α | |
|---|---|---|
| | TPA | OA |
| **1a** | 158 | 49 |
| **1b** | 248 | 47 |
| **1c** | 138 | 46 |
| **1d** | 166 | 138 |
| **1e** | 161 | 78 |
| **1f** | 147 | 47 |
| **2a** | 107 | 91 |
| **2b** | 124 | 48 |
| **2c** | 132 | 47 |
| **2d** | 149 | 50 |
| **2e** | 105 | 59 |
| **2f** | 672 | 6 |
| **2g** | 125 | 93 |
| **3a** | 116 | 55 |
| **3b** | 86 | 51 |
| **3c** | 140 | 49 |
| **3d** | 258 | 60 |
| **3e** | 146 | 111 |
| **3f** | 146 | 66 |
| **4** | 294 | 34 |
| **5** | 138 | 77 |
| **6g** | 159 | 99 |
| Thalidomide | 164 | 53 |

*4.1.3. General procedure for the synthesis of glutarimides **1***

A mixture of the corresponding anhydride **9** (1.00 mmol) and $(NH_4)_2CO_3$ (0.62 mmol) was heated for 15–30 min at 190–250 °C. Then, the mixture was cooled to room temperature and the crude was recrystallized from glacial acetic acid.

*4.1.3.1. Synthesis of 2-(2,6-dioxopiperidin-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione **1a**.* Following the general procedure for the synthesis of glutarimides, from **9a** (0.26 g, 0.85 mmol), 0.12 g (45%) of **1a** were obtained as a white solid (mp >300 °C dec). $^1$H NMR (DMSO-$d_6$) δ 2.02–2.09 (m, 1H, $CH_2$), 2.52–2.67 (m, 2H, $CH_2$), 2.89–3.02 (m, 1H, $CH_2$), 5.85 (dd, 1H, CH, $J_1 = 11.6$ Hz, $J_2 = 5.5$ Hz), 7.88–7.95 (m, 2H, Ar), 8.47 (d, 1H, Ar, $J = 7.3$ Hz), 8.52 (d, 2H, Ar, $J = 8.5$ Hz), 8.58 (d, 1H, Ar, $J = 6.7$ Hz), 11.10 (bs, 1H, NH). $^{13}$C NMR (DMSO-$d_6$) δ 21.4, 30.9, 50.5, 121.5, 121.8, 127.4, 127.4, 127.5, 131.0, 131.3, 131.6, 134.9, 134.9, 162.6, 163.5, 170.3, 172.9. IR (KBr) 3200, 1700, 1660. MS (ESI), *m/z* 331 $[M + Na]^+$ Anal. ($C_{17}H_{12}N_2O_4$) C, H, N.

*4.1.3.2. Synthesis of 2-(2,6-dioxopiperidin-3-yl)-5-nitro-1*H*-benzo-[*de*]isoquinoline-1,3(2*H*)-dione **1b**.* **C**ompound **3b** (1.49 g, 4.01 mmol) was heated for 15 min at 250 °C, then cooled to room temperature, and finally disgregated in AcOEt. The suspension was filtered and the solid was purified by recrystallization from glacial acetic acid to afford **1b** (0.42 g, 30%) as a yellow solid (mp >298 °C). $^1$H NMR (DMSO-$d_6$) δ 2.02–2.10 (m, 1H, $CH_2$), 2.58–2.64 (m, 2H, $CH_2$), 2.89–3.02 (m, 1H, $CH_2$), 5.87 (dd, 1H, CH, $J_1 = 11.9$ Hz, $J_2 = 5.8$ Hz), 8.07–8.14 (m, 1H, Ar), 8.66 and 8.77 (d, d, 1H, Ar, $J = 7.3$ Hz), 8.85 (d, 1H, Ar, $J = 8.6$ Hz), 8.93 and 9.04 (d, d*, 1H, Ar, $J = 2.5$ Hz, $J^* = 1.8$ Hz), 9.56 (bs, 1H, Ar), 11.10 (bs, 1H, NH). $^1$H NMR (DMSO-$d_6$, 60 °C) δ 2.06–2.13 (m, 1H, $CH_2$), 2.55–2.68 (m, 2H, $CH_2$), 2.88–3.00 (m, 1H, $CH_2$), 5.84 (dd, 1H, CH, $J_1 = 11.6$ Hz, $J_2 = 5.5$ Hz), 8.08 (t, 1H, Ar, $J = 7.9$ Hz), 8.72 (bs, 1H, Ar), 8.81 (d, 1H, Ar, $J = 7.9$ Hz), 9.01 (bs, 1H, Ar), 9.48 (d, 1H, Ar, $J = 2.4$ Hz), 10.90 (bs, 1H, NH). $^{13}$C NMR (DMSO-$d_6$) δ 21.3, 21.4, 30.9, 50.9, 50.9, 122.0, 122.3, 123.4, 123.4, 123.6, 123.8, 129.4, 129.5, 129.5, 130.2, 130.3, 131.0, 134.2, 134.8, 136.9, 146.0, 161.6, 162.0, 162.4, 162.8, 170.1, 172.9. IR (KBr) 3180, 1700, 1665, 1530, 1335, 1325. MS (EI), *m/z* 353 ($M^+$, 33), 323 (48), 242 (100), 212 (79). Anal. ($C_{17}H_{11}N_3O_6 \cdot 0.2CH_3COOH$) C, H, N.

*4.1.3.3. Synthesis of 5-amino-2-(2,6-dioxopiperidin-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione **1c**.* A solution of **1b** (0.20 g, 0.57 mmol) in DMF (30 mL) was treated with 10% Pd/C (0.07 g) and hydrogenated for 4.5 h at 50 psi. The mixture was filtered through a celite pad. The solvent was removed under reduced pressure, the residue was disgregated in AcOEt and filtered to afford **1c** (0,16 g, 88%) as a red solid (mp >299 °C). $^1$H NMR (DMSO-$d_6$) δ 1.99–2.03 (m, 1H, $CH_2$), 2.50–2.60 (m, 2H, $CH_2$), 2.87–2.98 (m, 1H, $CH_2$), 5.80 (dd, 1H, $J_1 = 11.9$ Hz, $J_2 = 5.8$ Hz), 6.07 (bs, 2H, $NH_2$), 7.33 (bs, 1H, Ar), 7.61–7.68 (m, 1H, Ar), 7.90–8.16 (m, 3H, Ar), 11.00 (s, 1H, NH). $^1$H NMR (DMSO-$d_6$, 60 °C) δ 2.00–2.09 (m, 1H, $CH_2$), 2.58–2.63 (m, 2H, $CH_2$), 2.85–2.98 (m, 1H, $CH_2$), 5.78 (dd, 1H, CH, $J_1 = 11.3$ Hz, $J_2 = 5.8$ Hz), 5.91 (bs, 2H, $NH_2$Ar), 7.35 (s, 1H, Ar), 7.64 (t, 1H, Ar,

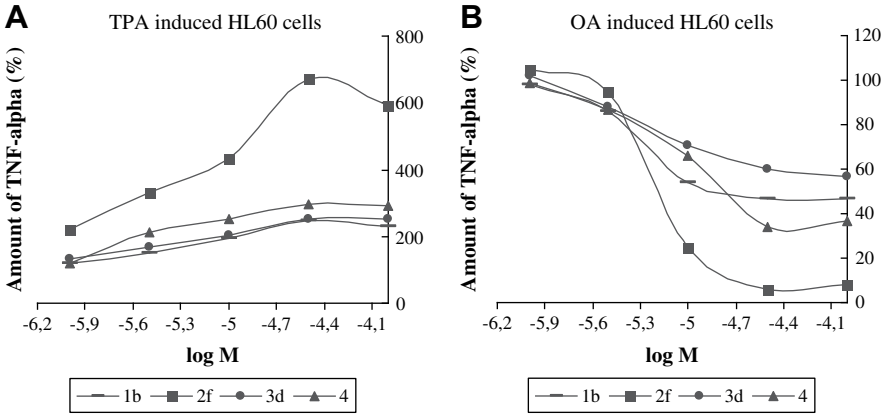


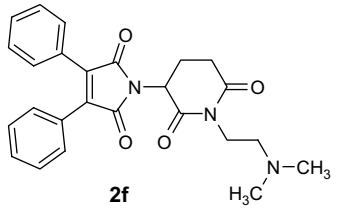

**Fig. 3.** Dose–response curves of most active compounds. Horizontal scale: concentration of added test compound. Vertical scale: amount of TNF-α (%). A) TPA-induced TNF-α production-enhancing activity. HL-60 cells were treated with 10 nM TPA in the presence of a test compound. TNF-α concentration in treatments without test compounds (only with TPA) was defined as 100%. B) OA-induced TNF-α production-inhibiting activity. HL-60 cells were treated with 50 nM OA in the presence of a test compound. TNF-α concentration in treatments without test compounds (only with TPA) was defined as 100%.

$J = 7.9$ Hz), 7.95–8.15 (m, 2H, Ar), 8.06 (d, 1H, Ar, $J = 8.5$ Hz), 10.82 (bs, 1H, NH). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.4, 30.9, 50.4, 112.2, 112.2, 120.5, 120.6, 121.2, 121.6, 121.7, 122.0, 122.3, 122.4, 125.6, 126.2, 127.1, 127.1, 132.0, 132.1, 133.6, 148.0, 148.0, 162.8, 163.0, 163.6, 163.8, 170.3, 172.9. IR (KBr) 3450, 3360, 3200, 1690, 1655. MS (ESI), *m*/*z* 324 $[M + H]^+$ Anal. ($C_{17}H_{13}N_3O_4 \cdot 0.6H_2O$) C, H, N.

*4.1.3.4. Synthesis of 2-(2,6-dioxopiperidin-3-yl)-1*H*-benzo[f]isoindole-1,3(2*H*)-dione* ***1d***. Following the general procedure for the synthesis of glutarimides, from **9d** (1.00 g, 3.23 mmol), 0.48 g (48%) of **1d** were obtained as a white solid (mp >298 °C). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.08–2.15 (m, 1H, $CH_2$), 2.55–2.66 (m, 2H, $CH_2$), 2.87–2.99 (m, 1H, $CH_2$), 5.24 (dd, 1H, CH, $J_1 = 12.8$ Hz, $J_2 = 5.5$ Hz), 7.81 (dd, 2H, Ar, $J_1 = 6.1$ Hz, $J_2 = 3.1$ Hz), 8.30 (dd, 2H, Ar, $J_1 = 6.1$ Hz, $J_2 = 3.7$ Hz), 8.60 (s, 2H, Ar), 11.18 (s, 1H, NH). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.9, 30.9, 49.1, 125.0, 126.9, 129.5, 130.4, 135.1, 166.8, 169.8, 172.8. IR (KBr) 3200, 1760, 1710. MS (EI), *m*/*z* 308 ($M^+$, 84), 197 (100), 154 (40), 126 (93). Anal. ($C_{17}H_{12}N_2O_4$) C, H, N.

*4.1.3.5. Synthesis of 2-(2,6-dioxopiperidin-3-yl)-1*H*-benzo[e]isoindole-1,3(2*H*)-dione* ***1e***. Following the general procedure for the synthesis of glutarimides, from **9e** (1.00 g, 3.23 mmol), 0.53 g (53%) of **1e** were obtained as a yellow solid (mp >280 °C). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.10–2.14 (m, 1H, $CH_2$), 2.54–2.66 (m, 2H, $CH_2$), 2.87–2.98 (m, 1H, $CH_2$), 5.22 (dd, 1H, CH, $J_1 = 12.8$ Hz, $J_2 = 5.5$ Hz), 7.78–7.88 (m, 2H, Ar), 7.96 (d, 1H, Ar, $J = 8.6$ Hz), 8.22 (d, 1H, Ar, $J = 8.6$ Hz), 8.48 (d, 1H, Ar, $J = 7.9$ Hz), 8.79 (d, 1H, Ar, $J = 8.6$ Hz), 11.18 (s, 1H, NH). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 22.2, 31.1, 49.0, 118.5, 123.8, 126.3, 127.1, 129.1, 129.2, 130.1, 130.8, 135.8, 136.2, 167.7, 168.4, 170.2, 172.9. IR (KBr) 3200, 1770, 1710. MS (EI), *m*/*z* 308 ($M^+$, 85), 197 (100), 154 (40), 126 (66). Anal. ($C_{17}H_{12}N_2O_4$) C, H, N.

*4.1.3.6. Synthesis of 3-(2,5-dioxo-3,4-diphenyl-2,5-dihydro-1*H*-pyrrol-1-yl)piperidine-2,6-dione* ***1f***. A mixture of **3f** (412 mg, 1.09 mmol), acetic anhydride (1.1 mL), and acetyl chloride (1.1 mL) was refluxed for 24 h. After cooling, the formed solid was collected by filtration and recrystallized from glacial acetic acid to afford **1f** (0.22 g, 55%) as a yellow solid (mp 288–290 °C). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.07–2.12 (m, 1H, $CH_2$), 2.45–2.64 (m, 2H, $CH_2$), 2.84–2.96 (m, 1H, $CH_2$), 5.14 (dd, 1H, CH, $J_1 = 12.8$ Hz, $J_2 = 4.9$ Hz), 7.40–7.45 (m, 10H, Ar), 11.15 (s, 1H, NH). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 22.0, 30.8, 49.2, 128.2, 128.5, 129.5, 129.9, 136.1, 169.4, 169.9, 172.7. IR (KBr) 3280, 1770, 1710. MS (EI), *m*/*z* 360.




**2f**

**Fig. 4.** Compound **2f**.

*4.1.4. General procedure for the synthesis of* N*-substituted glutarimides* ***2***

*4.1.4.1. Procedure A.* To a mixture of the corresponding anhydride **9** (10.00 mmol) and DMF (60 mL) was added *N*,*N*-dimethylethylendiamine (10.00 mmol) at room temperature until a precipitate appeared. Then, the precipitate was collected by filtration, treated with 14 mL of a mixture of acetic anhydride:acetyl chloride (1:1), and refluxed. After cooling, the solid was collected by filtration, disgregated in AcOEt and filtered. The solid obtained was recrystallized from DMF–AcOEt.

*4.1.4.1.1. Synthesis of 2-(3-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-2,6-dioxopiperidin-1-yl)-*N,N*-imethylethanaminium chloride* ***2a***. Following the general procedure A for the synthesis of *N*-substituted glutarimides, from **9a** (2.00 g, 6.47 mmol), refluxed for 16 h, 1.58 g (59%) of **2a** were obtained as a pale solid (mp 273–275 °C). $^1$H NMR (DMSO $d_6$) $\delta$ 2.08–2.14 (m, 1H, $CH_2$), 2.52–2.67 (m, 1H, $CH_2$), 2.73–2.89 (m, 1H, $CH_2$), 2.81 (s, 6H, 2 × $CH_3$), 3.05–3.17 (m, 1H, $CH_2$), 3.23 (t, 2H, $CH_2N$, $J = 6.1$ Hz), 4.08 (t, 2H, $CH_2N$, $J = 6.1$ Hz), 6.03 (dd, 1H, CH, $J_1 = 12.2$ Hz, $J_2 = 6.1$ Hz), 7.88–7.97 (m, 2H, Ar), 8.47 (d, 1H, Ar, $J = 7.3$ Hz), 8.54 (d, 2H, Ar, $J = 8.6$ Hz), 8.61 (d, 1H, Ar, $J = 7.3$ Hz), 10.23 (bs, 1H, NH). $^{13}$C NMR (DMSO $d_6$) $\delta$ 20.5, 31.2, 34.6, 42.6, 51.0, 54.0, 121.5, 121.7, 127.4, 127.5, 131.1, 131.4, 131.7, 135.1, 162.8, 163.3, 170.3, 172.1. IR (KBr) 2540, 1740, 1690, 1670. MS (EI), *m*/*z* 379 ($M^+$, 4), 126 (6), 71 (35), 58 (100). Anal. ($C_{21}H_{22}ClN_3O_4$) C, H, N.

*4.1.4.1.2. Synthesis of* N,N*-dimethyl-2-(3-(5-nitro-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-2,6-dioxopiperidin-1-yl)ethanaminium chloride* ***2b***. Following the general procedure A for the synthesis of *N*-substituted glutarimides, from **9b** (1.00 g, 2.82 mmol), refluxed for 48 h, 0.44 g (37%) of **2b** were obtained as a pale solid (mp >270 °C dec). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.08–2.13 (m, 1H, $CH_2$), 2.51–2.84 (m, 2H, $CH_2$), 2.80 (bs, 6H, 2 × $CH_3$), 3.04–3.16 (m, 1H, $CH_2$), 3.20 (t, 2H, $CH_2N$, $J = 5.8$ Hz), 4.06 (t, 2H, $CH_2N$, $J = 5.8$ Hz), 6.04 (dd, 1H, CH, $J_1 = 12.2$ Hz, $J_2 = 5.5$ Hz), 8.05–8.13 (m, 1H, Ar), 8.64 and 8.77 (d, d*, 1H, Ar, $J = 6.7$ Hz, $J^* = 7.3$ Hz), 8.84 (d, 1H, Ar, $J = 7.9$ Hz), 8.91 and 9.03 (d, d, 1H, Ar, $J = 1.8$ Hz), 9.55 (s, 1H, Ar), 10.27 (bs, 1H, NH). $^1$H NMR (DMSO-$d_6$, 60 °C) $\delta$ 2.08–2.17

(m, 1H, $CH_2$), 2.55–2.89 (m, 2H, $CH_2$), 2.80 (s, 6H, 2 × $CH_3$), 3.06–3.14 (m, 1H, $CH_2$), 3.23 (t, 2H, $CH_2N$, $J = 6.1$ Hz), 4.09 (t, 2H, $CH_2N$, $J = 6.1$ Hz), 6.05 (dd, 1H, CH, $J_1 = 12.2$ Hz, $J_2 = 5.5$ Hz), 8.10 (t, 1H, Ar, $J = 7.3$ Hz), 8.74 (bs, 1H, Ar), 8.84 (d, 1H, Ar, $J = 8.5$ Hz), 9.00 (bs, 1H, Ar), 9.51 (s, 1H, Ar), 10.36 (bs, 1H, NH). $^{13}$C NMR ($D_2O$) $\delta$ 22.8, 22.8, 32.8, 33.4, 37.9, 45.5, 46.5, 54.3, 54.4, 57.9, 123.5, 123.8, 125.0, 125.3, 126.2, 126.8, 131.5, 131.6, 132.0, 132.0, 132.9, 132.9, 133.0, 133.1, 137.4, 138.0, 139.6, 139.8, 147.9, 148.0, 165.3, 165.5, 166.0, 166.3, 174.1, 177.0. IR (KBr) 2480, 1730, 1705, 1670, 1530, 1330. MS (ESI), *m*/*z* 425 $[M + H]^+$. Anal. ($C_{21}H_{21}ClN_4O_6 \cdot 0.25H_2O$) C, H, N.

*4.1.4.1.3. Synthesis of 2-(3-(5-amino-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-2,6-dioxopiperidin-1-yl)-*N,N*-dimethylethanaminium chloride **2c***. A mixture of **2b** (110 mg, 0.24 mmol) in DMF/MeOH (35 mL, 6/1, v/v) was treated with 10% Pd/C (0.04 g) and hydrogenated for 4.5 h at 50 psi. The mixture was filtered through a celite pad. The solvent was removed under reduced pressure, the residue was disgregated in AcOEt and filtered to afford **2c** (0,94 g, 91%) as a orange solid (mp >268 °C dec). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.06–2.09 (m, 1H, $CH_2$), 2.49–2.64 (m, 1H, $CH_2$), 2.75–2.77 (m, 1H, $CH_2$), 2.80 (s, 6H, 2 × $CH_3$), 3.03–3.15 (m, 1H, $CH_2$), 3.20 (t, 2H, $CH_2N$, $J = 5.8$ Hz), 4.06 (t, 2H, $CH_2N$, $J = 5.8$ Hz), 5.98 (dd, 1H, CH, $J_1 = 11.9$ Hz, $J_2 = 5.8$ Hz), 6.10 (bs, 1H, $NH_2$), 6.12 (bs, 1H, $NH_2$), 7.35 (s, 1H, Ar), 7.61–7.70 (m, 1H, Ar), 7.91–8.19 (m, 3H, Ar), 10.20 (bs, 1H, NH). $^1$H NMR (DMSO-$d_6$, 60 °C) $\delta$ 2.06–2.13 (m, 1H, $CH_2$), 2.50–2.65 (m, 1H, $CH_2$), 2.73–2.85 (m, 1H, $CH_2$), 2.81 (s, 6H, 2 × $CH_3$), 3.00–3.30 (m, 3H, $CH_2 + CH_2N$), 4.07 (t, 2H, $CH_2N$, $J = 6.1$ Hz), 5.94 (bs, 2H, $NH_2$), 5.95 (dd, 1H, CH, $J_1 = 12.5$ Hz, $J_2 = 5.8$ Hz), 7.36 (s, 1H, Ar), 7.61–7.67 (m, 1H, Ar), 7.92 (bs, 1H, Ar), 8.07 (d, 1H, Ar, $J = 7.9$ Hz), 8.15 (bs, 1H, Ar). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.6, 31.2, 34.7, 42.6, 50.9, 54.0, 112.3, 120.6, 121.2, 121.5, 121.8, 122.0, 122.2, 122.4, 125.8, 126.3, 127.1, 127.2, 133.2, 133.6, 148.0, 148.1, 163.0, 163.2, 163.5, 163.7, 170.4, 172.1. IR (KBr) 3380, 3320, 3200, 2550, 1730, 1680, 1650. MS (ESI), *m*/*z* 395 $[M + H]^+$. Anal. ($C_{21}H_{23}ClN_4O_4 \cdot 1.25H_2O$) C, H, N.

*4.1.4.2. Procedure B.* To a mixture of the corresponding anhydride **9** (10.00 mmol) and DMF (60 mL) was added *N,N*-dimethylethylendiamine (10.00 mmol) and the mixture was stirred for 24 h at room temperature. Then, the solvent was evaporated under reduced pressure and the resulting residue was treated with 14 mL of a mixture of acetic anhydride:acetyl chloride (1:1), and refluxed. After cooling, the reaction mixture was concentrated in vacuo, and the crude product was recrystallized from DMF–AcOEt.

*4.1.4.2.1. Synthesis of 2-(3-(1,3-dioxo-1*H*-benzo[f]isoindol-2(3*H*)-yl)-2,6-dioxopiperidin-1-yl)-*N,N*-dimethylethanaminium **2d***. Following the general procedure B for the synthesis of *N*-substituted glutarimides, from **9d** (1.00 g, 3.23 mmol), refluxed for 24 h, 0.64 g (47%) of **2d** were obtained as a pale solid (mp 283–284 °C). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.14–2.18 (m, 1H, $CH_2$), 2.62–2.87 (m, 2H, $CH_2$), 2.80 (s, 6H, 2 × $CH_3$), 3.01–3.13 (m, 1H, $CH_2$), 3.21 (t, 2H, $CH_2N$, $J = 6.1$ Hz), 4.05 (t, 2H, $CH_2N$, $J = 6.1$ Hz), 5.40 (dd, 1H, CH, $J_1 = 12.8$ Hz, $J_2 = 5.5$ Hz), 7.81–7.84 (m, 2H, Ar), 8.29–8.33 (m, 2H, Ar), 8.61 (s, 2H, Ar), 10.23 (bs, 1H, NH). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.1, 31.4, 34.8, 42.3, 49.7, 53.7, 125.1, 126.9, 129.6, 130.4, 135.1, 166.7, 169.9, 172.1. IR (KBr) 2500, 1770, 1710, 1680. MS (ESI), *m*/*z* 380 $[M + H]^+$. Anal. ($C_{21}H_{22}ClN_3O_4 \cdot 0.75H_2O$) C, H, N.

*4.1.4.2.2. Synthesis of 2-(3-(1,3-dioxo-1*H*-benzo[e]isoindol-2(3*H*)-yl)-2,6-dioxopiperidin-1-yl)-*N,N*-dimethylethanaminium chloride **2e***. Following the general procedure B for the synthesis of *N*-substituted glutarimides, from **9e** (1.66 g, 5.38 mmol), refluxed for 24 h, 1.58 g (71%) of **2e** were obtained as a yellow solid (mp 243–245 °C). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.16–2.21 (m, 1H, $CH_2$), 2.63–2.89 (m, 2H, $CH_2$), 2.78 (bs, 6H, 2 × $CH_3$), 3.02–3.14 (m, 1H, $CH_2$), 3.18 (t, 2H, $CH_2N$, $J = 5.5$ Hz), 4.04 (t, 2H, $CH_2N$, $J = 5.8$ Hz), 5.37 (dd, 1H, CH, $J_1 = 13.1$ Hz, $J_2 = 5.2$ Hz), 7.78–7.90 (m, 2H, Ar), 7.96 (d, 1H, Ar, $J = 7.9$ Hz), 8.23 (d, 1H, Ar, $J = 8.6$ Hz), 8.49 (d, 1H, Ar, $J = 8.6$ Hz), 8.79 (d, 1H, Ar, $J = 8.6$ Hz), 10.28 (bs, 1H, NH). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.4, 31.5, 34.8, 42.3, 49.6, 53.7, 118.5, 123.8, 126.3, 127.1, 129.1, 129.2, 130.1, 130.7, 135.9, 136.2, 167.5, 168.3, 170.1, 172.2. IR (KBr) 2600, 1765, 1730, 1700, 1680. MS (EI), *m*/*z* 379 ($M^+$, 3), 126 (6), 71 (17), 58 (100). Anal. ($C_{21}H_{22}ClN_3O_4 \cdot 0.9H_2O$) C, H, N.

*4.1.4.2.3. Synthesis of 2-(3-(2,5-dioxo-3,4-diphenyl-2,5-dihydro-1*H*-pyrrol-1-yl)-2,6-dioxopiperidin-1-yl)-*N,N*-dimethylethanaminium chloride **2f***. Following the general procedure B for the synthesis of *N*-substituted glutarimides, from **9f** (0.50 g, 1.38 mmol), refluxed for 2.5 h, 0.57 g (88%) of **2f** were obtained as a yellow solid (mp >259 °C dec). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.13–2.17 (m, 1H, $CH_2$), 2.50–2.66 (m, 1H, $CH_2$), 2.75–2.84 (m, 1H, $CH_2$), 2.79 (s, 6H, 2 × $CH_3$), 3.00–3.12 (m, 1H, $CH_2$), 3.19 (t, 2H, $CH_2N$, $J = 5.8$ Hz), 4.03 (t, 2H, $CH_2N$, $J = 5.8$ Hz), 5.29 (dd, 1H, CH, $J_1 = 13.1$ Hz, $J_2 = 5.2$ Hz), 7.43 (bs, 10H, Ar), 10.33 (bs, 1H, NH). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.2, 31.4, 34.8, 42.3, 49.9, 53.8, 128.2, 128.7, 129.6, 130.0, 136.3, 169.4, 169.9, 172.0. IR (KBr) 2600, 1770, 1710, 1680. MS (EI), *m*/*z* 431 ($M^+$, 3), 178 (8), 71 (17), 58 (100). Anal. ($C_{25}H_{26}ClN_3O_4$) C, H, N.

*4.1.4.2.4. Synthesis of 2-(3-(1,3-dioxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)-*N,N*-dimethylethanaminium chloride **2g***. Following the general procedure B for the synthesis of *N*-substituted glutarimides, from **9g** (2.00 g, 7.72 mmol), refluxed for 24 h, 1.83 g (65%) of **2g** were obtained as a white solid (mp 238–240 °C). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.11–2.16 (m, 1H, $CH_2$), 2.52–2.67 (m, 1H, $CH_2$), 2.74–2.89 (m, 1H, $CH_2$), 2.78 (s, 6H, 2 × $CH_3$), 3.01–3.13 (m, 1H, $CH_2$), 3.19 (t, 2H, $CH_2N$, $J = 5.8$ Hz), 4.03 (t, 2H, $CH_2N$, $J = 6.1$ Hz), 5.34 (dd, 1H, CH, $J_1 = 13.1$ Hz, $J_2 = 5.2$ Hz), 7.89–7.97 (m, 4H, Ar), 10.50 (bs, 1H, NH). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.1, 31.4, 34.8, 42.4, 49.6, 53.8, 123.5, 131.2, 135.0, 167.1, 169.9, 172.0. IR (KBr) 2540, 1780, 1720, 1685. MS (EI), *m*/*z* 329 ($M^+$, 3), 76 (6), 71 (13), 58 (100), Anal. ($C_{17}H_{20}ClN_3O_4$) C, H, N.

*4.1.5. Synthesis of 2,6-bis(2,6-dioxopiperidin-3-yl)pyrrolo[3,4-f]isoindole-1,3,5,7(2*H*,6*H*)-tetraone **4***

A mixture of the benzene-1,2,4,5-tetracarboxylic dianhydride (0.10 g, 0.46 mmol), 3-aminopiperidine-2,6-dione (0.15 g, 0.92 mmol) and sodium acetate (0.08 mg, 1.01 mmol) was heated at reflux in an anhydrous atmosphere for 16 h. The solution was allowed to cool to room temperature and concentrated in vacuo. Water was then added to the residue with vigorous stirring to give a precipitate, which was collected by filtration, washed with water and dried to offer **4** (0.17 mg, 83%) as a grey solid (mp >300 °C). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.10–2.13 (m, 2H, 2 × $CH_2$), 2.50–2.66 (m, 4H, 2 × $CH_2$), 2.86–2.98 (m, 2H, 2 × $CH_2$), 5.27 (dd, 2H, 2 × CH, $J_1 = 12.5$ Hz, $J_2 = 5.2$ Hz), 8.36 (s, 2H, Ar), 11.21 (s, 2H, 2 × NH). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.9, 30.9, 49.5, 118.3, 136.8, 165.5, 169.6, 172.8. IR (KBr) 3240, 1780, 1720. MS (ESI), *m*/*z* 437 $[M - H]^+$. Anal. ($C_{20}H_{14}N_4O_8$) C, H, N.

*4.1.6. Synthesis of 2,2′-(3,3′-(1,3,5,7-tetraoxopyrrolo[3,4-f]isoindole-2,6(1*H*,3*H*,5*H*,7*H*)-diyl)bis(2,6-dioxopiperidine-3,1-diyl))bis(*N,N*-dimethylethanaminium) chloride **5***

To a mixture of anhydride **11** [34] (1.00 g, 2.27 mmol) and dry DMF (15 mL) was added *N,N*-dimethylethylendiamine (0.5 mL, 4.55 mmol) and the mixture was stirred for 24 h at room temperature. Then, the solvent was evaporated under reduced pressure and the resulting residue was treated with 10 mL of a mixture of acetic anhydride:acetyl chloride (1:1), and refluxed for 24 h. After cooling, the solid was collected by filtration and recrystallized from DMF–AcOEt to afford **5** (0,78 g, 52%) as a white solid (mp >280 °C dec). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.15–2.19 (m, 2H, 2 × $CH_2$), 2.54–2.69 (m, 2H, 2 × $CH_2$), 2.73–2.89 (m, 2H, 2 × $CH_2$), 2.78 (s, 12H, 4 × $CH_3$), 3.03–3.15 (m, 2H, 2 × $CH_2$), 3.20 (t, 4H, 2 × $CH_2N$, $J = 6.1$ Hz), 4.05 (t, 4H, 2 × $CH_2N$, $J = 6.1$ Hz), 5.45 (dd, 2H, 2 × CH, $J_1 = 13.1$ Hz, $J_2 = 5.2$ Hz), 8.38 (s, 2H, Ar), 10.51 (bs, 2H, 2 × NH). $^{13}$C NMR

(DMSO-$d_6$) $\delta$ 21.0, 31.4, 34.9, 42.4, 50.1, 53.8, 118.3, 136.8, 165.3, 169.6, 172.0. IR (KBr) 2600, 1775, 1720, 1680. MS (EI), *m*/*z* 580 ($M^+$, 5), 71 (4), 58 (100). Anal. ($C_{28}H_{34}Cl_2N_6O_8 \cdot 1.5H_2O$) C, H, N.

*4.1.7. General procedure for the synthesis of HCl salt* N-*substituted bis-glutarimides* ***6***

To a solution of the corresponding anhydride **9** (1.62 mmol) and dry DMF (10 mL) was added *N,N*-(2-aminoetil)-*N*-metiletilendiamine (0.81 mmol) and the mixture was stirred for 48 h at room temperature. After evaporation in vacuo water (2 mL) was added, and the pH value was adjusted to 1 by adding 10% HCl. Then, the solvent was evaporated under reduced pressure and the resulting residue was treated with 4 mL of a mixture of acetic anhydride:acetyl chloride (1:1), and refluxed. After cooling, the solid was collected by filtration, washed thoroughly with $Ac_2O$ and $Et_2O$ and filtered to afford HCl salt-6. The solid was recrystallized from DMF.

*4.1.7.1. Synthesis of 2,2′-(1,1′-(2,2′-(methylazanediyl)bis(ethane-2,1-diyl))bis(2,6-dioxopiperidine-3,1-diyl))bis(1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione)* ***6a***. Following the general procedure for the synthesis of *N*-substituted bis-glutarimides, from **9a** (0.50 g, 1.62 mmol), refluxed for 72 h, 0.48 g (81%) of HCl salt-**6a** were obtained. This salt was converted into the free base using a saturated 5% $NaHCO_3$ solution which was filtered and washed with hot EtOH. The solid was purified by recrystallization from $CHCl_3/Et_2O$ to afford **6a** (0.38 g, 68%) as a pale solid (mp >154 °C dec). $^1$H NMR (DMSO-$d_6$ + TFA) $\delta$ 2.02–2.15 (m, 2H, 2 × $CH_2$), 2.50–2.72 (m, 2H, 2 × $CH_2$), 2.76–3.10 (m, 4H, 2 × $CH_2$), 3.01 (s, 3H, $CH_3$), 3.36–3.46 (m, 4H, 2 × $CH_2$N), 4.14–4.16 (m, 4H, 2 × $CH_2$N), 5.94–6.00 (m, 2H, 2 × CH), 7.80–7.95 (m, 4H, Ar), 8.44–8.62 (m, 8H, Ar). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.6, 31.1, 36.3, 42.3, 51.0, 53.4, 121.4, 121.7, 121.8, 127.3, 127.4, 131.1, 131.3, 131.3, 131.6, 134.9, 162.7, 163.3, 170.0, 171.8. IR (KBr) 1730, 1680, 1660. MS (ESI), *m*/*z* 700 $[M+H]^+$. Anal. ($C_{39}H_{33}N_5O_8 \cdot 0.6CHCl_3$) C, H, N.

*4.1.7.2. Synthesis of 2-(3-(1,3-dioxo-1*H*-benzo[f]isoindol-2(3*H*)-yl)-2,6-dioxopiperidin-1-yl)-*N*-(2-(3-(1,3-dioxo-1*H*-benzo[f]isoindol-2(3*H*)-yl)-2,6-dioxopiperidin-1-yl)ethyl)-*N*-methylethanaminium chloride* ***6d***. Following the general procedure for the synthesis of HCl salt *N*-substituted bis-glutarimides, from **9d** (0.50 g, 1.62 mmol), refluxed 48 h, 0.48 g (81%) of HCl salt-**6d** were obtained as a pale solid (mp > 272 °C dec). $^1$H NMR (DMSO-$d_6$) $\delta$ 2.11–2.20 (m, 2H, 2 × $CH_2$), 2.60–2.73 (m, 2H, 2 × $CH_2$), 2.81–2.90 (m, 2H, 2 × $CH_2$), 2.89 (bs, 3H, $CH_3$), 2.99–3.09 (m, 2H, 2 × $CH_2$), 3.25 (bs, 4H, 2 × $CH_2$N), 4.08 (bs, 4H, 2 × $CH_2$N), 5.34–5.43 (m, 2H, 2 × CH), 7.81 (dd, 4H, Ar, $J_1$ = 6.1 Hz, $J_2$ = 3.1 Hz), 8.29 (dd, 4H, Ar, $J_1$ = 6.1 Hz, $J_2$ = 3.1 Hz), 8.60 (bs, 4H, Ar), 10.33 (bs, 1H, NH). $^{13}$C NMR (DMSO-$d_6$ + TFA) $\delta$ 21.2, 31.4, 34.5, 41.1, 49.7, 52.6, 125.2, 127.0, 129.7, 130.6, 135.3, 166.9, 169.9, 172.2. IR (KBr) 2500, 1770, 1710, 1685. MS (ESI), *m*/*z* 700 $[M+H]^+$. Anal. ($C_{39}H_{34}ClN_5O_8 \cdot 0.6H_2O$) C, H, N.

*4.1.7.3. Synthesis of 2-(3-(2,5-dioxo-3,4-diphenyl-2,5-dihydro-1*H*-pyrrol-1-yl)-2,6-dioxopiperidin-1-yl)-*N*-(2-(3-(2,5-dioxo-3,4-diphenyl 2,5-dihydro-1*H*-pyrrol-1-yl)-2,6-dioxopiperidin-1-yl)ethyl)-*N*-methyl-ethanaminium chloride* ***6f***. Following the general procedure for the synthesis of HCl salt *N*-substituted bis-glutarimides, from **9d** (0.50 g, 1.39 mmol), refluxed 16 h, 0.43 g (74%) of HCl salt-**6f** were obtained as a yellow solid (mp > 261 °C dec). $^1$H NMR (DMSO-$d_6$, 60 °C) $\delta$ 2.12–2.17 (m, 2H, 2 × $CH_2$), 2.50–2.65 (m, 2H, 2 × $CH_2$), 2.78–2.88 (m, 2H, 2 × $CH_2$), 2.85 (bs, 3H, $CH_3$), 2.96–3.07 (m, 2H, 2 × $CH_2$), 3.26–3.32 (m, 4H, 2 × $CH_2$N), 4.05 (bs, 4H, 2 × $CH_2$N), 5.24 (dd, 2H, 2 × CH, $J_1$ = 12.8 Hz, $J_2$ = 5.5 Hz), 7.41 (bs, 20H, Ar), 10.28 (NH, 1H). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.2, 31.3, 34.3, 40.2, 49.9, 52.2, 128.2, 128.7, 129.6, 130.0, 136.3, 169.4, 169.9, 172.0. IR (KBr) 2500, 1770, 1710, 1690. MS (ESI), *m*/*z* 805 $[M+H]^+$. Anal. ($C_{47}H_{42}ClN_5O_8$) C, H, N.

*4.1.7.4. Synthesis of 2-(3-(1,3-dioxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)-*N*-(2-(3-(1,3-dioxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)ethyl)-*N*-methylethanaminium chloride* ***6g***. Following the general procedure for the synthesis of HCl salt *N*-substituted bis-glutarimides, from **9g** (0.50 g, 1.93 mmol), refluxed 24 h, 0.25 g (40%) of HCl salt-**6d** were obtained as a white solid (mp > 210 °C dec). $^1$H NMR ($D_2O$) $\delta$ 1.85 (bs, 2H, 2 × $CH_2$), 2.26–2.41 (m, 2H, 2 × $CH_2$), 2.62–2.65 (m, 4H, 2 × $CH_2$), 2.83 (s, 3H, $CH_3$), 3.27 (bs, 4H, 2 × $CH_2$N), 3.97 (bs, 4H, 2 × $CH_2$N), 4.96 (dd, 2H, 2 × CH, $J_1$ = 12.4 Hz, $J_2$ = 5.2 Hz), 7.52–7.54 (m, 8H, Ar). $^{13}$C NMR ($D_2O$) $\delta$ 23.5, 33.5, 38.1, 44.4, 52.4, 57.6, 126.3, 133.3, 137.7, 171.5, 174.4, 177.0. IR (KBr) 2500, 1780, 1715, 1680. MS (ESI), *m*/*z* 600 $[M+H]^+$. Anal. ($C_{31}H_{30}ClN_5O_8 \cdot 0.7DMF$) C, H, N.

*4.2. Biological assays*

*4.2.1. Cell and measurement of TNF-*$\alpha$

HL-60 cells were incubated at 37 °C in a 5% $CO_2$ atmosphere using RPMI 1640 medium (Gibco, Grand Island, NY, U.S.A.) supplemented with 10% v/v BFS (Bovine Foetal Serum), 2 mM L-glutamine, 100 U/mL penicillin and 100 μL/mL streptomycin. Cells in exponential growth ($5 \times 10^5$ cells/mL) were then treated with OA (50 nM) or TPA (10 nM) and with or without each compound (30 μM), during 16 h in 24 well plates. Then cell number and cell morphology were evaluated microscopically. Cells were collected by centrifugation (2000 rpm during 10 min.). TNF-$\alpha$ supernatant concentration was quantified using a human TNF-$\alpha$ ELISA (Amersham Co.) following supplier protocol. The TNF-$\alpha$ concentration in those treatments without thalidomide or derivate compounds (only with TPA or OA) was defined as the 100%. All experiments were performed at least three times.

## Acknowledgment

We thank Dirección General de Estudios Superiores (Grant No. PB98-055, CYTED), Laboratorios Knoll (BASF Pharma), Universidad CEU-San Pablo for financial support and Brian Crilly for linguistic assistance. L. Añorbe acknowledges Comunidad de Madrid for a predoctoral fellowship.

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