Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 3067–3073

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Anti-Inflammatory Activity of Phthalimide Derivatives, Designed as New Thalidomide Analogues

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Received 3 December 2001; accepted 16 January 2002

Abstract—This paper describes the synthesis and anti-inflammatory activity of new *N*-phenyl-phthalimide sulfonamides (**3a–e**) and the isosters *N*-phenyl-phthalimide amides (**4a–e**), designed as hybrids of thalidomide (**1**) and aryl sulfonamide phosphodiesterase inhibitor (**2**). In these series, compound **3e** (LASSBio 468), having a sulfonyl-thiomorpholine moiety, showed potent inhibitory activity on LPS-induced neutrophil recruitment with $ED_{50} = 2.5\,mg\,kg^{-1}$, which was correlated with its inhibitory effect on TNF-α level.

Introduction

Tumor necrosis factor-α (TNF-α), is a cytokine considered to be a primary mediator of the inflammatory response.[1] At lung level, it has been demonstrated that the release of TNF-α favours the sequestration and migration of neutrophils which play a critical role in the pathogenesis of lung inflammation.[2–4] Using different models, it has been shown that adenosine cyclic monophosphate (cAMP) elevating-agents can also regulate the induction of TNF-α synthesis at the transcriptional levels.[5,6] Over production of TNF-α is associated with a wide range of pathological conditions, this has led to much recent effort in finding ways to downregulate its production or inhibit its effects.[7–9]

Cyclic-AMP or GMP are ubiquitous second messengers involved in a vast array of cellular responses to different inflammatory stimuli.[10] Inside the cell, cyclic nucleotide phosphodiesterase (PDE) represents a group of 11 distinct proteins, differentially expressed depending on the tissue, whose function is to hydrolyse cyclic nucleotides.[10,11] Since the last decade, studies on the control of cAMP or cGMP levels through the action of PDE increased and its relevance can be demonstrated by the number of clinical trials with selective inhibitors of PDE for different inflammatory disorders such as asthma.[12,13] Trying to reduce side effects, much effort have been expended to discover new substances that could selectively block members of the PDE family, such as PDE-4, the isoform found in all pro-inflammatory and immunocompetent cells.[14,15]

It is well documented that elevated levels of cAMP inhibit TNF-α production in activated monocytes and peripheral blood mononuclear cells,[22] and inhibition of PDE-4 has been shown to be a successful method for modulating TNF-α activity.

Thalidomide (**1**), a drug used to treat different inflammatory diseases is also an inhibitor of TNF-α expression.[16–21] It has been approved by the Food and Drug Administration (FDA) in July 1998 for a treatment of erythema nodosum leprosum. After that, the interest in this drug has been intensified because of its effective immunomodulatory and anti-inflammatory properties.[23] However, given the problems in administering an effective, nontoxic oral dose of thalidomide (**1**), there is concern about the design of new compounds that are based on the thalidomide structure, but which have greater anti-TNF-α activity, less toxicity and greater stability than the prototype (**1**).

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0968-0896/02/$ - see front matter
PII: S0968-0896(02)00152-9

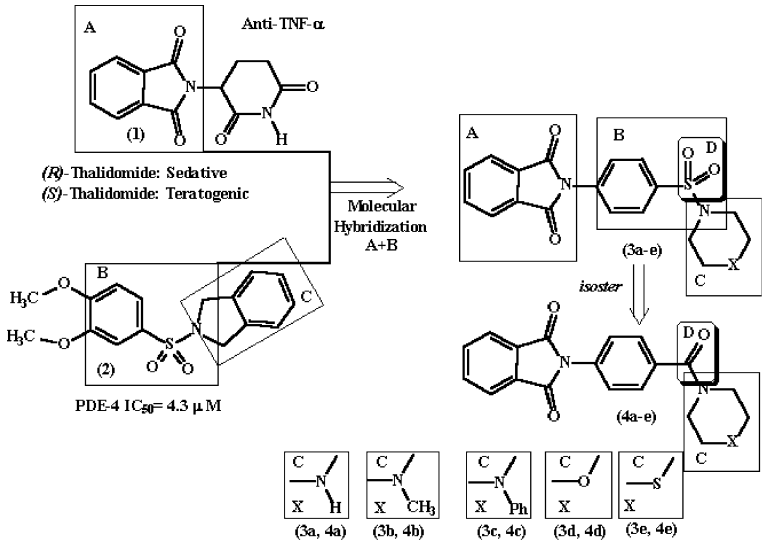


Chart 1.

As part of a research program aiming at the synthesis and pharmacological evaluation of novel anti-inflammatory lead-candidates, we describe in the present paper the synthesis and anti-inflammatory profile of new *N*-substituted phthalimides derivatives (**3a**–**e**) and (**4a**–**e**), structurally designed as hybrids of thalidomide (**1**) and aryl sulfonamides, described as selective PDE-4 inhibitors, like active compound (**2**)[24] (Chart 1). The rational basis to design the new derivatives **3** was founded to promote a dual anti-inflammatory profile acting at TNF-α and phosphodiesterase levels. For instance, this symbiotic activity[25] was anticipated to be possible in a compound having the phthalimide ring of **1** (A, Chart 1) and the phenylsulfonamide moiety of **2** (B, Chart 1), raising the new derivatives **3a**–**e**.

In order to investigate the contribution of the C framework of **2** (Chart 1) in the anti-inflammatory activity, the isoindoline group was substituted by a hexahydropyrazine moiety in the derivative **3a**. Next, we decide to vary the *N*-piperazinyl ring substituent as methyl (**3b**), the large phenyl group (**3c**) and by isosteric morpholine and thiomorpholine nucleous, providing derivatives **3d** and **3e**, respectively (Chart 1).

Chemistry

Among several obvious synthetic routes to the new thalidomide analogues **3a**–**e**, we decided to explore in our approach the sulfonylchloride derivative (**7**) as the key intermediate. This compound could be easily obtained, in high yield, by the synthetic sequence depicted in Scheme 1. The condensation of phthalic anhydride (**5**) with aniline at reflux, furnished the *N*-phenylphthalimide (**6**) in 86% yield.[26]

The second step in the synthesis of **7** was based on a regioselectivy eletrophilic aromatic substitution employing a mixture of chlorosulfonic acid and phosphorous pentachloride,[27] at 50 °C, obtaining **7** in 70% yield. The synthetic route to the new derivatives **3a**–**e** was accomplished by classical functional group interconversion. Thus, the target compounds **3a**–**e** could be prepared by condensation of **7** with corresponding piperazine derivatives,[28] as showed in Scheme 1.

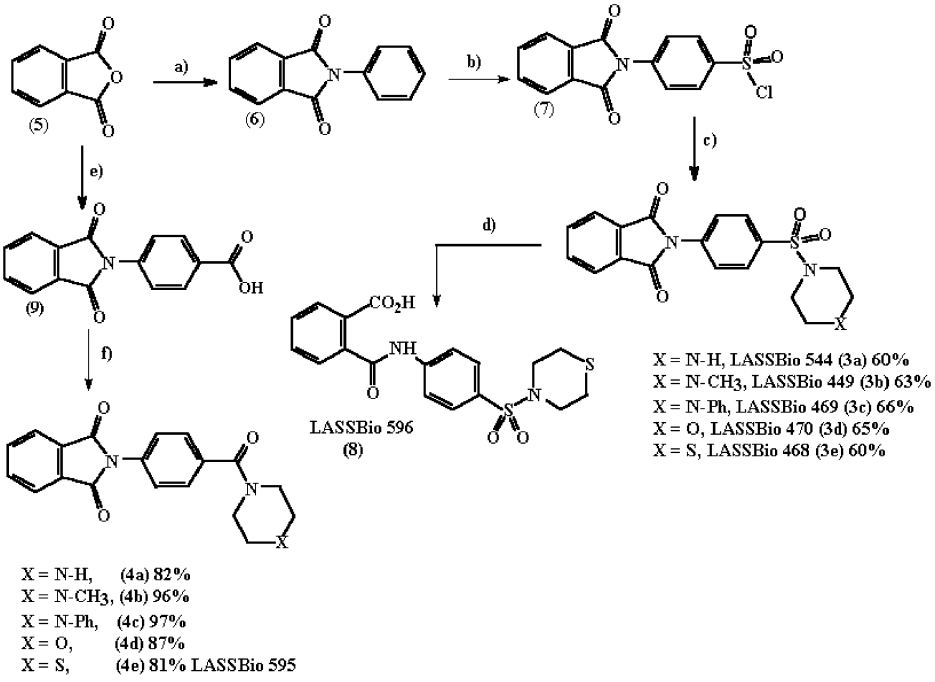
Finally, the bioisosteric series **4a**–**e**, possessing a carbonyl group instead of the sulfonyl unit of derivatives **3a**–**e** (Chart 1), was synthesized by the route illustrated in Scheme 1. The amide derivatives **4a**–**e** were prepared from functionalized phthalimide **9**, in excellent yields, exploring classical methodology, using thionyl chloride to the more electrophilic acid chloride, followed by treatment with functionalized piperazines in the presence of dichlorometane, at room temperature.[29]

Results and Discussion

Thalidomide analogues (**3a**–**e**) were screened for their ability to inhibit the acute inflammatory response, measured by inhibition of LPS-induced TNF-α and neutrophil infiltration into mice lungs, with thalidomide as standard. The results obtained are shown in Figure 1.

In the series of sulfonamide derivatives (**3a**–**e**), the most active compound (**3e**), which possesses the thiomorpholine ring at the aryl moiety of the 4-sulfonylphenylphthalimide framework, was selected to study the dose-dependent response for this new bioactive compound. Compound **3e** (LASSBio 468) inhibited the neutrophil infiltration induced by LPS with ED_{50} 2.5 mg kg^{-1}.

In order to investigate the importance of phthalimide ring in the anti-inflammatory activity, compound **3e** (LASSBio 468) was hydrolised by treatment with lithium hydroxide in a mixture of THF/MeOH/H_2O,[29] to furnish compound **8** in 77% yield (Scheme 1). The pharmacological evaluation of this compound (**8**) revealed a remarkable decrease in anti-inflammatory activity (Table 1).



Scheme 1. (a) $C_6H_5NH_2$, reflux, 1 h; 86%; (b) $ClSO_3H$, PCl_5, 50 °C, 30 min, 70%; (c) functionalized piperazines, CH_2Cl_2, rt, 30 min, 60–66%; (d) LiOH, THF, MeOH, H_2O, rt, 10 min, 77%; (e) 4-$CO_2HC_6H_4NH_2$, AcOH, reflux, 1 h, 91%; (f) (1) SO_2Cl, DMF (cat), reflux, 1 h; (2) CH_2Cl_2, functionalized piperazines, rt, 30 min, 81–97%.

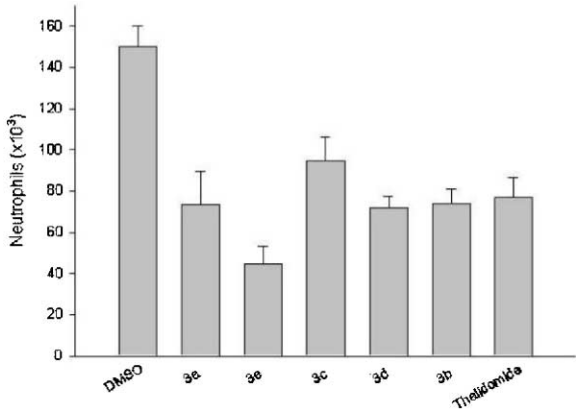


Figure 1. Effect of compounds **3a**–**e** and thalidomide on neutrophil influx induced by LPS into BALF of mice lungs.

In order to study the role of the sulfonyl group present in the aryl-sulfonamide moiety found in the prototype (**2**), we were able to replace this group by an isosteric carbonyl unit, as in compounds **4a**–**e**. The pharmacological evaluation of amide compound **4e** (LASSBio 596), designed upon the bioassays results from **3e**, revealed a significant loss of activity when compared with the original compound **3e** (Table 1), suggesting the crucial role of the phenyl-sulfonyl-piperazine framework in the investigated bioactivity.

To correlate the anti-inflammatory activity of compound **3e** (LASSBio 468) with a possible effect on TNF-α production, the cytokine levels were also evaluated as shown in Figure 2. This result revealed the ability of compound **3e** (LASSBio 468) to inhibit TNF-α levels in BALF of mice lungs treated with LPS.

Table 1. Inhibitory effects of compounds **1**, **3e**, **4e**, and **8** on neutroplihs recruitment induced by LPS[a]

Compd	% Inhibition
DMSO (vehicle)	0.0±0.0
Thalidomide (**1**)	50.0±7.3
LASSBio 468 (**3e**)	72.0±8.6
LASSBio 595 (**4e**)	48.0±11.7
LASSBio 596 (**8**)	50.0±7.3

[a]Neutrophils number was determined by BALF CTR = animals pretreated with vehicle. Results are expressed as mean±SEM of five animals.[4]

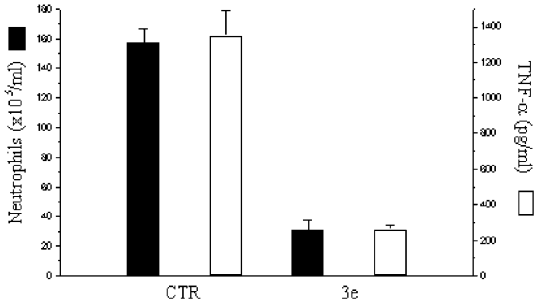


Figure 2. Effect of compound **3e** (LASSBio 468) on TNF-α levels and neutrophil influx into the BALF of mice lungs.

It is well-known that elevation of intracellular levels of cAMP in leukocytes is accompanied by significant inhibition of the production of TNF-α and is associated with inhibition of PDE-4 activity.[6,30,31] Therefore, the new thalidomide analogues **3e, 4e** and **8** were also evaluated, in vitro, as PDE-4 and PDE-3 inhibitors. The results obtained with PDE's from bovine aorta assay,[32]

indicated that none of the compounds assayed presented a significative inhibitory effect on PDE-4 and PDE-3 activity. For instance, **3e** (LASSBio 468) presented only 40% of PDE-4 inhibition at 300 μM concentration and was ineffective on PDE-3. Compound **8** (LASSBio 596) and **4e** (LASSBio 595) presented the same poor profile of bioactivity (data not shown).

In conclusion, in an effort to discover new thalidomide analogues with anti-inflammatory activity we found a novel lead-compound candidate **3e** (LASSBio 468). The preliminary SAR with this compound revealed the importance of the sulfonyl group, the nature of the N-terminal piperazine ring, and also indicated the role of the phthalimide ring in the anti-inflammatory activity of this compound (**3e**). Further, **3e** presented a great inhibitory activity on neutophil recruitment and on TNF-α level in BALF of mice lungs treated with LPS. Moreover, the ability of compound **3e** (LASSBio 468) to inhibit TNF-α production seems to not correlate with activity awards PDE-4 or PDE-3, major isoforms found in all pro-inflammatory and immunocompetent cells.

Experimental

Chemistry

Melting points were determined with a Buchi 540 apparatus and are uncorrected. Proton magnetic resonance (^{1}H NMR), unless otherwise stated, was determined in deuterated dimethylsulfoxide containing ca. 1% tetramethylsilane as an internal standard with Bruker AC 200, Bruker DRX 300 spectrometers at 200 and 300 MHz, respectively. Splitting patterns are as follows: s, singlet; d, doublet; t, triplet; q, quartet; qt, quintet; dd, double doublet; br, broad; m, multiplet. Carbon magnetic resonance (^{13}C NMR) was determined in the same spectrometers described above at 50 and 75 MHz, respectively, using deuterated dimethylsulfoxide as internal standard. Infrared (IR) spectra were obtained with Nicolet-550 Magna spectrophotometer using potassium bromide plates. The mass spectra (MS) were obtained by electron impact (70 eV) with a GC/VG Micromass 12 spectrometer.

The progress of all reactions was monitored by tlc performed on 2.0 × 6.0 cm aluminum sheets precoated with silica gel 60 (HF-254, Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under ultraviolet light at 254 nm. For column chromatography Merck silica gel (70–230 mesh) was used.

2-Phenyl-1,3-isoindolinedione (6).[26] A mixture of 0.5 g (3.38 mmol) of phthalic anhydride in 0.4 mL (4.06 mmol) of aniline was refluxed for 30 min. The *N*-phenyl phthalimide (**6**) separates out on cooling. A nearly white powder appeared which was filtered through a Büchner funnel and washed twice with 20 mL of water, to give the desired 2-phenyl-1,3-isoindolinedione (**6**) in 86% yield, mp 204–205 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.46 (m, H2′-H6′), 7.80 (m, H5 and H6), 7.96 (m, H4 and H7). ^{13}C NMR (50 MHz, $CDCl_3$) δ 123.91 (C4 and C7), 126.74 (C2′ and C6′), 128.26 (C4′), 129.28 (C3′ and C5′), 131.86 (C1′), 131.94 (C3a–C7a), 134.56 (C5–C6), 167.44 (C1 and C3).

4-(1,3-Dioxo-2,3-dihydro-1H-2-isoindolyl)-1-benzenesulfonyl chloride (7).[27] To a solution of 0.16 g (0.09 mL, 1,35 mmol) of chlorosulfonic acid and 0.14 g (0.67 mmol) of phosphorus pentachloride that had been stirred for 10 min, was added 0.15 g (0.67 mmol) of *N*-phenyl-phthalimide (**6**) in small portion. The resulting mixture was stirred and heated at 50 °C for 30 min. The reaction mixture was poured onto ice and extracted with chloroform. The organic phase was separated, washed with brine, dried over anhyd Na_2SO_4 and evaporated at reduced pressure to furnish compound (**7**) in 70% yield, as a white solid mp 180–182 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.41 (d, J = 8.25 Hz, H2–H6′), 7.74 (d, J = 8.23 Hz, H3′ and H5′), 7.91 (m, H4–H7); ^{13}C NMR (50 MHz, DMSO-d_6) δ 124.05 (C4 and C7), 126.66 (C2′ and C6′), 127.34 (C3′ and C5′), 132.08 (C3a–C7a), 132.67 (C1′), 135.37 (C5–C6), 147.97 (C4′), 167.50 (C1 and C3).

General procedures for the preparation of sulfonamides 3a–e[28]

To a solution of sulfonyl chloride derivative (**7**) (0.5 g; 1.56 mmol) in 50 mL of methylene chloride, were added the functionalized piperazine derivatives (3.12 mmol). The reaction mixture was stirred for about 30 min, at room temperature, when the end of reaction was observed by TLC. Next, the phthalimide derivatives **3a**–**e** were isolated by addition of 50 mL of methylene chloride and extraction with 10% aq HCl and brine. The organic layer was dried over anhyd Na_2SO_4 and evaporated at reduced pressure to give the sulfonamide derivatives **3a**–**e** in good yields.

2-(4-Hexahydro-1-pyrazinylsulfonylphenyl)-1,3-isoindolinedione (3a). The title compound was obtained as a white powder, in 60% yield, by condensing **7** with piperazine, as described above, mp > 250 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.21 (sl, H1″ and H2″ and H3″ and H4″), 7.82 (d, J = 8.58 Hz, H2′ and H6′), 7.97 (d, J = 8.62 Hz, H3′ and 5′), 7.99 (s, H5–H6 and H4–H7). ^{13}C NMR (50 MHz, DMSO-d_6) δ 42.59 (C3″ and C4″), 43.30 (C1″ and C2″), 124.13 (C7 and C4), 128.18 (C2′ and C6′), 128.89 (C3′ and C5′), 131.93 (C7a and C3a), 133.73 (C6 and C5), 135.45 (C1′), 137.04 (C4′), 166.96 (C1 and C3). IR (KBr) 3495 and 3363 (ν NH), 1787 and 1766 (ν C=O), 1747 and 1720 (ν C=O), 1381 (ν C–N–C), 1343 (ν SO_2) cm^{-1}. Anal. calcd for $C_{18}H_{17}N_3O_4S$: C, 58.21; H, 4.61; N, 11.31; O, 17.23; S, 8.63. Found: C, 58.19; H, 4.60; N, 11.33; O, 17.22; S, 8.62.

2-[4-(4-Methylhexahydro-1-pyrazinylsulfonyl)phenyl]-1,3-isoindolinedione (3b). The title compound was obtained as a white powder, in 63% yield, by condensing **7** with 1-methylpiperazine, as described above, mp 210–213 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.21 (sl, H1″ and H2″ and H3″ and H4″), 7.82 (d, J = 8.58 Hz, H2′ and H6′), 7.97 (d, J = 8.62 Hz, H3′ and 5′), 7.99 (s, H5–H6 and H4–H7). ^{13}C NMR (50 MHz, DMSO-d_6) δ

42.59 (C3″ and C4″), 43.30 (C1″ and C2″), 124.13 (C7 and C4), 128.18 (C2′ and C6′), 128.89 (C3′ and C5′), 131.93 (C7a and C3a), 133.73 (C6 and C5), 135.45 (C1′), 137.04 (C4′), 166.96 (C1 and C3). IR (KBr) 3495 and 3363 (ν NH), 1787 and 1766 (ν C=O), 1747 and 1720 (ν C=O), 1381 (ν C–N–C), 1343 (ν SO_2) cm^{-1}. Anal. calcd for $C_{19}H_{19}N_3O_4S$: C, 59.21; H, 4.97; N, 10.90; O, 16.60; S, 8.32. Found: C, 59.20; H, 4.96; N, 10.88; O, 16.59; S, 8.31.

2-[4-(4-Phenylhexahydro-1-pyrazinylsulfonyl)phenyl]-1,3-isoindolinedione (3c). The title compound was obtained as a white powder, in 65% yield, by condensing **7** with 1-phenylpiperazine, as described above, mp 231–233 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.26 (s, H1″ and H2″ and H3″ and H4″), 6.90 (m, H3‴-H5‴), 7.27 (dd, J = 7.58 and 8.25 Hz, H2‴ and H6‴), 7.76 (d, J = 8.74 Hz, H2′ and H6′), 7.86 (m, H5 and H6), 7.94 (d, J = 8.77 Hz, H3′ and 5′), 8.00 (m, H4 and H7). ^{13}C NMR (50 MHz, $CDCl_3$) δ 46.10 (C3″ and C4″), 49.28 (C1″ and C2″), 117.05 (C2‴ and C5‴), 120.95 (C4‴), 124.09 (C7 and C4), 126.31 (C2′ and C6′), 128.66 (C3′ and C5′), 129.24 (C3‴ and C5‴), 131.41 (C7a and C3a), 134.44 (C6 and C5), 134.88 (C1′), 136.12 (C4′), 150.68 (C1‴), 166.54 (C1 and C3). IR (KBr) 1789 and 1745 (ν C=O), 1714 (ν C=O), 1384 (ν C–N–C), 1348 (ν SO_2) cm^{-1}. Anal. calcd for $C_{24}H_{21}N_3O_4S$: C, 64.42; H, 4.73; N, 9.39; O, 14.30; S, 7.16. Found: C 64.43; H 4.72; N, 9.40; O, 14.29; S, 7.17.

2-[4-(1,4-Oxazinan-4-ylsulfonyl)phenyl]-1,3-isoindolinedione (3d). The title compound was obtained as a white powder, in 66% yield, by condensing **7** with morpholine, as described above, mp 218–220 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.05 (t, J = 4.70 Hz, H1″ and H2″), 3.77 (t, J = 4.71 Hz, H3″ and H4″), 7.76 (d, J = 8.76 Hz, H2′ and H6′), 7.85 (m, H5 and H6), 7.90 (d, J = 8.77 Hz, H3′ and 5′), 8.00 (m, H4 and H7) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 46.04 (C1″ and C2″), 66.16 (C3″ and C4″), 124.19 (C7 and C4), 126.42 (C2′ and C6′), 128.75 (C3′ and C5′), 131.43 (C7a and C3a), 134.08 (C6 and C5), 135.01 (C1′), 136.22 (C4′), 166.65 (C1 and C3) ppm. IR (KBr): 1743 and 1716 (ν C=O), 1595 (ν C–O–C), 1373 (ν C–N–C), 1352 (ν SO_2) cm^{-1}. Anal. calcd for $C_{18}H_{16}N_2O_5S$: C, 58.06; H 4.33; N, 7.52; O, 21.48; S, 8.61. Found: C, 58.05; H 4.31; N, 7.53; O, 21.49; S, 8.60.

2-[4-(1,4-Thiazinan-4-ylsulfonyl)phenyl]-1,3-isoindolinedione (3e). The title compound was obtained as a white powder, in 60% yield, by condensing **7** with thiomorpholine, as described above, mp 190–192 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.73 (t, J = 5.22 Hz, H3′ and H4′), 3.40 (t, J = 5.06 Hz, H1′ and H2′), 7.74 (d, J = 8.58 Hz, H2′ and H6′), 7.85 (m, H5 and H6), 7.88 (d, J = 8.65 Hz, H3′ and 5′), 7.99 (m, H4 and H7). ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.95 (C3″ and C4″), 47.48 (C1″ and C2″), 123.65 (C7 and C4), 126.07 (C2′ and C6′), 127.79 (C3′ and C5′), 130.93 (C7a and C3a), 134.55 (C6 and C5), 135.25 (C1′), 135.63 (C4′), 166.55 (C1 and C3). IR (KBr) 1786 and 1766 (ν C=O), 1745 and 1716 (ν C=O), 1379 (ν C–N–C), 1337 (ν SO_2) cm^{-1}. Anal. calcd for $C_{18}H_{16}N_2O_4S_2$: C, 55.66; H 4.15; N, 7.21; O, 16.47; S, 16.51. Found: C, 55.67; H 4.15; N, 7.22; O, 16.48; S, 16.50.

2-[4-(1,4-Thiazinan-4-ylsulfonyl)phenylcarbamoyl]benzoic acid (8).[29] To a solution of 0.2 g (0.51 mmol) of the phthalimide derivative (**3e**) in a solution of 6 mL of tetrahydrofuran/methanol/water (3:1:1) was added 0.1 g (4.12 mmol) LiOH 98% and the resulting solution was stirred at room temperature for 2 h. The reaction mixture was diluted with ethyl ether (20 mL) and carefully acidified with 2 N aq HCl. The organic layer was washed with brine, dried over anhyd Na_2SO_4 and evaporated at reduced pressure to give the acid derivative (**9**) in 77% yield, as a white solid, mp 187–189 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.68 (sl, H3″ and H4″), 3.18 (sl, H1″ and H2″), 7.61 (d, J = 8.14 Hz, H2′ and H6′), 7.67 (d, J = 8.10 Hz, H3′ and 5′), 7.96 (m, H4 and H5), 7.95 (m, H3 and H6), 10.80 (s, CONHAr), 12.41 (sl, ArCO_2H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 26.91 (C3″ and C4″), 48.28 (C1″ and C2″), 119.77 (C2′ and C6′), 124.13 (C3), 128.42 (C6), 128.94 (C3′ and C5′), 130.15 (C5), 130.34 (C1), 130.94 (C2), 132.39 (C4), 138.89 (C4′), 144.20 (C1′), 167.71 (ArCONHR), 168.55 (ArCO_2H).

4-(1,3-Dioxo-2,3-dihydro-1*H*-2-isoindolyl)benzoic acid (9).[26] Dissolve 1.0 g (7.29 mmol) of 4-aminobenzoic acid and 1.0 g (6.75 mmol) of phthalic anhydride in 10 mL of glacial acetic acid and reflux for 1 h. The *N*-substituted phthalimide (**9**) separates out on cooling. A nearly white powder appeared that was filtered through a Büchner funnel and washed twice with 30 mL of water, to give the desired 4-(1,3-dioxo-2,3-dihydro-1*H*-2-isoindolyl)benzoic cid (**9**) in 91% yield, as white powder, mp > 250 °C. ^{1}H NMR (200 MHz, DMSO-d_6/40 °C) δ 7.70 (d, J = 8.70 Hz, H2′–H6′), 7.90 (m, H5–H6), 7.99 (m, H4–H7), 8.08 (d, J = 8.70 Hz, H3′-H5′), 13.02 (sl, RCO_2H). ^{13}C NMR (50 MHz, DMSO-d_6/40 °C) δ 123.42 (C4 and C7), 126.78 (C2′ and C6′), 129.75 (C3′ and C5′), 129.98 (C4′), 131.36 (C3a and C7a), 134.71 (C5 and C6), 135.73 (C1′), 166.51 (ArCO_2H), 166.62 (C1 and C3). IR (KBr) 3547 (ν OH), 1782 and 1748 (ν C=O), 1746 (ν C=O), 1700 (ν C=O), 1428 (ν N–C–O), 1380 (ν C–N–C) cm^{-1}.

General procedures for the preparation of amides 4a–e[29]

A solution of 0.2 g (0.75 mmol) of the acid (**9**) in 3 mL of freshly distilled thionyl chloride and catalytic amount of dimethylformamide was vigorously stirred under reflux for 1 h. After this time, the solvent was carefully evaporated at reduced pressure and a solution of 0.97 mmol of the respective amine in 10 mL of methylene chloride was added. The reaction mixture was stirred for 30 min at room temperature, then poured in 20 mL of water and extracted with methylene chloride (3 × 15 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated at reduced pressure to give the amides derivatives (**4a–e**) in excellent yields.

2-(4-Hexahydro-1-pyrazinylcarbonylphenyl)-1,3-isoindolinedione (4a). The title compound was obtained as a white powder, in 82% yield, by condensing **9** with piperazine, as described above, mp > 250 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.60 (sl, H1″ and H2″), 3.72 (sl, H3″ and H4″), 7.59 (m, H2′-6′ and H3′-H5′), 7.84 (m,

H5 and H6), 7.97 (m, H4 and H7). IR (KBr): 3470 (ν NH), 1739 and 1713 (ν C=O), 1621 (ν C=O), 1466 (ν N–C–O), 1389 (ν C–N–C) cm^{-1}. Anal. calcd for $C_{19}H_{17}N_3O_3$: C, 68.05; H 5.11; N, 12.53; O, 14.31; Found: C, 68.06; H 5.10; N, 2.56; O, 14.32.

2-[4-(4-Methylhexahydro-1-pyrazinylcarbonyl)phenyl]-1,3-isoindolinedione (4b). The title compound was obtained as a white powder, in 96% yield, by condensing **9** with 1-methylpiperazine, as described above, mp 140–142 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26 (R_2NCH_3), 3.05 (sl, H1″-H2″, 3.14 (sl, H3″-H4″), 7.55 (s, H2′-H6′ and H3′-H5′), 7.82 (m, H5 and H6), 7.98 (m, H4 and H7); ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.87 (R_2NCH_3), 35.59 (C1″ and C2″), 39.85 (C3″ and C4″), 124.05 (C4 and C7), 126.37 (C2′ and C6′), 128.14 (C3′ and C5′), 131.80 (C3a and C7a), 133.03 (C1′), 134.76 (C5 and C6), 135.82 (C4′), 167.15 (C1 and C3), 170.95 (Ar*C*ONR$_2$). IR (KBr) 2923 (ν CH_3), 1788 and 1712 (ν C=O), 1617 (ν C=O), 1491 (ν N–C–O), 1388 (ν C–N–C) cm^{-1}. Anal. calcd for $C_{20}H_{19}N_3O_3$: C, 68.75; H 5.48; N, 12.03; O, 13.74. Found: C, 68.76; H 5.49; N, 12.05; O, 13.75.

2-[4-(4-Phenylhexahydro-1-pyrazinylcarbonyl)phenyl]-1,3-isoindolinedione (4c). The title compound was obtained as a yellow powder, in 97% yield, by condensing **9** with 1-phenylpiperazine, as described above, mp 242–244 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.21 (sl, H1″–H2″), 3.80 (m, H3″–H4″), 6.95 (d, J = 8.58 Hz, H2‴ and H6‴), 7.44 (m, H3‴, H4‴ and H5‴), 7.58 (s, H2′–H6′ and H3′–H5′), 7.82 (m, H5 and H6), 7.99 (m, H4 and H7); ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.83 (C1″ and C2″), 49.93 (C3″ and C4″), 116.92 (C2‴ and C6‴), 120.82 (C4‴), 124.04 (C4 and C7), 126.52 (C2′ and C6′), 128.17 (C3′ and C5′), 129.41 (C3‴ and C5‴), 131.74 (C3a and C7a), 133.26 (C4′), 134.77 (C5 and C6), 135.13 (C1′), 151. 06 (C1‴), 167.09 (C1 and C3), 169. 61 (Ar*C*ONR$_2$). IR (KBr) 1780 and 1760 (ν C=O), 1736 and 1711 (ν C=O), 1633 (ν C=O), 1461 (ν N–C–O), 1379 (ν C–N–C) cm^{-1}. Anal. calcd for $C_{25}H_{21}N_3O_3$: C, 72.98; H 5.14; N, 10.21; O, 11.67. Found: C, 72.97; H 5.12; N, 10.22; O, 11.65.

2-[4-(1,4-Oxazinan-4-ylcarbonyl)phenyl]-1,3-isoindolinedione (4d). The title compound was obtained as a white powder, in 87% yield, by condensing **9** with morpholine, as described above, mp 158–160 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.62 (sl, H1″–H2″ and H3″–H4″), 7.56 (s, H2′–H6′ and H3′–H5′), 7.95 (m, H4–H7 and H5–H6); ^{13}C NMR (50 MHz, DMSO-d_6) δ 66.38 (C1″ and C2″), 66.56 (C3″ and C4″), 123.98 (C4 and C7), 127.67 (C2′ and C6′), 128.13 (C3′ and C5′), 132.00 (C3a and C7a), 133.37 (C4′), 135.26 (C5 and C6), 135.54 (C1′), 167.29 (C1 and C3), 168.90 (Ar*C*ONR$_2$). IR (KBr) 1786 and 1764 (ν C=O), 1740 and 1708 (ν C=O), 1625 (ν C=O), 1518 (ν C–O–C), 1440 (ν N–C–O), 1380 (ν C–N–C) cm^{-1}. Anal. calcd for $C_{19}H_{16}N_2O_4$: C, 67.85; H 4.79; N, 8.33; O, 19.03; Found: C, 67.86; H 4.79; N, 8.31; O, 19.04.

2-[4-(1,4-Thiazinan-4-ylcarbonyl)phenyl]-1,3-isoindolinedione (4e). The title compound was obtained as a white powder, in 81% yield, by condensing **9** with thiomorpholine, as described above, mp 197–199 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.64 (sl, H3″–H4″), 3.85 (sl, H1″–H2″), 7.54 (s, H2′–H6′ and H3′–H5′), 7.81 (m, H5 and H6), 7.96 (m, H4 and H7); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.40 (C3″ and C4″), 44.24 (C1″), 49.70 (C2″), 123.52 (C4 and C7), 126.10 (C2′ and C6′), 127.33 (C3′ and C5′), 131.20 (C3a and C7a), 132.71 (C4′), 134.30 (C5 and C6), 134.80 (C1′), 166.55 (C1 and C3), 169.43 (Ar*C*ONR$_2$). IR (KBr) 1780 and 1761 (ν C=O), 1739 and 1715 (ν C=O), 1635 (ν C=O), 1459 (ν N–C–O), 1379 (ν C–N–C) cm^{-1}. Anal. calcd for $C_{19}H_{16}N_2O_3S$: C, 64.76; H 4.58; N, 7.95; O, 13.62; S, 9.10; Found: C, 64.77; H 4.59; N, 7.97; O, 13.61; S, 9.09.

Biological assays

Male BALB/c mice weighing 25–30 g (UFF, RJ, BR.) were used throughout this study. Animals were maintained in a temperature-controlled room and received water and food ad libitum. During all experiments mice were care in accordance with published guidelines for animal use in laboratory.[33] Mice were pretreated with saline (vehicle) or 10 mg/kg of each compound ip, 45 min before LPS inhalation. The inhalation procedure has been done as previously described.[4] After different time intervals, airspaces were washed with saline to provide 4 mL of bronchoalveolar lavage fluid (BALF). Aliquots were used for evaluate the neutrophil number and for TNF-α assay. TNF-α levels were determined by a highly specific ELISA with a detection limit of 50 pg/mL. Results are expressed as mean ± SEM of five animals.[4]

Acknowledgements

Thanks are due to CNPq (#52.0033/98–5), FAPERJ (E-26/151.868/2000) and FUJB-UFRJ (BR.) for the financial support of this work and to CNPq and CAPES (BR.) for fellowships (LML, PC, ALM, CAMF, VLGM and EJB). We are grateful to Analytical Center of NPPN (UFRJ-BR) and Instituto de Química (UFRJ-BR).

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