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PII: S0960-894X(14)00504-6
DOI: http://dx.doi.org/10.1016/j.bmcl.2014.05.017
Reference: BMCL 21630

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 18 March 2014
Revised Date: 30 April 2014
Accepted Date: 5 May 2014

Please cite this article as: Yamasaki, P.R., do Nascimento, D.C., Chelucci, R.C., Belone, a.d.F., Rosa, P.S., Diório, S.M., de Melo, T.R.F., Barbieri, K.P., Placeres, M.C.P., Carlos, I.Z., Chung, M.C., dos Santos, J.L., Synthesis and Evaluation of Novel Dapsone–Thalidomide Hybrids for the Treatment of Type 2 Leprosy Reactions, *Bioorganic & Medicinal Chemistry Letters* (2014), doi: http://dx.doi.org/10.1016/j.bmcl.2014.05.017

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Synthesis and Evaluation of Novel Dapsone–Thalidomide Hybrids for the Treatment of Type 2 Leprosy Reactions

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Abstract

We synthesized a series of novel dapsone–thalidomide hybrids (**3a–i**) by molecular hybridization and evaluated their potential for the treatment of type 2 leprosy reactions. All of the compounds had analgesic properties. Compounds **3c** and **3h** were the most active antinociceptive compounds and reduced acetic acid-induced abdominal constrictions by 49.8% and 39.1%, respectively. The hybrid compounds also reduced tumor necrosis factor-α levels in lipopolysaccharide-stimulated L929 cells. Compound **3i** was the most active compound; at concentrations of 15.62 μM and 125 μM, compound **3i** decreased tumor necrosis factor-α levels by 86.33% and 87.80%, respectively. In nude mice infected with *Mycobacterium leprae in vivo*, compound **3i** did not reduce the number of bacilli compared with controls. Compound **3i** did not have mutagenic effects in *Salmonella typhimurium* strains TA100 and TA102, with or without metabolic activation (S9 mixture). Our results indicate that compound **3i** is a novel lead compound for the treatment of type 2 leprosy reactions.

Keywords: Leprosy; *M. leprae*; anti-inflammatory; analgesic; TNFα; dapsone; thalidomide; molecular hybridization; erythema nodosum leprosum.

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Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* (*M. leprae*) that is a serious public health problem in several developing countries, including India and Brazil.[1] In 2012, 232,857 patients worldwide were newly reported to have leprosy.[2] The disease affects peripheral nerves, especially Schwann cells, as well as skin and multiple internal organs. Its clinical manifestations are related to the patient's level of cell-mediated immunity.[3]

Multidrug therapy consisting of combinations of dapsone, rifampicin, and clofazimine are usually used to treat multibacillary leprosy, while dapsone in combination with rifampicin is usually used to treat paucibacillary leprosy.[4] Although Multidrug therapy shows good effectiveness, many patients experience reactional episodes (RE), potentially serious events that may occur during or after treatment. REs are classified either as reversal reactions or as erythema nodosum leprosum (ENL).[5]

ENL, a type 2 reaction caused by a humoral immune response to *M. Leprae*, is characterized by fever and painful subcutaneous nodules in the patient's face and/or upper and lower limbs. Damage to the peripheral nerves causes painful recurrent episodes.[6] It has been estimated that 30% of patients with lepromatous leprosy have ENL.[7] The levels of proinflammatory cytokines such as tumor necrosis factor-α (TNFα) are increased in serum and in the cutaneous lesions of patients with ENL, contributing to their pain and inflammatory disorders.[8]

Thalidomide is frequently used to treat ENL because of its ability to reduce the levels of TNFα.[9,10] It was also reported that thalidomide reduces reactional iritis, iridocyclitis, neuritis, and symptoms associated with ENL.[11] Thalidomide also reduces the expression of intercellular adhesion molecule-1 and other cytokines (e.g. interferon-γ,

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interleukin [IL]-12, IL-1β, IL-6, and IL-10), and suppresses the dermal infiltration of polymorphonuclear leukocytes and T cells.[12,13] Despite these beneficial effects, thalidomide is associated with a variety of adverse effects, including teratogenicity, neurotoxicity, and peripheral neuropathy, that limit its clinical use.[14,15] In addition, it has some pharmacokinetic limitations related to its poor water solubility, compromising its bioavailability.[14,16] Therefore, there is an urgent need to identify new drugs that are more effective and safer than thalidomide for the treatment of type 2 leprosy reactions.

Dapsone (4,4'-diamino-diphenylsulfone) is a bacteriostatic drug that inhibits dihydrofolic acid synthesis by competition with para-aminobenzoic acid.[17] Several studies have demonstrated that dapsone has bacteriostatic and anti-inflammatory properties comparable with those of nonsteroidal anti-inflammatory drugs. Dapsone can inhibit cellular adhesion, chemotaxis, and the expression of prostaglandin, leukotrienes, IL-8, and TNFα.[18] It was also reported that dapsone dose-dependently decreases TNFα levels at the protein and mRNA levels.[19]

In this study, molecular hybridization was used to develop new derivatives of thalidomide and dapsone (**3a–i**) for treating ENL in patients with leprosy. Because the phthalimide subunit is a pharmacophore able to inhibit TNFα production, we selected this as one of the molecular components (Scheme 1).[20] The aryl-sulfonyl subunit derivative of dapsone was chosen as the second component because this pharmacophore has anti-inflammatory effects and inhibits *M. leprae* activity. Our aim was to combine the analgesic, anti-inflammatory, and *M. leprae* inhibitory activities in the same molecule for the effective treatment of REs in patients with leprosy.

INSERT SCHEME 1

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The phthalimide derivatives (**3a–i**) were synthesized via condensation reactions between phthalic anhydride and amines, which were previously selected in acetic acid, under reflux for 2–3 h (Scheme 2). After recrystalization in ethanol, the compounds were obtained with yields of 70%–90%.[21] The structures of all compounds were established by mass spectroscopy, elemental analysis, infrared spectroscopy, and ^{1}H- and ^{13}C-nuclear magnetic resonance. All of the compounds were analyzed by high-performance liquid chromatography and their purities were > 98.5%.

INSERT SCHEME 2

The antinociceptive profiles of compounds **3a–i** were evaluated using acetic acid-induced abdominal constrictions in mice.[22] All of the compounds had analgesic effects in this model after an oral dose of 100 μmol/kg body weight (Table 1). Compounds **3c** and **3h** had the greatest antinociceptive effects, by reducing acetic acid-induced abdominal constrictions by 49.8% and 39.1%, respectively. Compound **3c** also had greater antinociceptive effects than thalidomide, which inhibited the number of abdominal constrictions by 42.2%. The antinociceptive effects of compounds **3d** and **3i** were similar to that of dypirone, which was used as a control.

INSERT TABLE 1

Cytotoxicity studies confirmed that the cell viability was > 90% for all compounds administered at concentrations of up to 150 μM (data not shown).[23] TNFα levels were measured in the supernatants of L929 cells.[24,25] Compounds **3a–i** were added to the cultured cells at concentrations of 15.62 μM and 125 μM. Thalidomide was used as the positive control for anti-inflammatory effects. The TNFα levels were 143.19 ± 18.85 and 133.10 ± 20.35 pg/mL in cells treated with 15.62 μM and 125 μM thalidomide, respectively (Table 2). Compounds **3a–i** dose-dependently inhibited lipopolysaccharide-induced TNFα

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production. The inhibition rates ranged from 0% to 86.33% at 15.62 μM and from 7.24% to 87.8% at 125 μM. Compound **3i** had the greatest effect on TNFα production; at concentrations of 15.62 μM and 125 μM, compound 3i reduced tumor necrosis factor-α levels by 86.33% and 87.80%, respectively. At both concentrations, compounds **3e–i** were more potent than thalidomide in terms of the decrease in TNFα levels. Compound **3a** had the weakest effect in terms of inhibiting proinflammatory cytokine production. The structure–activity relationships showed that substitution of the sulfonyl group with a pyridine (**3c**) or pyrimidine ring (**3f**, **3g**, and **3i**) achieved greater anti-inflammatory effects. The greatest effect was observed when the pyrimidine ring at 4 position was monosubstituted with one methyl group (**3i**).

INSERT TABLE 2

The antimycobacterial activities of each compound was evaluated in nude mice that were previously inoculated in both hind footpads with *M. leprae* using Shepard's method.[26,27] Compound **3i** was evaluated in this model because of the remarkable reduction in TNFα levels and its analgesic effects. Infected mice were orally administered with 200 mg/kg of compound **3i** or thalidomide for 4 weeks (5 days/weeks), starting 10 months after infection. After treatment, the mean number of bacilli in the control group, and in mice treated with thalidomide and compound **3i** were 3.56×10^6, 4.6×10^6, and 2.5×10^6 cells, respectively (Figure 1), which were not significantly different. It has been reported that substitution of dapsone and other sulfonamides at the amino group could decrease or abolish the inhibitory effects of these compounds on *M. leprae*.[28, 29]

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INSERT FIGURE 1

We finally examined the potential mutagenic effects of compound **3i** using *Salmonella typhimurium* strains TA100 and TA102, with or without metabolic activation (S9 mixture).[30-32] Table 3 shows the number of revertants per plate, the standard deviation, and the mutagenic index (MI) of both strains of *S. typhimurium* treated with compound **3i** or thalidomide. At all of the tested concentrations of compound **3i**, the MI was < 2. There were no significant differences in the numbers of revertents per plate in the presence or absence of S9 in both strains. Like thalidomide[32], compound **3i** was not considered mutagenic because the MI was < 2 for all tested concentrations of compound **3i**.[32-34]

INSERT TABLE 3

In conclusion, we synthesized a novel series of thalidomide–dapsone hybrids (**3a-j**) and evaluated their potential for treating the inflammatory conditions associated with type 2 leprosy reactions. Compound **3i** had analgesic properties and achieved superior reductions in TNFα levels compared with thalidomide. Although compound **3i** did not inhibit *M. leprae*, it did not increase the number of bacilli and it did not have mutagenic effects on *S. typhimurium* strains TA100 and TA102, with or without metabolic activation (S9 mixture). Interestingly, the molecular hybridization strategy yielded compounds whose analgesic and anti-inflammatory properties were superior to those of thalidomide. Based on these results, compound **3i** is a novel lead compound for the treatment of type 2 leprosy reactions.

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Acknowlegments

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) ref. Process: 07/54983-4, 07/57055-0 and 12/50359-2. Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) and Programa de Apoio ao Desenvolvimento das Ciências Farmacêuticas (PADC-FCF UNESP).

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27. *Anti mycobacterial activity*: Nude mice (n= 6) were inoculated in the both hind footpads with 0.03 mL of bacterial suspension containing 1×10^4 *M. leprae.* After inoculation, the animals were divided in the following groups: control, thalidomide and compound **3i**. Infected mice were orally administered with 200 mg/kg of compound **3i** or thalidomide for 4 weeks (5 days/weeks), starting 10 months after infection. The control group received a suspension of arabic gum 5%. At 12^{th} month after inoculation, mice were euthanized by carbonic monoxide inhalation. The footpads were removed aseptically and homogenized in 2 mL of Hank's balanced solution. The mycobacteria numbers were counted. Positive multiplication occurred in footpads that contained at least 10^5 bacilli.

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Captions

Scheme 1. Molecular hybridization between dapsone and thalidomide.

Scheme 1. Preparation of compounds **3a-i**.

Table 1. Antinociceptive effect of dypirone, thalidomide and compounds (**3a-i**) using acetic acid-induced abdominal constrictions.

Table 2. TNFα Production in LPS-stimulated L929 cells by thalidomide and compounds **3a-i**.

Table 3. Mutagenic activity expressed as the mean and standard deviation of the number of revertants/plate in bacterial strains TA100 and TA102 exposed to compound **3i** at various doses, with (+S9) or without (-S9) metabolic activation.

Figure 1. Anti *M.leprae* activity of compound **3i** and thalidomide.

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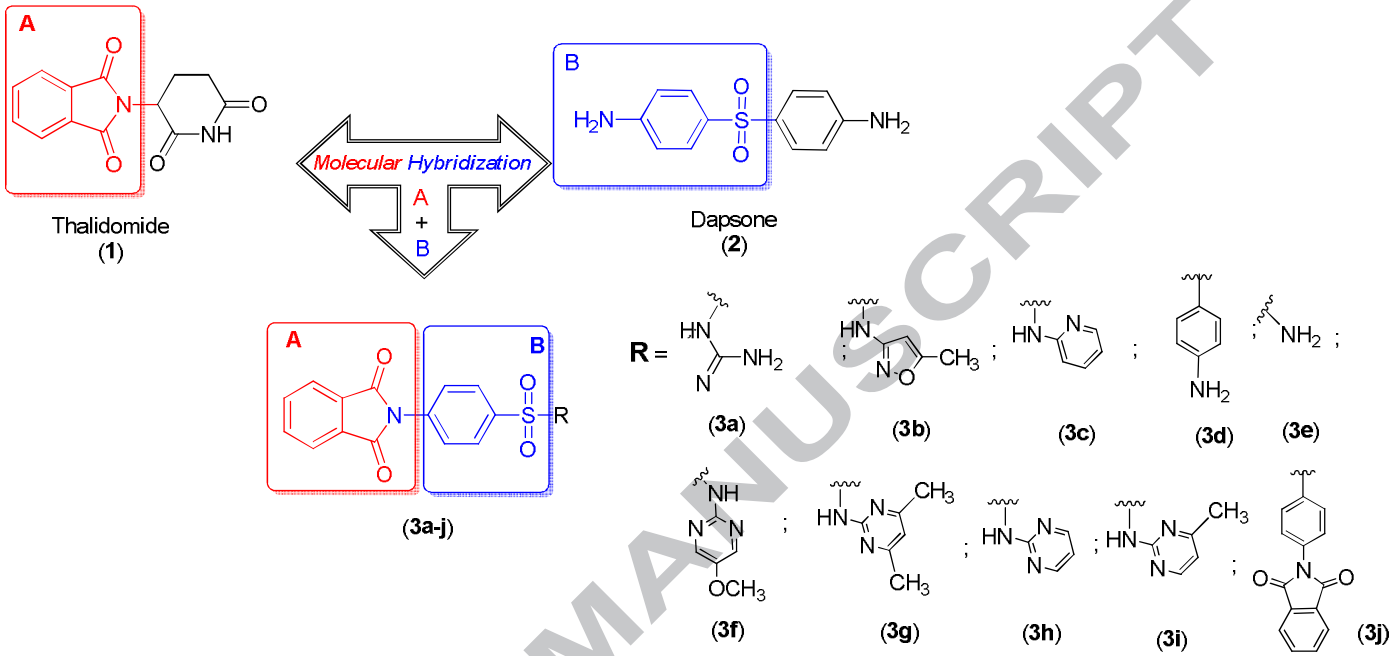
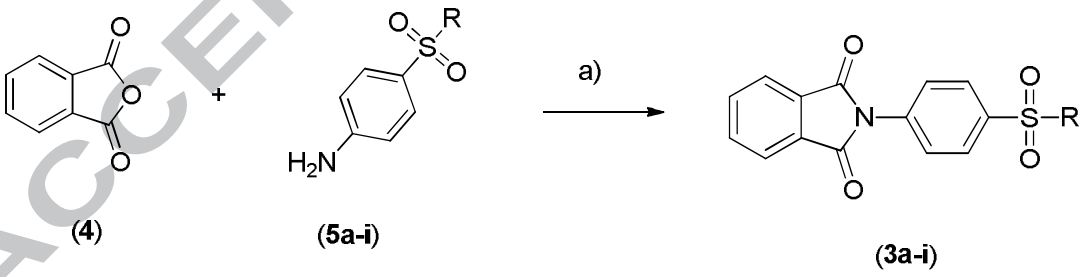
Shemes

Scheme 1.



Scheme 2.



a) acetic acid, reflux, 2h, 70-90%

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Tables

Table 1.

Compounds	Dose (μM/Kg)	*n*	Constrictions number	Inhibition (%)
Vehicle control[a)]	100	6	81.00 ± 2.31	-
Thalidomide	100	6	34.18 ± 3.08	42.20
Dipyrone	100	6	53.83 ± 2.40	33.50
3a	100	6	57.00 ± 4.77	29.63*
3b	100	6	66.50 ± 5.89	17.90
3c	100	6	40.66 ± 6.19	49.80*
3d	100	6	52.50 ± 2.56	35.20*
3e	100	6	59.33 ± 5.22	26.75*
3f	100	6	58.66 ± 2.24	27.57*
3g	100	6	58.83 ± 4.21	27.37*
3h	100	6	49.33 ± 3,36	39.10*
3i	100	6	53.33 ± 3.46	34.15*

[a)] arabic gum 5% *P<0,005 (ANOVA followed by Dunnett's Multiple Comparison Test)

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Table 2.

Compound	125 μM		15.625 μM	
	TNFα (pg/mL)	Inhibition (%)	TNFα (pg/mL)	Inhibition (%)
Control (-)	5.14 ± 1.01	-	5.14 ± 1.01	-
Control (+) (LPS)	232.75 ± 3.2	-	232.75 ± 3.19	-
Thalidomide	133.10 ± 20.35*	42.81	143.19 ± 18.85*	35.38
3a	215.90 ± 1.89	7.24	238.64 ± 7.59	0.00
3b	153.41 ± 11.42*	34.09	185.60 ± 15.66	16.25
3c	77.65 ± 5.14*	66.64	162.88 ± 4.95	26.50
3d	138.26 ± 32.43*	40.60	157.77 ± 18.78*	28.81
3e	68.18 ± 4.52*†	70.71	104.17 ± 22.01*	52.99
3f	44.67 ± 4.56*†	80.81	58.71 ± 4.06*†	73.51
3g	43.56 ± 0.96*†	81.28	102.27 ± 10.99*	53.85
3h	38.03 ± 4.97*†	79.31	154.31 ± 5.70*	33.70
3i	28.41 ± 0.44*†	87.79	30.30 ± 2.03*†	86.33

*P<0,005 (ANOVA followed by Tukey's Multiple Comparison Test). The asterisks (*) denote the levels of significance between control (-) and compounds. The cross mark (†) denote the level of significance between the standard thalidomide group and compounds.

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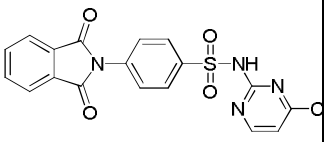
Table 3.

Compound	Concentration µmol/plate	Revertants/plate in *Salmonella typhimurium* strains			
		TA 100		TA 102	
		+S9	-S9	+S9	-S9
 (3i)	0	128.9 ± 7.3	136.8 ± 3.4	327.8 ± 9.1	301 ± 5.2
	2	140.1 ± 5.8 (1.08)	147 ± 8.5 (1.07)	352.7 ± 6.6 (1.07)	327.9 ± 5.7 (1.08)
	4	156.7 ± 6.6 (1.21)	163.2 ± 4.5 (1.19)	384.2 ± 8.2 (1.17)	365.1 ± 6.9 (1.22)
	8	172.5 ± 6.9 (1.33)	182.5 ± 7.1 (1.33)	407.8 ± 5.9 (1.24)	380.3 ± 7.3 (1.26)
	16	211.3 ± 8.1 (1.64)	220.1 ± 6.2 (1.61)	432.3 ± 8.7 (1.31)	410.6 ± 6.2 (1.36)
	32	205.6 ± 4.8 (1.59)	218.4 ± 7.3 (1.59)	455.1 ± 6.8 (1.38)	422.7 ± 6.8 (1.40)
Positive control	-	2540 ± 71.9*	2210 ± 67.3*	843 ± 33.7*	902 ± 38.3*

0 = negative control (DMSO–100 µL/plate). *P<0.01 (ANOVA followed by Tukey's Multiple Comparison Test). The values in parenthesis = mutagenic index. Numbers represent averages of triplicates from the three different experiments ± the standard deviation. ***Positive control: sodium azide (1.25 µg/plate) for TA100 (-S9), daunomycin (3 µg/plate) for TA102 (-S9) and 2-anthramine (1.25 µg/plate) for TA100 (+S9) and 2-aminofluorene (1.25 µg/plate) for TA102 (+S9).

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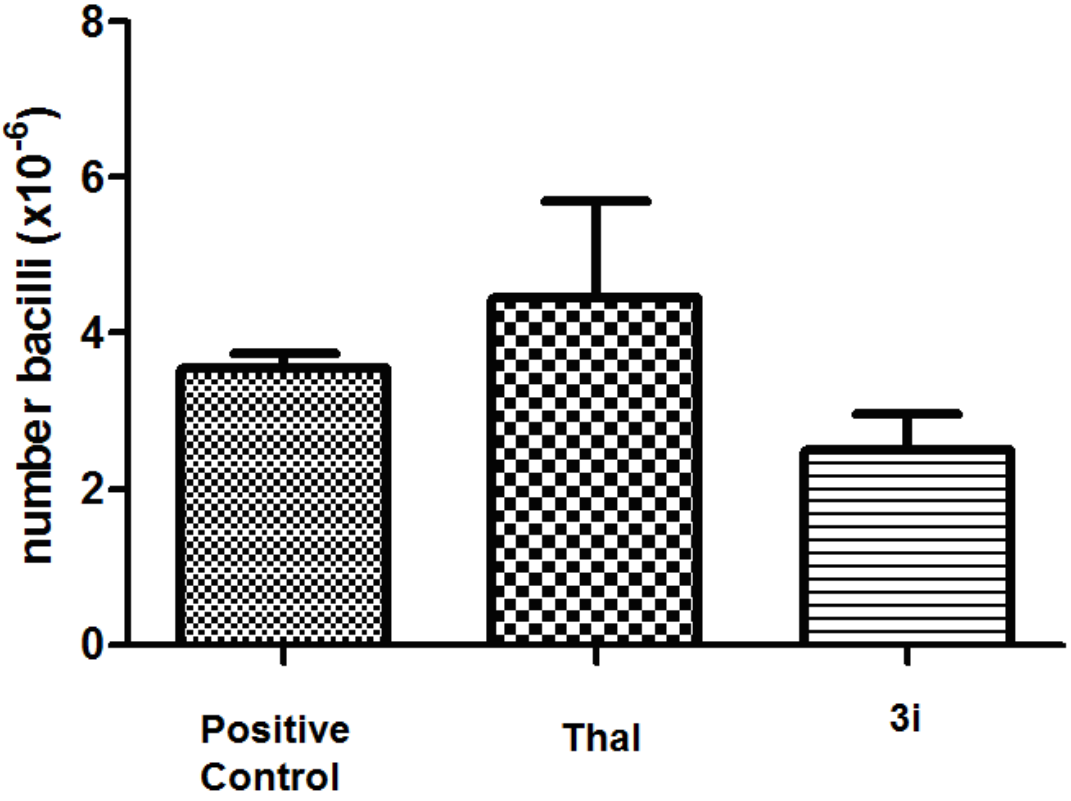
Figures

Figure 1.



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Graphical Abstract

Novel Dapsone-Thalidomide Hybrid Compounds Useful to Treat a Type 2 Leprosy Reactions

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Paulo Renato Yamasaki[1], Dejair Caetano do Nascimento[2], Rafael Consolin Chelucci[1], Andréa de Faria Fernandes Belone[2], Patrícia Sammarco Rosa[2], Suzana Madeira Diório[2], Thais Regina Ferreira de Melo[1], Karina Pereira Barbieri[1], Marisa C.P. Placeres[1], Iracilda Zepone Carlos[2] , Man Chin Chung[1] and Jean Leandro dos Santos[1]*

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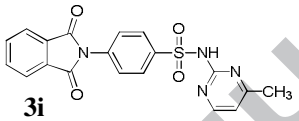

3i

- *Analgesic activity;*
- *TNF-α inhibitor;*
- *No-mutagenic effect;*

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