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Synthesis and evaluation of novel dapsone–thalidomide hybrids for the treatment of type 2 leprosy reactions

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

[a] Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista—UNESP, Rodovia Araraquara Jaú Km 01, 14801-902 Araraquara, SP, Brazil
[b] Instituto Lauro de Souza Lima, Rodovia Comandante João Ribeiro de Barros, Km 225/226, 17034-971 Bauru, SP, Brazil

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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 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.

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, 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

* Corresponding author. Tel.: +55 (16)33016972; fax: +55 (16)33016960.
E-mail address: santosjl@fcfar.unesp.br (J.L. dos Santos).

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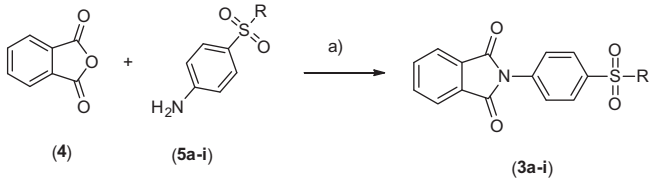
limitations related to its poor water solubility, compromising its bioavailability.[14,16] Therefore, there is an urgent need to identify new drugs that are more effective and safer than thalidomide for the treatment of type 2 leprosy reactions.

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.

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%.

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.



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

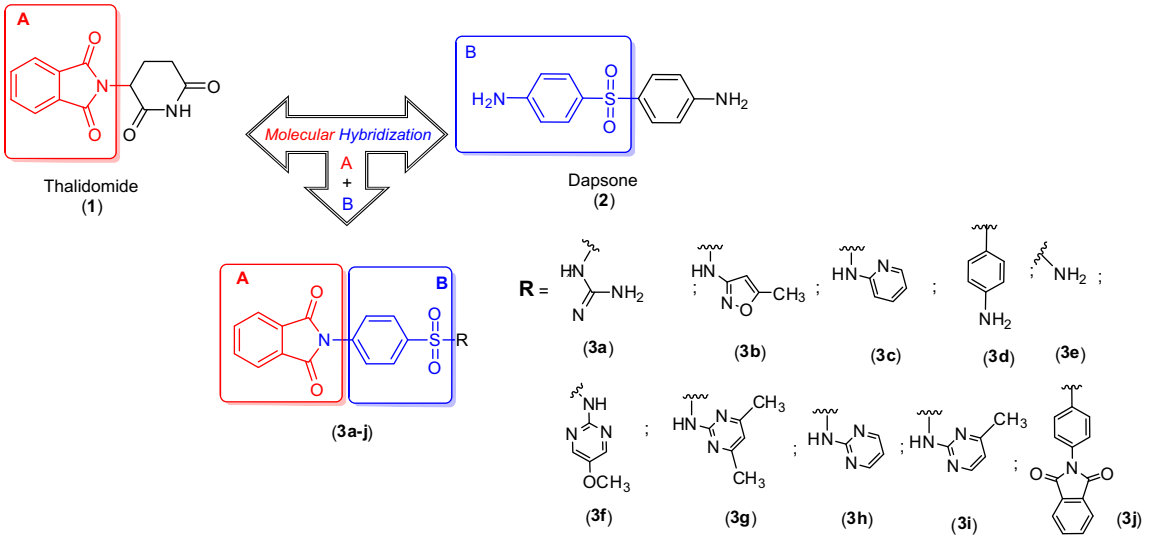
Scheme 2. Preparation of compounds **3a–i**.

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

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).

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 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 and 125 μM thalidomide, respectively (Table 2). Compounds **3a–i** dose-dependently inhibited lipopolysaccharide-induced TNFα 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 and 125 μM, compound **3i** reduced tumor necrosis factor-α levels



Scheme 1. Molecular hybridization between dapsone and thalidomide.