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Thalidomide analogues: Tumor necrosis factor-alpha inhibitors and their evaluation as anti-inflammatory agents

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ABSTRACT

A series of related thalidomide derivatives (**2**–**9**) were synthesized by microwave irradiation and evaluated for anti-inflammatory activity. Such activity was assessed in vivo and ex vivo. Compounds **2**, **8** and **9** showed the highest levels of inhibition of TNF-α production. On rat paw edema and hyperalgesia assays, compound **9**, (1,4-phthalazinedione) demonstrated the highest in vivo anti-inflammatory activity. Thus, compound **9** can be considered as a promising compound to be subjected to further modification to obtain new agents for the treatment of inflammatory diseases.

1. Introduction

Thalidomide (2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione), compound **1,** is a synthetic glutamic acid derivative developed as a sedative hypnotic agent to treat emesis during pregnancy (Fig. 1). Despite its early successful clinical results, this compound had to be withdrawn from the market due to its teratogenic effects (Ito et al., 2011). Nevertheless, over the past years, the interest in this drug has resurged due to its potential usefulness in the treatment of erythema nodosum leprosum (Sampaio et al., 1993; Haslett et al., 2005), multiple myeloma (Singhal et al., 1999), AIDS as well as various cancers (Jacobson et al., 1999; Singhal and Mehta, 2001). Thalidomide has been found to have several biological activities, including, the inhibition of tumor necrosis factor-α (TNF-α) production (Sampaio et al., 1991, Moreira et al., 1993), as well as anti-inflammatory, anti-angiogenic (D'Amato et al., 1994), and cyclooxygenase inhibitory activities (Sano et al., 2005).

TNF-α was originally described as a circulating factor that can cause necrosis of tumors. Later, it has been identified as a key regulator of the inflammatory response. The central role of TNF-α in inflammation has been demonstrated through the ability of TNF-α blocking agents to treat a wide range of inflammatory conditions.

TNF-α production inhibitory activity was initially considered to be one of the key thalidomide action mechanisms. This cytokine plays a critical role in several physiological immunological processes, causing severe damage when produced in excess.

A number of phthalimide derivatives have been previously synthesized employing different strategies in order to obtain molecules that can act as modulators of the over production of TNF-α (Niwayama et al., 1998; Muller et al., 1998; Lima et al., 2002; Cupertino Da Silva et al., 2010; Stewart et al., 2007; Zhu et al., 2003; Machado et al., 2005; Chaulet et al., 2011; Tweedie et al., 2011). In an attempt to maintain the beneficial properties while avoiding its side effects, new achiral synthetic thalidomide analogues have been designed.

In this work we optimized the synthesis process and evaluated the anti-inflammatory activity of a series of phthalimides that are, structurally related to thalidomide (Fig. 1). To test the anti-inflammatory activity in vivo (rat paw edema, hyperalgesia and myeloperoxidase activity) and ex vivo (TNF-α production) assays were employed. A phthalazinone derivative was synthesized and its anti-inflammatory activity was also evaluated.

2. Materials and Methods

Melting points (uncorrected) were determined in a capillary with an Electrothermal 9100 SERIES-Digital apparatus. The microwave-assisted reaction was carried out in Monowave 300 Anton Paar. Reactions were monitored by thin-layer chromatography (TLC) in silica gel plates (F245 Merck) and the products visualized under ultraviolet light (254 and 365 nm).

IR spectra were recorded with a FT Perkin Elmer Spectrum One employing KBr discs. ^{1}H- and ^{13}C-NMR spectra were determined in DMSO-d_6 and $CDCl_3$ solutions using a Bruker 300 MHz and Bruker Biospin 600 MHz AVIII600 spectrometers at room temperature with

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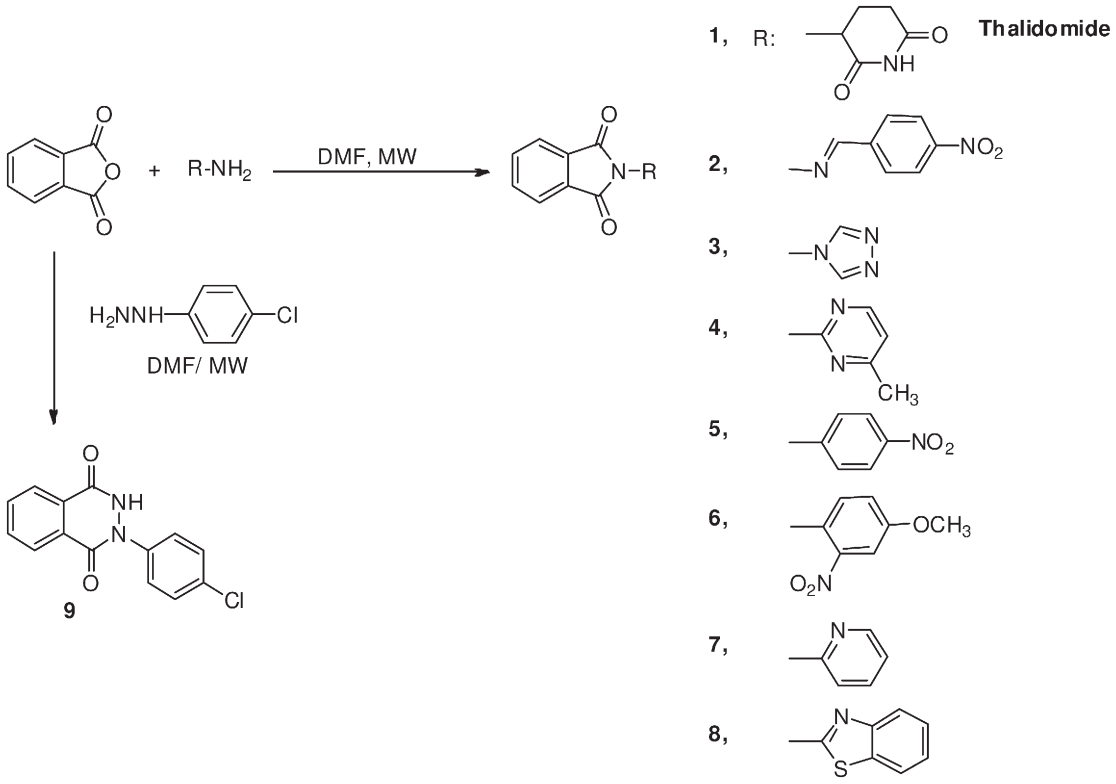

Fig. 1. Structure of thalidomide (compound **1**). Synthesis of phthalimide derivatives (compounds **2**–**9**) from phthalic anhydride and the corresponding amines.

tetramethylsilane as internal standard. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hertz.

2.1. Chemistry

2.1.1. General Procedure for the Synthesis of Phthalimide Derivatives

A mixture of phthalic anhydride (2.5 mmol, 0.37 g), 2.5 mmol of the corresponding amine and 0.1 mL of DMF were subjected to microwave irradiation (Fig. 1). Reaction time and temperature varies for each derivative being synthesized. The reaction was monitored by TLC employing Cl_2CH_2: MeOH (8:2) as mobile phase. After complete conversion, the reaction mixture was triturated with ethanol and the solid product was recrystallized from a solvent mixture (EtOH: H_2O, 2:1).

2.1.1.1. (E)-2-((4-Nitrobenzylidene)amino)isoindoline-1,3-dione ***(2)***. Reaction temperature: 170 °C, reaction time: 2 min. A pale yellow solid was obtained after recrystallization. Yield: 0.45 g (62%). Mp: 260–264 °C. Mp Lit. (Hearn and Lucero, 1982; Salman and Ray, 1981): 301–305 °C and 290 °C. IR (KBr, ν cm^{-1}): 1724 (CO), 1597 (CN), 1513 and 1345 (NO_2), 837 (C—H). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.48 (s, 1 H, *H*CN), 8.28 (d, 2 H, *J* = 8.2 Hz, Ar*H*), 7.76–7.84 (m, 3 H, Ar*H*), 7.50 (dd, 3 H, *J* = 7.8 1.6 Hz, Ar*H*).

^{13}C NMR (151 MHz, DMSO-d_6): δ 163.9, 148.5, 136.7, 135.6, 129.5, 129.3, 125.3, 124.7, and 124.1.

2.1.1.2. 2-(4 H-1,2,4-Triazol-4-yl)isoindoline-1,3-dione ***(3)***. Reaction temperature: 170 °C, reaction time: 4:00 min. A white solid was obtained after recrystallization. Yield: 0.52 g (87%). Mp: 266–269 °C. Mp Lit. (Sena et al., 2003): 269.7–270.4 °C. ^{1} H NMR (300 MHz, DMSO-d_6): δ 8.44 (s, 2 H, *H-3′*, *H-5′*), 7.84 (d, 2 H, *J* = 7.5 Hz, Ar*H*), 7.74 (d, 2 H, *J* = 7.5 Hz, Ar*H*). ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.9, 141.2, 135.5, 133.8, 132.9, 128.9, 119.4, and 118.7.

2.1.1.3. 2-(4-Methylpirimidin-2-yl)isoindolin-1,3-dione ***(4)***. Reaction temperature: 160 °C, reaction time: 4:00 min. A white solid was obtained after recrystallization. Yield: 0.13 g (22%). Mp: 178–180 °C. Mp Lit. (Cingolani et al., 1976): 188–190 °C. ^{1} H NMR (300 MHz, DMSO-d_6): δ 8.78 (d, 1 H, *J* = 6.0 Hz, *H-6′*), 7.99–7.97 (m, 2 H, Ar*H*), 7.84–7.80 (m, 2 H, Ar*H*, *H-5′*), 7.25 (dt, 1 H, *J* = 6.0, 0.51 Hz, Ar*H*), 2.89 (s, 3 H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 168.8, 167.6, 158.5, 135.2, 133.9, 132.9, 128.9, 126.1, and 21.1.

2.1.1.4. 2-(4-Nitrophenyl)isoindolin-1,3-dione ***(5)***. Reaction temperature: 160 °C, reaction time: 2:00 min. A yellow solid was obtained after recrystallization. Yield 0.54 g (82%). Mp: 273–275 °C. Mp. Lit. (Aliabadi et al., 2014): 265 °C. IR (KBr, ν cm^{-1}): 1738 (CO), 1523 and 1346 (NO_2), 837 (C—H). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.42 (d, 2 H, *J* = 8.6 Hz, Ar*H*), 8.02 (dt, 2 H, *J* = 7.6, 3.9 Hz, Ar*H*), 7.95 (dd, 2 H, *J* = 5.5, 3.1 Hz, Ar*H*), 7.81 (d, 2 H, *J* = 8.6 Hz, Ar*H*).^{13}C NMR (151 MHz, DMSO-d_6): δ 166.8, 146.6, 138.2, 135.4, 131.9, 128.2, 124.6, and 124.2.

2.1.1.5. 2-(4-Methoxy-2-nitrophenyl)isoindolin-1,3-dione ***(6)***. Reaction temperature: 160 °C, reaction time: 4:00 min. A yellow solid was obtained after recrystallization. Yield: 0.65 g (88%). Mp: 132–134 °C. Mp Lit. (Davood et al., 2013): 148–153 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.03 (dd, 2 H, *J* = 5.5, 3.0 Hz, Ar*H*), 7.97 (td, 2 H, *J* = 5.2, 2.1 Hz, Ar*H*), 7.76 (d, 1 H, *J* = 2.9 Hz, Ar*H*), 7.70 (d, 1 H, *J* = 8.8 Hz, Ar*H*), 7.53 (dd, 1 H, *J* = 8.8, 2.9 Hz, Ar*H*), 3.94 (s, 3 H, OCH_3). ^{13}C NMR (151 MHz, DMSO-d_6): δ 166.9, 160.2, 146.8, 135.7, 132.8, 131.8, 124.3, 120.8, 117.5, 110.9, and 56.8.

2.1.1.6. 2-(Pyridin-2-yl)isoindolin-1,3-dione ***(7)***. Reaction temperature: 170 °C, Reaction time: 4:00 min. A white solid was obtained after recrystallization. Yield 0.49 g (88%). Mp: 227–230 °C. Mp Lit. (Guirado et al., 1997): 225–226 °C. IR (KBr, ν cm^{-1}): 1713 (CO), 1586 and 1571 (CC and CN), 717 (C—H).^{1}H NMR (600 MHz, DMSO-d_6): δ 8.66 (dd, 1 H, *J* = 7.1, 3.1 Hz, Ar*H*), 8.06 (t, 1 H, *J* = 7.0 Hz, Ar*H*), 8.01 (dd, 2 H, *J* = 7.1, 3.1 Hz, Ar*H*), 7.95 (dd, 2 H, *J* = 7.3, 3.0 Hz, Ar*H*), 7.58–7.53 (m, 2 H, Ar*H*). ^{13}C NMR (151 MHz, DMSO-d_6): δ 166.9, 149.8, 146.3, 139.1, 135.4, 131.8, 124.5, 124.1, and 123.5.