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SYNTHESIS OF 3-ALKYLIDENE-ISOINDOLINONES VIA SULPHIDE CONTRACTION

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Abstract- Isoindolinones are essential moieties in numerous natural products, chemical research tools and therapeutics. Syntheses of such isoindoline containing agents may occur through precursors, particularly via 3-alkylidene-isoindolinones. An alternate approach be can achieved via sulphide contraction, described herein. As an example, phthalimide was thionated with Lawesson's reagent to give monothiophthalimide (**4**), which on stirring with α-bromoketones in the presence of base led to the Eschenmoser coupling reaction. In this manner, compounds (**5**, **6a** and **6b**) were formed by alkylation of monothiophthalimide with various α-bromoketones, followed by elimination of sulfur. For the compounds (**6a** and **6b**), (*Z*)-3-alkylidene-isoindolinones were generated as a single product and no (*E*)-isomer was separated from the reaction mixture.

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The isoindolinone (**1**) moiety is an integral part of several naturally occurring products, as exemplified by magallanesine (**2**), the first known isoindolobenzazocine alkaloid isolated from *Berberis darwinii* Hook (Darwin's Barberry).[1] In this regard, numerous compounds containing an isoindolinone moiety are of biological importance, possessing anxiolytic, antiviral, antileukemic, anti-inflammatory, antipsychotic and antiulcer pharmacological activity. [2, 3]

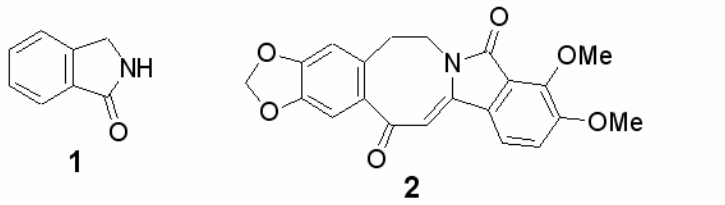
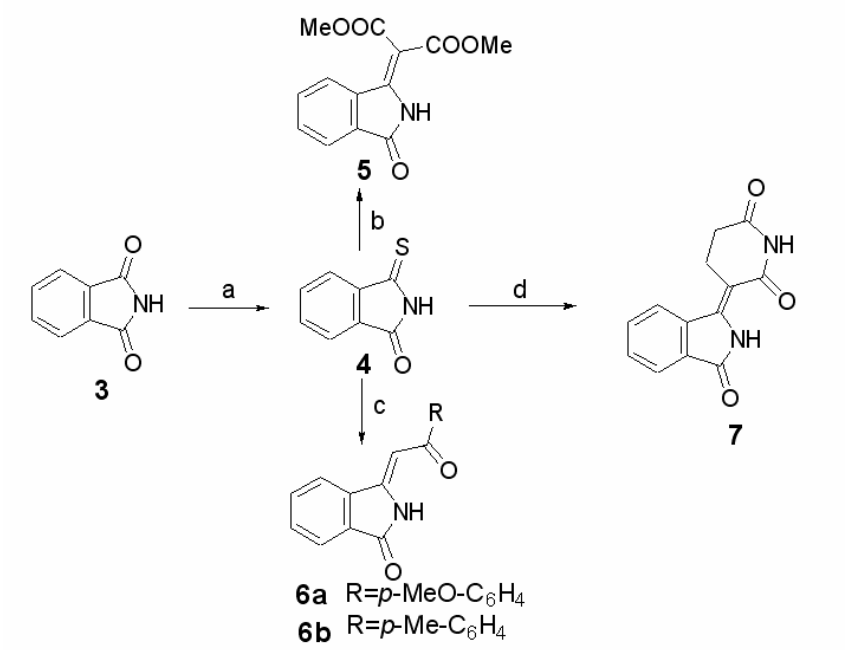


Figure 1. Structures of compounds (**1** and **2**)

The isolable natural products as well as pharmacological action of these 3-substituted isoindolinone derivatives have generated significant synthetic interest. This is particularly true for 3-alkylidene isoindolinones, as simple analogues of these are suitable as precursors in the total synthesis of natural products as well as compounds with important biological properties.



Scheme 1: (a) LR, PhMe; (b) $BrCH(COOMe)_2$, K_2CO_3, 81%; (c) $BrCH_2COAr$, K_2CO_3, 89% for **6a** and 62% for **6b**; (d) α-bromoglutarimide, K_2CO_3, 58%.

Several methods for the synthesis of the 3-alkylidene isoindolinones have been reported. These include, (i) the palladium catalyzed reaction of 2-iodobenzamides with trimethylsilylacetylene followed by Friedel-Crafts reaction with acid chlorides;[4] (ii) the preparation from *N*-acyl-2-bromobenzamides that

have undergone metal-halogen exchange with n-BuLi to form *N*-acyl-2-lithiobenzamides and then cyclized,[5] and finally, (iii), Meyer-Schuster rearrangement of ω-alkynyl-ω-carbinol lactams.[6] Due to the limitations of these methods, in this paper we report an alternate approach to the synthesis of 3-alkylidene isoindolinone via sulphide contraction.

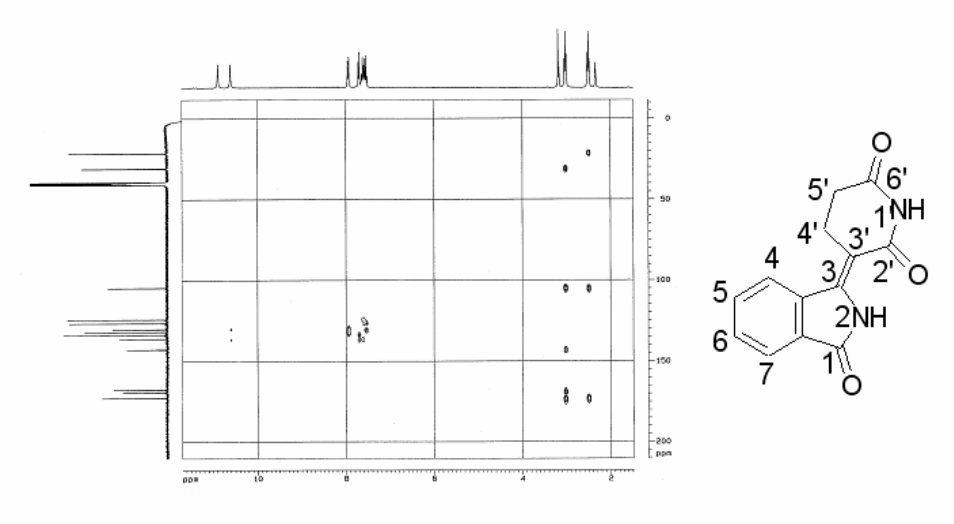


Figure 2: HMBC spectrum of compound (**7**)

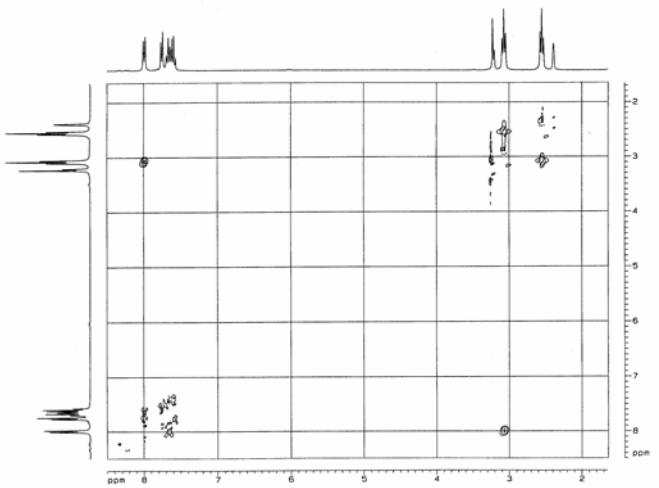


Figure 3: NOESY spectrum of compound (**7**)

As depicted in Scheme 1, phthalimide was thionated with Lawesson's reagent (LR) to give monothiophthalimide (**4**) as a major product[7]. Compound (**4**) was stirred with α-bromoketones in the presence of base to lead to the Eschenmoser coupling reaction. Thus, compounds (**5**, **6a** and **6b**) were formed by alkylation of monothiophthalimide with various α-bromoketones, followed by elimination of sulfur. For the compounds (**6a** and **6b**), (*Z*)-3-alkylidene isoindolinones were generated as a single product and no (*E*)-isomer was separated from the reaction mixture,[8] as identified by NOESY spectrum. In light of the success of this synthetic scheme, we applied this methodology in the synthesis of compound (**7**), which has a novel heterocycle system as well as a similar structure to thalidomide.

In this regard, monothiophthalimide was refluxed with 3-bromoglutarimide in THF in the presence of Na_2CO_3 to afford compound **7** in the yield of 58%. Its structure was then identified by 1D and 2D NMR. The chemical shifts were assigned by ^{1}H NMR, ^{13}C NMR, DEPT, COSY and HMBC (Figure 2). ^{1}HNMR (DMSO-d_6): 11.05 (s, 1 H, H-1'), 10.29 (s, 1 H, H-2), 8.13 (d, 1 H, H-4), 7.89 (d, 1 H, H-7), 7.80 (m, 1 H, H-6), 7.73 (m, 1 H, H-5), 3.20 (t, 2 H, H-4'), 2.67 (t, 2 H, H-5'). ^{13}C NMR (DMSO-d_6): 172.6 (C-6'), 169.0 (C-2'), 167.3 (C-1), 142.7 (C-3), 136.1 (H-7a), 134.3 (C-6), 131.7 (C-5), 130.1 (C-4a), 126.4 (C-4), 124.1 (C-7), 104.6 (C-3'), 21.2 (C-5'), 11.7 (C-4'). The Z-geometry about the alkene was established from the NOESY cross peak of H-4/H-4' (Figure 3).

Thalidomide (*N*-α-phthalimidoglutarimide), originally developed as a sedative hypnotic that was later withdrawn from clinical use due to tertatogenicity,[9] comprises of phthalimide and glutarimide moieties. From a pharmacological perspective, the agent is being increasingly used in the clinical management of a wide spectrum of immunologically-mediated and infectious diseases due to its inhibitory actions on TNF-α.[10-12] Compound (**7**), likewise, contains phthalimidine and glutarimide moieties, which are connected by a double bond; and hence was assessed for effects on TNF-α alongside thalidomide. Specifically, TNF-α levels were quantified in lipopolysacharide (LPS) stimulated peripheral blood mononuclear cells (PBMC) in cell culture in the presence and absence of increasing concentrations of compound (**7**) and thalidomide. TNF-α levels were measured by ELISA assay and cell viability was quantified to differentiate reductions in TNF-α secretion from that associated with toxicity. Interestingly, compound (**7**) proved to be more potent than thalidomide in the inhibition of LPS-induced TNF-α production in PMBCs. Whereas thalidomide totally lacked activity at 30 μM, requiring a concentration of 100 μM for significant activity, compound (**7**) possessed 37% inhibition at 30 μM.

As the *in vitro* action of thalidomide to lower TNF-α levels is not particularly potent, with an IC_{50} (concentration required to induce a 50% reduction) value of 200 μM,[12] compound (**7**) may therefore represent an interesting lead for additional medicinal chemistry to further augments its TNF-α inhibitory action, potentially by thionation, as described herein. From a clinical perspective, TNF-α is a validated therapeutic target to alleviate the symptoms of rheumatoid arthritis and Crohn's disease, as exemplified by the drugs, Remicade (Centocor, Malvern, PA/Schering-Plough, Orange, NJ) and Enbril (Amgen, Thousand Oaks, CA/Wyeth, Princeton, NJ), which, unlike the small molecules compound (**7**) and thalidomide, are injectable proteins.

EXPERIMENTAL

Melting points were determined with a Fisher-Johns apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker AC-300 spectrometer. Mass spectra and high resolution mass spectra (HRMS) were recorded on VG 7070 mass spectrometer and Finnigan-1015D mass spectrometer. All

exact mass measurements show an error of less than 5 ppm. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

Dimethyl 2-(3-oxo-2,3-dihydroisoindol-1-ylidene)-propandionate (5). The mixture of thiophthalimide (20 mg, 0.12 mmol), dimethyl bromomalonate (25 mg, 0.12 mmol), and potassium carbonate (150 mg) in anhydrous THF (4 mL) was stirred at 60°C in an oil bath for 4 h. TLC showed that the starting materials had disappeared and ethyl acetate (20 mL) and water (10 mL) then were added. The organic layer thereafter was separated, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by chromatography on silica gel with CH_2Cl_2, and then with CH_2Cl_2/EtOAc (10:1) to give the desired product (25 mg, 81%) as white crystals: mp 137°C; ^{1}H NMR ($CHCl_3$) δ 10.03 (s, 1H), 7.90-7.87 (m, 1H), 7.63-7.7.60 (m, 2H), 7.54-7.24 (m, 1H), 3.96 (s, 3H, 3.85 (s, 3H); MS (DEI) m/z 261 $[M]^+$; HRMS (DEI) m/z calcd for $C_{13}H_{11}NO_5$ 261.0637, found 261.0641. Anal. Calcd for $C_{13}H_{11}NO_5$: C, 59.77; H, 4.24; N, 5.36. Found: C, 59.81; H, 4.22; N, 5.21.

3-[2-(4-Methoxyphenyl)-2-oxo-ethylidene]-2,3-dihydro-isoindol-1-one (6a). The mixture of thiophthalimide (15 mg, 0.092 mmol), 2-bromo-4-methoxyacetophenone (24 mg, 0.105 mmol), and potassium carbonate (150 mg) in THF/CH_3OH (5:1, 6 mL) was stirred at 80°C in an oil bath for 2 h. TLC showed that the starting materials had disappeared and ethyl acetate (20 mL) and water (10 mL) were added. The organic layer was separated, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by chromatography on silica gel with CH_2Cl_2 and then CH_2Cl_2/EtOAc (10:1) to give the desired product (23 mg, 89%) as a yellow solid: mp 165°C (EtOAc/Et_2O); ^{1}H NMR ($CHCl_3$) δ 10.62 (s, 1H), 8.03 (d, J = 8.9 Hz, 2H), 7.91-7.81 (m, 2H), 7.68-7.63 (m, 2H), 6.98 (d, J = 8.9 Hz, 2H), 6.85 (s, 1H), 3.90 (s, 3H). MS (DEI) m/z 279 $[M]^+$; HRMS (DEI) m/z calcd for $C_{17}H_{13}NO_3$ 279.0895, found 279.0897.

3-(2-(4-Tolyl)-2-oxo-ethylidene]-2,3-dihydroisoindol-1-one (6b). The mixture of thiophthalimide (16 mg, 0.1 mmol), 2-bromo-4-methylacetophenone (21 mg, 0.1 mmol), and potassium carbonate (200 mg) in THF (10 mL) was refluxed for 10 h. TLC showed that the starting materials had disappeared and ethyl acetate (20 mL) and water (10 mL) then were added. The organic layer was separated, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by chromatography on silica gel with CH_2Cl_2 to give the desired product (16 mg, 62%) as purple crystals: mp 194°C; ^{1}H NMR ($CHCl_3$) δ 7.95-7.81 (m, 4H), 7.67-7.62 (m, 2H), 7.32-7.29 (m, 2H), 6.86 (s, 1H), 2.43 (s, 3H). MS (DEI) m/z 263 $[M]^+$; HRMS (DEI) m/z calcd for $C_{17}H_{13}NO_2$ 263.0946, found 263.0954.

3-(3-Oxo-2,3-dihydroisoindol-1-ylidene)-piperidine-2,6-dione (7). The mixture of monothiophthalimide (16 mg, 0.1 mmol), α-bromoglutarimide (19 mg, 0.1 mmol), and potassium carbonate (100 mg) in anhydrous THF was refluxed for 7 h. TLC showed that the starting materials had

disappeared and ethyl acetate (20 mL) and water (10 mL) then were added. Subsequently, the organic layer was separated, dried over Na_2SO_4 and concentrated under vacuum. The residue then was purified by chromatography on silica gel with petroleum ether/ethyl acetate (first 2:1, and then 1:2) to give **7** (14 mg, 58%) as yellow crystals: mp 295°C; ^{1}HNMR (DMSO-d_6): 11.05 (s, 1 H), 10.29 (s, 1 H), 8.13 (d, J = 7.6 Hz, 1 H), 7.89 (d, J = 7.2 Hz, 1 H), 7.80 (m, 1 H), 7.73 (m, 1 H), 3.20 (t, J = 7.0 Hz, 2 H), 2.67 (t, J = 7.0 Hz, 2 H). ^{13}C NMR (DMSO-d_6): 172.6, 169.0, 167.3, 142.7, 136.1, 134.3, 131.7, 130.1, 126.4, 124.1, 104.6, 21.2, 11.7. MS (DEI) m/z 242 $[M]^+$; HRMS (DEI) m/z calcd for $C_{13}H_{10}N_2O_3$ 242.0691, found 242.0687.

Cell Culture Studies: Freshly prepared PBMCs were utilized. Explicitly, a blood sample, 40 mL, was drawn from a volunteer, immediately mixed with sodium heparin (50 U/mLl) and then was diluted to a total volume of 50 mL with sterile physiological buffered saline (PBS). Samples (20 mL) of this preparation were then layered onto an equal volume of Ficoll-Paque and were centrifuged (800g, 20 min, 4^{o}C). The Ficoll/plasma interface, containing the PBMCs, was then collected, diluted to 200 ml with PBS, and was centrifuged (800g, 15 min, 4^{o}C) to pellet the cells. Next, the recovered pellet was re-suspended in 37^{o}C tissue culture medium (RPMI/1 mM sodium pyruvate/10% heat inactivated FBS/2mM Glutamax) and was placed on ice. Finally, recovered cells were counted, pipetted (1 x 10^5 cells in 200 μL) into 96 well plates, and incubated for 60 min (37^{0}C, 5% CO_2). Thereafter, appropriate concentrations of compound (**7)**, thalidomide or vehicle (10 μL DMSO) were added to duplicate wells. Following an additional 60 min of incubation, a 10 μL sample of lipopolysaccharide (LPS) (100 ng/mL in supplemented medium) or vehicle was added to induce stimulated and unstimulated cells, respectively, which were then incubated for 16 h. Supernatants, thereafter, were collected for TNF-α measurement by ELISA assay (Pierce-Endogen human TNF-α mini kit, Rockford, IL) using the monoclonal antibodies, M303E and M302B (Pierce-Endogen), for capture and detection, respectively. Finally, ELISA plates were read at 450 nm λ and TNF-α levels were determined from a calibration curve of six-points that was run concurrently with the experimental samples. The effect of compound (**7)** and thalidomide on PBMC viability was determined by MTS assay (Promega, Madison, WI) utilizing the cells from which TNF-α levels in supernatant samples were measured, as described.

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