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# Noble 2-[3-(Cyclopentyloxy)-4-Methoxyphenyl]-1-Isoindolinone Derivatives. Part I: Synthesis and SAR Studies for the Inhibition of TNF-α Production

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This study describes the synthesis and *in vitro* evaluation of noble 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone derivatives for the inhibition of TNF-α production. Among these compounds, 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-3-methyl-1-isoindolinone (**5**) was the most potent in inhibitory activity of TNF-α production in LPS-stimulated RAW264.7 cells.

**Key words:** 2-[3-(Cyclopentyloxy)-4-methoxyphenyl]-3-methyl-1-isoindolinone, TNF-α inhibitor, Structure-activity relationship, RAW264.7 cells

## INTRODUCTION

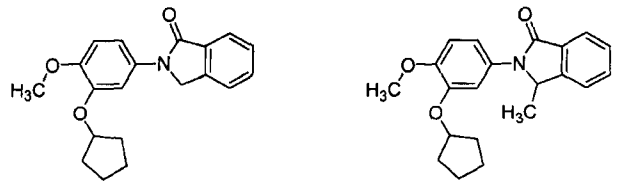
Tumor necrosis factor-α (TNF-α) is a key cytokine in the inflammatory cascade. Excessive TNF-α levels have been found to be associated with a number of inflammatory and autoimmune conditions including rheumatoid arthritis, Crohn's disease, aphthous ulcers, erythema nodosum leprosum in leprosy, septic, cachexia, graft versus host disease, asthma, ARDS, and AIDS (Eigler *et al.*, 1997). Thus, control of TNF-α levels could be a key to the treatment of a wide range of diseases. The validity of this approach has recently been demonstrated by the clinical benefit observed in the treatment of rheumatoid arthritis and Crohns disease by TNF-α antibodies (Infliximab) and TNF-α soluble receptors (Etanercept), which approved by FDA (Harriman *et al.*, 1999; Garrison and McDonnell, 1999). Hence, the effort to develop the more potent and safe TNF-α production inhibitor is progressing in a lot of laboratory (Newton and Decicco, 1999).

In our previous report (Park *et al.*, 2000a,b), 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone (**DWP 205190**) was selected as a lead compound for inhibitory activity of TNF-α production.

As our continuous effort to develop more potent inhibitor of TNF-α production, we accomplished synthesis and structure-activity relationships of derivatives on 3-C position of 1-isoindolinone moiety (Fig. 1).

## MATERIALS AND METHODS

Unless otherwise noted, materials were obtained from commercial suppliers and were without purification. All reactions requiring anhydrous conditions were performed in oven-dried glassware under $N_2$ atmosphere. Tetrahydrofuran (THF) was distilled from sodium-benzophenon immediately prior to use. Thin layer chromatography (TLC) was carried out using E. Merck Silica Gel 60 precoated plates. Products were purified by open column chromatography on Merck 60 (230-400 mesh) silica gel. Melting points were determined by the capillary method on electrothermal IA9200 digital melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) data for $^1$H-NMR were taken on Bruker AMX 300 and are reported in (ppm) downfield from tetramethylsilane (TMS).




**DWP205190** **5**

**Fig. 1.** Structure of TNF-α production inhibitory compounds

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**2-[3-(Cyclopentyloxy)-4-methoxyphenyl]-1,3-isoindolindione (3)**

In briefly (Park *et al.*, 2000b), cyclopentylation of 2-methoxy-5-nitrophenol and then reduction with Pd-C was to give the 3-cyclopentyloxy-4-methoxyaniline (**1**, 77%). The coupling of the aniline (**1**) and phthalic anhydride (**2**) was to afford the title compound (**3**, 91%).

**3-Methyl-3-hydroxy-2-(3-cyclopentyloxy-4-methoxyphenyl)-1-isoindolinone (4)**

To a solution of 2-(3-cyclopentyloxy-4-methoxyphenyl)-1,3-isoindolindione (**3**, 0.5 g, 1.48 mmol) in anhydrous THF (10 ml) was slowly added 3.0 M methylmagnesium bromide (0.2 ml, 4.44 mmol) at 0. The reaction mixture was warmed to room temperature (rt), stirred for 20 minutes, quenched with saturated ammonium chloride solution, and diluted with ethyl acetate. The organic layer was washed with distilled water, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. To the residue was added ether, stirred at rt, and filtered to give the title compound (**4**, 0.35 g) as a white solid.

**3-Methyl-2-(3-cyclopentyloxy-4-methoxyphenyl)-1-isoindolinone (5)**

To a solution of 3-methyl-3-hydroxy-2-(3-cyclopentyloxy-4-methoxyphenyl)-1-isoindolinone (**4**, 0.52 g, 1.48 mmol) in dichloromethane (10 ml) were added triethylsilane (1.29 ml, 1.78 mmol) and trifluoroacetic acid (0.14 ml, 1.78 mmol). The reaction mixture was stirred for 4 h at rt, evaporated *in vacuo*, and then diluted with ethyl acetate. The organic layer was washed with distilled water, dried over $MgSO_4$, filtered, concentrated *in vacuo*, and recrystallized from ether to give the title compound (**5**, 0.46 g) as a white solid.

**3-Methylene-2-(3-cyclopentyloxy-4-methoxyphenyl)-1-isoindolinone (6)**

To a solution of 3-methyl-3-hydroxy-2-(3-cyclopentyloxy-4-methoxyphenyl)-1-isoindolinone (**4**, 0.52 g, 1.48 mmol) in benzene (10 ml) was added *p*-toluenesulfonic acid (0.28 g, 1.48 mmol). The reaction mixture was stirred for 4 h at rt, diluted with ethyl acetate. The organic layer was washed with distilled water, dried over $MgSO_4$, filtered, and evaporated *in vacuo* to remove solvent. The residue was purified by flash chromatography (EtOAc -Hexane=1:2) to afford the title compound (**6**, 0.52 g) as a white solid.

**3-Hydroxy-2-(3-cyclopentyloxy-4-methoxyphenyl)-1-isoindolinone (10)**

To a solution of 2-(3-cyclopentyloxy-4-methoxyphenyl)-1,3-isoindolindione (**3**, 0.5 g, 1.48 mmol) in methanol (10 ml) was added sodium borohydride (0.06 g, 1.48 mmol) at 0°C. The reaction mixture was treated with ice-water, extracted with ethyl acetate, dried over $MgSO_4$, filtered, concentrated *in vacuo*, and recrystallized from ether to give the title compound (**10**, 0.48 g) as a white solid.

**3-Methoxy-2-(3-cyclopentyloxy-4-methoxyphenyl)-1-isoindolinone (11)**

To a solution of 3-hydroxy-2-(3-cyclopentyloxy-4-methoxyphenyl)-1-isoindolinone (**10**, 0.5 g, 1.48 mmol) in DMF (10 ml) were added potassium carbonate (0.4 g, 2.96 mmol) and iodomethane (0.42 g, 2.96 mmol). The reaction mixture was stirred for 12 h at 60°C, cooled to rt, added distilled water (10 ml), and extracted twice with ether. The organic layer was dried over $MgSO_4$, filtered, concentrated *in vacuo*, and recrystallized from ether to give the title compound (**11**, 0.48 g) as a white solid.

**3-Azido-2-(3-cyclopentyloxy-4-methoxyphenyl)-1-isoindolinone (14)**

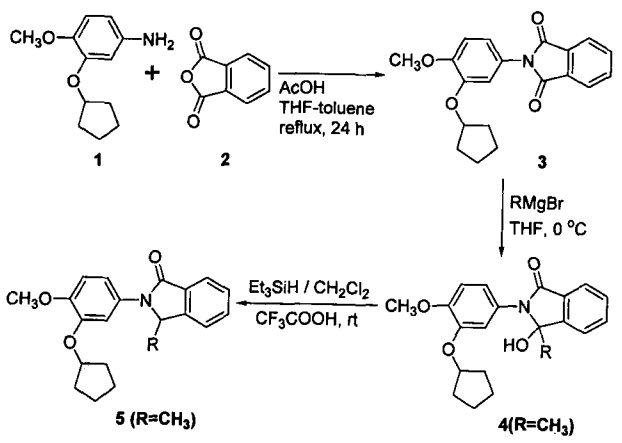
To a solution of 3-hydroxy-2-(3-cyclopentyloxy-4-methoxyphenyl)-1-isoindolinone (**10**, 0.5 g, 1.48 mmol) in anhydrous toluene (10 ml) were added diphenylphosphorylazide (0.49 g, 1.78 mmol) and 1,8-diazabicyclo [5,4,0] undec-7-ene (0.27 g, 1.78 mmol). The reaction mixture was stirred for 4 h at rt, added ethyl acetate, washed twice with distilled water, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (EtOAc-Hexane=1:1) to afford the title compound (**14**, 0.5 g) as a white solid.

**3-Amino-2-(3-cyclopentyloxy-4-methoxyphenyl)-1-isoindolinone (15)**

The title compound was prepared following the procedures described in synthesis of 3-cyclopentyloxy-4-methoxyaniline (**1**) with 3-azido-2-(3-cyclopentyloxy-4-methoxyphenyl)-1-isoindolinone (0.54 g, 1.48 mmol) as a white solid (0.47 g, 94%).

**Chemistry**

3-Substituted-2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone derivatives were synthesized by the procedure shown in scheme 1. Representatively, 2-cyclopentyloxy-1-methoxy-4-nitro-benzene, prepared from 2-methoxy-5-nitrophenol and cyclopentyl bromide, was reduced to 3-(cyclopentyloxy)-4-methoxy aniline (**1**) with palladium charcoal and ammonium formate, and then treated with phthalic anhydride to give a 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1,3-isoindolindione (**3**) (Park *et al.*, 2000b). Grignard reaction of this product with alkyl magnesium bromide in THF afforded the 2-(3-cyclopentyloxy-4-methoxy-phenyl)-3-hydroxy-3-methyl-2,3-di-




**Scheme 1.** Synthesis of 2-[3-(cyclopentyloxy)-4-methoxyphenyl] -3-methyl-1-isoindolinone (**5**)

hydro-1-isoindolinone (**4**). Finally, dehydroxylation of the compound **4** by using triethylsilane and trifluoroacetic acid was given to 2-(3-cyclopentyloxy-4-methoxy-phenyl)-3-methyl-2,3-dihydro-1-isoindolinone (**5**). Some chiral compounds were not separated and the mixtures were assayed.

### TNF-α *in vitro* assay

After cancer cell line of mouse macrophage (RAW 264.7) is diluted with RPMI 1640 medium (containing 10% FBS), then plated out in 24 well plates at $1 \times 10^6$ cells/ml. Then, the culture is incubated for 18 h at 5% $CO_2$ and 37°C. 1 μ M of compound and 1 μg/ml of lipopoly-saccharide (LPS) are added to the plate and the culture is incubated for 6 hours at 37°C. After incubated, the culture is centrifuged and supernatants are collected. The supernatants are stored at -20°C till measurement. The measurement of TNF-α in the media is performed with a mouse TNF-α kit (Amersham, UK). And the procedure is in accordance with the guidance provided by Amersham. Inhibition percentage of each compound is calculated by comparison of amount of TNF-α, released in the well treated with compound, with that in the well without any treatment.

## RESULTS AND DISCUSSION

Compounds prepared in this study were tested for their ability to inhibit TNF-α production in LPS-stimulated RAW264.7 cells. TNF-α production inhibitory data for these compounds are summarized in Table I.

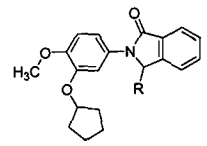
Initially, the hydroxy-methyl intermediate **4** was deprived the activity to inhibit TNF-α production. Thus, the derivatives of this intermediate were not studied.

**Table I.** Physical and spectral data of compounds **3-15**

| No. | mp (°C) | Yield* (%) | $^1$H-NMR($CDCl_3$, δ=ppm) |
|---|---|---|---|
| **4** | 136-137 | 67 | 1.15-1.91(m, 8H), 1.56(s, 3H), 3.86(s, 3H), 4.43(s, 1H), 4.58(m, 1H), 6.75(d, *J*=8.7Hz, 1H), 6.90(dd, *J*=8.7, 2.4Hz, 1H), 6.99(d, *J*=2.4Hz, 1H), 7.28-7.56(m, 4H) |
| **5** | 98-100 | 63 | 1.49(d, *J*=6.3Hz, 3H), 1.64-2.05(m, 8H), 3.92(s, 3H), 4.87(m, 1H), 5.15(q, *J*=6.3Hz, 1H), 6.97(s, 2H), 7.34(s, 1H), 7.56-7.62(m, 3H), 7.96(d, *J*=7.5Hz, 1H) |
| **6** | 116-117 | 61 | 1.64-2.05(m, 8H), 3.91(s, 3H), 4.78(m, 1H), 4.82(d, *J*=1.9Hz, 1H), 5.24(d, *J*=1.9Hz, 1H), 6.88-7.00(m, 3H), 7.57-7.66(m, 2H), 7.77(d, *J*=7.5Hz, 1H), 7.94(d, *J*=7.5Hz, 1H) |
| **7** | 94-96 | 62 | 0.51(t, *J*=7.3Hz, 3H), 1.62-2.05(m, 10H), 3.87(s, 3H), 4.83(m, 1H), 5.19(t, *J*=4.1Hz, 1H), 6.93(s, 2H), 7.30(s, 1H), 7.46-7.62(m, 3H), 7.92(d, *J*=7.5Hz, 1H) |
| **8** | 92-94 | 59 | 0.46(d, *J*=6.8Hz, 3H), 1.21(d, *J*=7.1Hz, 3H), 1.62-2.06(m, 8H), 2.31(m, 1H), 3.89(s, 3H), 4.82(m, 1H), 5.19(d, *J*=3.1Hz, 1H), 6.95(s, 2H), 7.28(s, 1H), 7.52-7.58(m, 3H), 7.95(d, *J*=7.5Hz, 1H) |
| **9** | 146-148 | 56 | 1.58-2.00(m, 8H), 3.81(s, 3H), 4.68(m, 1H), 6.00(s, 1H), 6.79(d, *J*=8.5Hz, 1H), 6.97(dd, *J*=8.5, 2.1Hz, 1H), 7.20-7.32(m, 7H), 7.52(m, 2H), 7.98(d, *J*=2.1Hz, 1H) |
| **10** | 123-125 | 96 | 1.60-2.00(m, 8H), 3.84(s, 3H), 4.76(m, 1H), 6.30(s, 1H), 6.87(d, *J*=8.5Hz, 1H), 7.43(d, *J*=2.1Hz, 1H), 7.50-7.80(m, 4H) |
| **11** | 109-111 | 92 | 1.60-2.05(m, 8H), 2.97(s, 3H), 3.89(s, 3H), 4.86(m, 1H), 6.39(s, 1H), 6.94(d, *J*=8.5Hz, 1 H), 7.24(dd, *J*=8.5, 2.1Hz, 1H), 7.54(d, *J*=2.1Hz, 1H), 7.55-7.95(m, 4H) |
| **12** | 105-106 | 87 | 0.95(d, *J*=6.1Hz, 3H), 1.01(d, *J*=6.1Hz, 3H), 1.60-2.05(m, 8H), 3.59(m, 1H), 3.89(s, 3H), 4.85(m, 1H), 6.37(s, 1H), 6.93(d, *J*=8.7Hz, 1H), 7.20(dd, *J*=8.5, 2.1Hz, 1H), 7.47(d, *J*=2.4Hz, 1H), 7.57-7.92(m, 4H) |
| **13** | 98-100 | 82 | 1.62-2.03(m, 8H), 2.09(s, 3H), 3.88(s, 3H), 4.82(m, 1H), 6.92(d, *J*=8.5Hz, 1H), 7.00(dd, *J*=8.5, 2.1Hz, 1H), 7.25(d, *J*=2.1Hz, 1H), 7.28(s, 1H), 7.61-7.93(m, 4H) |
| **14** | 86-88 | 81 | 1.60-2.05(m, 8H), 3.90(s, 3H), 4.80(m, 1H), 6.00(s, 1H), 6.96(d, *J*=8.5Hz, 1H), 7.08(dd, *J*=8.5, 2.1Hz, 1H), 7.38(d, *J*=2.1Hz, 1H), 7.60-7.97(m, 4H) |
| **15** | 125-127 | 76 | 1.60-2.05(m, 8H), 3.89(s, 3H), 4.84(m, 1H), 5.87(s, 1H), 6.88(d, *J*=8.5Hz, 1H), 6.96(dd, *J*=8.5, 2.1Hz, 1H), 7.31(d, *J*=2.1Hz, 1H), 7.55-7.92(m, 4H) |

*indicates the total isolated yield from reaction with the compound **3**.

**Table II.** Inhibitory activity on TNF-α production in LPS-stimulated RAW264.7 cells



| Compound | R | TNF-α $IC_{50}$ [μM] |
|---|---|---|
| DWP205190 | H | 0.14 ± 0.023 |
| 3 | oxo | 0.68 ± 0.072 |
| 4 | OH, $CH_3$ | 10.2 ± 1.89 |
| 5 | $CH_3$ | 0.095 ± 0.015 |
| 6 | methenyl | 10.7 ± 2.24 |
| 7 | ethyl | 45.9 ± 6.8%[a] |
| 8 | isopropyl | 26.1 ± 2.65 |
| 9 | phenyl | 356 ± 26.5 |
| 10 | OH | 34.5 ± 4.2% |
| 11 | $OCH_3$ | 0.68 ± 0.25 |
| 12 | $OCH(CH_3)_2$ | 28.9 ± 4.58 |
| 13 | $OCOCH_3$ | 20.0 ± 3.21 |
| 14 | $N_3$ | 3.25 ± 0.43 |
| 15 | $NH_2$ | 31.4 ± 10.1% |

The RAW264.7 ($1 \times 10^6$ cells/ml) cells stimulated with 1 μg/ml of LPS produced about 65 ng/ml of TNF-α and contained 0.5 ng/ml to 1 ng/ml of TNF-α as a basal level (Cho *et al.*, 1998). Assays were performed in triplicate at three to four different concentrations, the mean of the determinations at each concentration was plotted, and the $IC_{50}$ values were determined graphically. $IC_{50}$ values presented are from representative experiments.
[a]indicates percent inhibitory activity at 1 μg/ml.

With our surprising, dehydroxylation product **5** of the intermediate **4** was more potent than the lead compound (**DWP205190**) and dioxo derivatives **3**. As second step, we synthesized the number of alkyl derivatives. The methenyl derivative **6** having same size but different geometry was highly less potent. Also, the derivatives **7**, **8**, and **9** having long and large substituent were not active. In this case, the smaller the substituent was the better for inhibiting TNF-α production.

In next step, we synthesized a variety of hydroxy- or amino-related derivatives **10-15**. Among the hydroxy derivatives, the methoxy compound **11** was the most potent and the activity was comparable with the oxo compound **3**. This result suggests importantly that the substituent having lipophilic character and small size (1 or 2 carbon length) is optimizing as a substituent on 3-C position of 1-isoindolinone moiety for inhibiting TNF-α production. The inhibitory activity of isopropoxy **12** and methyl ester compound **13** was comparable with ethyl **7** and isopropyl compound **8** and more potent than phenyl derivative **9**. This result indicates that the size character was more important than hydrophilicity. A similar result was shown in case of amino **15** and azide derivative **14**. However, the azide compound **14** was 10 times more potent than amine compound **15**. This result indicates that lipophilic substituent was more preferable for inhibiting TNF-α production.

Based on these results, we found the methyl group is a best substituent and small and lipophilic substituent can be a good substituent on 3-C position of 1-isoindolinone moiety for inhibiting TNF-α production.

## REFERENCES

Cho, J. Y., Park, J., Yoo, E. S., Yoshikawa, K., Baik, K. U., Lee, J. S., and Park, M. H. Inhibitory effect of lignans from the rhizomes of Coptis japonica var. dissecta on tumor necrosis factor-alpha production in lipopolysaccharide-stimulated RAW264.7 cells. *Arch. Pharm. Res.*, 21, 12-16 (1998).

Eigler A., Sinha B., Hartmann G., and Endres S. Taming TNF: strategies to restrain this proinflammatory cytokine. *Immunology Today*, 28, 487-492 (1997).

Garrison, L. and McDonnell, N. D. Etanercept: therapeutic use in patients with rheumatoid arthritis. *Ann. Rheum. Dis.*, 58, Suppl 1, 165-169 (1999).

Harriman, G., Harper, L. K., and Schaible, T. F. Summary of clinical trials in rheumatoid arthritis using infliximab, an anti-TNF alpha treatment. *Ann. Rheum. Dis.*,58, Suppl 1, 161-164 (1999).

Newton, R. C. and Decicco, C. P. Therapeutic potential and strategies for inhibiting tumor necrosis factor-alpha. *J. Med. Chem.*, 42, 2295-2314 (1999).

Park, J. S., Baik, K. U., Son, H. J., Cho, J. Y., Park, J., Lee, J. H., Lee, S. J., Yoo, E. S., and Park, M. H. Syntheses and biological evaluation of novel compounds for the inhibition of TNF-α production. *Kor. J. Med. Chem.*, 10, 6-9 (2000a).

Park, J. S., Baik, K. U., Son, H. J., Cho, J. Y., Park, J., Lee, J. H., Lee, S. J., Yoo, E. S., Byun, Y. S., and Park, M. H. Synthesis and structure-activity relationships of novel compounds for the inhibition of TNF-α production. *Arch. Pharm. Res.*, 23, 332-337 (2000b).