Arch Pharm Res Vol 25, No 2, 137-142, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

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

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(Received October 10, 2001)

This study describes the synthesis and *in vitro* evaluation of 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone derivatives substituted on benzene moiety of isoindoline ring for the inhibition of TNF-α production. From this study, we have found the 6-C position on isoindolinone ring is an optimal derivatization site. Among the compounds synthesized, 6-amino-2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone (**6**) was the most potent in inhibitory activity of TNF-α production in LPS-stimulated RAW264.7 cells.

Key words : 6-Amino-2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone, TNFα production inhibitor, Structure-activity relationships, RAW264.7

INTRODUCTION

Tumor necrosis factor-α (TNF-α) is an inflammatory cytokine with a multitude of biological activities linked to pathology of autoimmune diseases such as rheumatoid arthritis, Crohns disease, systemic lupus erythematosus, multiple sclerosis, septic shock, asthma, 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), TNF-α soluble receptors (Etanercept) approved by FDA (Harriman, *et al.*, 1999; Garrison and McDonnell. ,1999), and Abotts anti-TNF-α human monoclonal antibody (Breedveld, *et al.*, 2000).

Up to date, low molecular weight compounds such as rolipram, thalidomide, and adenosine derivatives have been known to inhibit the production of TNF-α (Newton and Decicco, 1999). Hence, efforts to develop more potent and safe TNF-α production inhibitors are progressing in many labs. In our previous report (Park, *et al.*, 2000a, b), 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone (**DWP205190**) was selected as a lead compound for the inhibitory activity of TNF-α production. From our continuous effort to develop more potent inhibitor of TNF-α production, we accomplished the synthesis and structure-activity relationship study of derivatives on benzene ring of 1-isoindolinone moiety (Fig. 1).

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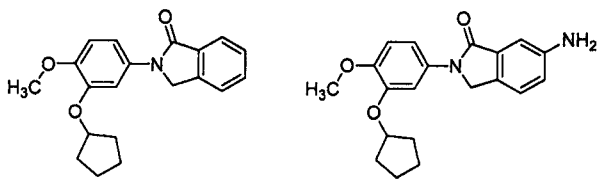
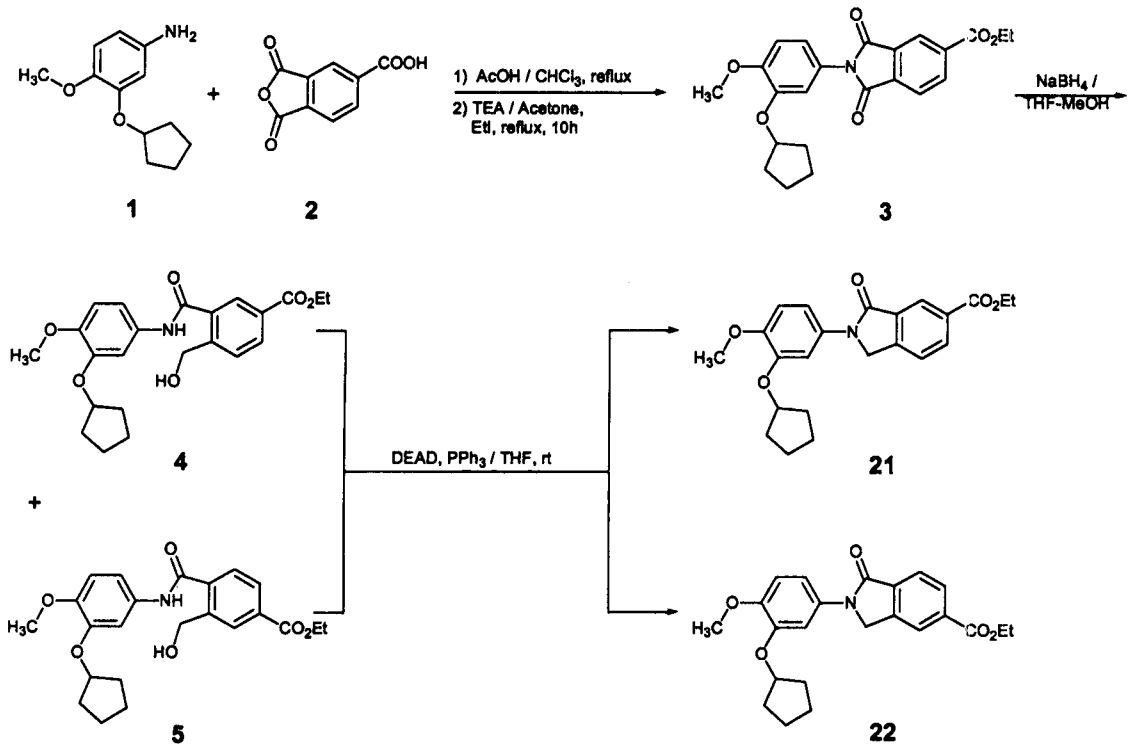
MATERIALS AND METHODS

Unless otherwise noted, materials were obtained from commercial suppliers and used 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



DWP205190 **6**

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



Scheme 1. Synthetic method for 2-(3-cyclopentyloxy-4-methoxyphenyl)-3-oxo-2,3-dihydro-1*H*-isoindole-5-carboxylic acid ethyl ester (**21**) and its isomer (**22**)

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).

2-(3-Cyclopentyloxy-4-methoxyphenyl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic acid ethyl ester (3)

To a solution of 3-cyclopentyloxy-4-methoxyaniline (**1**, 5.0 g, 24.2 mmol) which was synthesized as reported previously (Park *et al.*, 2000b) in chloroform (100 ml) was added 1,3-dioxo-1,3-dihydro-isobenzofuran-5-carboxylic acid (**2**, 4.6 g, 24.2 mmol). The reaction mixture was stirred for 0.5 h at room temperature (rt), treated with acetic acid (100 ml), refluxed for 4 h, cooled to rt, and then concentrated *in vacuo*. The residue was recrystallized from methanol to furnish 2-(3-cyclopentyloxy-4-methoxyphenyl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic acid (8.4 g, 91%) as a brown solid. To a solution of 2-(3-cyclopentyloxy-4-methoxyphenyl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic acid (4.6 g, 12.1 mmol) in anhydrous acetone (50 ml) was added triethylamine (5.0 ml, 36.3 mmol). The reaction mixture was stirred at rt for 10 min, treated with iodoethane (5.7 g, 36.3 mmol), refluxed for 10 h, cooled to rt, evaporated *in vacuo*, and treated with methanol. The resultant solid was filtered to afford the title compound (**3**, 4.7 g, 95%) as a pale yellow solid.

^{1}H-NMR ($CDCl_3$) 1.48 (t, J=7.1 Hz, 3H), 1.61-1.65 (m, 2H), 1.83-1.99 (m, 6H), 3.92 (s, 3H), 4.49 (q, J=7.1 Hz, 2H), 4.80 (m, 1H), 6.99 (m, 3H), 8.05 (d, J= .5 Hz, 1H), 8.51 (dd, J=6.4, 1.4 Hz, 1H), 8.61 (s, 1H)

N-(3-Cyclopentyloxy-4-methoxyphenyl)-4-hydroxymethyl-isophthalamic acid ethyl ester (4) and *N*-(3-Cyclopentyloxy-4-methoxyphenyl)-3-hydroxymethyl-terephthalamic acid ethyl ester (5)

To a solution of 2-(3-cyclopentyloxy-4-methoxyphenyl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-5-carboxylic acid ethyl ester (**3**, 6.1 g, 14.8 mmol) in THF (100 ml) and methanol (100 ml) was added slowly sodium borohydride (2.3 g, 59.2 mmol) at ice-water bath temperature. The reaction mixture was stirred for 1 h, quenched with saturated ammonium chloride solution, evaporated *in vacuo*, dissolved in EtOAc, and washed with water. The resultant organic layer was dried over $MgSO_4$, filtered, concentrated *in vacuo*, and then purified by column chromatography on silica (EtOAc: Hexane=1:2) to give the title compounds (**4**,

2.8 g, 45% and **5**, 2.9 g, 48%) as a white solid.

4: ^{1}H-NMR ($CDCl_3$) 1.42 (t, *J*=7.1 Hz, 3H), 1.62-1.65 (m, 2H), 1.89-2.00 (m, 6H), 3.86 (s, 3H), 4.42 (q, *J*=7.1 Hz, 2H), 4.73 (d, *J*=6.7 Hz, 2H), 4.81 (m, 1H), 6.86 (d, *J*=8.7 Hz, 1H), 7.06 (dd, *J*=6.2, 2.4 Hz, 1H), 7.41 (d, *J*=2.4 Hz, 1H), 7.52 (d, *J*=7.9 Hz, 1H), 8.14 (dd, *J*=6.2, 1.7 Hz, 1H), 8.30 (s, 1H), 8.36 (d, *J*=1.6 Hz, 1H)

5: 1.43 (t, *J*=7.1 Hz, 3H), 1.61-1.65 (m, 2H), 1.89-2.01 (m, 6H), 3.87 (s, 3H), 4.41 (q, *J*=7.1 Hz, 2H), 4.77 (d, *J*= 6.7 Hz, 2H), 4.79 (m, 1H), 6.86 (d, *J*=8.7 Hz, 1H), 7.04 (dd, *J*=6.2, 2.4 Hz, 1H), 7.45 (d, *J*=2.4 Hz, 1H), 7.81 (d, *J*= 7.9 Hz, 1H), 8.08 (m, 2H), 8.52 (br, 1H)

2-(3-Cyclopentyloxy-4-methoxyphenyl)-3-oxo-2,3-dihydro-1*H*-isoindole-5-carboxylic acid ethyl ester (21)

To a solution of *N*-(3-cyclopentyloxy-4-methoxyphenyl)-4-hydroxymethyl-isophthalamic acid ethyl ester (**4**, 2.4 g, 5.85 mmol) in anhydrous THF (50 ml) were added triphenylphosphine (1.9 g, 7.1 mmol) and diethylazodicarboxylate (1.1 ml, 7.1 mmol) at rt. The reaction mixture was stirred for 1 h at rt, and evaporated *in vacuo*. The residue was recrystalized from isopropyl alcohol-ethyl acetate to give the title compound (2.1 g, 89%) as a white solid.

6-Amino-2-(3-cyclopentyloxy-4-methoxyphenyl)-2,3-dihydro-1-isoindolinone (6)

To a solution of 2-(3-cyclopentyloxy-4-methoxyphenyl)-6-nitro-2,3-dihydro-1-isoindolinone (**25**, 0.89 g, 2.42 mmol) in methanol (10 ml) were added ammonium formate (0.48 g, 7.26 mmol) and 10% Pd-C (0.05 g). The reaction mixture was refluxed for 1 h, cooled to rt, filtered through Celite, and evaporated *in vacuo*. The residue was dissolved in ether, washed twice with water, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the title compound (0.74 g, 91%) as a white solid.

2-(3-Cyclopentyloxy-4-methoxyphenyl)-6-hydroxyamino-1- isoindolinone (14)

A solution of hydroxyamine hydrogen chloride (1.68 g, 24.2 mmol) in water (10 ml) was basified to pH 8 with 1N NaOH solution, and methanol (20 ml) was added. The mixture was treated with 2-(3-cyclopentyloxy-4-methoxyphenyl)-3-oxo-2,3-dihydro-1*H*-5-isoindolinyl]methyl methanesulfonate (1.0 g, 2.42 mmol) as described in our previous world patent (WO9842666, 1998), and refluxed for 3 h. The reaction mixture was evaporated *in vacuo*, and water was added. It was extracted three times with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the title compound (0.47 g, 52%) as a white solid.

2-(3-Cyclopentyloxy-4-methoxyphenyl)-6-methylamino-isoindolin-1-one (16)

To a solution of 6-amino-2-(3-cyclopentyloxy-4-methoxyphenyl)-isoindolin-1-one (**6**, 0.2 g, 0.59 mmol) in dichloromethane (10 ml) was added methyltrifluoromethanesulfonate (0.2 g, 1.18 mmol). The reaction mixture was stirred for 2 h at rt, and washed twice with distilled water. The organic layer was dried over $MgSO_4$, filtered, concentrated *in vacuo*, and purified by flash chromatography on silica (EtOAc: Hexane=1:1) to give the title compound (0.19 g, 42%) as a white solid.

2-(3-Cyclopentyloxy-4-methoxyphenyl)-3-oxo-5-isoindolinecarboxylic acid (19)

To a solution of 2-(3-cyclopentyloxy-4-methoxyphenyl)-3-oxo-2,3-dihydro-1*H*-isoindole-5-carboxylic acid ethyl ester (**21**, 0.96 g, 2.42 mmol) in ethanol (10 ml) was added the solution of lithium hydroxide (0.25 g, 6.05 mmol) in water (10 ml). The reaction mixture was stirred for 1 h at 80, cooled to rt, and acidified to pH 1 with concentrated HCl solution. The resultant solid was filtered to afford the title compound (0.88 g, 98%) as a white solid.

2-(3-Cyclopentyloxy-4-methoxyphenyl)-3-oxo-2,3-dihydro-1*H*-isoindole-5-carboxylic acid amide (23)

A mixture of 2-(3-cyclopentyloxy-4-methoxyphenyl)-3-oxo-2,3-dihydro-1*H*-isoindole-5-carboxylic acid ethyl ester (**21**, 0.96 g, 2.42 mmol), concentrated ammonium hydroxide solution (5 ml), and ammonium chloride (0.5 g) was stirred for 2 h at rt, filtered, washed with ethanol, and recrystalized from ethanol-ether to give the title compound (0.71 g, 80%) as a white solid.

TNF- *in vitro* assay

Cancer cell line of mouse macrophage (RAW264.7) was diluted with RPMI 1640 medium (containing 10% FBS), then plated out in 24 well plates at 1×10^6 cells/ml. Then, the culture was incubated for 18 hours at 5% CO_2 and 37°C. 1 μM of compound and 1 g/ml of lipopolysaccharide (LPS) were added to the plate and the culture was incubated for 6 h at 37. After being incubated, the culture was centrifuged and supernatants were collected. The supernatants were stored at -20°C till measurement. The measurement of TNF-α in the media was performed with a mouse TNF-α kit (Amersham, UK). And the procedure was in accordance with the guidance provided by Amersham. Inhibition percentage of each compound was calculated by comparison of the amount of TNF-α released in the well treated with compound with that in the well without any treatment.

RESULTS AND DISCUSSION

Substituted 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone derivatives were synthesized by the procedure described in our previous report (Park, *et al.*, 2000b) and the spectral and physical data for the synthetic compounds are summarized in Table II. The compounds were tested for their ability to inhibit TNF-α production in LPS-stimulated RAW264.7 cells (Cho, *et al.*, 1998a). TNF-α production inhibitory data for these compounds are summarized in Table I.

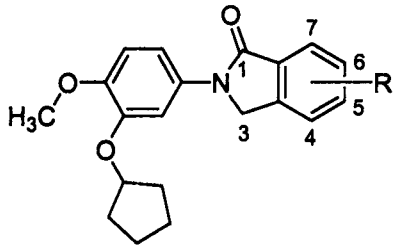
Our first step for developing more potent TNF-α production inhibitor is the searching the substituent position in benzene ring of 1-isoindolinone moiety. In compounds **6-9**, the amino substituent was hydrophilic (-π) and electron-donating (-σ) character. The best position for TNF-α inhibitory activity was C-6 whether all the compounds were held in some activity except C-7 position. Also in hydroxy compounds **10-13**, the similar result was showed which the C-7 hydroxy compound (**12**) was one hundred times less active than C-6 compound (**10**). This result suggested that the amino and hydroxy group of C-7 position might form intra-molecular hydrogen bonding and this formation would contribute to the loss of activity and also the free oxygen of 1-isoindolinone moiety was essential for retaining the activity (Panunto, *et al.*, 1987). This idea was supported by the melting point data in table III. Melting points of compound **6** and **10** which cannot form intra-molecular hydrogen bonding were much higher, that is ranging between 70°C to 110°C, than those of compound **8** and **12**. To confirm this result, we checked melting points for some intermediates of amino and hydroxy compound **6**, **8** and **12** in Table III. As suggested the above, the melting point of 7-amino and hydroxy intermediates (**31**, **32**) was lower than 6-derivatized intermediates (**30**, **33**). From these results, we concluded that 7-NH_2 and 7-OH compound could be forming intra-molecular hydrogen bonding with 1-carbonyl oxygen and this formation was contributed the decreased inhibitory activity of TNF-α production (Kubicni, *et al.*, 2000). Especially, compound **6** was three times more potent than the lead compound **DWP205190**. This high *in vitro* activity of compound **6** was comparable to that of our oxime compounds having the same dialkoxyphenyl moiety previously submitted (Cho, *et al.*, 2001). In the oxime derivatives, the *cis*-propenal oxime compound was more potent than *trans*-compound having different geometric hydrogen bonding site, that is the exact position of hydrogen bonding site is essential for inhibitory activity of TNF-α production. Thereafter we prepared another derivatives (**25-28**) having different electronic character such as nitro group. All the compound did not showed good activity. However, the activity profile was similar to that of the amino and hydroxy derivatives, indicating C-6 position is the most active whether the nitro group could not form intramolecular hydrogen bonding like amino and hydroxy group. From these results, we decided the most effective position of substitution was C-6.

Other substituent in this class (-π, -σ) was hydroxy amino (**14**) group. The hydroxy amino compound was less potent than the amino compound **6** but was more potent than the hydroxy compound **10**.

The next class of compounds has lipophilic (+π) and electron-donating (-σ) character (i.e. methyl, *N*-methylamino, *N,N*-dimethylamino). The compounds of this class (**15-18**) were somewhat less potent than the first class of compounds. Interestingly the methyl substituted compound (**15**) was comparable to the lead compound in inhibitory activity of TNF-α production. However, methylation to amino substituent decreased the activity from mono- (**16**) to di-methylation (**17**, **18**). This result suggests that small lipophilic hydrocarbon and free amino group are optimal to inhibit TNF-α production.

The third class of substituent was the hydrophilic (-π) and electron-withdrawing (+σ) (i.e. carboxylic acid, ethyl acetate,

Table I. Inhibitory effect on TNF-α production in LPS-stimulated RAW264.7 cells



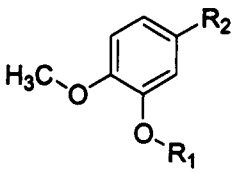
No.	R	TNF-α IC_{50}[μM]	No.	R	TNF-α IC_{50}[μM]
DWP205190	H	0.14	17	6-$N(CH_3)_2$	20.9%*
6	6-NH_2	0.047	18	5-$N(CH_3)_2$	2.5%*
7	5-NH_2	1.58	19	6-COOH	18.9%*
8	7-NH_2	< 100	20	5-COOH	12.4%*
9	4-NH_2	0.63	21	6-$CO_2C_2H_5$	27.5%*
10	6-OH	0.87	22	5-$CO_2C_2H_5$	10.7%*
11	5-OH	0.65	23	6-$CONH_2$	2.85
12	7-OH	87.5	24	6-OCH_3	30.9%*
13	4-OH	18.9	25	6-NO_2	8.26
14	6-NHOH	0.29	26	5-NO_2	13.2%*
15	6-CH_3	0.19	27	7-NO_2	12.8%*
16	6-$NHCH_3$	0.72	28	4-NO_2	25.8%*

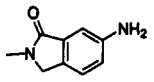
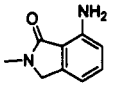
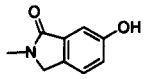
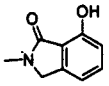
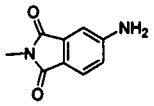
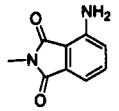
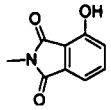
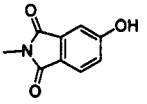
The RAW264.7 (1×10^6 cells/ml) cells stimulated with 1 μg/ml of LPS produced about 65 ng/ml of TNF-α and contained 0.5 ng/ml to 1 ng/ml of TNF-α as a basal level (Cho, *et al.*, 1998). Assays were performed in triplicate at three to four different concentrations, the mean of the determinations at each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

Table II. Physical and spectral data for synthetic compounds

No.	Mp (°C)	^{1}H-NMR ($CDCl_3$) = δppm
DWP 205190	130-132	1.61-1.64 (m, 2H), 1.86-2.07 (m, 6H), 3.88 (s, 3H), 4.84 (s, 2H), 4.88 (m, 1H), 6.91 (d, *J*=8.6 Hz, 1H), 7.03 (d, *J*=8.6 Hz, 1H), 7.52 (d, *J*=6.1 Hz, 2H), 7.59 (d, *J*=6.9 Hz, 1H), 7.92 (s, 2H)
6	228-229	1.60-1.67 (m, 2H), 1.87-2.08 (m, 6H), 3.88 (s, 3H), 4.75 (s, 2H), 4.88 (m, 1H), 6.89-6.94 (m, 2H), 7.03 (dd, *J*=6.2, 2.5 Hz, 1H), 7.18 (d, *J*=2.0 Hz, 1H), 7.30 (s, 2H), 7.90 (d, *J*=2.5 Hz, 1H)
7	232-234	1.60-1.65 (m, 2H), 1.82-2.06 (m, 6H), 3.87 (s, 3H), 4.71 (s, 2H), 4.88 (m, 1H), 6.72-6.76 (m, 2H), 6.88 (d, *J*=8.7 Hz, 1H), 6.96 (dd, *J*=6.2, 2.5 Hz, 1H), 7.68 (d, *J*=8.1 Hz, 1H), 7.92 (d, *J*=2.4 Hz, 1H)
8	110-111	1.61-1.66 (m, 2H), 1.83-2.08 (m, 6H), 3.87 (s, 3H), 4.74 (s, 2H), 4.86 (m, 1H), 4.94 (br, 2H), 6.61 (d, *J*=8.0 Hz, 1H), 6.75 (d, *J*=7.3 Hz, 1H), 6.89 (d, *J*=8.7 Hz, 1H), 7.00 (dd, *J*=6.3, 2.4 Hz, 1H), 7.31 (d, *J*=7.7 Hz, 1H), 7.77 (d, *J*=2.4 Hz, 1H)
9	119-121	1.60-1.63(m, 2H), 1.85-2.03 (m, 6H), 3.87 (s, 3H), 4.65 (s, 2H), 4.88 (m, 1H), 6.87-6.90 (m, 2H), 7.02 (dd, *J*=6.1, 2.6 Hz, 1H), 7.32-7.38 (m, 2H), 7.88 (d, *J*=2.5 Hz, 1H)
10	181-182	1.65-1.69 (m, 2H), 1.88-2.07 (m, 6H), 3.90 (s, 3H), 4.88 (m, 1H), 4.97 (s, 2H), 6.93 (d, *J*= 8.8 Hz, 1H), 7.05 (dd, *J*=6.2, 2.5 Hz, 1H), 7.72 (d, *J*=8.3 Hz, 1H), 7.83 (d, *J*=2.5 Hz, 1H), 8.50 (dd, *J*=6.1, 2.1 Hz, 1H), 8.77 (d, *J*=2.0 Hz, 1H)
11	196-197	1.64-1.67 (m, 2H), 1.84-2.06 (m, 6H), 3.89 (s, 3H), 4.85 (m, 1H), 4.89 (s, 2H), 6.92 (d, *J*=8.7 Hz, 1H), 7.05 (dd, *J*=6.1, 2.6 Hz, 1H), 7.84 (d, *J*=2.5 Hz, 1H), 8.06-8.09 (m, 1H), 8.41-8.43 (m, 2H)
12	112-113	1.65-1.69 (m, 2H), 1.85-2.05 (m, 6H), 3.88 (s, 3H), 4.87 (m, 1H), 4.95 (s, 2H), 6.91 (d, *J*=8.7 Hz, 1H), 7.04 (dd, *J*=6.2, 2.5 Hz, 1H), 7.48 (d, *J*=2.0 Hz, 1H), 7.65 (d, *J*=7.5 Hz, 1H), 7.85 (m, 2H)
13	135-137	1.64-1.67 (m, 2H), 1.84-2.06 (m, 6H), 3.87 (s, 3H), 4.85 (m, 1H), 4.89 (s, 2H), 6.92 (d, *J*=8.7 Hz, 1H), 7.05 (dd, *J*=6.1, 2.6 Hz, 1H), 7.75 (d, *J*=2.5 Hz, 1H), 7.86 (d, *J*=2.5 Hz, 1H), 8.15 (dd, *J*=6.2, 2.1 Hz, 1H), 8.76 (d, *J*=2.0 Hz, 1H)
14	189-190	1.62-1.67 (m, 2H), 1.80-2.07 (m, 6H), 3.89 (s, 3H), 4.88 (m, 1H), 4.92 (s, 2H), 6.91 (d, *J*= 8.8 Hz, 1H), 7.05 (dd, *J*= 6.2, 2.5 Hz, 1H), 7.65 (d, *J*=7.9 Hz, 1H), 7.85 (d, *J*=2.5 Hz, 1H), 8.37 (dd, *J*=6.5, 1.5 Hz, 1H), 8.68 (s, 1H)
15	128-129	1.62-1.67 (m, 2H), 1.80-2.06 (m, 6H), 3.89 (s, 3H), 4.88 (m, 1H), 4.91 (s, 2H), 6.92 (d, *J*=8.8 Hz, 1H), 7.04 (dd, *J*=6.2, 2.5 Hz, 1H), 7.86 (d, *J*=2.5 Hz, 1H), 8.19 (d, *J*= 8.0 Hz, 1H), 8.32 (m, 2H)
16	185-187	1.33 (t, *J*=8.8 Hz, 3H), 1.62-1.67 (m, 2H), 1.80-2.07 (m, 6H), 3.87 (s, 3H), 4.31 (q, *J*=6.2 Hz, 2H), 4.88 (m, 1H), 4.92 (s, 2H), 6.92 (d, *J*=8.8 Hz, 1H), 7.05 (dd, *J*=6.2, 2.5 Hz, 1H), 7.65 (d, *J*=7.9 Hz, 1H), 7.85 (d, *J*=2.5 Hz, 1H), 8.37 (dd, *J*=6.5, 1.5 Hz, 1H), 8.68 (s, 1H)
17	142	1.33 (t, *J*=8.8 Hz, 3H), 1.62-1.67 (m, 2H), 1.80-2.06 (m, 6H), 3.89 (s, 3H), 4.31 (q, *J*=6.2 Hz, 2H), 4.88 (m, 1H), 4.91 (s, 2H), 6.92 (d, *J*=8.8 Hz, 1H), 7.04 (dd, *J*=6.2, 2.5 Hz, 1H), 7.86 (d, *J*=2.5 Hz, 1H), 8.19 (d, *J*=8.0 Hz, 1H), 8.32 (m, 2H)
18	153-154	1.58-1.74 (m, 6H), 1.91-1.93 (m, 2H), 3.74 (s, 3H), 4.81 (m, 1H), 4.95 (s, 2H), 6.99 (d, *J*=8.9 Hz, 1H), 7.23 (dd, *J*=6.4, 2.4 Hz, 1H), 7.51 (d, *J*=8.0 Hz, 1H), 7.71 (d, *J*=2.3 Hz, 1H), 8.16 (dd, *J*=6.5, 1.9 Hz, 2H)
19	205-206	1.63-1.68 (m, 2H), 1.81-2.08 (m, 6H), 3.89 (s, 3H), 4.86-4.90 (m, 1H), 4.93 (s, 2H), 6.92 (d, *J*=8.8Hz, 1H), 7.05 (dd, *J*=8.8, 2.5Hz, 1H), 7.65 (d, *J*=7.9Hz, 1H), 7.86 (d, *J*=2.5Hz, 1H), 8.37 (dd, *J*=7.9, 1.5Hz, 1H), 8.69 (d, *J*=1.5Hz, 1H).
20	224-225	1.70-1.96 (m, 8H), 3.72 (s, 3H), 4.76 (m, 1H), 4.82 (s, 1H), 6.95 (d, *J*=8.8 Hz, 1H), 7.01-7.04 (m, 2H), 7.18 (d, *J*=6.5 Hz, 1H), 7.40 (d, *J*=7.9 Hz, 1H), 7.65 (s, 1H)
21	108-109	1.45 (t, *J*=7.1Hz, 3H), 1.62-1.66 (m, 2H), 1.83-2.07 (m, 6H), 3.89 (s, 3H), 4.43 (q, *J*=7.1Hz, 2H), 4.86-4.89 (m, 1H), 4.90 (s, 2H), 6.92 (d, *J*=8.7Hz, 1H), 7.05 (dd, *J*=8.7, 2.5Hz, 1H), 7.88 (d, *J*=2.5Hz, 1H), 7.97 (d, *J*=8.3Hz, 1H), 8.21 (m, 2H)
22	114-115	1.44 (t, *J*=7.1Hz, 3H), 1.56-1.67 (m, 2H), 1.83-2.08 (m, 6H), 3.89 (s, 3H), 4.43 (q, *J*=7.1Hz, 2H), 4.86-4.89 (m, 1H), 4.90(s, 2H), 6.92 (d, *J*=8.7Hz, 1H), 7.04 (dd, *J*=8.7, 2.5Hz, 1H), 7.61 (d, *J*=7.9Hz, 1H), 7.87 (d, *J*=2.5Hz, 1H), 8.31 (dd, *J*=7.9, 1.5Hz, 1H), 8.60 (s, 1H)
23	289	1.58-1.64 (m, 2H), 1.86-2.05 (m, 6H), 2.48 (s, 3H), 3.87 (s, 3H), 4.80 (s, 2H), 4.88 (m, 1H), 6.90 (d, *J*=8.8 Hz, 1H), 7.03 (d, *J*=8.1 Hz, 1H), 7.40 (s, 2H), 7.72 (s, 1H), 7.89 (s, 1H)
24	137-139	1.61-1.64 (m, 2H), 1.85-2.08 (m, 6H), 2.92 (s, 3H), 3.88 (s, 3H), 4.74 (s, 2H), 4.89 (m, 1H), 6.83-6.91 (m, 2H), 7.02 (dd, *J*=6.2, 2.5 Hz, 1H), 7.10 (d, *J*=2.13 Hz, 1H), 7.30 (s, 1H), 7.92 (d, *J*=2.4 Hz, 1H)
25	176-177	1.63-1.66 (m, 2H), 1.83-2.08 (m, 6H), 3.90 (s, 3H), 4.88 (m, 1H), 4.97 (s, 2H), 6.93 (d, *J*=8.7 Hz, 1H), 7.05 (dd, *J*=6.2, 2.5 Hz, 1H), 7.73 (d, *J*=8.3 Hz, 1H), 7.82 (d, *J*=2.5 Hz, 1H), 8.50 (d, *J*=6.1 Hz, 1H), 8.77 (d, *J*=2.0 Hz, 1H)
26	184-185	1.64-1.66 (m, 2H), 1.84-2.06 (m, 6H), 3.89 (s, 3H), 4.87 (m, 1H), 4.96 (s, 2H), 6.92 (d, *J*=8.7 Hz, 1H), 7.05 (dd, *J*=6.2, 2.5 Hz, 1H), 7.84 (d, *J*=2.5 Hz, 1H), 8.08 (d, *J*=8.7 Hz, 1H), 8.41 (m, 2H)
27	115-117	1.62-1.67 (m, 2H), 1.81-2.08 (m, 6H), 3.03 (s, 3H), 3.86 (s, 3H), 4.73 (s, 2H), 4.86 (m, 1H), 6.91 (d, *J*=8.7 Hz, 1H), 7.04 (dd, *J*=6.2, 2.5 Hz, 1H), 7.20 (d, *J*=2.2 Hz, 1H), 7.69 (d, *J*=2.4 Hz, 1H), 8.50 (d, *J*=6.1 Hz, 1H), 8.77 (d, *J*=2.0 Hz, 1H)
28	124-126	1.63-1.66 (m, 2H), 1.83-2.08 (m, 6H), 3.04 (s, 3H), 3.88 (s, 3H), 4.88 (m, 1H), 5.29 (s, 2H), 6.94 (d, *J*=8.7 Hz, 1H), 7.07 (dd, *J*=8.8, 1.7 Hz, 1H), 7.67-7.77 (m, 2H), 8.20 (d, *J*=07.5 Hz, 1H), 8.40 (d, *J*=8.2 Hz, 1H)

Table III. Melting point for some amino and hydroxy compounds



No.	R1	R2	Mp (°C)
6	cyclopentyl		228-229
8	cyclopentyl		110-111
10	cyclopentyl		181-182
12	cyclopentyl		112-113
30	cyclopentyl		181-182
31	cyclopentyl		104-105
32	cyclopentyl		108-109
33	cyclopentyl		183-185

and amino aceto) group. The compounds of this class (**19-23**) were almost deprived of the activity. Likewise the above classes, C-6 position was more preferable to C-5 position.

The last class of substituent was the hydrophilic (-π) and electron-withdrawing (+σ) (i.e. methoxy and nitro) group. These compounds (**24-28**) were also inactive.

From these results, we concluded that C-6 position is the best optimal position to substitute the benzene ring of 1-isoindolinone moiety and carbonyl group of isoindolinone ring should be free to act as a hydrogen bonding acceptor. Also free amino group is the best substituent in our ongoing study to develop potent TNF-α production inhibitors. In the future, we will report the SAR studies for 6-amine and other derivatives of 1-isoindolinone moiety.

REFERENCES

Baik, K., Yoo, E., Byun, Y., Lee, S., Jang, B., Son, H., Lee, J., Cho, J., Lee, S., Moon, S., Park, J., and Lee, B. Novel 3,4-dialkoxyphenylderivatives and their therapeutic use thereof. Patent: WO9842666, 1998.

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).

Cho, J. Y., Park, J., Son, H. J., Park, J. S., Yoo, E. S., Baik, K. U., and Park, M. H. Effects of dialkoxylphenyl compounds with oxime group on activated macrophages and lymphocytes. Submitted (2001).

Eigler A., Sinha B., Hartmann G., Endres S. Taming TNF: strategies to restrain this proinflammatory cytokine. *Immunology Today*, **18**, 487-492 (1997).

Espat, N. J., Copeland, E. M. and Moldawer, L. L. Tumor necrosis factor and cachexia: a current perspective. *Surg. Oncol.*, 3, 255-262 (1994).

Garrison, L. and McDonnell, N. D. Etanercept: therapeutic use in patients with rheumatoid arthritis. *Ann. Rheum. Dis.*, Suppl 1, I65-169 (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.*, Suppl 1, I61-164 (1999).

Jones, R. E. and Moreland, L. W. Tumor necrosis factor inhibitors for rheumatoid arthritis. *Bull. Rheum. Dis.*, 48, 1-4 (1999).

Kubicki, M., Borowiak, T., Antkowiak W. The Effect of Different Hydrogen Bonding Schemes on Melting Points of Two Isomeric 2-Hydroxy-bomane-3-carboxylic Acids. *Z. Naturforsch. B*, 55, 671-676 (2000).

Muller, G. W., Shire, M. G., Wong, H., Corral, L. G., Patterson, R. T., Chen, y. and Stirling, D. I. Thalidomide analogs and PDE4 inhibition. *Bioorg. Med. Chem. Lett.*, 8, 2669-2674 (1998).

Newton, R. C. and Decicco, C. P. Therapeutic Potential and Strategies for Inhibiting Tumor Necrosis Factor-α. *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., 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., 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).

Thomas P., Zofia, U., Ruth, J., Margaret, E.. Hydrogen-Bond Formation in Nitroanilines: The First Step in Designing Acentric Materials. *J. Am. Chem. Soc.*, 109, 7786-7797 (1987).

van de Putte, LBA, Rau, R., Breedveld, F. One year efficacy results of the fully human anti-TNF antibody D2E7 in rheumatoid arthritis. Program and abstracts from the 64th Annual Scientific Meeting of the American College of Rheumatology, Philadelphia, Pennsylvania. Abstract 1218 (2000).