Arch Pharm Res Vol 23, No 4, 332-337, 2000 Medicinal Chemistry & Natural Products

Synthesis and Structure-Activity Relationships of Novel Compounds for the Inhibition of TNF-α Production

Joon Seok Park, Kyong Up Baik, Ho Jung Son, Jae Ho Lee, Se Jong Lee, Jae Youl Cho, Jisoo Park, Eun Sook Yoo, Young Seok Byun, and Myung Hwan Park

R&D Center, Daewoong Pharm. Co. Ltd., Sungnam, Kyunggi-do 462-120, Korea

(Received January 21, 2000)

This study describes the synthesis, *in vitro* evaluation and molecular modeling study of novel compounds for the inhibition of TNF-α production. Among these compounds, 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone (**9**) was selected as a lead compound and its pyridine derivative **10** was more potent in activity and safer than rolipram.

Key words: 2-[3-(Cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone, TNF-α production inhibitor, PDE 4 inhibition, Structure-activity relationship

INTRODUCTION

Tumor necrosis factor-α (TNF-α) is an important cytokine produced by activated monocytes/macrophages. There is a widely accepted belief that inhibition or modification of TNF-α overproduction in different inflammatory diseases like endotoxemia, toxic shock syndrome (van der Poll and Lowry, 1995), rheumatoid arthritis (Jones and Moreland, 1999) or cachexia (Espat, *et al.*, 1994) would be of benefit in the treatment of some of these conditions. Therefore, inhibitor of TNF-α production is now being studied extensively for therapeutics against the above diseases. In recent years, a soluble receptor (Etanercept) for TNF-α and an anti-TNF antibody (Infliximab) were approved by FDA for use in rheumatoid arthritis (Harriman, *et al.*, 1999; Garrison and McDonnell, 1999).

Rolipram is a protypic phosphodiesterase (PDE) 4 inhibitor and inhibit the production of TNF-α mediated by cyclic adenosine monophosphate (cAMP) elevation (Prabhakar, *et al.*, 1994). Although rolipram has CNS side effects like nausea and vomiting which correlated with [^{3}H] rolipram binding activity (Duplantier, *et al.*, 1996), it provides an active pharmacophore currently being studied as novel derivatives for the inhibition of TNF-α production (Christensen, *et al.*, 1995).

Thalidomide, which is a drug for a hypnotic/sedative agent and had to be withdrawn from the market because of its teratogenicity, specifically inhibits the production of TNF-α from human monocytes upon stimulation with bacterial lipopolysaccharide (LPS) (Sampaio, *et al.*, 1991). So, Muller and co-workers have developed a lot of thalidomide analogs focusing on glutarimide ring hydrolysis and reported that a good correlation between TNF-α inhibition and PDE 4 inhibition was observed for the majority of compounds (Muller, *et al.*, 1998).

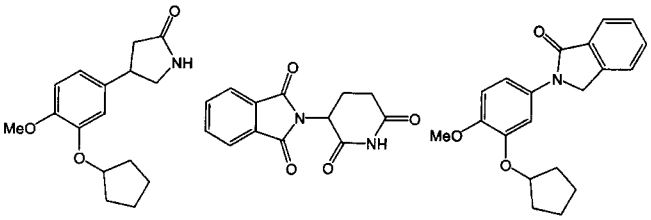


Fig. 1. Structure of TNF-α inhibitory compounds

In this paper, we would like to report that our novel compounds combining rolipram and thalidomide block the production of TNF-α by which may inhibit PDE 4 and would be more safe and potent than rolipram and thalidomide (Fig. 1).

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. Tetrahydro-

Correspondence to: Joon Seok Park, R&D Center, Daewoong Pharm. Co. Ltd., 223-23 Sangdaewon-dong, Joongwon-ku, Sungnam, Kyunggi-do 462-120, Korea
E-mail: joonchem@hanmail.net

furan (THF) was distilled from sodium-benzophenone immediately prior to use. Thin layer chromatography (TLC) was carried out using E. Merck Silica Gel 60 pre-coated 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).

General procedure for the preparation of 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone derivatives

3-Cyclopentyloxy-4-methoxyaniline (ii)

To a solution of 2-methoxy-5-nitrophenol (3.0 g, 17.7 mmol) in DMF (30 ml) were added cyclopentyl bromide (4.0 g, 26.6 mmol) and potassium carbonate (5.0 g, 35.4 mmol). The reaction mixture was stirred at 60°C for 15 h, cooled to room temperature, treated with distilled.water (20 ml), and extracted twice with ether. The ether layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the title compound, 3-cyclopentyloxy-4-methoxynitrobenzene (**i**) (4.0 g, 95%) as a pale yellow solid. ^{1}H-NMR ($CDCl_3$, ppm) δ 1.65-1.68 (m, 2H), 1.86-2.02 (m, 6H), 3.95 (s, 3H), 4.87 (m, 1H), 6.9 (d, *J*=8.9 Hz, 1H), 7.74 (d, *J*=2.6 Hz, 1H), 7.87-7.91 (dd, *J*=2.6, 8.9 Hz, 1H).

To a solution of 3-cyclopentyloxy-4-methoxynitrobenzene (**i**, 4.2 g, 17.7 mmol) in methanol (30 ml) were added ammonium formate (3.5 g, 53.2 mmol) and 10% Pd/C (0.3 g). The reaction mixture was refluxed for 2 h, cooled to room temperature, filtered through Celite, and evaporated *in vacuo* to remove solvent. The residue was dissolved in ether, washed twice with distilled water, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the title compound (3.0 g, 81%) as pale brown liquid. ^{1}H-NMR ($CDCl_3$, ppm) δ 1.60-1.63 (m, 2H), 1.85-1.95 (m, 6H), 3.05 (br, 2H), 3.78 (s, 3H), 4.73 (m, 1H), 6.24 (dd, *J*=2.6, 8.4 Hz, 1H), 6.33 (d, *J*=2.6 Hz, 1H), 6.73 (d, *J*=8.4 Hz, 1H).

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

To a solution of 3-cyclopentyloxy-4-methoxyaniline (**ii**, 0.52 g, 2.42 mmol) in chloroform (10 ml) was added phthalic anhydride (0.36 g, 2.43 mmol). The reaction mixture was stirred for 0.5 h at room temperature, treated with acetic acid (10 ml), refluxed for 4 h, cooled to room temperature, and then concentrated *in vacuo* to remove chloroform and acetic acid. The residue was crystallized from methanol to afford the title compound (0.75 g, 91%) as a white solid. mp 154-156°C; ^{1}H-NMR ($CDCl_3$, ppm) δ 1.60-1.64 (m, 2H), 1.82-1.96 (m, 6H), 3.90 (s, 3H), 4.78 (m, 1H), 6.96-7.00 (m, 3H), 7.77-7.80 (m, 2H), 7.93-7.96 (m, 2H).

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

white solid (89%); mp 86-88°C; ^{1}H-NMR ($CDCl_3$, ppm) δ 1.62-1.64 (m, 2H), 1.83-1.99 (m, 6H), 3.81 (s, 3H), 4.77 (s, 3H), 6.79 (d, *J*= 8.1 Hz, 1H), 7.01 (d, *J*=9.2 Hz, 1H), 7.71 (d, *J*=2.7 Hz, 2H), 7.85 (d, *J*=2.9 Hz, 2H).

1-[3-(Cyclopentyloxy)-4-methoxyphenyl]-2,5-pyrrolidinedione (3)

white solid (82%); mp 176-179°C; ^{1}H-NMR ($CDCl_3$, ppm) δ 1.57-1.60 (m, 2H), 1.84-1.92 (m, 6H), 2.90 (s, 4H), 3.87 (s, 3H), 4.75 (m, 1H), 6.78 (s, 1H), 6.81 (d, *J*= 8.8 Hz, 1H), 6.94 (d, *J*= 8.3 Hz, 1H).

2-[3-(Cyclopentyloxy)-4-methoxyphenyl]-3a,4,7,7a-tetrahydro-1,3-isoindolindione (4)

white solid (78%); mp 112-114°C; ^{1}H-NMR ($CDCl_3$, ppm) δ 1.53-1.64 (m, 2H), 1.78-1.90 (m, 6H), 2.54 (d, *J*=8.1 Hz, 1H), 2.58 (d, *J*=4.5 Hz, 1H), 3.11 (s, 2H), 3.83 (s, 3H), 4.78 (m, 1H), 5.84 (m, 2H), 6.47 (d, *J*=8.5 Hz, 1H), 7.08 (d, *J*=8.4 Hz, 1H), 7.28 (s, 1H).

2-[3-(Cyclopentyloxy)-4-methoxyphenyl]-*4H*-1,3-isoquinolinedione (5)

To a solution of 2,3-naphthalene diacid chloride (1.22 g, 4.83 mmol) in dichloromethane (30 ml) was added a solution of 3-cyclopentyloxy-4-methoxyaniline (**B**, 1.0 g, 4.83 mmol) in dichloromethane (20 ml) and TEA (1.23 g, 12.1 mmol). The reaction mixture was heated at reflux for 16 h, cooled to room temperature, and then concentrated *in vacuo* to remove dichloromethane, added water, extracted with ethyl acetate, washed with sodium bicarbonate solution, dried over $MgSO_4$, filtered, and concentrated *in vacuo*, recrystallized from methanol to give the title compound (1.6 g, 86%) as a white solid. mp 228-230°C; ^{1}H-NMR ($CDCl_3$, ppm) δ 1.57 (s, 2H), 1.80-2.06 (m, 6H), 3.89 (s, 3H), 4.24 (s, 2H), 4.74 (m, 1H), 6.70 (s, 1H), 6.75 (d, *J*=8.8 Hz, 1H),6.98 (d, *J*=8.5 Hz, 1H),7.36 (d, *J*=7.5 Hz, 1H), 7.49 (t, *J*=8.2 Hz, 1H), 7.65 (t, *J*=7.9 Hz, 1H), 8.26 (d, *J*=7.8 Hz, 1H).

2-[3-(Cyclopentyloxy)-4-methoxyphenyl]-3-thioxo-1-isoindolinone (6)

To a solution of 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1,3-isoindolindione (**1**, 0.5 g, 1.48 mmol) in toluene (20 ml) was added the Lawesson's reagent (0.3 g, 0.74 mmol) at room temperature. The mixture was heated at reflux for 2 h, cooled to room temperature, and then concentrated *in vacuo* to remove toluene, and purified by flash chromatography (EtOAc-Hexane=1:1) to give the title compound (0.23 g, 43%) as a orange solid. mp 152-154 °C; ^{1}H-NMR ($CDCl_3$, ppm) δ 1.57-1.60 (m, 2H), 1.84-1.94 (m, 6H), 3.91 (s, 3H), 4.74 (m, 1H), 6.86-6.93 (m, 2H), 7.01 (d, *J*=8.5 Hz, 1H), 7.77-7.80 (m, 2H), 7.87-7.89 (m, 1H), 8.06-8.08 (m, 1H).

2-[3-(Cyclopentyloxy)-4-methoxyphenyl]-1,3-isoindoline dithione (7)

yellowish soild (38%); mp 156-158°C; ^{1}H-NMR ($CDCl_3$, ppm) δ 1.57-1.60 (m, 2H), 1.85-1.92 (m, 6H), 3.92 (s, 3H), 4.72 (m, 1H), 6.78-6.87 (m, 2H), 7.01 (d, *J*=8.5 Hz, 1H), 7.76-7.80 (m, 2H), 7.95-7.98 (m, 2H).

2-[3-(Cyclopentyloxy)-4-methoxyphenyl] isoindoline (8)

To a solution of 3-cyclopentyloxy-4-methoxyaniline (**ii**, 0.52 g, 2.42 mmol) in DMF (10 ml) was added α, α'-dibromo-*o*-xylene (0.64 g, 2.42 mmol) and potassium carbonate (1.67 g, 12.1 mmol) at room temperature. The reaction mixture was stirred for 3 h at room temperature, added water, extracted with ethyl acetate, washed with saturated ammonium chloride solution and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*, recrystallized from methanol to give the title compound (0.75 g, 91%) as a yellowish solid. mp 118-121°C; ^{1}H-NMR ($CDCl_3$, ppm) δ 1.57-1.64 (m, 2H), 1.88-1.99 (m, 6H), 3.82 (s, 3H), 4.62 (s, 4H), 4.87 (m, 1H), 6.89-6.98 (m, 2H), 7.07-7.09 (m, 1H), 7.32-7.40 (m, 3H), 7.57-7.60 (m, 1H).

2-[3-(Cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone (9)

To a solution of 2-[3-(Cyclopentyloxy)-4-methoxyphenyl]-1,3-isoindolindione (**1**, 0.5 g, 1.48 mmol) in THF (10 ml) methanol (10 ml) was added sodium borohydride (0.23 mg, 5.92 mmol) at 0°C. The reaction mixture was stirred for 1 h, quenched with saturated ammonium chloride solution, evaporated *in vacuo*, dissolved in EtOAc, extracted with water. Then, the resultant organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 3-(cyclopentyloxy)-4-methoxy-1-[2-(hydroxymethyl)phenyl] carboxamidobenzene (**iii**, 0.49 g, 97%). To a solution of 3-(cyclopentyloxy)-4-methoxy-1-[2-(hydroxymethyl) phenyl] carboxamidobenzene (0.4 g, 1.17 mmol) in anhydrous THF (10 ml) were added triphenylphosphine (0.37 g, 1.41 mmol) and diethylazodicarboxylate (0.25 g, 1.41 mmol) at room temperature. The reaction mixture was stirred for 1 h at room temperature, evaporated *in vacuo*, treated with 6N HCl solution (10 ml), and extracted with ethyl acetate. The aqueous layer was adjusted to pH 8-9 with 6N NaOH solution, and extracted with ethyl acetate. Then, the resultant organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the title compound (0.34 g, 89%) as a white solid. mp 130-132°C; ^{1}H-NMR ($CDCl_3$, ppm) δ 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); *Anal.* Calcd for $C_{20}H_{21}NO_3$: C, 74.30; H, 6.50; N, 4.33. Found: C, 74.03; H, 6.27; N, 4.13.

2-(3-Cyclopentyloxy-4-methoxyphenyl)-2,3-dihydro-pyrrolo[3,4-c]pyridin-1-one (10)

This compound was purified from its three different isomers by column chromatography (EtOAc-Hexane=2:1) and recrystallization. yellowish solid (24%); mp 139-141°C; ^{1}H-NMR ($CDCl_3$, ppm) δ1.60-2.05 (m, 8H), 3.88 (s, 3H), 4.85 (m, 1H), 4.87 (s, 2H), 6.91 (d, *J*=8.5 Hz, 1H), 7.02 (dd, *J*=2.1, 8.5 Hz, 1H), 7.51 (d, *J*=5.1 Hz, 1H), 7.82 (d, *J*=2.2 Hz, 1H), 8.82 (d, *J*=5.1 Hz, 1H), 9.18 (s, 1H); *Anal.* Calcd for $C_{19}H_{20}N_2O_3$: C, 70.37; H, 6.17; N, 8.64. Found: C, 70.08; H, 6.07; N, 8.52.

Chemistry

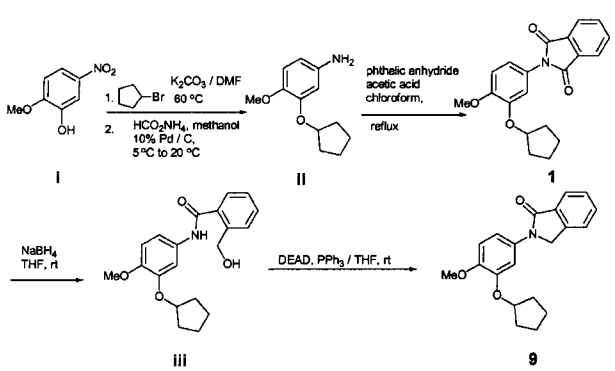
Derivatives of 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone (**9**) (Baik, *et al.*, 1998) were synthesized by the procedure shown in scheme 1. Representatively, 3-(cyclopentyloxy)-4-methoxy-1-*N*-substituted aniline, prepared from the reaction 2-methoxy-5-nitrophenol (**i**) and cyclopentyl bromide, was reduced to 3-(cyclopentyloxy)-4-methoxy aniline (**ii**) with palladium charcoal and ammonium formate, and then treated with phthalic anhydride to give a 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1,3-isoindolindione (**1**). Reduction of this compound with sodium borohydride in THF afforded the mixture of 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-3-hydroxy-1-isoindo-linone and *N*-3-(cyclopentyloxy)-4-methoxyphenyl)-2-hydro-xymethyl-benzamide (**iii**). The partially reduced mixture was fully reduced to give ring opened form with additional sodium borohydride. Finally, the 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone (**9**) was prepared by the Mitsunobu reaction with DEAD and triphenyl phosphine in THF.

Molecular modeling study

The molecular modeling of **1**, **6**, **7**, **9**, **10**, and rolipram was performed with Sybyl (version 6.3) on an Indigo 2 R10000 workstation.

TNF-α *in vitro* assay

After cancer cell line of mouse macrophage (RAW264.7) is diluted with RPMI 1640 medium (containing 10% FBS), then plated out in 24 well plates at 1×10^6 cells/ml.



Scheme 1. Synthesis of 2-[3-(cyclopentyloxy)-4-methoxyphenyl] -1-isoindolinone (**9**)

Then, the culture is incubated for 18 h at 5% CO_2 and 37°C. 1 μM of compound and 1 g/ml of lipopolysaccharide (LPS) are added to the plate and the culture is incubated for 6 h 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.

Assay for PDE 4 inhibitory activity

PDE 4 activity was determined by using partially purified PDE 4 from human monocyte (U937) and [^{3}H]-cAMP (1 μM) as the substrate. Human monocyte PDE 4 was isolated as described by Torphy *et al.* (1994b). Synthetic compounds and rolipram were tested at seven concentrations from 10^{-9} to 10^{-3} M in duplicate. The test compounds and the substrate with U937 cells was incubated at 37°C for 30 min. The product of reaction ([^{3}H] 5'AMP) was separated from the substrate by elution on cation-exchange columns and radioactivity was determined with a liquid scintillation counter (LS 1701, Beckman) using a liquid scintillation cocktail. IC_{50} values were determined by non-linear regression analysis of the competition curves.

[^{3}H]Rolipram binding assay

[^{3}H]Rolipram binding was measured in mouse brain membrane as described by Duplantier *et al.* Membrane homogenates were suspended in 50 mM tris-HCl buffer (pH 8.0) containing 1.2 mM $MgCl_2$ and incubated with [^{3}H] rolipram for 60 min at 4°C in a total volume of 1ml. Nonspecific binding was defined in the presence of 10^{-5} M rolipram. Following incubation, the content of each tube was rapidly filtered under vacuum through Whatman GF/B glass filters. The membranes were then washed three times with 3 ml of ice-cold buffer using a Brandel cell harvester. Bound radioactivity was measured with a scintillation counter (LS 6000, Beckman) using a liquid scintillation cocktail.

Using a fixed protein concentration (2 nM), a saturation experiment was performed with increasing concentrations of [^{3}H] rolipram ranging from 0.2 to 10 nM to determine the K_D (affinity) and B_{max} (maximum binding site) values. Using fixed protein and [^{3}H] rolipram (equal to its K_D value) concentrations, competition experiments were performed with unlabeled rolipram and test compounds for which the IC_{50} (concentration causing a half-maximum inhibition of specific binding) value was determined. Each compound was tested at seven concentrations in duplicate ranging from 10^{-10} to 10^{-4} M for its ability to inhibit the specific [^{3}H] rolipram binding.

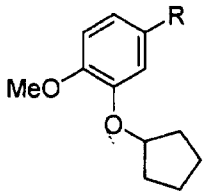
RESULTS AND DISCUSSION

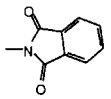
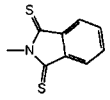
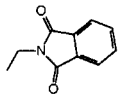
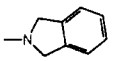
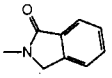
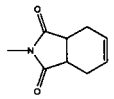
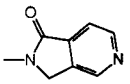
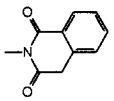
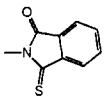
Compounds prepared in this study were tested for their ability to inhibit TNF-α production in LPS-stimulated RAW264.7 cells (Park, *et al.*, 2000; Cho, *et al.*, 1998a,b). Among these compounds, potent compounds for inhibiting TNF-α production were also evaluated their inhibitory activity against PDE 4 to test their mechanism (Torphy, *et al.*, 1992a). Also, to estimate the side effect of our compounds, selected compounds (**9**, **10**) were examined for the [^{3}H] rolipram binding assay (RBA) and compared the ratio of PDE 4 IC_{50} to RBA IC_{50} with rolipram. TNF-α production inhibitory data for these compounds are summarized in Table I.

Data given in Table I show that the distance between dialkoxyphenyl ring and phthalimide ring is an important factor for inhibiting TNF-α production. The phenyl compound **1** directly linked to phthalimide ring was 10 times more potent than the benzyl compound **2** and was almost comparable to rolipram in its inhibitory effect of TNF-α production. To identify the requirement of phenyl ring of phthalimide moiety were designed compound **3** & **4** and both of these compounds were found to be not effective. Compound **5** was designed to identify the effect of the ring size of phthalimde but was turned out inactive. These results clearly indicate that our novel compounds combining rolipram and thalidomide could be potent inhibitors of TNF-α production in the condition that only five-membered and phthalimide ring is linked directly to rolipram moiety to maintain the inhibitory effect of TNF-α production. In the next step, compound **6**, **7**, and **8** having low formal charge and weak hydrogen bonding intensity by replacing the oxygen of compound **1** with sulfur or removing it were synthesized to identify the requirement and intensity of hydrogen bonding. Interestingly, all the compounds had no activity. From this result, we assumed that hydrogen bond acceptor is essential to maintain the activity and also the oxygen atom is more effective than sulfur atom. Thereafter, monoketo compound **9** was designed. This compound was five times more potent than diketo phthalimide compound **1** in its inhibitory effect of TNF-α production. And also compound **10** that is a pyridine derivative of compound **9** was comparable to its isostere in its inhibitory effect of TNF-α production.

In order to determine the reason for the difference in activity between rolipram, monoketo compound **9** and **10**, diketo compound **1**, and sulfur compound **6** and **7**, we calculated the distance between the carbonyl oxygen of the phthalimide ring and the oxygen of the 4-methoxy group. We calculated dihedral angle between dialkoxy phenyl ring and phthalimde ring, and formal charge of the carbonyl oxygen or sulfur by using molecular model-

Table I. Synthetic yield and inhibitory effect on TNF-α production in LPS-stimulated RAW264.7 cells



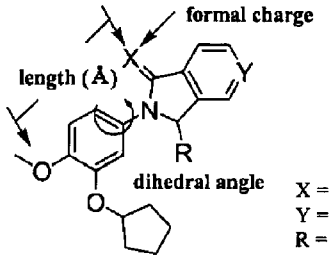
Compound	R	TNF-α IC_{50}[μM]	Compound	R	TNF-α IC_{50} [μM]
1		0.68	**7**		>100
2		7.0	**8**		9% @1 μg/ml
3		33.0	**9**		0.13
4		1% @1 μg/ml	**10**		0.18
5		>100	thalidomide		194
6		>100	rolipram		0.10

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 1ng/ml of TNF-α as a basal level. 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.

ing software program (Table II).

Our compounds have activity comparable to rolipram in inhibitory effect of TNF-α production even though they have shorter distance between the carbonyl oxygen of the phthalimide ring and the oxygen of the 4-methoxy group than rolipram, namely, these results indicate that the position of carbonyl oxygen was not an essential factor to maintain the inhibitory activity of TNF-α production. It was dihedral angle that differentiates compounds in Table II. Sulfur compounds **6** and **7** have greater dihedral angle than oxygen compounds such as rolipram, **1**, **9**, and **10**, so that they have less inhibitory activity because it may have not appropriate angle to fit enzyme pocket. Also, as sulfur compounds have less formal charge than oxygen compounds, they have less intensity of hydrogen bonding than oxygen compounds. From this study, we found that these molecular modeling data correlate with the biological activity, that is, the less dihedral angle and the stronger hydrogen bonding acceptor in our compound series explain better activity in inhibiting TNF-α production.

Table II. Molecular modeling data of selected compound and rolipram.



Compound	Distance(Å)	Dihedral Angle	Formal Charge
1	6.5	39.8	-0.36
6	6.5	51.4	-0.20
7	6.5	51.7	-0.20
9	6.4	37.6	-0.38
10	6.4	35.2	-0.37
rolipram	8.6	27.9	-0.40

Table III. Result of PDE 4 inhibitory activity and [^{3}H] rolipram binding assay

Compound	PDE 4 IC_{50} [μM]	RBA IC_{50} [μM]	Ratio PDE 4/ RBA
9	0.78	NT	-
10	0.053	0.014	3.78
rolipram	0.26	0.001	260

NT: not tested

Based on these results, we decided the monoketo dialkoxyphenyl compound **9** as a lead compound for inhibitor of TNF-α production. Especially compound **10** was five times more potent than rolipram for its inhibitory potency against PDE 4 (Table III). These results indicate the most potent PDE 4 inhibitory compound **10** in this study was highly improved in terms of the PDE 4/ [^{3}H]rolipram ratio and would be safer PDE 4 inhibitor than rolipram. This result suggests that our compounds inhibit TNF-α production by PDE 4 inhibition and would be safer than rolipram. Also, it suggest that SAR for PDE 4 inhibition may be somewhat different from that of inhibition of TNF -α production in our compound series. To understand the exact mechanism of inhibition of TNF-α production in our compounds, other studies such as nuclear factor-κB (NF-κB) inhibition (Newton and Decicco, 1999), cAMP quantitation, and inhibition of TNF-α mRNA must be required.

In conclusion, our novel 2-[3-(cyclopentyloxy)-4-methoxyphenyl]-1-isoindolinone (**9**) and its pyridine derivative **10** combining thalidomide and rolipram moiety were potent inhibitor of TNF-α production. The ability of **9** and **10** to block TNF-α production was found to be due to the inhibition of PDE 4. The most potent PDE 4 inhibitory compound **10** displayed a highly improved ratio with respect to the [^{3}H]rolipram specific binding site and is being evaluated *in vivo* as potential anti-inflammatory agent.

REFERENCES

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

Cho, J. Y., Park, J., Yoo, E. S., Baik, K. U., Lee, J. S. and Park, M. H. Inhibitory effect of sesquiterpene lactones from Saussurea lappa on tumor necrosis factor-alpha production in murine macrophage-like cells. *Planta Med.*, 64, 594-597 (1998a).

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 (1998b).

Christensen, S. B. and Webb, K. S. Compounds, Compositions and Treatment of Allergies and Inflammation. Patent: WO9509624, 1995.

Duplantier, A. J., Biggers, M. S., Chambers, R. J., Cheng, J. B., Damon, D. B., Eggler, J. F., Kraus, K. G., Marfat, A., Masamune, H., Pillar, J. S., Shirley, J. T., Umland, J. P. and Watson, J. W. Biarylcarboxylic acids and -amides: inhibition of phosphodiesterase type IV versus [^{3}H]rolipram binding activity and their relationship to emetic behavior in the ferret. *J. Med. Chem.*, 39, 120-125 (1996).

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).

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., and Park, M. H. The Discovery of Novel Lead Compound for the Inhibition of Tumor Necrosis Factor-α Production. *Korean J. Med. Chem.*, 10, 6-9 (2000).

Prabhakar, U., Lipshutz. D., Bartus, J. O., Slivjak, M. J., Smith, E. F. 3rd, Lee, J. C. and Esser, K. M. Characterization of cAMP-dependent inhibition of LPS-induced TNF alpha production by rolipram, a specific phosphodiesterase IV (PDE IV) inhibitor. *Int. J. Immunopharmacol.*, 10, 805-16 (1994).

Sampaio, E. P., Sarno, E. N., Galilly, R., Cohn, Z. A. and Kaplan, G. Thalidomide selectively inhibits tumor necrosis factor alpha production by stimulated human monocytes. *J. Exp. Med.*, 173, 699-703 (1991).

Torphy, T. J., Stadel, J. M., Burman, M., Cieslinski, L. B., Mclaughlin, M. M., White, J. R. and Livi, G. P. Coexpression of human cAMP-specific phosphodiesterase activity and high affinity rolipram binding in yeast. *J. Biol. Chem.*, 267, 1798-1804 (1992a).

Torphy, T. J., Zhou, H. L., and Cieslinski, L. B. Stimulation of beta adrenoceptors in a human monocyte cell line (U937) up-regulates cyclic AMP-specific phosphodiesterase activity. *J. Pharmacol. Exp. Ther.*, 263, 1195-1205 (1992b).

Van der Poll, T. and Lowry, S. F. Tumor necrosis factor in sepsis: mediator of multiple organ failure or essential part of host defense? *Shock*, 3, 1-12 (1995).