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# Synthesis of 1,5-diarylhaloimidazole analogs and their inhibitory activities against $PGE_2$ production from LPS-treated RAW 264.7 cells

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ABSTRACT

A number of 1,5-diarylimidazole analogs were synthesized and evaluated their inhibitory activities of cyclooxygenase-2 catalyzed prostaglandin $E_2$ production. Reactions of 1,5-diarylimidazoles with halogenating reagents (NCS, NBS, NIS) afforded halogenated analogs. Among the analogs tested, compounds **Ib**, **IIa**, **IIb** and **IIe** exhibited significantly improved inhibitory activities against COX-2-mediated $PGE_2$ production from LPS-induced RAW 264.7 cells compared to those of the parent 1,5-diarylimidazoles. Especially, the analogs **Ib** ($IC_{50}$ = 0.55 μM) and **IIa** ($IC_{50}$ = 0.58 μM) showed best results. Halogenation on the 1,5-diarylimidazole ring enhanced inhibitory activities against COX-2 catalyzed $PGE_2$ production, however, inhibitory activities were significantly varied by position(s) and species of the substituted halogen(s).

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## 1. Introduction

Several chemicals and biologicals such as eicosanoids and tissue degradation enzymes are involved in inflammatory process. Cyclooxygenase (COX), is the key proinflammatory enzyme which catalyses the conversion of arachidonic acid to prostaglandins (PGs). Cyclooxygenase exists in two isoforms. Cyclooxygenase-1 (COX-1) is a constitutive enzyme processing homeostasis function, while cyclooxygenase-2 (COX-2) is an inducible one and known as a major isoform found in inflammatory lesions.[1]

During the past two decades, efforts have been focused on the development of selective COX-2 inhibitors. Modification of the central heterocycle in tricyclic series has been a popular area of research. As a result, diverse heterocycles have been explored.[2] It has been observed from previous SAR studies that COX-2 selective inhibitors require a 4-methylsulfonylphenyl or a 4-sulfonamidophenyl group attached to an unsaturated ring system in which an additional vicinal lipophilic moiety is needed to possess good activity and selectivity (Fig. 1).

Also it was well acknowledged that the nature of central scaffold is very important for activity. Therefore, choice of the central scaffold is crucial for activity as well as selectivity. As central scaffold, diverse regioisomeric imidazoles such as 1,2-,[3] 1,5-,[4–8] 4,5-[9,10] and 2,4- diarylimidazoles[11] have been explored. However, only 1,5-diarylimidazole made success and was pursued in further development. Our previous results related with 1,5-diarylimidazoles also revealed very similar results.[6,8] Other important SAR results were observed from a precedent literature,[4] the analogs with halogen substitution at the 2- or 4-position of 1,5-diarylimidazoles exhibited significantly improved biological activity. These results led us to conduct SAR study of 1,5-diarylimidazoles halogenated at 2- or/and 4-position(s). To decipher halogen effects on COX-2 catalyzed $PGE_2$ production from LPS-induced RAW 264.7 cell, two analogs (2,4-difluorophenyl or 3,4-difluorophenyl at N1, 4-methylsulfonylphenyl at C5; Fig. 2) were selected for further investigation based on our previous results.[8] We planned to observe the result whether the halogen substitutions enhance the moderate inhibitory activity of the parent compounds (84% and 55% at 10 μM). In this study, several halogenated 1,5-diarylimidazoles at 2- or/and 4-position(s) were synthesized and evaluated their inhibitory activities against COX-2 catalyzed $PGE_2$ production from LPS-induced RAW 264.7 cells.

## 2. Chemistry & pharmacology

### 2.1. Synthesis of 1,5-diarylimidazole analogs

Halogenated 1,5-diarylimiazole analogs were prepared following the procedures and conditions as shown in Scheme 1. 4-Halo- and 2,4-dihalo-1,5-diarylimidazoles (**Ib**, **Ic**, **IIc**, **IId**, **IIe**) were prepared by reacting the 1,5-diarylimidazoles **I** and **II** with halogenating

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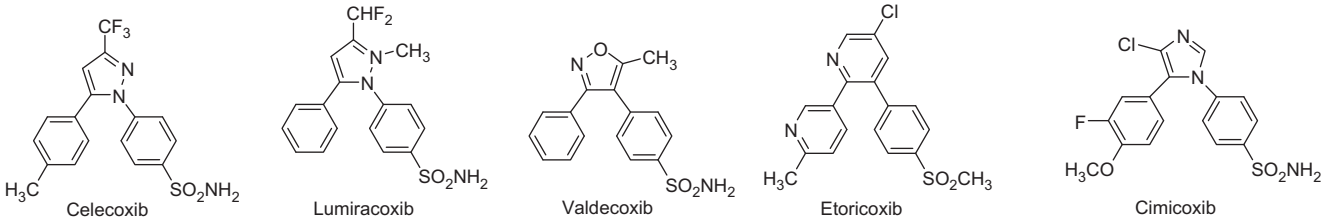



**Figure 1.** Chemical structures of known COX-2 selective inhibitors.

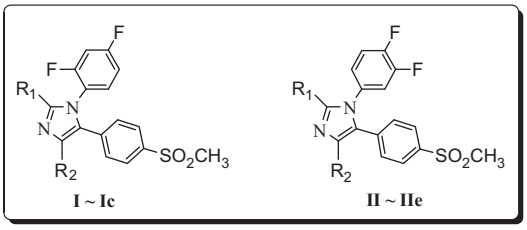
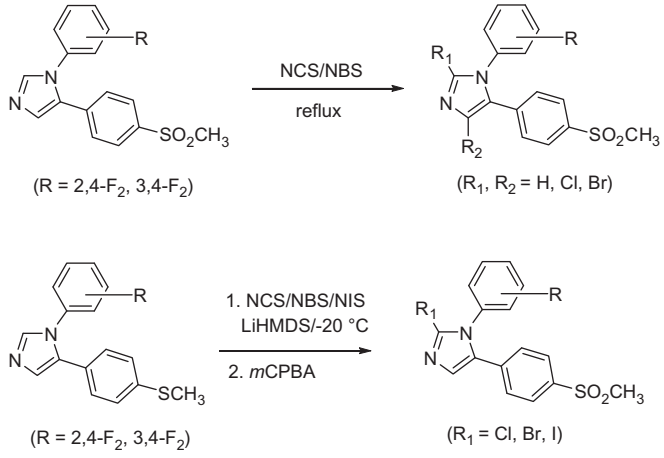



**Figure 2.** 1,5-Diarylimidazoles (**I** and **II**) and their halogenated derivatives.




**Scheme 1.** Synthetic pathway for halogenated 1,5-diarylimidzole derivatives.

reagents (NCS, NBS) in $CHCl_3$ at reflux condition for 2–5 h gave 4-halo and 2,4-dihalo products.[12,13] The halogenation reactions with NIS in reflux conditions gave decomposed products. 2-Halo-1,5-diarylimidazoles (**Ia**, **IIa**, **IIb**) were prepared from (methylthiophenyl)imidazoles in two steps. Reaction of (methylthiophenyl)imidazoles, halogenating reagents (NCS, NBS, NIS) and lithium hexamethyldisilazide (LiHMDS) in THF followed by oxidation with *m*-chloroperbenzoic acid (*m*CPBA) provided 2-halogeno-1,5-diarylimidazoles in crude form. Purification of the crude products by column chromatography yielded pure 2-halogeno-1,5-diarylimidazole analogs.

## 2.2. RAW 264.7 cell culture and measurement of $PGE_2$ concentrations

RAW 264.7 cells obtained from American Type Culture Collection (ATCC, Rockville, MD) were cultured in DMEM supplemented with 10% FBS and 1% antibiotics under 5% $CO_2$ at 37 °C based on the previously described procedures.[14] Briefly, cells were plated in 96-well plates ($2 \times 10^5$ cells/well). After pre-incubation with the test compounds for 1 h, LPS (1 μg/mL) were added and incubated for 24 h. $PGE_2$ concentration in the medium was measured using ELISA kit for $PGE_2$ (Cayman Chem. Co.) according to the manufacturer's recommendation. Cell viability was assessed with MTT assay. All tested compounds showed no or less than 10% reduction of MTT assay at the tested concentrations, indicating that they were not significantly cytotoxic to RAW 264.7 cells in the presence or absence of LPS. Therefore, the inhibition of $PGE_2$ production by 1,5-diarylimidazoles might be not associated with their cytotoxicity.

## 3. Results and discussion

Halogenated 1,5-diarylimidazole analogs on the imidazole ring were prepared via two different synthetic pathways. Mono-substitution (Br, I) at 2-position increased the inhibitory activity of $PGE_2$ production (**IIa** and **IIb**), whereas 2-chlorination decreased inhibitory activity of $PGE_2$ production (**Ia**) compared to those of the parent compounds. Di-bromination at 2- and 4-positions decreased the inhibitory activity of $PGE_2$ production (**Ic**), but di-chlorination increased the inhibitory activity of $PGE_2$ production (**Ib** and **IIe**) compared to those of the parent compounds. To monitor the relationship between physicochemical properties (electron density/lipophilicity) and biological activity of 1,5-diarylhaloimidazole analogs, $pK_a$ and log*P* values of each compound were obtained and compared with bioactivity data. The active compounds Ib($pK_a$ = 1.07) and IIa($pK_a$ = 4.05) were observed to possess different electronic density. To observe the effects of lipophilicity, log*P* values of active compounds (Ib = 3.64; IIa = 3.70; IIb = 3.88; IIe = 4.44) and inactive compounds (Ic = 4.64; IId = 3.72) were compared. Thus, no relationship between electron density/lipophilicity and bioactivity was observed. However, in some cases, halogenation on the 1,5-diarylimidazole ring enhanced inhibitory activities of synthesized imidazole analogs against COX-2 catalyzed $PGE_2$ production. In detail, inhibitory activities were significantly varied by halogenation position(s) and species of the substituted halogen(s) in some cases as shown in Table 1. Two analogs exhibited much stronger inhibitory activities of $PGE_2$ production (**Ib/**

**Table 1**
Yields of each compounds and % inhibitory activities of halogenated 1,5-diarylimidazoles against COX-2 catalyzed $PGE_2$ production from LPS-induced RAW 264.7 cells[a]

| Compounds | $R^1/R^2$ | Yields (%) | % Inhibition[b,c] ($IC_{50}$ μM) |
|---|---|---|---|
| I | $R_1 = R_2 = H$ | | 84 (6.52) |
| Ia | $R_1 = Cl, R_2 = H$ | 60 | 42 |
| Ib | $R_1 = R_2 = Cl$ | 80 | 98 (0.55) |
| Ic | $R_1 = R_2 = Br$ | 55 | 15 |
| II | $R_1 = R_2 = H$ | | 55 |
| IIa | $R_1 = Br, R_2 = H$ | 40 | 99 (0.58) |
| IIb | $R_1 = I, R_2 = H$ | 85 | 90 |
| IIc | $R_1 = H, R_2 = Cl$ | 55 | 56 |
| IId | $R_1 = H, R_2 = Br$ | 50 | 7 |
| IIe | $R_1 = R_2 = Cl$ | 74 | 81 |

[a] All compounds were treated at 10 μM. Treatment of LPS to RAW cells increased $PGE_2$ production (10.0 μM) from the basal level of 0.5 μM.

[b] % Inhibition = 100 × [1−($PGE_2$ of LPS with the flavones treated group−$PGE_2$ of the basal)/($PGE_2$ of LPS treated group−$PGE_2$ of the basal)].

[c] All data are the arithmetic means ($n$ = 3).

$IC_{50}$ = 0.55 μM and **IIa**/0.58 μM) than those of the parent compounds (**I** and **II**). Our present results imply that structural modification at the imidazole ring system plays very important roles to possess strong bioactivity. Further SAR study with large number of halogenated imidazole analogs is under progress.

## 4. Experimental

### 4.1. Materials and methods

All chemicals were obtained from commercial suppliers, and used without further purification. All solvents used for reaction were freshly distilled from proper dehydrating agent under nitrogen gas. All solvents used for chromatography were purchased and directly applied without further purification. $^1H/^{13}C$ NMR spectra were recorded on a Varian Gemini 2000 instrument (200 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm) downfield relative to tetramethylsilane as an internal standard. Peak splitting patterns were abbreviated as m (multiplet), s (singlet), br s (broad singlet), d (doublet), br d (broad doublet), t (triplet) and dd (doublet of doublets). Mass spectra were recorded on a Autospec M363. Melting points were recorded on a Fisher-Johns microscopic scale melting point apparatus. Analytical thin-layer chromatography (TLC) was performed using commercial glass plate with silica gel $60F_{254}$ purchased from Merck. Chromatographic purification was carried out by flash chromatography using Kieselgel 60 (230–400 mesh, Merck).

### 4.2. General synthetic conditions for 4-halogeno and 2,4-dihalogeno-1,5-diarylimidazoles

4-Halogeno and 2,4-dihalogeno-1,5-diarylimiazole analogs were prepared from 1,5-diarylimidazoles (**I** and **II**) following the procedure as shown in Scheme 1. To the solution of **I** or **II** (0.5 mmol) in $CHCl_3$ (4 mL) was added NCS or NBS (0.75 mmol). The mixture was refluxed for 5 h, extracted with DCM, washed with aqueous $NaHSO_3$ and brine and dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography to give 4-halogeno and 2,4-dihalogeno imidazoles. Reactions with excess halogenating reagents (3.0 equiv) and extended reaction time (3–12 h) in chloroform or acetonitrile exclusively provided 2,4-di-halogeno products.[10,11] After the time required in each case, the solvent was evaporated and ether was added. The ethereal phase was washed with aqueous $NaHSO_3$ solution, water, brine, dried with anhydrous $MgSO_4$ and evaporated to dryness. The residue was purified by column chromatography with hexane:acetone as the mobile phase to get title products.

### 4.3. 1-(2,4-Difluorophenyl)-2,4-dichloro-5-(4-methylsulfonyl phenyl)imidazole (Ib)

80%; White solid; mp 207 °C; $R_f$ = 0.70 (hexane:acetone = 1:1); $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.87 (d, $J$ = 8 Hz, 2H, Ar-H), 7.38 (d, $J$ = 8 Hz, 2H, Ar-H), 7.28–7.17 (m, 1H, Ar-H), 7.03–6.95 (m, 2H, Ar-H), 3.05 (s, 3H, $SO_2CH_3$); HRMS (EI) *m/z* Calcd for $C_{16}H_{10}Cl_2F_2N_2O_2S$ $[M]^+$ 401.9808. Found 401.9882.

### 4.4. 1-(2,4-Difluorophenyl)-2,4-dibromo-5-(4-methylsulfonyl phenyl)imidazole (Ic)

55%; White solid; mp 239–240 °C; $R_f$ = 0.55 (hexane:acetone = 1:1); $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.87 (d, $J$ = 8.4 Hz, 2H, Ar-H), 7.40 (d, $J$ = 8.4 Hz, 2H, Ar-H), 7.28–7.16 (m, 1H, Ar-H), 7.03–6.93 (m, 2H, Ar-H), 3.06 (s, 3H, $SO_2CH_3$); HRMS (EI) *m/z* Calcd for $C_{16}H_{10}Br_2F_2N_2O_2S$ $[M]^+$ 489.8798. Found 489. 8725.

### 4.5. 1-(3,4-Difluorophenyl)-4-chloro-5-(4-methylsulfonyl phenyl)imidazole (IIc)

55%; White solid; mp 191–192 °C; $R_f$ = 0.50 (hexane:acetone = 1:1); $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.94–7.88 (m, 2H, Ar-H), 7.63 (s, 1H, $H_2$-imidazole), 7.44–7.36 (m, 2H, Ar-H), 7.30–7.17 (m, 1H, Ar-H), 7.09–6.98 (m, 1H, Ar-H), 6.94–6.88 (m, 1H, Ar-H), 3.07 (s, 3H, $SO_2CH_3$); HRMS (EI) *m/z* Calcd for $C_{16}H_{11}ClF_2N_2O_2S$ $[M]^+$ 368.0198. Found 368.0155.

### 4.6. 1-(3,4-Difluorophenyl)-4-bromo-5-(4-methylsulfonyl phenyl)imidazole (IId)

50%; White solid; mp 195–196 °C; $R_f$ = 0.40 (hexane:acetone = 1:1); $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.90 (d, $J$ = 8.2 Hz, 2H, Ar-H), 7.67 (s, 1H, $H_2$-imidazole), 7.42 (d, $J$ = 8.2 Hz, 2H, Ar-H), 7.29–7.16 (m, 1H, Ar-H), 7.06–6.96 (m, 1H, Ar-H), 6.92–6.84 (m, 1H, Ar-H), 3.08 (s, 3H, $SO_2CH_3$); HRMS (EI) *m/z* Calcd for $C_{16}H_{11}BrF_2N_2O_2S$ $[M]^+$ 411.9693. Found 411.9659.

### 4.7. 1-(3,4-Difluorophenyl)-2,4-dichloro-5-(4-methylsulfonyl phenyl)imidazole (IIe)

74%; White solid; mp 201 °C; $R_f$ = 0.65 (hexane:acetone = 1:1); $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.89–7.85 (m, 2H, Ar-H), 7.38–7.33 (m, 2H, Ar-H), 7.28–7.20 (m, 1H, Ar-H), 7.13–7.04 (m, 1H, Ar-H), 6.99–6.93 (m, 1H, Ar-H), 3.05 (s, 3H, $SO_2CH_3$); HRMS (EI) *m/z* Calcd for $C_{16}H_{10}Cl_2F_2N_2O_2S$ $[M]^+$ 401.9808. Found 401.9819.

### 4.8. General synthetic conditions for 2-halogeno-1,5-diarylimidazoles

To the solution of (methylthiophenyl)imidazoles (0.4 mmol) in THF (3 mL) was added, at −20 °C, LiHMDS (1 M in THF, 1.2 mL) dropwise. The mixture was stirred for 0.5 h, then halo genating reagent (NCS, NBS or NIS) (1.6 mmol) in THF (3 mL) was added. The mixture was stirred for 0.5 h at −20 °C and 6 h at room temperature. Aqueous $NH_4Cl$ was added to the mixture, and the reaction mixture was extracted with EtOAc. The organic layer was washed with aqueous $NaHSO_3$ and brine, dried over anhydrous $MgSO_4$ and concentrated. To the solution of crude intermediates (1 mmol) in DCM (10 mL) was added, at 0 °C, *m*-chloroperbenzoic acid (0.56 g, 2.5 mmol). The mixture was stirred for 2 h, added more DCM, washed with aqueous $Na_2S_2O_3$, $NaHCO_3$ and brine and dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography with hexane–EtOAc mixture (8:1).

### 4.9. 1-(2,4-Difluorophenyl)-2-chloro-5-(4-methylsulfonyl phenyl) imidazole (Ia)

60%; White solid; mp 183 °C; $R_f$ = 0.55 (hexane:acetone = 1:1); $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.88 (d, $J$ = 8.6 Hz, 2H, Ar-H), 7.59 (s, 1H, $H_4$-imidazole), 7.40 (d, $J$ = 8.6 Hz, 2H, Ar-H), 7.29–7.18 (m, 1H, Ar-H), 7.00–6.90 (m, 2H, Ar-H), 3.06 (s, 3H, $SO_2CH_3$); HRMS (EI) *m/z* Calcd for $C_{16}H_{11}ClF_2N_2O_2S$ $[M]^+$ 368.0198. Found 368.0184.

### 4.10. 1-(3,4-Difluorophenyl)-2-bromo-5-(4-methylsulfonyl phenyl)imidazole (IIa)

40%; White solid; mp 169–170 °C; $R_f$ = 0.45 (hexane:acetone = 1:1); $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.83 (d, $J$ = 8.4 Hz, 2H, Ar-H), 7.36 (s, 1H, $H_4$-imidazole), 7.33–7.22 (m, 1H, Ar-H), 7.24 (d, $J$ = 8.4 Hz, 2H, Ar-H), 7.18–6.90 (m, 2H, Ar-H), 3.04 (s, 3H, $SO_2CH_3$); HRMS (EI) *m/z* Calcd for $C_{16}H_{11}BrF_2N_2O_2S$ $[M]^+$ 411.9693. Found 411.9675.

### 4.11. 1-(3,4-Difluorophenyl)-2-iodo-5-(4-methylsulfonyl phenyl)imidazole (IIb)

85%; White solid; mp 208–209 °C; $R_f$ = 0.35 (hexane:acetone = 1:1); $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.82 (d, $J$ = 8.4 Hz, 2H, Ar-H), 7.41 (s, 1H, $H_4$-imidazole), 7.37–7.21 (m, 1H, Ar-H), 7.23 (d, $J$ = 8.4 Hz, 2H, Ar-H), 7.13–6.98 (m, 2H, Ar-H), 3.03 (s, 3H, $SO_2CH_3$); HRMS (EI) $m/z$ Calcd for $C_{16}H_{11}F_2IN_2O_2S$ $[M]^+$ 459.9554. Found 459.9512.

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## References and notes

1. Needleman, P.; Isakson, P. C. *J. Rheumatol., Suppl.* **1997**, *49*, 6.
2. Black, W. C. *Annu. Rep. Med. Chem.* **2004**, *39*, 125.
3. Khanna, I. K.; Weier, R. M.; Yu, Y.; Xu, X. D.; Koszyk, F. J.; Collins, P. W.; Veenhuizen, A. W.; Perkins, W. E.; Casler, J. J.; Masferrer, J. L.; Zhang, Y. Y.; Gregory, S. A.; Seibert, K.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1634.
4. Almansa, C.; Alfón, J.; de Arriba, A. F.; Cavalcanti, F. L.; Escamilla, I.; Gómez, L. A.; Miralles, A.; Soliva, R.; Bartroli, J.; Carceller, E.; Melos, M.; Garcia-Rafanell, J. *J. Med. Chem.* **2003**, *46*, 3463.
5. Almansa, C.; Bartrolí, J.; Belloc, J.; Cavalcanti, F. L.; Gómez, L. A.; Ramis, I.; Carceller, E.; Merlos, M.; García-Rafanell, J. *J. Med. Chem.* **2004**, *47*, 5579.
6. Truong, N. T.; Sin, K.-S.; Kim, H. P.; Park, H. *Arch. Pharm. Res.* **2005**, *28*, 1013.
7. Chegaev, K.; Lazzarato, L.; Tosco, P.; Cena, C.; Marini, E.; Rolando, B.; Carrupt, P. A.; Fruttero, R.; Gasco, A. *J. Med. Chem.* **2007**, *50*, 1445.
8. Che, H.; Truong, N. T.; Kim, H. P.; Park, H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4035.
9. Barta, T. E.; Stealey, M. A.; Collins, P. W.; Weier, R. M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3443.
10. Biersack, B.; Muthukumar, Y.; Schobert, R.; Sasse, F. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6270.
11. Yang, S.-W.; Smotryski, J.; Matasi, J.; Ho, G.; Tulshian, D.; Greenlee, W. J.; Brusa, R.; Beltramo, M.; Cox, K. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 182.
12. Laughton, M. J.; Evans, P. J.; Moroney, M. A.; Hoult, J. R. S.; Halliwell, B. *Biochem. Pharmacol.* **1991**, *51*, 1673.
13. Carrenõ, M. C.; Ruano, J. L. G.; Sanz, G.; Toledo, M. A.; Urbano, A. *Tetrahedron Lett.* **1996**, *37*, 4081.
14. Chi, Y. S.; Cheon, B. S.; Kim, H. P. *Biochem. Pharmacol.* **2001**, *61*, 1195.