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Synthesis and Biological Evaluation of Curcumin Analogs as Antiplatelet Inhibitor

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The curcuminoid natural products have long been recognized for their medicinal properties and utilized for the treatment of many diseases. Curcuminoids are the major constituents of turmeric (*Curcuma longa* L.) and some other *Curcuma* species, including a number of other plant species. Curcuminoids include curcumin (~77%), demethoxycurcumin (~18%), and bisdemethoxycurcumin (~5%),[1,2] and they exhibit many interesting biological activities such as antioxidant, anti-inflammatory, anticancer, and antiplatelet activities.[3] The cause of thrombosis is commonly associated with platelet aggregation. The effect of curcumin has been reported to reduce platelet aggregation and to increase prostacyclin (PGI_2) as the main factor, and it inhibited the induction of platelet aggregation for adenosine diphosphate (ADP), epinephrine (adrenaline), and collagen. Platelets play a main role in hemostasis in the main defense mechanism in biological systems (*in vivo*). However, hyper-activated platelets increase thrombosis and could develop various cardiovascular diseases such as coronary artery disease, myocardial infarction, and stroke. Conventional anti-platelet drugs such as aspirin, clopidogrel bisulfate, and abciximab have already been proven to treat and prevent cardiovascular diseases, but these drugs also have side effects like bleeding. We decided to use curcumin and its analogs as starting materials in order to prevent side effects. However, curcumin has poor absorption *in vivo*, thus we studied improving its physical properties. It remains unknown whether the activity of curcumin is based on its presumably promiscuous scaffold, or if it results from the Michael acceptor properties of the α,β-unsaturated 1,3-diketone moiety central to its structure. The keto-enol tautomerism and *E/Z* enol stereoisomerism of curcumin result in dynamic equilibria of isomers, which hinder the evaluation of the structure-activity relationships (SARs) of substituted curcumins. We studied the relationship between curcumin-derived pyrazoles of the 1,3-dicarbonyl with various hydrazines[4-7] and curcumin-ester[8,9] of the phenol position. Curcumin-derived pyrazoles were synthesized in order to minimize the metal chelation properties of curcumin. We designed and synthesized new curcumin derivatives. First, known compounds (curcumin, bisdemethoxycurcumin, curcumin-derived pyrazole, and bisdemethoxycurcumin-derived pyrazole) were separated

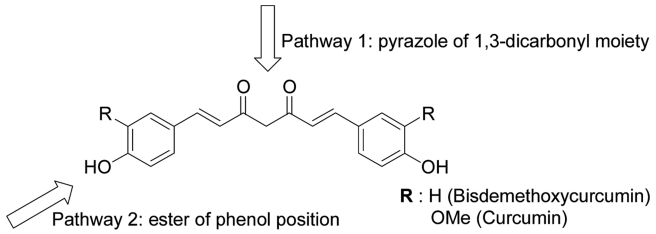
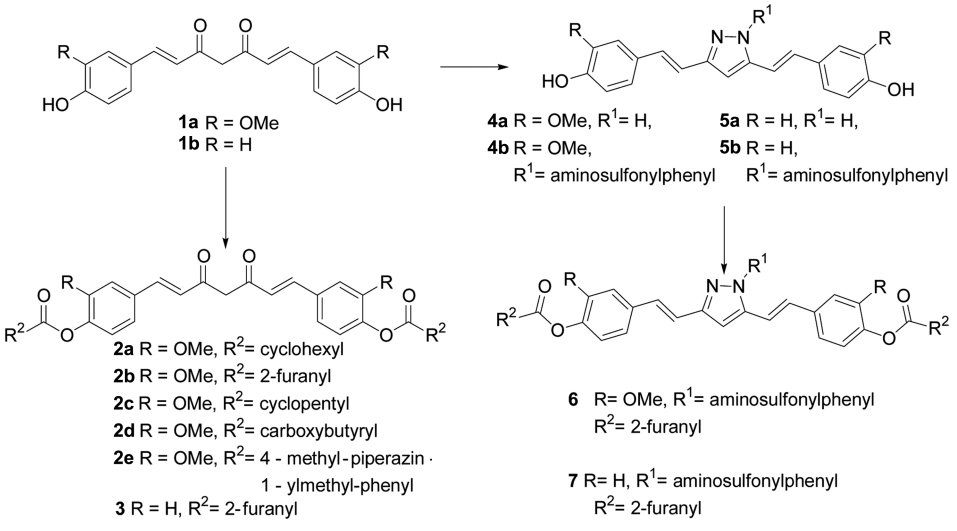


Figure 1. Approaches of curcumin analogs.



Scheme 1. Synthesis of curcumin derivatives.

and synthesized. The main functional group of each compound was substituted and compared in regard to the platelet aggregation inhibition effect. There were two pathways in the approach of the reaction. In one pathway, di-ketone was cyclized to pyrazole with hydrazine derivatives, and the other pathway involved esterification at the phenol position (Figure 1).

The metal chelating properties of diketone can reduce active Fe^{2+} in the blood[10] and the bisphenol influences the reduction of active oxygen. Thus we synthesized three types of compounds. The first type of compound was cyclized to pyrazol, and for the second type esterification was added to the first type of product. The third type of compound was only subjected to esterification. First, curcumin and bisdemethoxycurcumin were separated from commercial curcuminoids with ethanol, and these compounds were used as the control compounds in pharmacological evaluation.

The separated compounds were reacted with appropriate amounts of hydrazine to afford pyrazol derivatives **4a**, **4b**, **5a**, and **5b** in 80-90% yield. The substitution to the phenol position of curcumin and pyrazole derivatives would result in **2a-3** and **6-7** with 30-80% yield. The overall synthetic route is summarized in Scheme 1. The synthesized compounds were biologically evaluated against thrombin, ADP, and collagen using an aggregometer. We used washed platelets from SD rat, and all tests were performed using each compound. As shown in Table 1, compound **2c** showed higher thrombin inhibition than **1a**, **1b**, **4a**, and **5a** as control compounds, and compound **2d** showed high inhibition against thrombin, ADP, and collagen. In case of pyrazole-derives, compound **4b** and **5b** with aromatic group showed lower all inhibitions than unsubstituted pyrazole compound **4a** and **5a**. As a result, hydrogen bonding donor property of pyrazole was required for the inhibition. Also 5-membered ring compound **2c** and 6-membered ring compound **2a** of esterification on the phenol showed the difference in the inhibitory activity.

In summary, new curcumin derivatives were designed and synthesized with a convenient method. We measured the inhibition rate of thrombin, ADP, and collagen. Based on this study, we will study extensive SARs (structure-activity relationships) of the synthesized compounds to obtain better pharmacological profiles with in vitro activity.

Table 1. Inhibitory activities of curcumin derivatives

Compound no.	Thrombin inhibition (%)	ADP inhibition (%)	Collagen Inhibition (%)
1a	26.83	85.12	36.08
1b	77.57	21.75	0.56
2a	17.58	96.2	35.3
2b	23.17	27.8	0.00
2c	100.0	16.4	54.41
2d	90.65	79.16	66.26
2e	12.2	8.8	77.94
3	7.7	15.04	41.4
4a	75.67	23.75	66.67
4b	26.03	8.12	41.89
5a	87.84	38.23	75.14
5b	11.07	15.16	58.66
6	26.03	8.12	47.37
7	16.21	18.65	55.06

Experimental

Preparation of Platelets. Whole blood for experiment was collected from abdominal aorta in male SD rat. And it was anticoagulated with 1 mL of 3.2% sodium citrate solution for the preparation of platelet rich plasma (PRP) and pletelets were activated in the presence of 100 μL of PGE_1 for the preparation of washed platelets (WPs). PRP was prepared by centrifugation for 15 min at 150 g, and platelet-poor plasma was obtained from the precipitated fraction of PRP by centrifugation for 20 min at 2000 g. The platelet count in PRP was adjusted to 1×10^8 cells/mL by using platelet-poor plasma. For preparation of WPs, PRP was centrifuged for 10 min at 500 g, and platelet pellets were washed in Tyrode buffer.

Measurement of Aggregation. Washed platelets were adjusted to 1×10^8 cells/mL with suspension buffer . After incubation with 10 mM of each compound for 3 min at 37 °C, 500 μL of suspention buffer was loaded on the aggregometer and stimulated 20 μL of thrombin (20 u/mL), 20 μL of ADP (1 mM) and 20 μL of collagen (1 mg/mL). Platelet aggregation was measured by light transmission.

Purification of Curcumin and Bisdemethoxycurcumin from Commercial Curcuminoids. A solution of curcuminoids (5.0 g) in ethanol (50 mL) was heated under reflux for 1 h and the mixture was cooled to room temperature and filtered and washed with a small amount of ethanol. 2.6 g of curcumin was obtained and the mother liquid was concentrated under reduced pressure. 50 mL of dichloromethane was added to the residue and heated under reflux for 1 h. The mixture was cooled to room temperature, filtered, and washed with a small amount of dichloromethane. 1.1 g of bisdemethoxycurcumin was obtained.

Synthesis of (1*E*,6*E*)-1,7-Bis(4-cyclopentyloxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (2c) (General procedure). Cyclopentanecarbonyl chloride (1.0 mL, 8.14 mmol) was added to a solution of curcumin (1.0 g, 2.71 mmol) and TEA (1.1 mL, 8.14 mmol) in acetone (10 mL). The reaction mixture was stirred at 0 °C for 0.5 h. The mixture was quenched with water (30 mL) and extracted with EtOAc (30 mL). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. EtOH was added to the residue and stirred overnight at room temperature. The formed solid was filtered and washed with a small amount of EtOH and dried under vacuum at 40 °C. 0.9 g of the title compound was obtained as a dark yellow solid. (yield 66%) ^{1}H NMR (400 MHz DMSO-d_6) δ 7.66 (d, J = 16.0 Hz, 2H), 7.52 (s, 2H), 7.33 (dd, J = 1.6 and 1.2 Hz, 2H), 7.15 (d, J = 8.0 Hz, 2H), 7.00 (d, J = 16.0 Hz, 2H), 6.20 (s, 1H), 3.84 (s, 6H), 3.08-3.01 (m, 2H), 2.00-1.84 (m, 8H), 1.70-1.64 (m,

8H); ^{13}C NMR δ 183.7, 174.4, 151.7, 141.6, 140.3, 134.1, 125.0, 123.7, 121.9, 122.5, 102.2, 56.5, 43.2, 30.0, 25.9; Mass *m/z* 561.6 [M+1].

Synthesis of Pentanedioic Acid Mono-(4-{7-[4-(4-carboxy-butyryl-oxy)-3-methoxy-phenyl]-3,5-dioxo-hepta-1,6-dienyl}-2-methoxyphenyl) (2d) (General procedure). Glutaric anhydride (0.93 g, 8.14 mmol) was added to a solution of curcumin (1.0 g, 2.71 mmol) and TEA (1.1 mL, 8.14 mmol) in dichloromethane (10 mL). The reaction mixture was stirred overnight at room temperature and then concentrated under reduced pressure. EtOAc (20 mL) and water (20 mL) were added to the residue, and the pH of the mixture was adjusted to 4 by dropwise addition of conc. HCl. The combined organic layer extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. IPA (10 mL) was added to the residue and water was added dropwise until a solid was formed. The mixture was stirred overnight at room temperature. The formed solid was filtered and washed with a small amount of IPA and dried under vacuum at 40 °C. 1.0 g of the title compound was obtained as a dark yellow solid. (yield 31%) ^{1}H NMR (400 MHz DMSO-d_6) δ 12.15 (brs, 2H), 7.66 (d, *J* = 16.0 Hz, 2H), 7.33 (dd, *J* = 1.6 and 1.2 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 2H), 7.00 (d, *J* = 16.0 Hz, 2H), 6.21 (s, 1H), 3.87 (s, 1H), 2.63 (t, *J* = 7.2 Hz, 4H), 2.37 (t, *J* = 7.2 Hz, 4H), 1.87 (p, *J* = 7.2 Hz, 4H); ^{13}C NMR δ 183.7, 174.5, 171.2, 151.6, 141.4, 140.3, 134.2, 125.1, 123.8, 121.9, 112.5, 54.5, 32.9, 29.8, 20.5; Mass *m/z* 597.3 [M+1].

Synthesis of 4-{3,5-Bis-[2-(4-hydroxy-3-methoxy-phenyl)-vinyl]-pyrazol-1-yl}-benzenesulfonamide (4b) (General procedure). Catalytic amount of acetic acid was added to a solution of curcumin (10.0 g, 27.1 mmol) and 4-hydrazino-benzenesulfonamide hydrochloride (12.0 g, 54.3 mmol) in EtOH (100 mL). The reaction mixture was heated under reflux for 6 h and cooled to room temperature. The formed solid was filtered and washed with a small amount of EtOH and dried overnight under vacuum at 40 °C. 13.7 g of the title compound was obtained as a yellow solid. (Yield 82%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00 (d, *J* = 8.8 Hz, 2H), 7.77 (d, *J* = 8.8 Hz, 2H), 7.50 (s, 2H), 7.25-7.15 (m, 4H), 7.12 (s, 1H), 7.05 (d, *J* = 16.0 Hz, 1H), 7.02-6.99 (m, 2H), 6.88 (d, *J* = 16.0 Hz, 1H), 6.80 (dd, *J* = 1.2 and 1.6 Hz, 2H), 3.85 (s, 3H), 3.81 (s, 3H); ^{13}C NMR δ 152.33, 148.4, 148.3, 148.0, 147.5, 143.3, 142.9, 142.2, 134.1, 131.9, 128.9, 128.7, 128.2, 127.5, 125.1, 124.9, 121.0, 120.9, 117.5, 111.8, 111.3, 110.1, 102.2, 56.2, 56.1; Mass *m/z* 520.5 [M+1].

(1*E*,6*E*)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-hepta-diene-3,5-dione (1a): ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68 (brs, 2H), 7.55 (d, *J* = 15.6 Hz, 2H), 7.33 (d, *J* = 1.6 Hz, 1H), 7.16 (d, *J* = 8.4 Hz, 2H), 6.83 (d, *J* = 8.4 Hz, 2H), 6.76 (d, *J* = 16.0 Hz, 2H), 6.06 (s, 1H), 3.84 (s, 6H); ^{13}C NMR δ 183.7, 149.8, 148.4, 141.2, 126.8, 123.6, 121.5, 116.2, 111.8, 101.3, 56.1.

(1*E*,6*E*)-1,7-Bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione (1b): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.06 (brs, 2H), 7.58-7.53 (m, 6H), 6.82 (d, *J* = 8.4 Hz, 4H), 6.70 (d, *J* = 16.0 Hz, 2H), 6.04 (s, 1H); ^{13}C NMR δ 183.7, 160.3, 140.8, 130.8, 126.3, 121.2, 116.4, 101.5.

(1*E*,6*E*)-1,7-Bis(4-cyclohexyloxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (2a): ^{1}H NMR (400 MHz $CDCl_3$) δ 7.61 (d, *J* = 16.0 Hz, 2H), 7.16-7.02 (m, 6H), 6.56 (d, *J* = 16.0 Hz, 2H), 5.85 (s, 1H), 3.86 (s, 6H), 2.65-2.58 (m, 2H), 2.17-2.06 (m, 4H), 1.85-1.82 (m, 4H), 1.71-1.58 (m, 6H), 1.42-1.32 (m, 6H); ^{13}C NMR δ 183.1, 173.9, 151.5, 141.7, 140.0, 133.7, 124.1, 123.3, 122.2, 121.1, 111.5, 101.8, 55.9, 43.0, 29.0, 25.8, 25.3; Mass *m/z* 589.6 [M+1].

(1*E*,6*E*)-1,7-Bis-(4-furyloxy-3-methoxyphenyl)-1,6-hepta-diene-3,5-dione (2b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (s, 2H), 7.64 (d, *J* = 16.0 Hz, 2H), 7.41 (d, *J* = 3.6 Hz, 2H), 7.18 (s, 4H), 7.16 (s, 2H), 6.60-6.57 (m, 4H), 5.87 (s, 1H), 3.87 (s, 1H); ^{13}C NMR δ 183.1, 156.2, 151.6, 147.2, 143.7, 140.7, 139.9, 134.3, 124.4, 123.4, 121.1, 119.8, 112.3, 111.6, 101.9, 56.0; Mass *m/z* 557.7 [M+1].

1,7-Bis-[4-(4-4-methyl-piperazin-1-ylmethyl-benzoyloxy)-3-methoxyphenyl]-hepta-1,6-diene-3,5-dione (2e): ^{1}H NMR (400 MHz $CDCl_3$) δ 8.17 (d, *J* = 8.0, 4H), 7.67 (d, *J* = 16.0, 2H), 7.50 (d, *J* = 8.0, 4H), 7.28-7.18 (m, 6H), 6.61 (d, *J* = 16.0, 2H), 5.90 (s, 1H), 3.88 (s, 3H), 3.62 (s, 4H), 2.53 (brs, 16H), 2.34 (s, 6H); ^{13}C NMR δ 183.1, 164.5, 151.7, 144.7, 141.6, 140.0, 134.0, 130.4, 129.1, 127.9, 124.3, 123.5, 121.2, 111.6, 101.9, 62.6, 56.0, 55.1, 53.0, 46.0; Mass *m/z* 801.4 [M+1].

(1*E*,6*E*)-1,7-Bis(4-cyclopentyloxyphenyl)-1,6-heptadiene-3,5-dione (3): ^{1}H NMR (400 MHz DMSO-d_6) δ 7.72 (d, *J* = 8.4 Hz, 4H), 7.65 (d, *J* = 16.0 Hz, 2H), 7.19 (d, *J* = 8.8 Hz, 4H), 6.83 (d, *J* = 16.0 Hz, 2H), 6.20 (s, 1H), 3.09-3.01 (m, 2H), 2.05-1.88 (m, 8H), 1.78-1.66 (m, 8H); ^{13}C NMR δ 183.6, 174.5, 152.7, 139.7, 132.9, 129.7, 125.2, 122.5, 101.5, 44.0, 30.0, 25.8; Mass *m/z* 497.4 [M+1].

4-{(1*E*,1'*E*)-2-[5-(4-Hydroxy-3-methoxystyryl)-1*H*-pyrazole-3-yl]vinyl}-2-methoxyphenol (4a): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.82 (brs, 1H), 9.18 (brs, 2H), 7.14 (s, 2H), 7.04 (d, *J* = 16.0 Hz, 2H), 6.95-6.91 (m, 4H), 6.77 (d, *J* = 8.0 Hz, 2H), 6.62 (s, 1H), 3.83 (s, 6H); ^{13}C NMR δ 157.8, 148.3, 147.2, 130.0, 129.7, 128.8, 128.2, 120.5, 116.1, 109.9, 99.7, 56.0.

4-{(1*E*,1'*E*)-2-[5-(4-Hydroxy-styryl)-1*H*-pyrazole-3-yl]-vinyl}-phenol (5a): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.83 (s, 1H), 9.62 (brs, 2H), 7.38 (d, *J* = 8.8 Hz, 4H), 6.96 (dd, *J* = 14.8 and 16.0 Hz, 4H), 6.79 (d, *J* = 8.4 Hz, 4H), 6.63 (s, 1H); ^{13}C NMR δ 157.7, 130.1, 129.3, 128.0, 118.5, 116.2, 112.9, 99.6.

4-{(1*E*,1'*E*)-2-[5-(4-Hydroxy-styryl)-1-(4-aminosulfonyl-phenyl)-1*H*-pyrazole-3-yl]vinyl}-phenol (5b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.6 (brs, 2H), 8.01 (dd, *J* = 1.6 and 1.6 Hz, 2H), 7.75 (d, *J* = 1.6 and 2.0 Hz, 2H), 7.71 (d, *J* = 8.8 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.22 (t, *J* = 16.0 Hz, 2H), 7.14 (s, 1H), 7.07 (d, *J* = 8.8 Hz, 2H), 6.97 (d, *J* = 16.8 Hz, 2H), 6.82-6.78 (m, 4H); ^{13}C NMR δ 158.6, 158.1, 152.3, 148.8, 143.2, 143.0, 142.1, 136.6, 133.7, 131.6, 128.8, 128.4, 128.1, 127.5, 127.4, 125.1, 117.1, 116.2, 113.8, 112.0, 102.0; Mass *m/z* 460.3 [M+1].

4-{3,5-[2-(4-Furoyloxy-3-methoxyphenyl)-vinyl]-pyrazol-1-yl}-benzenesulfonamide (6): ^{1}H NMR (400 MHz, Acetone-

d_6) δ 8.12 (d, J = 8.4 Hz, 2H), 7.96 (d, J = 1.2 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H), 7.49-7.48 (m, 3H), 7.41-7.37 (m, 3H), 7.30-7.20 (m, 5H), 6.78-6.77 (m, 4H), 3.94 (s, 3H), 3.85 (s, 3H); ^{13}C NMR δ 169.8, 155.9, 151.8, 147.9, 147.8, 143.9, 142.9, 142.6, 142.3, 139.5, 139.1, 136.5, 135.9, 132.6, 130.5, 127.4, 124.7, 123.3, 123.2, 120.7, 119.5, 119.4, 115.8, 112.4, 111.0, 110.1, 102.6, 55.5, 48.9; Mass *m/z* 708.3 [M+1].

4-{3,5-Bis-[2-(4-furoyloxy-phenyl)-vinyl]-pyrazol-1-yl}-benzenesulfonamide (7): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (t, J = 1.6 Hz, 2H), 8.03 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 8.8 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H), 7.69 (d, J = 8.4 Hz, 2H), 7.61-7.58 (m, 2H), 7.53 (s, 2H), 7.43-7.24 (m, 8H), 7.08 (d, J = 16.4 Hz, 2H), 6.83-6.81 (m, 2H); ^{13}C NMR δ 156.8, 151.8, 150.3, 149.9, 149.2, 143.3, 142.8, 141.9, 135.1, 134.6, 130.6, 128.6, 128.2, 127.6, 125.3, 122.8, 120.8, 115.8, 113.3; Mass *m/z* 648.4 [M+1].

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References

1. Jadhav, B. K.; Mahadik, K. R.; Paradkar, A. R. *Chromatographia* **2007**, *65*, 483.
2. Guddadarangavvanahally, K.; Jayaprakasha.; Lingamullu, J. M. R.; Kunnumpurath, K.; Sakariah. *J. Agric. Food. Chem*. **2002**, *50*, 3668.
3. Singh, R.; Rai, D.; Yadav, D.; Bhargava, A.; Balzarrini, J.; Clercq, E. D. *Eur. J. Med. Chem.* **2010**, *45*, 1078.
4. Rajeshwar, N.; Marcus, P.; Stefanie, L.; Karheinz, B.; Sabin, K.; Thomas, D.; Sascha, W.; Eckhard, M.; Boris, S. *ChemMedChem*. **2008**, *3*, 165.
5. Claramunt, R. M.; Bouissane, L.; Cornago, M. P.; Elguero, J.; Radziwon, A.; Medina, C. *Bioorg. Med. Chem*. **2009**, *17*, 1290.
6. Changtam, C.; Hongmanee, P.; Suksamrarn, A. *Eur. J. Med. Chem*. **2010**, *45*, 4446.
7. Shim, J. S.; Kim, D. H.; Jung, H. J.; Kim, J. H.; Lim, D. Y.; Lee, S. K.; Kim, K. W.; Ahn, J. W.; Yoo, J. S.; Rho, J. R.; Shin, J. H.; Kwon, J. R. *Bioorg. Med. Chem*. **2002**, *10*, 2987.
8. Changtam, C.; Koning, H. P.; Ibrahim, H.; Sajid, M. S. *Eur. J. Med. Chem.* **2010**, *45*, 941.
9. Mishra, S.; Narain, U.; Mishra, R.; Misra, K. *Bioorg. Chem.* **2005**, *13*, 1477.
10. Chen, S. Y.; Chen, Y.; Li, Y. P.; Chen, S. S.; Tan, J. H.; Ou, T. M.; Gu, L. Q.; Huang, Z. S. *Bioorg. Med. Chem.* **2011**, *19*, 5596.