Efficient Synthesis and *In Vitro* Biological Evaluation of 2,5-Diaryloxazoles as Potential Nitric Oxide Production Inhibitors

Ha Young Jang,[†] Kongara Damodar,[†] Jin-Kyung Kim,[‡] and Jong-Gab Jun[†,*]

[†]*Department of Chemistry and Institute of Applied Chemistry, Hallym University, Chuncheon 24252, Korea. *E-mail: jgjun@hallym.ac.kr*
[‡]*Department of Biomedical Science, College of Natural Science, Catholic University of Daegu, Gyeungsan-Si 38430, South Korea*
Received June 28, 2017, Accepted October 31, 2017, Published online November 23, 2017

An efficient first synthesis of 2,5-diaryloxazoles **1**–**5** was accomplished from commercially inexpensive precursors and in overall yields of 38–48%. The synthesis proceeds via α-aminoketones and cyclodehydration (Robinson–Gabriel reaction) as key step. Next, these oxazoles were examined for their inhibitory effect against nitric oxide (NO) production in lipopolysaccharide (LPS)-induced RAW 264.7 cells and were found to display concentration-dependent inhibition of NO production without cytotoxicity. Of note, compound **3** (70.7%; IC_{50} = 2.33 μM) was identified as a potent inhibitor in view of its comparable inhibitory effect with the positive control, N^G-monomethyl-L-arginine acetate (L-NMMA) (79.3%; IC_{50} = 4.51 μM) followed by compounds **5** (68.3%; IC_{50} = 2.30 μM) and **2** (53.9%; IC_{50} = 6.31 μM). As a whole, compound **3** may hold great promise for further development of NO production targeted anti-inflammatory agent.

Keywords: 2,5-Diaryloxazoles, α-Aminoketones, Robinson–Gabriel reaction, Nitric oxide, RAW-264.7 macrophages, Anti-inflammatory

Introduction

Inflammation is a protective and appropriate physiological response induced by endogenous and exogenous insults such as infection or injury. However, dysregulation of this response may cause various pathophysiological conditions including cardiovascular diseases, rheumatoid arthritis, bronchitis, diabetes, neurodegenerative disorders, and cancer.[1,2] Activated macrophages, lymphocytes, and neutrophils are important that have been involved in the pathogenesis of acute and chronic inflammatory diseases. Activated macrophages secrete a number of potent bioactive inflammatory mediators, including nitric oxide (NO), leukotrienes (LTs), prostaglandins (PGs), and cytokines such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor α (TNF-α).[3,4] Among these, NO, a gaseous free radical, produced by nitric oxide synthase (endothelial-NOS, neuronal-NOS, and inducible-NOS) has a key role in the pathogenesis of inflammation and its role in this process is tightly depended upon its concentration. Under normal physiological conditions, NO has anti-inflammatory effects. However, excess production of NO by inducible-NOS is implicated in the pathogenesis of afore mentioned inflammatory diseases.[5] Hence, tissue and/or site-specific inhibition of NO will open up new avenues for the future management of inflammation-based diseases.

Current strategies to alleviate inflammation chiefly relied on corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), selective COX-2 inhibitors (COXIBs), and antihistamines, which work either by interrupting the synthesis or action of mediators that drive the host's response to injury. Despite notable successes from these small-molecule inhibitors, still they are not without their shortcomings such as gastric ulceration, renal toxicity, joint destruction, and cardiovascular disorders.[6] Thus, there exist an unmet need for novel anti-inflammatory drugs.

Oxazole, a five-membered heterocyclic aromatic compound, is one of the important scaffolds existing in a wide variety of biologically active compounds and natural products.[7,8] In recent years, its research has gained considerable attention from both industry and academia in view of its manifold pharmacological properties.[9] Some oxazoles also exhibited enzyme inhibitory activity. [9,10] It is reported that substituted oxazoles can be used as agrochemicals, fluorescent dyes, corrosion inhibitors and find applications in photography and polymer industries.[9] In addition, they can serve as important synthons in organic synthesis.[11] Particularly, 2,5-substituted aryl or alkyl oxazoles are privileged scaffolds found in numerous natural products and pharmaceutical agents.[12] They have been investigated as antidiabetic, anticancer, and antibacterial agents.[9]

2,5-Diaryloxazoles (**1**–**5**) under the current study are depicted in Figure 1. Compounds **2** and **4** were isolated from the roots of *Oxytropis lanata*[13] and compound **2** displayed the most potent trypanocidal activity (IC_{50} 1.0 μM) whereas compounds **1**, **3**, and **5** are new synthetic 2,5-diaryloxazoles.

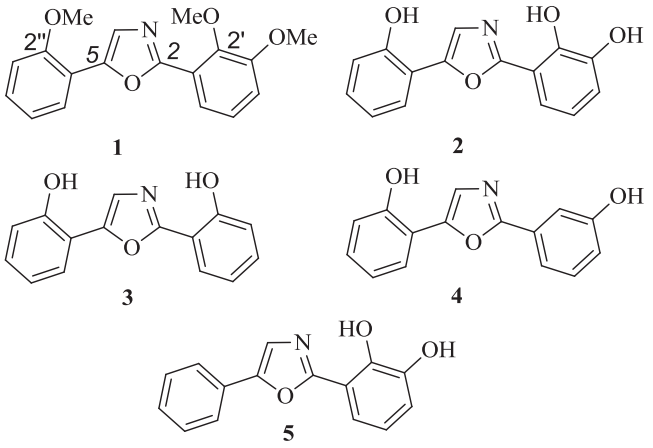


Figure 1. Structures of 2,5-diaryloxazoles (**1**–**5**).

In continuation of our research efforts directed towards the synthesis of bioactive natural products and their analogues as NO inhibitors,[14] herein, we report an efficient synthesis and *in vitro* biological assessment of oxazoles **1**–**5**.

Experimental

All chemicals were purchased from local sources and were used without further purification unless noted otherwise. All solvents used for reactions were freshly distilled from proper dehydrating agents under nitrogen atmosphere. All solvents used for chromatography were purchased and directly used without further purification. Thin-layer chromatography (TLC) was performed on DC-Plastikfolien 60, F_{254} (layer thickness 0.2 mm; Merck, Darmstadt, Germany) plastic-backed *silica* gel plates and visualized by UV light (254 nm) or staining with *p*-anisaldehyde and phosphomolybdic acid (PMA) stain. Chromatographic purification was carried out using Kieselgel 60 (60–120 mesh; Merck). ^{1}H NMR spectra were recorded at Varian Mercury-300 MHz FT-NMR (Varian, Inc., Palo Alto, CA, USA) and 75 MHz for ^{13}C, with the chemical shift (δ) reported in parts per million (ppm) downfield relative to tetramethylsilane and the coupling constants (J) quoted in hertz (Hz). Peak splitting patterns were abbreviated as s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublet), and m (multiplet) and $CDCl_3$/CD_3COCD_3 was used as a solvent and an internal standard. High-resolution mass spectra electrospray ionization (HRMS-ESI) was obtained on an Agilent 6220 TOF LC/MS spectrometer (Agilent Technologies, Santa Clara, CA, USA) spectrometer. Melting points were measured on a MEL-TEMP II (Triad Scientific, Manasquan, NJ, USA) apparatus and were uncorrected.

2-Bromo-1-(2-Methoxyphenyl)Ethanone (7a)**.** Copper (II) bromide (2.97 g, 6.66 mmol) was placed in a two-necked round-bottomed flask fitted with a reflux condenser. EtOAc (10.0 mL) was added to it and the mixture was stirred at 70°C for 5 min under nitrogen atmosphere. 1-(2-Methoxyphenyl)ethanone (**6**) (0.50 g, 3.33 mmol) in $CHCl_3$ (10.0 mL) was slowly added to it and then the mixture was refluxed for 8 h. After completion of the reaction, it was cooled to room temperature, filtered through Celite® pad, and washed with EtOAc (20 mL). The filtrate was concentrated under reduced pressure. The crude was purified by column chromatography (EtOAc:hexane = 1:4) to afford the pure product **7a** (0.73 g, 96%) as brown liquid. R_f = 0.43 (EtOAc/hexane = 1/4); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.81 (1H, dd, J = 7.8, 1.8 Hz), 7.52 (1H, td, J = 7.8, 1.8 Hz), 7.05–6.96 (2H, m), 4.61 (2H, s), 3.94 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 192.3, 158.8, 134.9, 131.6, 124.9, 121.2, 111.7, 56.0, 38.0.

General Procedure for Delépine Reaction. To a stirred solution of 2-methoxyphenacyl bromide (**7a**)/phenacyl bromide (**7b**) (1 mmol, 1 equiv) in diethyl ether (13 mL) was added hexamethylenetetramine (1 mmol, 1 equiv) all at once and the mixture was stirred for 12 h at room temperature (solid formation observed). The resulting solid was filtered, washed with diethyl ether (15 mL), and dried under reduced pressure to afford the quaternary salt, which was next placed in a two-necked round-bottomed flask fitted with reflux condenser and EtOH (22 mL) was added to it. Concentrated HCl (0.6 mL) was added to it and the mixture was refluxed for 3 h (solid formed). After cooling to room temperature, the solid was filtered, washed with EtOH (20 mL), and dried under vacuum to afford pure 2-methoxybenzoylmethylammonium chloride (**8a**)/benzoylmethylammonium chloride (**8b**) salt.

*2-Amino-1-(2-methoxyphenyl)ethanone hydrochloride (**8a**)*: Yield: 88%; brown solid; mp 102–104°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26 (1H, d, J = 8.7 Hz), 7.11 (1H, t, J = 7.2 Hz), 4.33 (2H, q, J = 5.4 Hz), 3.94 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 192.1, 159.6, 135.9, 130.0, 120.7, 112.9, 56.1, 48.7.

*2-Amino-1-phenylethanone hydrochloride (**8b**):* Yield: 97%; brown solid; mp 180–182°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 (3H, br s), 7.84 (1H, d, J = 7.8 Hz), 7.67 (1H, t, J = 7.8 Hz), 7.26 (1H, d, J = 8.7 Hz), 7.11 (1H, t, J = 7.2 Hz), 4.33 (2H, q, J = 5.4 Hz), 3.94 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 192.1, 159.6, 135.9, 130.0, 120.7, 112.9, 56.1, 48.7.

General Procedure for Synthesis of 2,5-Diaryloxazole. First step: To a stirred solution of substituted benzoic acid (**9a/9b/9c**) (1.0 mmol) in anhydrous DMF (6 mL) was added thionyl chloride (3.43 mL, 2.0 mmol) dropwise at 0°C under nitrogen atmosphere. The reaction mixture was then warmed to at 60°C and stirred for 2 h. After completion of the reaction, cooled to room temperature, solvent and excess thionyl chloride was removed under reduced pressure. The crude compound was dried under vacuum for 1 h and was used in the next step.

Second step: Pyridine (4 mL) was added to 2-methoxybenzoylmethylammonium chloride (**8a**)/benzoylmethylammonium chloride (**8b**) salt (0.5 mmol) and stirred for 10 min at room temperature. To this mixture, above

corresponding acid chloride was added slowly and stirred for 1 h under nitrogen atmosphere. After completion of the reaction, water (4 mL) was added, neutralized with 3 N HCl, and extracted with EtOAc (2 × 20 mL). The combined organic layer was washed with water (2 × 15 mL), brine (15 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. To this crude, acetic anhydride (3 mL) followed by conc. H_2SO_4 (0.1 mL) were added at room temperature and the mixture was stirred at 90°C for 1 h. After completion of the reaction, cooled to room temperature, H_2O (5 mL) was added. The mixture was neutralized with saturated aqueous $NaHCO_3$ and extracted with EtOAc (2 × 25 mL). The combined organic layer was washed with water (2 × 15 mL), brine (15 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude was purified by column chromatography (EtOAc:hexane = 1:3) to afford the pure oxazole product.

*2-(2,3-Dimethoxyphenyl)-5-(2-methoxyphenyl)oxazole (**1**)*: Yield: 59%; pale yellow solid; mp 146–148°C; R_f = 0.56 (EtOAc/hexane = 1/1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (1H, dd, *J* = 7.8, 1.5 Hz), 7.68 (1H, s), 7.63 (1H, dd, *J* = 7.8, 1.5 Hz), 7.29 (1H, td, *J* = 8.7, 1.8 Hz), 7.14 (1H, t, *J* = 8.1 Hz), 7.05 (1H, td, *J* = 7.8, 0.9 Hz), 6.96–7.01 (2H, m), 3.99 (3H, s), 3.98 (3H, s), 3.91 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.3, 155.7, 153.8, 147.8, 147.7, 129.0, 127.5, 125.9, 124.4, 122.2, 121.5, 120.9, 117.4, 114.1, 110.9, 61.4, 56.2, 55.7; HRMS-ESI *m/z* calcd. for $C_{18}H_{18}NO_4$ $[M + H]^+$: 312.1236, found 312.1229.

*2,5-bis(2-Methoxyphenyl)oxazole (**10a**)*: Yield: 61%; pale yellow solid; mp 110–112°C; R_f = 0.44 (EtOAc/hexane = 1/1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04 (1H, d, *J* = 7.5 Hz), 7.87 (1H, d, *J* = 7.5 Hz), 7.68 (1H, s), 7.42 (1H, t, *J* = 7.8 Hz), 7.29 (1H, t, *J* = 7.8 Hz), 7.08–7.03 (3H, m), 6.97 (1H, d, *J* = 8.1 Hz), 4.01 (3H, s), 3.97 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.4, 157.6, 155.7, 147.2, 131.5, 130.1, 128.9, 127.7, 126.0, 120.9, 120.7, 117.5, 116.7, 112.1, 110.9, 56.3, 55.7; HRMS-ESI *m/z* calcd. for $C_{17}H_{16}NO_3$ $[M + H]^+$: 282.1130, found 282.1136.

*5-(2-Methoxyphenyl)-2-(3-methoxyphenyl)oxazole (**10b**)*: Yield: 47%; pale yellow solid; mp 114–116°C; R_f = 0.75 (EtOAc/hexane = 1/1); ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.87 (1H, d, *J* = 7.5 Hz), 7.70 (1H, d, *J* = 7.8 Hz), 7.63 (2H, s), 7.40–7.24 (2H, m), 7.06 (1H, t, *J* = 7.5 Hz), 6.98 (2H, d, *J* = 8.1 Hz), 3.98 (3H, s), 3.89 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.0, 159.9, 155.8, 147.9, 130.0, 129.2, 128.9, 127.7, 125.9, 120.9, 118.9, 117.3, 116.8, 111.1, 111.0, 55.7; HRMS-ESI *m/z* calcd. for $C_{17}H_{16}NO_3$ $[M + H]^+$: 282.1130, found 282.1143.

*2-(2,3-Dimethoxyphenyl)-5-phenyloxazole (**10c**)*: Yield: 56%; pale yellow solid; mp 86–88°C; R_f = 0.48 (EtOAc/hexane = 1/1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71 (2H, d, *J* = 8.4 Hz), 7.61 (1H, dd, *J* = 8.1, 1.5 Hz), 7.48 (1H, s), 7.43 (1H, t, *J* = 7.2 Hz), 7.33 (1H, d, *J* = 7.5 Hz), 7.15 (1H, t, *J* = 8.1 Hz), 7.01 (1H, d, *J* = 8.1 Hz),4.01 (3H, s), 3.92 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.4, 153.9, 151.3, 147.8, 129.0, 128.5, 128.3, 124.4, 124.3, 123.4, 122.2, 121.4, 114.4, 61.5, 56.3; HRMS-ESI *m/z* calcd. for $C_{17}H_{16}NO_3$ $[M + H]^+$: 282.1130, found 282.1135.

General Procedure for Demethylation of 2,5-Diaryloxazole. To a stirred solution of oxazole (0.2 mmol) in anhydrous CH_2Cl_2 (5 mL) was added BBr_3 solution (1.0 M in CH_2Cl_2, 1.0 mL, 5.00 mmol, 5.0 equiv) slowly at 0°C under nitrogen atmosphere. The reaction was warmed to room temperature and stirred for 2–6 h. After completion of the reaction, excess BBr_3 was quenched by the slow addition of MeOH (1 mL), stirred for 20 min and then solvent was removed under reduced pressure. H_2O (5 mL) and CH_2Cl_2 (15 mL) were added to the crude and two layers separated. Aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to afford the product (*Note*: For compound **2** preparation from **1**, 10.0 equiv of BBr_3 was used instead of 5.0 equiv).

*2-(2′,3′-Dihydroxyphenyl)-5-(2″-hydroxyphenyl)oxazole (**2**)*: Yield: 95%; white solid; mp 174–176°C; R_f = 0.32 (EtOAc/hexane = 1/1); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 7.92 (1H, d, *J* = 7.2 Hz), 7.78 (1H, s), 7.54 (1H, d, *J* = 7.5 Hz), 7.26 (1H, t, *J* = 7.2 Hz), 6.99–7.01 (3H, m), 6.90 (1H, t, *J* = 7.8 Hz); ^{13}C NMR (75 MHz, CD_3COCD_3): δ 160.5, 154.6, 148.0, 146.7, 146.2, 130.3, 126.4, 125.2, 120.8, 120.5, 118.5, 117.0, 116.6, 115.5, 111.8; HRMS-ESI *m/z* calcd. for $C_{15}H_{12}NO_4$ $[M + H]^+$: 270.0766, found 270.0760.

*2,2′-(Oxazole-2,5-diyl)diphenol (**3**)*: Yield: 91%; white solid; mp 184–186°C; R_f = 0.30 (EtOAc/hexane = 1/1); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 11.21 (1H, s), 9.59 (1H, s), 8.04 (1H, d, *J* = 8.2 Hz), 7.93 (1H, d, *J* = 7.8 Hz), 7.77 (1H, s), 7.38 (1H, t, *J* = 7.8 Hz), 7.25 (1H, t, *J* = 7.5 Hz), 7.08–7.00 (4H, m); ^{13}C NMR (75 MHz, CD_3COCD_3): δ 160.2, 158.1, 154.6, 148.1, 133.0, 130.4, 126.7, 126.5, 125.3, 120.8, 120.3, 117.6, 116.6, 115.5, 111.7; HRMS-ESI *m/z* calcd. for $C_{15}H_{12}NO_3$ $[M + H]^+$: 254.0817, found 254.0806.

*2-(2-(3-Hydroxyphenyl)oxazol-5-yl)phenol (**4**)*: Yield: 98%; white solid; mp 240–242°C; R_f = 0.33 (EtOAc/hexane = 1/1); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 9.44 (1H, br s), 8.77 (1H, br s), 7.87 (1H, d, *J* = 8.1 Hz), 7.67 (2H, s), 7.63 (1H, d, *J* = 7.5 Hz), 7.36 (1H, t, *J* = 8.1 Hz), 7.21 (1H, t, *J* = 7.8 Hz), 6.97–7.06 (3H, m); ^{13}C NMR (75 MHz, CD_3COCD_3): δ 160.2, 158.5, 154.5, 148.9, 130.9, 129.8, 129.7, 127.9, 126.3, 120.8, 118.1, 116.6, 116.2, 113.5; HRMS-ESI *m/z* calcd. for $C_{15}H_{12}NO_3$ $[M + H]^+$: 254.0817, found 254.0823.

*3-(5-Phenyloxazol-2-yl)benzene-1,2-diol (**5**)*: Yield: 88%; white solid; mp 134–136°C; R_f = 0.25 (EtOAc/hexane = 1/1); ^{1}H NMR (300 MHz, CD_3COCD_3): δ 11.20 (1H, s), 8.02 (1H, s), 7.87 (2H, dd, *J* = 6.9, 1.5 Hz), 7.79 (1H, s), 7.52–7.48 (3H, m), 7.42 (1H, dd, *J* = 6.9, 1.5 Hz), 7.00 (1H, dd, *J* = 7.8, 1.2 Hz), 6.90 (1H, td, *J* = 7.8, 1.2 Hz); ^{13}C NMR (75 MHz, CD_3COCD_3): δ 161.7, 151.0, 146.7, 146.3, 129.8, 129.6, 128.1, 125.0, 122.2, 120.5, 118.7,

117.1, 111.7; HRMS-ESI *m/z* calcd. for $C_{15}H_{12}NO_3$ $[M + H]^+$: 254.0817, found 254.0810.

Results and Discussion

Our approach to synthesize the oxazole derivatives **1–5** is outlined in Scheme 1. α-Bromination of 2′-methoxyacetophenone (**6**) using $CuBr_2$ afforded 2′-methoxyphenacyl bromide (**7a**) in 96% yield. Next compound **7a** and phenacyl bromide (**7b**) were converted as α-aminoketones (Delépine reaction).[15] Treatment of bromo compounds **7a** and **7b** with hexamethylenetetramine gave the quaternary salts, which were subsequently underwent acid hydrolysis to furnish 4-methoxybenzoylmethylammonium chloride (**8a**) and benzoylmethylammonium chloride (**8b**) in 88 and 97% yields, respectively.

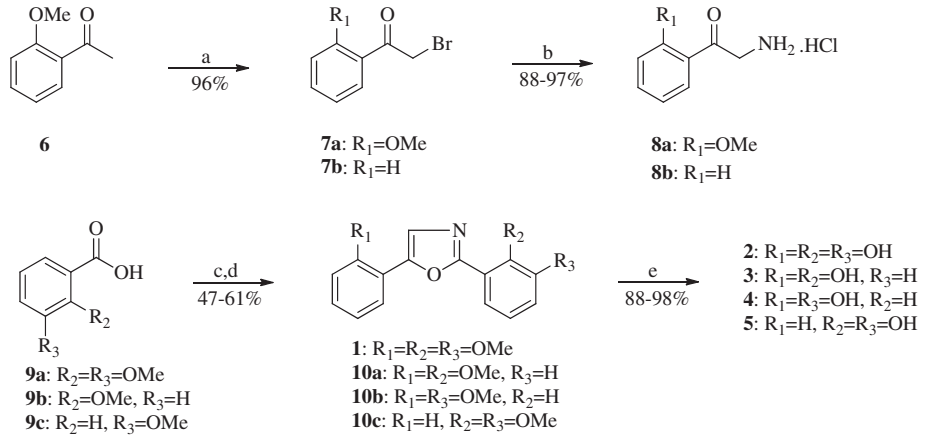
Next, substituted benzoyl chlorides obtained from their corresponding benzoic acids (**9a–9c**) reacted with either **8a** or **8b** in the presence of pyridine to give 2-acylamino-ketones, which subsequently underwent cyclodehydration (Robinson–Gabriel reaction)[16] to deliver the corresponding 2,5-diaryloxazoles **1** and **10a–10c** in 47–61% yields over three steps. Finally, addition of BBr_3 solution (1.0 M in CH_2Cl_2) to compounds **1** and **10a–10c** provided **2–5**, respectively, in 88–98% yields. All the target compounds **1–5** were settled from their NMR (^1H and ^{13}C) and MS data.

Inhibition of NO Production. The inhibitory effect of the prepared oxazoles **1–5** against NO production was analyzed by measuring the amount of NO in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells, as an indicator of anti-inflammatory activity, following our previous procedure.[14b] Indeed, quantification of NO is difficult intrinsically due to the existence of other scavenging molecules and transient nature of NO. Therefore, NO levels have been indirectly evaluated via analysis of its stable oxidation product, nitrite (NO_2^-) concentration in culture supernatant using the acidic Griess reagent. N^G-Monomethyl-L-arginine acetate (L-NMMA)[17] was used as a positive control.

Effect of compounds **1–5** on NO generation by induced macrophages was monitored (Table 1). When the cells were treated with LPS along with 1 and 10 μM concentration of compounds, concentration-dependent inhibition of NO production was observed. As shown in Table 1, the percentage of inhibition was impressive at the highest concentration (10 μM). Of note, compound **3** showed the highest percentage of inhibition (70.7%) at 10 μM followed by compounds **5** (68.3%) and **2** (53.9%) when compared to the positive control, L-NMMA (79.3%). However, trimethoxy analogue (**1**) of **2** and also compound **4** had almost no inhibitory effect at 10 μM also. Structure–activity relationships of these compounds against NO release indicated that C2-aryl group of oxazoles containing hydroxyl functionality (–OH) in the position 2 showed essential role for the inhibitory activity. Next, cell viability was determined with the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method to ensure that the observed NO inhibition was not due to cytotoxic effects. As a result, no cytotoxic effects were observed at the highest concentrations (10 μM) on RAW 264.7 cells. The IC_{50} values of compounds **1–5** were evaluated by GraphPad Prism 4.0 software and displayed >100, 6.31, 2.33, 24.83, and 2.30 μM, respectively (Table 1). From these pharmacological results, compound **3** strongly inhibits LPS-induced NO production without considerable cytotoxicity and hence appears as a promising NO production inhibitor candidate.

Conclusion

We have accomplished an efficient first synthesis of 2,5-diaryloxazoles **1–5** using commercially inexpensive precursors and in overall yields of 38–48%. The synthesis involves α-aminoketones as intermediates and cyclodehydration of α-acylamino ketones (Robinson–Gabriel reaction) as key step. Next, these oxazoles were examined for



Scheme 1. Synthesis of 2,5-diaryloxazoles **1–5**. *Reagents and conditions:* (a) $CuBr_2$, EtOAc/$CHCl_3$ (1:1), reflux, 8 h. (b) (i) Hexamethylenetetramine, ether, rt, overnight; (ii) conc. HCl, EtOH, reflux, 3 h. (c) $SOCl_2$, anhydrous DMF, 60°C, 2 h. (d) (i) **8a** (or **8b**), pyridine, rt, 1 h; (ii) H_2SO_4, Ac_2O, 90°C, 1 h. (e) BBr_3 (1.0 M in CH_2Cl_2), 0°C to rt, 2–6 h.

Table 1. NO production inhibitory effects of 2,5-diphenyloxazoles (**1**–**5**).

	No production (% inhibition)[a,b]		Proliferation[a]		
Compound	1 μM	10 μM	1 μM	10 μM	IC_{50} (μM)
Medium	15.8 ± 0.3 (74.2)	15.8 ± 0.3 (74.2)	100.0 ± 4.2	100.0 ± 4.2	
LPS	100.0 ± 6.4 (0.0)	100.0 ± 6.4 (0.0)			
1	127.0 ± 2.4 (−27.0)**	121.7 ± 2.6 (−21.7)*	109.4 ± 4.5	105.2 ± 4.2	>100.0
2	126.8 ± 3.1 (−26.8)**	46.1 ± 2.6 (53.9)***	104.7 ± 2.0	98.4 ± 1.8	6.31
3	128.0 ± 2.4 (−28.0)**	29.3 ± 1.0 (70.7)***	98.3 ± 3.3	91.0 ± 5.1	2.33
4	123.6 ± 2.9 (−23.6)*	108.7 ± 1.3 (−8.7)	86.5 ± 3.4	84.0 ± 2.2*	24.83
5	101.7 ± 4.1 (−1.7)	31.7 ± 2.6 (68.3)***	89.3 ± 4.1	79.4 ± 1.4*	2.30
L-NMMA	92.5 ± 0.2 (7.5)	20.7 ± 1.3 (79.3)***	102.2 ± 2.5	100.7 ± 1.8	4.51

[a] The results are reported as mean value ± SEM for $n = 3$. Statistical significance is based on the difference when compared with LPS-treated groups.
[b] % Inhibition is based on LPS as shown in parenthesis.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

their inhibitory effect against NO production in LPS-induced RAW 264.7 cells and were found to display concentration-dependent inhibition of NO production without cytotoxicity. Of note, compound **3** (70.7%; IC_{50} = 2.33 μM) was identified as a potent inhibitor, which was in close comparison with the positive control, L-NMMA (79.3%; IC_{50} = 4.51 μM) followed by compounds **5** (68.3%; IC_{50} = 2.30 μM) and **2** (53.9%; IC_{50} = 6.31 μM). As a whole, compound **3** may hold great promise for further development of NO production targeted anti-inflammatory agent.

Acknowledgments. This research was financially supported by industrial co-work program with SunChem (SunKyung Chemical Co., South Korea).

References

1. C. Nathan, A. Ding, *Cell* **2010**, *140*, 871.
2. P. Libby, *Nutr. Rev.* **2007**, *65*, 140.
3. M. D. Turner, B. Nedjai, T. Hurst, D. J. Pennington, *Biochim. Biophys. Acta* **2563**, *2014*, 1843.
4. R. Ostuni, I. Zanoni, F. Granucci, *Cell. Mol. Life Sci.* **2010**, *67*, 4109.
5. M. L. Lo Faro, B. Fox, J. L. Whatmore, P. G. Winyard, M. Whiteman, *Nitric Oxide* **2014**, *41*, 38.
6. D. W. Gilroy, T. Lawrence, M. Perretti, A. G. Rossi, *Nat. Rev. Drug Discov.* **2004**, *3*, 401.
7. D. C. Palmer, *Oxazoles: Synthesis, Reactions, and Spectroscopy, Part A*. Chemistry of Heterocyclic Compounds, John Wiley & Sons, Hoboken, NJ, **2003**, p. 60.
8. (a) V. S. C. Yeh, *Tetrahedron* **2004**, *60*, 11995. (b) Z. Jin, *Nat. Prod. Rep.* **2013**, *30*, 869.
9. A. Ibrar, I. Khan, N. Abbas, U. Farooqa, A. Khana, *RSC Adv.* **2016**, *6*, 93016.
10. J. Senger, J. Melesina, M. Marek, C. Romier, I. Oehme, O. Witt, W. ippl, M. Jung, *J. Med. Chem.* **2016**, *59*, 1545.
11. V. S. C. Yeh, *Tetrahedron* **2016**, *60*, 11995.
12. (a) A. Nishida, M. Fuwa, S. Naruto, Y. Sugano, H. Saito, M. Nakagawa, *Tetrahedron Lett.* **2000**, *41*, 4791. (b) T. Chatterjee, J. Y. Cho, E. J. Cho, *J. Org. Chem.* **2016**, *81*, 6995.
13. O. Banzragchgarav, T. Murata, G. Odontuya, B. Buyankhishig, K. Suganuma, B. Davaapurev, N. Inoue, J. Batkhuu, K. Sasaki, *J. Nat. Prod.* **2016**, *79*, 2933.
14. (a) H. Y. Jang, H. J. Park, K. Damodar, J. -K. Kim, J. -G. Jun, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 5438. (b) K. Damodar, J. -K. Kim, J. -G. Jun, *Tetrahedron Lett.* **2017**, *58*, 50.
15. Z. Wang, *Comprehensive Organic Name Reactions and Reagents, Delépine Reaction*, Vol. *1*, John Wiley & Sons, Hoboken, NJ, **2010**, p. 865.
16. M. Pulici, F. Quartieri, E. R. Felder, *J. Comb. Chem.* **2005**, *7*, 463.
17. (a) L. Salerno, V. Sorrenti, C. Di Giacomo, G. Romeo, M. A. Siracusa, *Curr. Pharm. Des.* **2002**, *8*, 177. (b) C. S. Kim, L. Subedi, J. Oh, S. Y. Kim, S. U. Choi, K. R. Lee, *J. Nat. Prod.* **2017**, *80*, 1134.

