MEDICINAL CHEMISTRY RESEARCH

CrossMark

ORIGINAL RESEARCH

Synthesis and inhibitory effects of triarylpyrazoles on LPS-induced NO and PGE$_2$ productions in RAW 264.7 macrophages

Byung-Jun Park[1] · Mohammed I. El-Gamal[2,3,4] · Woo-Suck Lee[5,6] · Ji-Sun Shin[5,6] · Kyung Ho Yoo[7] · Kyung-Tae Lee[5,6] · Chang-Hyun Oh[1,8]

Abstract The inhibition of nitric oxide and prostaglandin E$_2$ productions is a very interesting research topic in the field of anti-inflammatory drug development. In the current study, a new series of 1,3,4-triarylpyrazole derivatives was synthesized and evaluated for their capabilities to inhibit nitric oxide and prostaglandin E$_2$ productions in lipopolysaccharide-induced RAW 264.7 macrophages. Among all the target analogs, the diarylurea hydroxyl compounds **1f** and **1h** possessing phenyl and 3-(trifluoromethyl)phenyl terminal moiety, respectively, showed the highest inhibitory effect on the production of prostaglandin E$_2$. Both compounds exerted equal activity to the reference compound NS-398 at 3 μM concentration. This effect was due to inhibition cyclooxygenase-2 enzyme activity not inhibition of cyclooxygenase-2 protein expression. The IC$_{50}$ value of compound **1f** against lipopolysaccharide-induced prostaglandin E$_2$ production in the macrophages was 1.12 μM. In addition, compound **1j** with urea linker, hydroxyl group, and 3,5-bis(trifluoromethyl)phenyl terminal ring was the strongest nitric oxide inhibitor. Western blot study showed that it exerted that effect through inhibition of inducible nitric oxide synthase protein expression.

Byung-Jun Park and Mohammed I. El-Gamal have contributed equally to this work.

✉ Kyung-Tae Lee
   ktlee@khu.ac.kr

✉ Chang-Hyun Oh
   choh@kist.re.kr

[1] Center for Biomaterials, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea

[2] Department of Medicinal Chemistry, College of Pharmacy, University of Sharjah, Sharjah 27272, United Arab Emirates

[3] Sharjah Institute for Medical Research, University of Sharjah, Sharjah 27272, United Arab Emirates

[4] Department of Medicinal Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt

[5] Department of Pharmaceutical Biochemistry, College of Pharmacy, Kyung Hee University, 1 Hoegi-dong, Dongdaemun-gu, Seoul 130-701, Republic of Korea
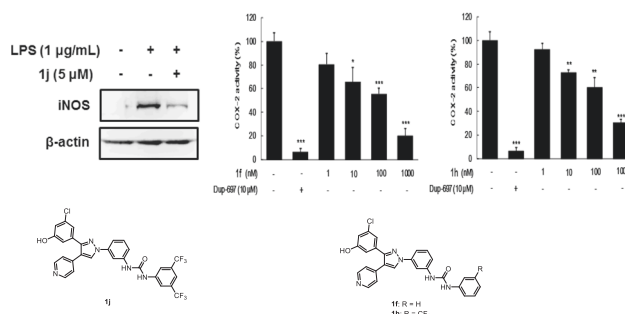
[6] Department of Life and Nanopharmaceutical Science, College of Pharmacy, Kyung Hee University, 1 Hoegi-dong, Dongdaemun-gu, Seoul 130-701, Republic of Korea

[7] Chemical Kinomics Research Center, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea

[8] Department of Biomolecular Science, University of Science and Technology, 113 Gwahangno, Yuseong-gu, Daejeon 305-333, Republic of Korea

Graphical Abstract

Keywords Anti-inflammatory · Diarylurea · Nitric oxide · PGE$_2$ · Pyrazole

Springer

Introduction

Inflammation is a complex physiological and pathological process associated with activation of the local vascular system, the immune system, and various cells within the damaged tissue (Coussens and Werb 2002). It is a normal protective response to tissue injury caused by physical trauma (cut, burn, or bruise), microbiological agents, noxious chemicals, or even autoimmune disorders. Acute inflammation is a part of the defense response by the organisms to remove injurious stimuli, such as pathogens, irritants, or physical injury, from tissues and to initiate the healing process. However, persistent and excessive immune response can promote tissue damage, resulting in chronic inflammation. This chronic inflammation is a part of many human diseases including arteriosclerosis (Qui et al. 2006), inflammatory bowel disease (Lee et al. 2010), arthritis (Hochberg 1990), cancer (Sung et al. 2011), and Alzheimer's disease (Sastre et al. 2011). In the inflammatory state, the activated immune cells such as macrophages, secrete large amounts of proinflammatory cytokines, nitric oxide (NO), and prostaglandin E_2 (PGE_2). However, high levels of NO and PGE_2 in a chronic inflammation state can result in various pathological conditions (Yun et al. 1996; Hinz and Brune 2002). Accordingly, control of the production of NO and PGE_2 in macrophages are currently interesting research topics for the development of new anti-inflammatory drugs.

In this study, we have synthesized a series of new compounds possessing 1,3,4-triarylpyrazole scaffold for the purpose of anticancer screening (will be carried out later and the results will be published in due course). Due to the presence of a vicinal diaryl heterocycle moiety which has been reported in selective cyclooxygenase (COX)-2 inhibitors such as celecoxib (Fig. 1), we decided to test their anti-inflammatory activity. Several pyrazole derivatives including celecoxib have been reported as anti-inflammatory agents (Norgard et al. 2004; Keche et al. 2012; El-Sayed et al. 2012; Ragab et al. 2013; Malvar et al. 2014; Kurumbail et al. 1996). The anti-inflammatory activities of our target compounds **1a–k** were evaluated through measuring their effects as inhibitors of lipopolysaccharide (LPS)-induced NO and PGE_2 productions in RAW 264.7 macrophages.

Experimental

General

Mass spectra (MS) were determined by liquid chromatography–mass spectrometry (LC-MS) analysis using the following system: Waters 2998 photodiode array detector, Waters 3100 mass detector, Waters SFO system
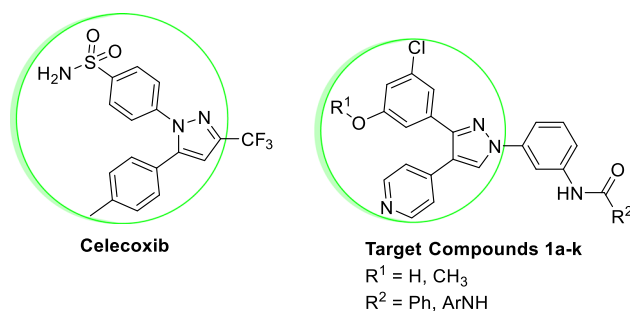

Fig. 1 Structures of celecoxib and the target compounds **1a–k**

fluidics organizer, Waters 2545 binary gradient module, Waters reagent manager, Waters 2767 sample manager, Sunfire™ C18 column (4.6 × 50 mm, 5 μm particle size); Solvent gradient = 95% A at 0 min, 1% A at 5 min; solvent A: 0.035% trifluoroacetic acid (TFA) in water; solvent B: 0.035% TFA in CH_3OH; flow rate of 3.0 mL/min. ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR analyses were carried out using a Bruker ARX-300, 300 MHz (Bruker Bioscience, Billerica, MA, USA) and a Bruker ARX-400, 400 MHz (Bruker Bioscience, Billerica, MA, USA) in NMR solvents containing tetramethylsilane as an internal standard. All the solvents and reagents were commercially available and used as such without further purification.

Synthesis of 4-(3-(3-chloro-5-methoxyphenyl)-1*H*-pyrazol-4-yl)pyridine (6)

It was synthesized by the previously reported 4-step method (Bennett et al. 2007; Choi and Oh 2009; El-Gamal et al. 2011). It was obtained as a white to off-white solid.

4-[3-(3-Chloro-5-methoxyphenyl)-1-(3-nitrophenyl)-1*H*-pyrazol-4-yl]pyridine (7)

A mixture of compound **6** (0.5 g, 1.7 mmol), 1-iodo-3-nitrobenzene (0.9 g, 3.5 mmol), K_2CO_3 (0.7 g, 5.2 mmol), CuI (0.033 g, 0.2 mmol), and ʟ-proline (0.04 g, 0.2 mmol) in DMSO (7 mL) was heated at 90 °C under nitrogen atmosphere for 8 h. The reaction mixture was cooled and then extracted between ethyl acetate (15 mL) and saturated saline (15 mL). The organic phase was washed with saturated saline solution (three times) and dried using anhydrous sodium sulfate. After evaporation of the organic solvent, the residue was purified by column chromatography (silica gel, hexane-ethyl acetate 1:2 v/v) to yield compound **7** (0.55 g, 59%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.28 (s, 1H), 8.79 (d, 1H, $J = 2.0$ Hz), 8.60 (d, 2H, $J = 4.4$ Hz), 8.46 (d, 1H, $J = 8.2$ Hz), 8.24 (d, 1H, $J = 8.2$ Hz), 7.87 (t, 1H, $J = 8.2$ Hz), 7.39 (d, 2H, $J = 4.2$ Hz), 7.16–7.14 (m, 2H), 6.99 (s, 1H), 3.75 (s, 3H).

4-[3-(3-Chloro-5-methoxyphenyl)-1-(3-aminophenyl)-1*H*-pyrazol-4-yl]pyridine (8)

A mixture of the nitro compound **7** (0.5 g, 1.2 mmol) and palladium over carbon 5% (0.5 g) in anhydrous tetrahydrofuran (5 mL) under hydrogen (50 psi) was stirred at room temperature for 2 h. The mixture was filtered through celite and the filtrate was evaporated under reduced pressure to give compound **8** (0.4 g, 87%) as an oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.56 (d, 2H, $J = 4.6$ Hz), 8.07 (s, 1H), 7.26–7.18 (m, 5H), 7.05 (d, 1H, $J = 6.7$ Hz), 6.92 (d, 2H, $J = 1.9$ Hz), 6.64 (d, 1H, $J = 6.4$ Hz), 3.72 (s, 3H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 160.3 (C=O), 150.0, 149.2, 147.8, 140.5, 140.4, 135.1, 135.0, 130.4, 127.3, 122.9, 121.0, 120.0, 114.6, 113.8, 112.6, 108.8, 106.0, 55.6 (OMe); LC-MS: 378.89 (M$^+$ + 2), 377.90 (M$^+$ +1), 376.91 (M$^+$).

General procedure for synthesis of diarylurea derivatives 1a–e

To a solution of compound **8** (50 mg, 0.1 mmol) in anhydrous tetrahydrofuran (THF) (1 mL), a solution of the appropriate aryl isocyanate (0.1 mmol) in THF (1 mL) was added dropwise at room temperature under N$_2$. The reaction mixture was stirred at room temperature for 12 h. After completion of the reaction, the mixture was evaporated under reduced pressure, and the residue was purified by column chromatography (silica gel, hexane-ethyl acetate 2:1 v/v then switching to 1:5 v/v) to yield the target compound **1a–e**.

*1-(3-(3-(3-Chloro-5-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-phenylurea (**1a**)*

White solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, 1H), 8.96 (s, 1H), 8.73 (s, 1H), 8.57 (d, 2H, $J = 6.0$ Hz), 8.17 (s, 1H), 7.53 (d, 1H, $J = 7.6$ Hz), 7.49–7.46 (m, 3H), 7.43 (d, 1H, $J = 4.8$ Hz), 7.38 (dd, 2H, $J = 4.8$ Hz, 1.2 Hz), 7.30 (t, 2H, $J = 7.8$ Hz), 7.11 (s, 1H), 7.11 (t, 1H, $J = 2.0$ Hz), 6.99 (d, 1H, $J = 7.2$ Hz), 6.97–6.96 (m, 1H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6 (C=O), 153.0 (C−OMe), 150.4 (pyridyl C−N−C), 148.7 (pyrazolyl C-3′), 141.5 (pyridyl C-4′), 140.2 (central phenyl Ar–C), 139.9 (C−Cl), 135.7 (Cl, OMe-phenyl Ar–C), 134.5 (terminal phenyl Ar–C), 130.4 (terminal phenyl Ar–C), 129.7 (pyrazolyl C-5′), 129.3 (central phenyl Ar–C), 123.2 (terminal phenyl Ar–C), 122.5 (terminal phenyl Ar–C), 120.6 (terminal phenyl Ar–C), 120.1 (central phenyl Ar–C), 118.9 (central phenyl Ar–C), 117.1 (Cl, OMe-phenyl Ar–C), 114.4 (Cl, OMe-phenyl Ar–C), 113.5 (Cl, OMe-phenyl Ar–C), 112.3 (pyrazolyl C-4′), 109.1 (central phenyl Ar–C), 56.1 (OMe); LC-MS: 496.0 (M$^+$ + 1).

*1-(3-(3-(3-Chloro-5-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-(3,4-dichlorophenyl)urea (**1b**)*

White solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H), 9.06 (s, 1H), 8.95 (s, 1H), 8.56 (d, 2H, $J = 6.0$ Hz), 8.16 (s, 1H), 7.90 (d, 1H, $J = 2.4$ Hz), 7.57–7.51 (m, 2H), 7.48–7.40 (m, 2H), 7.38–7.35 (m, 3H), 7.13–7.10 (m, 2H), 6.95 (dd, 1H, $J = 2.0$ Hz, 1.2 Hz), 3.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6 (C=O), 152.8 (C−OMe), 150.4 (pyridyl C−N−C), 148.7 (pyrazolyl C-3′), 141.1 (pyridyl C-4′), 140.3 (central phenyl Ar–C), 140.2 (Cl, OMe-phenyl Ar–C), 139.9 (Cl, OMe-phenyl Ar–C), 135.6 (terminal phenyl Ar–C), 134.5 (terminal phenyl Ar–C), 131.5 (pyrazolyl C-5′), 131.0 (central phenyl Ar–C), 130.5 (terminal phenyl Ar–C), 129.7 (terminal phenyl Ar–C), 123.8 (terminal phenyl Ar–C), 123.2 (central phenyl Ar–C), 120.6 (central phenyl Ar–C), 120.2 (terminal phenyl Ar–C), 120.0 (central phenyl Ar–C), 119.0 (Cl, OMe-phenyl Ar–C), 117.4 (Cl, OMe-phenyl Ar–C), 114.4 (Cl, OMe-phenyl Ar–C), 113.6 (Cl, OMe-phenyl Ar–C), 112.7 (pyrazolyl C-4′), 109.4 (central phenyl Ar–C), 56.1 (OMe); LC-MS: 564.9 (M$^+$ + 2), 563.9 (M$^+$ + 1).

*1-(3-(3-(3-Chloro-5-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-(3-(trifluoromethyl)phenyl)urea (**1c**)*

White solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (brs, 2H), 8.97 (s, 1H), 8.57 (d, 2H, $J = 6.0$ Hz), 8.17 (s, 1H), 8.03 (s, 1H), 7.62 (d, 1H, $J = 8.0$ Hz), 7.57–7.47 (m, 3H), 7.45 (s, 1H), 7.38 (d, 2H, 5.6 Hz), 7.32 (d, 1H, $J = 7.6$ Hz), 7.14 (s, 1H), 7.11 (t, 1H, $J = 1.8$ Hz), 6.96 (s, 1H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6 (C=O), 153.0 (C−OMe), 150.4 (pyridyl C−N−C), 148.7 (pyrazolyl C-3′), 141.1 (pyridyl C-4′), 140.9 (central phenyl Ar–C), 140.2 (Cl, OMe-phenyl Ar–C), 139.9 (terminal phenyl Ar–C), 135.7 (pyrazolyl C-5′), 134.5 (central phenyl Ar–C), 130.5 (terminal phenyl Ar–C), 130.4 (terminal phenyl Ar–C), 129.7 (terminal phenyl Ar–C), 123.2 (central phenyl Ar–C), 122.5 (Cl, OMe-phenyl Ar–C), 120.6 (terminal phenyl Ar–C), 120.1 (central phenyl Ar–C), 117.4 (CF$_3$), 114.8 (Cl, OMe-phenyl Ar–C), 114.4 (Cl, OMe-phenyl Ar–C), 113.5 (Cl, OMe-phenyl Ar–C), 112.7 (pyrazolyl C-4′), 109.4 (central phenyl Ar–C), 56.1 (OMe); LC-MS: 564.0 (M$^+$ + 1).

*1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(3-(3-(3-chloro-5-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl) urea (**1d**)*

White solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68 (brs, 2H), 8.95 (s, 1H), 8.56 (d, 2H, $J = 5.6$ Hz), 8.18 (s, 1H), 8.13 (d, 1H, $J = 2.4$ Hz), 7.71 (dd, 1H, $J = 8.4$ Hz, 1.6 Hz), 7.61 (d, 1H, $J = 8.8$ Hz), 7.55 (d, 1H, $J = 4.0$ Hz), 7.45

(d, 2H, J = 4.4 Hz), 7.38 (d, 2H, J = 6.0 Hz), 7.13–7.11 (m, 2H), 6.96 (s, 1H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 160.6 (C=O), 153.1 (<u>C</u>–OMe), 150.4 (pyridyl <u>C</u>–N–<u>C</u>), 148.7 (pyrazolyl C-3′), 141.2 (pyridyl C-4′), 140.2 (Cl, OMe-phenyl Ar–C), 139.9 (terminal phenyl Ar–C), 135.7 (pyrazolyl C-5′), 134.5 (central phenyl Ar–C), 132.4 (central phenyl Ar–C), 130.4 (terminal phenyl Ar–C), 129.7 (terminal phenyl Ar–C), 123.7 (central phenyl Ar–C), 123.2 (central phenyl Ar–C), 120.6 (terminal phenyl Ar–C), 120.1 (central phenyl Ar–C), 117.5 (CF$_3$), 117.4 (Cl, OMe-phenyl Ar–C), 114.4 (Cl, OMe-phenyl Ar–C), 113.5 (Cl, OMe-phenyl Ar–C), 112.7 (pyrazolyl C-4′), 109.5 (central phenyl Ar–C), 56.1 (OMe); LC-MS: 598.8 (M$^+$ + 2), 597.8 (M$^+$ + 1).

1-(3,5-Bis(trifluoromethyl)phenyl)-3-(3-(3-(3-chloro-5-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl) urea (1e)

White solid; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.53 (brs, 2H), 8.90 (s, 1H), 8.56 (d, 2H, J = 6.0 Hz), 8.22–8.20 (m, 3H), 7.58 (s, 1H), 7.51 (d, 2H, J = 6.4 Hz), 7.43 (d, 1H, J = 8.0 Hz), 7.38 (d, 2H, J = 5.6 Hz), 7.13–7.10 (m, 2H), 6.96 (s, 1H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 160.6 (C=O), 153.4 (<u>C</u>–OMe), 150.3 (pyridyl <u>C</u>–N–<u>C</u>), 148.7 (central phenyl Ar–C), 141.4 (pyrazolyl C-3′), 140.2 (pyridyl C-4′), 139.8 (Cl, OMe-phenyl Ar–C), 135.7 (Cl, OMe-phenyl Ar–C), 134.5 (terminal phenyl Ar–C), 131.2 (pyrazolyl C-5′), 130.9 (central phenyl Ar–C), 130.2 (central phenyl Ar–C), 129.6 (terminal phenyl Ar–C), 125.2 (terminal phenyl Ar–C), 123.1 (central phenyl Ar–C), 122.5 (central phenyl Ar–C), 120.6 (terminal phenyl Ar–C), 120.1 (central phenyl Ar–C), 118.3 (2 CF$_3$), 117.6 (Cl, OMe-phenyl Ar–C), 114.4 (Cl, OMe-phenyl Ar–C), 113.5 (Cl, OMe-phenyl Ar–C), 112.7 (pyrazolyl C-4′), 109.6 (central phenyl Ar–C), 56.1 (OMe); LC-MS: 632.9 (M$^+$ + 2), 631.9 (M$^+$ + 1).

General procedure for demethylation to the target hydroxyl derivatives 1f–j

Compound 1a–e (0.1 mmol) was dissolved in anhydrous dichloromethane (1 mL), and boron tribromide (0.08 mL of a 1 M solution in methylene chloride, 1.2 mmol) was added thereto dropwise at −78 °C under nitrogen atmosphere. The reaction mixture was stirred at the same temperature for 30 min. The temperature was raised to room temperature, and the mixture was stirred overnight at room temperature. The mixture was quenched with saturated aqueous sodium carbonate. Ethyl acetate (15 mL) was added, the mixture was stirred, and the organic layer was separated. The aqueous layer was extracted again with ethyl acetate (2 × 10 mL). The combined ethyl acetate extracts were washed with

saturated saline solution, and then dried with anhydrous Na$_2$SO$_4$. The organic solvent was evaporated under reduced pressure, and the remained crude product was purified by short column chromatography (silica gel, hexane-ethyl acetate 1:1 v/v then switching to 1:5 v/v) to yield the target compound 1f–j.

1-(3-(3-(3-Chloro-5-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-phenylurea (1f)

White solid; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.10 (s, 1H), 8.98 (s, 1H), 8.95 (s, 1H), 8.71 (s, 1H), 8.57 (s, 2H), 8.18 (t, 1H, J = 2.0 Hz), 7.54–7.49 (m, 1H), 7.48 (d, 1H, J = 1.2 Hz), 7.46 (d, 1H, J = 0.8 Hz), 7.43 (d, 1H, J = 8.0 Hz), 7.39–7.37 (m, 3H), 7.30 (t, 2H, J = 8.0 Hz), 6.99–6.98 (m, 2H), 6.87–6.85 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 159.0 (C=O), 153.0 (<u>C</u>–OH), 150.2 (pyridyl <u>C</u>–N–<u>C</u>), 149.0 (central phenyl Ar–C), 141.5 (pyrazolyl C-3′), 140.4 (pyridyl C-4′), 140.0 (Cl, OH-phenyl Ar–C), 135.6 (Cl, OH-phenyl Ar–C), 134.2 (terminal phenyl Ar–C), 130.4 (pyrazolyl C-5′), 129.7 (central phenyl Ar–C), 129.3 (central phenyl Ar–C), 123.2 (terminal phenyl Ar–C), 122.5 (terminal phenyl Ar–C), 120.0 (central phenyl Ar–C), 119.0 (central phenyl Ar–C), 118.9 (terminal phenyl Ar–C), 117.0 (central phenyl Ar–C), 115.8 (Cl, OH-phenyl Ar–C), 114.5 (Cl, OH-phenyl Ar–C), 112.3 (pyrazolyl C-4′), 109.0 (central phenyl Ar–C); LC-MS: 482.0 (M$^+$ + 1).

1-(3-(3-(3-Chloro-5-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-(3,4-dichlorophenyl)urea (1g)

White solid; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.11 (s, 1H), 9.18 (s, 1H), 9.10 (s, 1H), 8.94 (s, 1H), 8.56 (dd, 2H, J = 4.4 Hz, 1.6 Hz), 8.18 (t, 1H, J = 2.0 Hz), 7.91 (d, 1H J = 2.4 Hz), 7.56–7.52 (m, 2H), 7.46 (t, 1H, J = 8.0 Hz), 7.40–7.36 (m, 4 H), 6.98 (t, 1H, J = 1.6 Hz), 6.87 (dt, 2H, J = 6.8 Hz, 1.9 Hz); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 159.0 (C=O), 152.8 (<u>C</u>–OH), 150.4 (pyridyl <u>C</u>–N–<u>C</u>), 149.0 (central phenyl Ar–C), 141.1 (pyrazolyl C-3′), 140.3 (pyridyl C-4′), 140.2 (central phenyl Ar–C), 140.0 (Cl, OH-phenyl Ar–C), 135.6 (Cl, OH-phenyl Ar–C), 134.1 (terminal phenyl Ar–C), 131.5 (terminal phenyl Ar–C), 131.0 (pyrazolyl C-5′), 130.4 (central phenyl Ar–C), 129.7 (terminal phenyl Ar–C), 123.8 (terminal phenyl Ar–C), 123.1 (terminal phenyl Ar–C), 120.0 (central phenyl Ar–C), 119.9 (central phenyl Ar–C), 119.0 (terminal phenyl Ar–C), 118.9 (central phenyl Ar–C), 117.3 (Cl, OH-phenyl Ar–C), 115.8 (Cl, OH-phenyl Ar–C), 114.5 (Cl, OH-phenyl Ar–C), 112.6 (pyrazolyl C-4′), 109.3 (central phenyl Ar–C); LC-MS: 551.6 (M$^+$ + 2), 550.6 (M$^+$ + 1).

1-(3-(3-(3-Chloro-5-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-(3-(trifluoromethyl)phenyl)urea (1h)

White solid; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.10 (brs, 1H), 9.15 (d, 2H, $J = 6.8$ Hz), 8.95 (s, 1H), 8.57 (d, 2H, $J = 5.6$ Hz), 8.18 (s, 1H), 8.03 (s, 1H), 7.62 (d, 1H, $J = 8.0$ Hz), 7.55 (t 1H, $J = 6.4$ Hz), 7.51–7.40 (m, 3H), 7.38 (d, 2H, $J = 5.6$ Hz), 7.33 (d, 1H, $J = 7.6$ Hz), 6.98 (s, 1H), 6.86 (dd, 2H, $J = 4.8$ Hz, 2.0 Hz); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 159.0 (C=O), 153.0 (C–OH), 150.4 (pyridyl C–N–C), 149.0 (pyrazolyl C-3′), 141.1 (pyridyl C-4′), 140.9 (central phenyl Ar–C), 140.2 (Cl, OH-phenyl Ar–C), 140.0 (terminal phenyl Ar–C), 135.6 (pyrazolyl C-5′), 134.2 (central phenyl Ar–C), 130.5 (terminal phenyl Ar–C), 130.4 (terminal phenyl Ar–C), 129.7 (terminal phenyl Ar–C), 123.1 (central phenyl Ar–C), 122.5 (Cl, OH-phenyl Ar–C), 120.0 (Cl, OH-phenyl Ar–C), 119.0 (CF$_3$), 118.8 (Cl, OH-phenyl Ar–C), 117.4 (Cl, OH-phenyl Ar–C), 115.8 (Cl, OH-phenyl Ar–C), 114.8 (central phenyl Ar–C), 114.5 (Cl, OH-phenyl Ar–C), 112.6 (pyrazolyl C-4′), 109.3 (central phenyl Ar–C); LC-MS: 550.1 (M$^+$ + 1).

1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(3-(3-(3-chloro-5-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)urea (1i)

White solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32–10.28 (m, 3H), 8.90 (s, 1H), 8.53 (dd, 2H, $J = 4.6$ Hz, 1.4 Hz), 8.18–8.15 (m, 2H), 7.74 (d, 1H, $J = 8.8$ Hz), 7.59 (d, 1H, $J = 8.8$ Hz), 7.52–7.46 (m, 3H), 7.43 (d, 1H, $J = 8.0$ Hz), 7.38 (dd, 2H, $J = 4.4$ Hz, 1.6 Hz), 6.75–6.74 (m, 3H); LC-MS: 585.1 (M$^+$ + 2), 584.1 (M$^+$ + 1).

1-(3,5-Bis(trifluoromethyl)phenyl)-3-(3-(3-(3-chloro-5-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)urea (1j)

White solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (brs, 1H), 9.49 (brs, 1H), 9.36 (brs, 1H), 8.96 (s, 1H), 8.57 (d, 2H, $J = 4.8$ Hz), 8.17 (brs, 2H), 7.67 (s, 1H), 7.58 (d, 1H, $J = 7.2$ Hz), 7.50–7.44 (m, 2H), 7.38 (d, 2H, $J = 5.6$ Hz), 6.98 (s, 1H), 6.87–6.84 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 159.0 (C=O), 152.9 (C–OH), 150.4 (pyridyl C–N–C), 149.0 (pyrazolyl C-3′), 142.2 (pyridyl C-4′), 140.8 (Cl, OH-phenyl Ar–C), 140.2 (Cl, OH-phenyl Ar–C), 140.0 (terminal phenyl Ar–C), 135.6 (pyrazolyl C-5′), 134.2 (central phenyl Ar–C), 131.4 (central phenyl Ar–C), 130.5 (terminal phenyl Ar–C), 129.7 (terminal phenyl Ar–C), 123.1 (central phenyl Ar–C), 122.4 (central phenyl Ar–C), 120.0 (terminal phenyl Ar–C), 119.0 (central phenyl Ar–C), 118.7 (2 CF$_3$), 117.8 (Cl, OH-phenyl Ar–C), 115.8 (Cl, OH-phenyl Ar–C), 114.5 (Cl, OH-phenyl Ar–C), 113.0

(pyrazolyl C-4′), 109.7 (central phenyl Ar–C); LC-MS: 618.1 (M$^+$ + 1).

Synthesis of N-(3-(3-(3-chloro-5-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)benzamide (1k)

A mixture of the amine compound **8** (50 mg, 0.1 mmol), benzoic acid (25 mg, 0.2 mmol), HOBt (36 mg, 0.3 mmol), and EDCI (38 mg, 0.2 mmol) in DMF (1.0 mL) was cooled to 0 °C under nitrogen atmosphere. Triethylamine (0.03 mL, 0.2 mmol) was added to the reaction mixture at the same temperature. The mixture was then stirred at 80 °C for 12 h. The reaction mixture was cooled and then partitioned between saturated saline (10 mL) and ethyl acetate (10 mL). The organic layer was separated, and the aqueous layer was extracted again with ethyl acetate (3 × 5 mL). The organic extract was washed with saturated saline (3 × 15 mL), and dried using anhydrous sodium sulfate. The organic solvent was evaporated, and the crude residue was purified by column chromatography (silica gel, hexane-ethyl acetate 1:1 v/v) to yield the target compound as a white solid;. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (brs, 1H), 8.97 (s, 1H), 8.55 (dd, 2H, $J = 4.4$ Hz, 1.6 Hz), 8.45 (t, 1H, $J = 2.0$ Hz), 7.99 (d, 2H, $J = 6.8$ Hz), 7.83 (dd, 1H, $J = 8.0$ Hz, 1.2 Hz), 7.65 (dd, 1H, $J = 8.0$ Hz, 1.6 Hz), 7.62–7.58 (m, 1H), 7.55–7.51 (m, 3H), 7.36 (dd, 2H, $J = 4.4$ Hz, 1.6 Hz), 6.94 (s, 1H), 3.72 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.2 (C=O), 160.6 (C–OMe), 150.4 (pyridyl C–N–C), 148.8 (pyrazolyl C-3′), 140.9 (pyridyl C-4′), 140.2 (C–Cl), 139.7 (Cl, OMe-phenyl Ar–C), 135.6 (terminal phenyl Ar–C), 135.1 (terminal phenyl Ar–C), 134.5 (pyrazolyl C-5′), 132.3 (central phenyl Ar–C), 130.3 (central phenyl Ar–C), 129.7 (terminal phenyl Ar–C), 128.9 (terminal phenyl Ar–C), 128.2 (central phenyl Ar–C), 123.1 (central phenyl Ar–C), 120.6 (terminal phenyl Ar–C), 120.2 (central phenyl Ar–C), 119.1 (Cl, OMe-phenyl Ar–C), 114.4 (Cl, OMe-phenyl Ar–C), 114.0 (Cl, OMe-phenyl Ar–C), 113.6 (pyrazolyl C-4′), 111.3 (central phenyl Ar–C), 56.1 (OMe).

Biological evaluation

Cell culture and sample treatment, nitrite determination, PGE$_2$ assay, and MTT assay of cell viability were carried out following the procedure reported in the literature (Jang et al. 2014).

Western blot

RAW 264.7 cells were resuspended in PRO-PREP^{TM} protein extraction solution (Intron Biotechnology, Seoul, Korea) and incubated for 20 min at 4 °C. The cell debris was removed by microcentrifugation, and the supernatants were quickly frozen. The protein concentration was

determined using the Bio-Rad protein assay reagent according to the manufacture's instruction. The cell proteins were electroblotted onto a PVDF membrane after separation on 8–12% SDS-polyacrylamide gel electrophoresis. The immunoblot was incubated with a blocking solution (5% skim milk) at room temperature for 1 h, followed by incubation overnight with a primary antibody at 4 °C. The blots were washed four times with a Tween 20/Tris-buffered saline (T/TBS) and incubated with a 1:2000 dilution of horseradish peroxidase-conjugated secondary antibody at room temperature for 2 h. The blots were again washed three times with T/TBS, and then developed by enhanced chemiluminescence (GE healthcare, WI, USA).

COX-2 enzyme activity assay

Compounds **1f** and **1h** were evaluated for potency and selectivity of inhibition in vitro using COX Inhibitor Screening Assay (Cayman, MI, USA). Recombinant COX-2 proteins were pre-incubated with compounds **1f** or **1h** for 10 min in 37 °C. The reaction was started by the addition of 100 μM arachidonic acid and allowed to proceed for 2 min. The reaction was terminated by addition of HCl solution containing $SnCl_2$. The COX activity assay directly measures $PGF_{2\alpha}$ produced by $SnCl_2$ reduction of COX-derived PGH_2. The prostanoid product concentration was analyzed using EIA. Dup-697 (10 μM) was used as a reference COX-2 inhibitor.

Results and discussion

Chemistry

The target compounds **1a–k** were synthesized through the pathway illustrated in Scheme 1. Heating 3,5-dichlorobenzoic acid (**2**) with sodium methoxide (3 eq.) in hexamethylphosphoramide (HMPA) followed by acidification with HCl produced 3- chloro-5-methoxybenzoic acid (**3**). The acid **3** was esterified using methanol in the presence of acetyl chloride to produce the corresponding methyl ester **4** (Takahashi et al. 1985). Treatment of the ester **4** with 4-
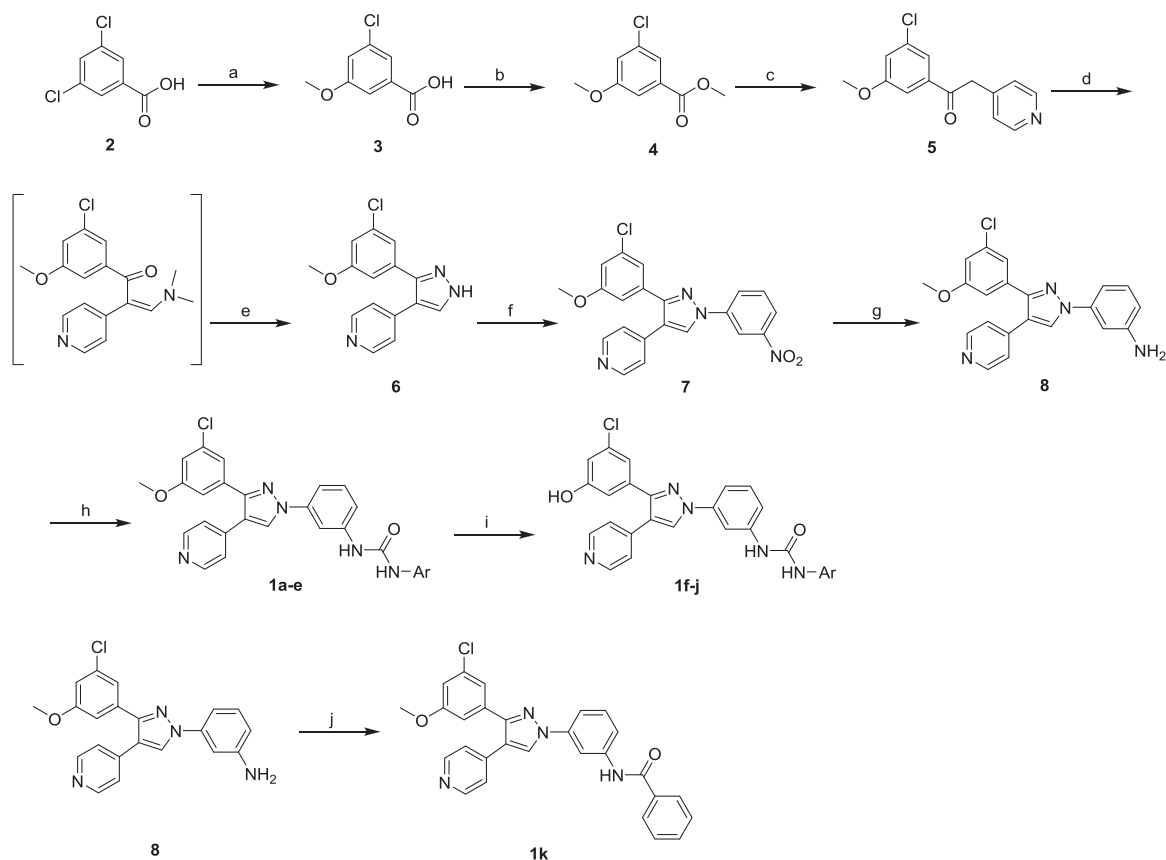
Scheme 1 Reagents and conditions: **a** sodium methoxide, HMPA, 115–120 °C, 15 h, 54%; **b** acetyl chloride, CH_3OH, rt, 15 h, 89%; **c** 4-picoline, LHMDS, THF, rt, overnight, 77%; **d** DMF-DMA, rt, 18 h; **e** hydrazine monohydrate, C_2H_5OH, rt, overnight, 72%; **f** 1-iodo-3-nitrobenzene, K_2CO_3, CuI, L-proline, DMSO, 90 °C, 8 h, 59%; **g** H_2, 10% Pd/C, THF, rt, 2 h, 87%; **h** appropriate aryl isocyanate, THF, rt, 12 h, 24~85%; **i** BBr_3, CH_2Cl_2, −78 °C, 30 min; rt, 1 h, 24~89%; **j** benzoic acid, HOBt, EDCI, TEA, DMF, 80 °C, 12 h, 65%

picoline in THF in the presence of lithium bis(trimethylsilyl)amide yielded the pyridyl ketide derivative **5**. Treatment of compound **5** with dimethylformamide dimethyl acetal (DMF-DMA) followed by treatment with hydrazine monohydrate led to formation of the cyclized 3,4-diarylpyrazole intermediate **6** (Bennett et al. 2007; Choi and Oh 2009; El-Gamal et al. 2011). N-Arylation of compound **6** using 1-iodo-3-nitrobenzene in the presence of potassium carbonate, copper(I)iodide, and L-proline as catalysts produced the *m*-nitrophenyl compound **7**. The nitro group of compound **7** was reduced to amino using Pd/C in H_2 atmosphere. The amine compound **8** was reacted with the appropriate aryl isocyanate to produce compounds **1a–e**. The methoxy group of compounds **1a–e** was demethylated in using boron tribromide to obtain the corresponding hydroxyl analogs **1f–j**. Compound **1k** was synthesized through condensation of the amino compound **8** with benzoic acid in presence of 1-hydroxybenzotriazole (HOBt), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), and triethylamine (TEA). The structures of the target compounds and their yield percentages are illustrated in Table 1.

Biological screening

Inflammation is a normal protective immune response against tissue damages that occur due to external factors in the body (Qui et al. 2006). But the inflammatory response can also cause considerable damage to the host. The microbial components such as LPS can induce the production of COX-2, inducible nitric oxide synthase (iNOS), and pro-inflammatory cytokines in the macrophages. In particular, if large amounts of NO and PGE_2 secreted by activated immune cells in the inflammatory condition, they can induce several pathological conditions. So inhibition of the production of inflammatory mediators is a potential avenue for treatment of acute and chronic inflammations. The target compounds **1a–k** were assessed for their abilities to inhibit the production of inflammatory mediators, NO and PGE_2, in LPS-induced RAW 264.7 macrophages (Moncada et al. 1991; Kim et al. 2008; Shin et al. 2014). N_6-(1-Iminoethyl)-L-lysine (L-NIL) and N-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide (NS-398) were utilized as reference compounds for screening of NO and PGE_2 production inhibitions, respectively. The cytotoxic effects of the triarylpyrazoles **1a–k** on RAW 264.7 macrophages were also evaluated using the MTT assay to test whether the inhibitory effects on the productions of NO and PGE_2 were caused by non-specific cytotoxicity (Table 2). All the target compounds showed IC_{50} values ≥5.86 μM. Compounds **1b** and **1e** did not inhibit 50% of RAW 264.7 macrophages growth up to 100 μM. After that, the target compounds were tested for NO and PGE_2 production inhibitions at 5 and 3 μM, respectively.

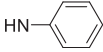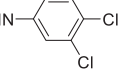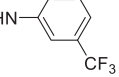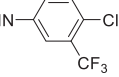**Table 1** Structures of the target compounds and their yield percentages
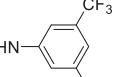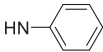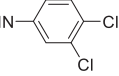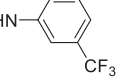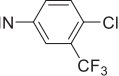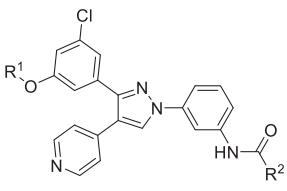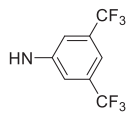
Compound no.	R^1	R^2	Yield (%)	Log P^a
1a	CH_3	(HN–phenyl)	24[b]	5.38
1b	CH_3	(HN–dichlorophenyl)	60[b]	6.50
1c	CH_3	(HN–CF_3 phenyl)	62[b]	6.30
1d	CH_3	(HN–Cl, CF_3 phenyl)	85[b]	6.86
1e	CH_3	(HN–diCF_3 phenyl)	82[b]	7.22
1f	H	(HN–phenyl)	24[c]	5.12
1g	H	(HN–dichlorophenyl)	89[c]	6.23
1h	H	(HN–CF_3 phenyl)	62[c]	6.04
1i	H	(HN–Cl, CF_3 phenyl)	79[c]	6.06

Table 1 continued

Compound no.	R^1	R^2	Yield (%)	Log P^a
1j	H		72^c	6.96
1k	CH$_3$		65^d	5.71

^{a} Calculated by ChemDraw Professional 15.0 software

^{b} Synthesized in step "h" as illustrated in Scheme 1

^{c} Synthesized in step "i" as illustrated in Scheme 1

^{d} Synthesized in step "j" as illustrated in Scheme 1

Table 2 Cytotoxicty (IC$_{50}$, MTT assay) of the target compounds **1a–k** over RAW 264.7 cells

Compound no.	IC$_{50}$^{a} (μM)
1a	23.42 ± 0.87
1b	>100
1c	23.26 ± 0.99
1d	82.12 ± 6.31
1e	>100
1f	15.86 ± 0.64
1g	19.32 ± 1.38
1h	18.71 ± 0.56
1i	10.29 ± 0.79
1j	19.46 ± 1.21
1k	48.38 ± 2.21
NS-398	>3

^{a} Data are presented as the means ± SD of three independent experiments

The target pyrazole compounds **1a–k** exerted varying inhibitory effects on the LPS-induced NO and PGE$_2$ productions (Table 3). Compounds **1h–j** possessing *m*-hydroxyl group on the aromatic ring on position 3 of the pyrazole ring showed higher inhibitory effect on NO production than the corresponding methoxy analogs **1c–e**. Similarly, the phenolic derivatives **1f**, **1h**, and **1i** were more active as PGE$_2$ production inhibitors than the corresponding methoxy

compounds **1a**, **1c**, and **1d**. So the phenolic OH group could be essential for anti-inflammatory activity of this series of compounds. It may act as hydrogen bond donor at the receptor site. Or its steric and/or electronic properties, which are different from those of the methoxy group, can strengthen the affinity at the receptor site. In addition, the increased lipophilicity seems to be unfavorable for PGE$_2$ production inhibitory activity.

Compound **1k** with amide linker was synthesized and biologically tested in order to compare the effect of the linker on the biological activity. It was found that the urea compound **1a** was more active than the amide analog **1k** in terms of NO production inhibition. This can be rationalized that the longer urea linker may help the compound fit appropriately at the receptor site. Or the extra terminal NH group of the urea linker can make additional hydrogen bond (s) at the receptor site. Any or both of these effects can help enhance the compound affinity at the receptor site, and hence higher activity. Due to the non-promising results of compound **1k** as NO production inhibitor, it was not tested for PGE$_2$ production inhibition.

The effect of the terminal aryl moiety on the biological activity was also investigated. Upon comparing the results of the methoxy compounds **1a–e** on NO production inhibitions, the mono-substituted phenylurea derivative **1a** was the most active than the other di- or tri-substituted phenyl derivatives **1b–e**. So the steric/electronic factor(s) may play a role in these activity differences. But upon comparing the hydroxyl derivatives **1f–j**, compound **1j** possessing 3,5-bis(trifluoromethyl)phenyl terminal moiety showed the best activity. Its IC$_{50}$ value over NO production was 4.35 μM. So that bulky terminal moiety may be optimal for activity. Since it is well-known that iNOS catalyzes the oxidative deamination of L-arginine to produce NO (Moncada et al. 1991), we determined the effect of compound **1j** on LPS-induced iNOS protein expression using Western blot. Compound **1j** significantly inhibited LPS-induced iNOS expression, suggesting the inhibitory action of compound **1j** on NO was due to inhibition on iNOS protein expression (Fig. 2).

Regarding the effect on PGE$_2$ production inhibition, the best results were obtained with compounds **1a** and **1f** with phenyl ring, and compound **1h** possessing 3-(trifluoromethyl)phenyl terminal moiety. Compounds **1f** and **1h** showed almost the same inhibition percentage as NS-398 and Celecoxib against PGE$_2$ at 3 μM concentration. So both phenyl and *m*-(trifluoromethyl)phenyl terminal rings are the optimum for PGE$_2$ production inhibitory effect of this series of compounds. During the inflammatory process, biosynthesis of PGE$_2$ requires transformation of arachidonic acid by COX-2 to PGH$_2$, which is subsequently converted by microsomal PGE synthases (mPGES-1) (Shin et al. 2014). To determine the mechanisms by which compound

Table 3 Inhibitory effects of the target compounds **1a–k** on LPS-induced NO and PGE$_2$ in RAW 264.7 cells

Compound no.	% Inhibition[a]	
	NO production at 5 μM	PGE$_2$ production at 3 μM
1a	34.22 ± 1.81	53.45 ± 4.86
1b	3.21 ± 0.09	–[b]
1c	12.74 ± 0.99	36.11 ± 2.38
1d	15.57 ± 0.71	13.89 ± 1.64
1e	24.29 ± 1.27	–[b]
1f	12.74 ± 1.03	92.68 ± 6.17 (1.12 ± 0.34)[c]
1g	0.71 ± 0.08	55.56 ± 3.83
1h	20.71 ± 1.03	92.41 ± 6.25
1i	25.88 ± 1.12	50.58 ± 4.32
1j	53.42 ± 1.91 (4.35 ± 0.62)[c]	50.74 ± 4.86
1k	2.50 ± 0.07	–
L-NIL (40 μM)	75.21 ± 3.67 (28.27 ± 0.13)[c]	–
NS-398 (3 μM)	–	93.27 ± 5.58 (6.75 ± 0.70)[d]

[a] Values represent means ± S.D. of three independent experiments

[b] No inhibition at less than the cytotoxic concentration

[c] Numbers in parenthesis are IC$_{50}$ values in μM

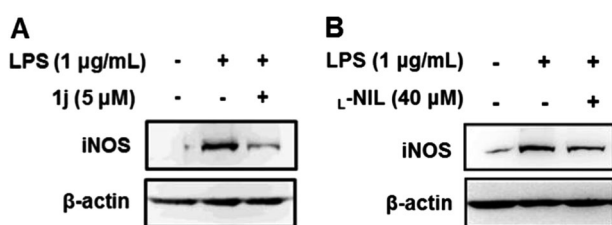[d] Numbers in parenthesis are IC$_{50}$ values in nM

Fig. 2 The inhibitory effect of compound **1j** on LPS-induced iNOS expression in RAW 264.7 cells. Total cellular proteins were obtained from cells stimulated with LPS (1 μg/mL) for 24 h in presence or absence of **1j** (a) or $_L$-NIL (b). iNOS was detected by Western blot using specific antibody. β-actin was used as an internal control. The experiments were repeated three times, and similar results were obtained

1f and **1h** blocked the PGE$_2$ production in macrophages, we first examined expression of both COX-2 and mPGES-1 by Western blot. As shown in Fig. 3a, compounds **1f** and **1h** had no effect on LPS-induced COX-2 and mPGES-1 protein expression. The COX-2 enzyme activity is extremely important for PGE$_2$ production, hence we next studied the effects of compounds **1f** and **1h** on COX-2 enzyme activities by performing screening assays using purified COX-2 enzymes. We found that compounds **1f** and **1h** exhibited inhibitory effect to COX-2 activity in a dose-dependent pattern. Compound **1f** was more potent than compound **1h**. Dup697 was used as a positive control for inhibition of COX-2 enzyme activity (Fig. 3b). These result indicated that compounds **1f** and **1h** reduced LPS-induced

PGE$_2$ production through inhibition of COX-2 enzyme activity.

Conclusion

A series of 1,3,4-triarylpyrazole derivatives was synthesized and studied in order to explore the relationship between their structures and their antiinflammatory activity. The hydroxyl compounds were generally more active than the corresponding methoxy derivatives. The urea moiety was found optimum linker for activity. The phenyl, *m*-(trifluoromethyl)phenyl, and 3,5-bis(trifluoromethyl)phenyl rings were the best terminal moieties. These together can represent the pharmacophore of this series of compounds. The highest PGE$_2$ production inhibition activities were obtained by compounds **1f** and **1h**. Both of them exerted that effect due to inhibition of COX-2 enzyme activity not COX-2 protein expression. Both compounds possess phenolic hydroxyl group and urea linker. Their terminal moieties are phenyl and *m*-(trifluoromethyl)phenyl rings, respectively. They showed almost equal inhibitory effect on PGE$_2$ production as NS-398 at the same concentration, 3 μM. In addition, the urea derivative **1j** containing hydroxyl group and 3,5-bis(trifluoromethyl)phenyl terminal ring showed the highest inhibitory effect on NO production, and this was due to inhibition of iNOS protein expression as concluded from the Western blot study. Further lead
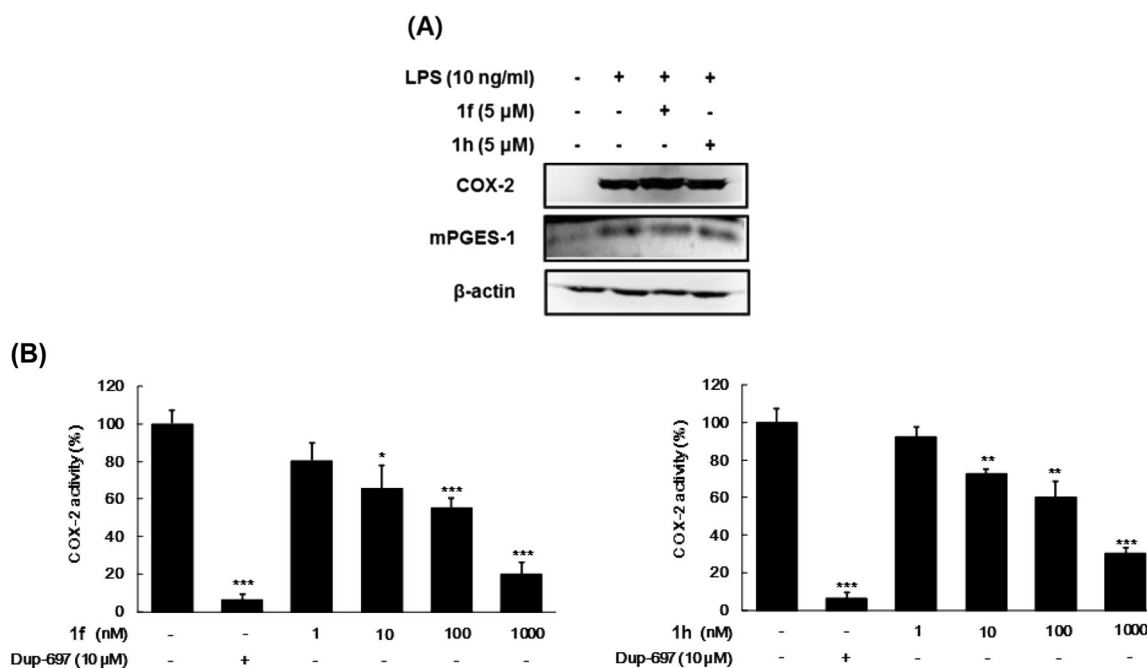
(A)

(B)

Fig. 3 Effect of compounds **1f** and **1h** on LPS-induced iNOS and mPGES expression in RAW 264.7 cells and COX-2 enzyme activities. **a** Total cellular proteins were obtained from cells stimulated with LPS (1 µg/mL) for 24 h in presence or absence of **1f** or **1h**. COX-2 and mPGES-1 were detected by Western blot using specific antibodies. β-actin was used as an internal control. The experiments were repeated three times, respectively, and similar results were obtained. **b** Recombinant COX-2 enzyme was in vitro treated with the indicated concentrations of **1f** or **1h** for 10 min. Dup-697 (10 µM) was used as a positive COX-2 inhibitor controls. Values shown are means ± SD of three independent experiments; $***p < 0.001$ vs. the 100% activity group

optimization is required for development of more active and more potent analogs based on the structure–activity relationship study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Bennett MJ, Cho-Schultz S, Deal JG, King SJ, Marrone TJ, Palmer CL, Romines WH, Rui EY, Sutton SC, Zehnder LR (2007) Pyrazole compounds. WO 2007/105058

Choi W-K, Oh C-H (2009) Synthesis and antiproliferative activities of 1-substituted-3-(3-chloro-5-methoxyphenyl)-4-pyridinylpyrazole derivatives against melanoma cell line. Bull Korean Chem Soc 30:2027–2031

Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420:860–867

El-Gamal MI, Sim TB, Hong JH, Cho J-H, Yoo KH, Oh C-H (2011) Synthesis of 1H-pyrazole-1-carboxamide derivatives and their antiproliferative activity against melanoma cell line. Arch Pharm Chem Life Sci 11:197–204

El-Sayed MA-A, Abdel-Aziz NI, Abdel-Aziz AA-M, El-Azab AS, ElTahir KEH (2012) Synthesis, biological evaluation and molecular modeling study of pyrazole and pyrazoline derivatives as selective COX-2 inhibitors and anti-inflammatory agents. Part 2. Bioorg Med Chem 20:3306–3316

Hinz B, Brune KJ (2002) Cyclooxygenase-2–10 years later. Pharmacol Exp Ther 300:367–375

Hochberg MC (1990) Changes in the incidence and prevalence of rheumatoid arthritis in England and Wales, 1970–1982. Semin Arthritis Rheum 19:294–302

Jang H-L, El-Gamal MI, Choi H-E, Cho H-Y, Lee K-T, Oh C-H (2014) Synthesis of tricyclic fused coumarin sulfonates and their inhibitory effects on LPS-induced nitric oxide and PGE₂ productions in RAW 264.7 macrophages. Bioorg Med Chem Lett 24:571–575

Keche AP, Hatnapure GD, Tale RH, Rodge AH, Kamble VM (2012) Synthesis, anti-inflammatory and antimicrobial evaluation of novel 1-acetyl-3,5-diaryl-4,5-dihydro (1H) pyrazole derivatives bearing urea, thiourea and sulfonamide moieties. Bioorg Med Chem Lett 22:6611–6615

Kim JY, Park SJ, Yun KJ, Cho YW, Park HJ, Lee KT (2008) Iso-liquiritigenin isolated from the roots of Glycyrrhiza uralensis inhibits LPS-induced iNOS and COX-2 expression via the attenuation of NF-kB in RAW 264.7 macrophages. Eur J Pharmacol 584:175–184

Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, Gildehaus D, Miyashiro JM, Penning TD, Seibert K, Isakson PC, Stallings WC (1996) Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature 384:644–648

Lee I-A, Bae E-A, Hyun Y-J, Kim D-H (2010) Dextran sulfate sodium and 2,4,6-trinitrobenzene sulfonic acid induce lipid peroxidation

by the proliferation of intestinal gram-negative bacteria in mice. J Inflamm 7:7

Malvar DDC, Ferreira RT, de Castro RA, dr Castro LL, Freitas ACC, Costa EA, Florentino IF (2014) Antinociceptive, anti-inflammatory and antipyretic effects of 1.5-diphenyl-1H-Pyr-azole-3-carbohydrazide, a new heterocyclic pyrazole derivative. Life Sci 95:81–88

Moncada S, Palmer RM, Higgs EA (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 43:109–142

Norgard B, Pedersen L, Johnsen SP, Tarone RE, McLaughlin JK, Friis S, Sorensen HT (2004) COX-2-selective inhibitors and the risk of upper gastrointestinal bleeding in high-risk patients with previous gastrointestinal diseases: a population-based casecontrol study. Aliment Pharmacol Ther 19:817–825

Qui H, Johansson A-S, Sjostrom M, Wan M, Schroder O, Palmblad J, Haeggstrom JZ (2006) Differential induction of BLT receptor expression on human endothelial cells by lipopolysacharide, cytokines, and leukotriene B4. Proc Natl Acad Sci USA 103:6913–6918

Ragab FA, Abdel Gawad NM, Georgey HH, Said MF (2013) Synth-esis of novel 1,3,4-trisubstituted pyrazoles as anti-inflammatory and analgesic agents. Eur J Med Chem 63:645–654

Sastre M, Richardson JC, Gentleman SM, Brooks DJ (2011) Inflam-matory risk factors and pathologies associated with Alzheimer's disease. Curr Alzheimer Res 8:132–141

Shin JS, Choi HE, Kim SD, Lee YS, Cho YW, Lee K-T (2014) Anti-inflammatory effects of 7-hydroxyl-1-methylindole-3-acetonitrile, a synthetic arvelexin derivative, on the macrophages through destabilizing mPGES-1 mRNA and suppressing NF-κB activa-tion. Chem Biol Interact 224C:68–77

Sung B, Prasad S, Yadav VR, Lavasanifar A, Aggarwal BB (2011) Cancer and diet: How are they related? Free Radic Res 45:864–879

Takahashi K, Shimizu S, Ogata M (1985) Synthesis of phenolic metabolites of an antifugal imidazole derivatives (cloconazole hydrochloride). Heterocycles 23:1483–1491

Yun HY, Dawson VL, Dawson TM (1996) Neurobiology of nitric oxide. Crit Rev Neurobiol 10:291–316