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Discovery of a novel COX-2 inhibitor as an orally potent anti-pyretic and anti-inflammatory drug: Design, synthesis, and structure–activity relationship

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ABSTRACT

Cyclooxygenase (COX) has been considered as a significant pharmacological target because of its pivotal roles in the prostaglandin biosynthesis and following cascades that lead to various (patho)physiological effects. Non-steroidal anti-inflammatory drugs (NSAIDs) that suppress COX activities have been used clinically for the treatment of fever, inflammation, and pain; however, nonselective COX inhibitors exhibit serious side-effects such as gastrointestinal damage because of their inhibitory activities against COX-1. Thus, COX-1 is constitutive and expressed ubiquitously and serves a housekeeping role, while COX-2 is inducible or upregulated by inflammatory/injury stimuli such as interleukin-1β, tumor necrosis factor-α, and lipopolysaccharide in macrophage, monocyte, synovial, liver, and lung, and is associated with prostaglandin E_2 and prostacyclin production that evokes or sustains systemic/peripheral inflammatory symptoms. Also, hypersensitivity of aspirin is a significant concern clinically. Hence, design, synthesis, and structure–activity relationship of [2-{[(4-substituted)-pyridin-2-yl]carbonyl}-(6- or 5-substituted)-1*H*-indol-3-yl]acetic acid analogues were investigated to discover novel acid-type COX-2 inhibitor as an orally potent new-class anti-pyretic and anti-inflammatory drug. As significant findings, compounds **1**–**3** demonstrated potent COX-2 inhibitory activities with high selectivities for COX-2 over COX-1 in human cells or whole-blood *in vitro*, and demonstrated orally potent anti-pyretic activity against lipopolysaccharide-induced systemic-inflammatory fever model in F344 rats. Also compound **1** demonstrated orally potent anti-inflammatory activity against edema formation and a suppressive effect against PGE_2 production in carrageenan-induced peripheral-inflammation model on the paw of SD rats. These results suggest that compounds **1**–**3** are potential agents for the treatment of inflammatory disease and are useful for further pharmacological COX-2 inhibitor investigations.

1. Introduction

In the human body the arachidonic acid (AA), that is released from phospholipids of cellular membrane by phospholipase A_2 (PLA_2) mediated-hydrolysis [1], is converted into prostaglandin (PG) H_2 (PGH_2) catalyzed by cyclooxygenase (COX, or prostaglandin G/H synthase or endoperoxidase; EC 1.14.99.1) [2–4] by way of the addition of two O_2 (oxygen) molecules onto AA to form PGG_2 followed by the reduction of the hydroperoxide group of PGG_2 to form PGH_2 using two distinctive catalytic sites, the cyclooxygenase and peroxidase sites in the COX protein [5,6]. Furthermore, PGH_2 is metabolized into various types of prostanoids (eicosanoids), namely, PGs such as PGE_2, PGD_2, and $PGF_{2\alpha}$, prostacyclin (PGI_2), and thromboxane (TX) A_2 (TXA_2) that is rapidly converted into TXB_2 [1,5]. These prostanoids bind with respective specific G-protein-coupled-receptors (GPCRs), and play various types of key roles for regulation of human physiology and pathophysiology; for example, PGE_2 closely associates with inflammation, fever, and pain, and PGI_2 closely associates with inflammation, pain, and vasodilation. Particularly in the inflammatory process, PGE_2 and PGI_2 play critical roles to cause increases in body temperature, vascular permeability, and edema as inflammatory-disease mediators [1,5,7–10]. Since COX is a key rate-limiting enzyme for PG production, and inhibition of COX activity is able to attenuate the levels of PGs that are involved in the inflammatory symptoms such as heat, swelling, flare, and pain, COX inhibition has been

Abbreviations: COX, cyclooxygenase (or endoperoxidase, or prostaglandin G/H synthase, EC 1.14.99.1); AA, arachidonic acid; PG, prostaglandin; PGH_2, prostaglandin H_2; PGG_2, prostaglandin G_2; PGE_2, prostaglandin E_2; PGI_2, prostacyclin; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_1\alpha$; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2; NSAID, non-steroidal anti-inflammatory drug; SPF, specific pathogen free; VAF, virus antibody free; F344 rat, Fischer 344 rat; SD rat, Sprague–Dawley rat; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6β, interleukin-6β; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; LPS, lipopolysaccharide; HUVEC, human umbilical vein endothelial cell; HWP, human washed platelet; RA, rheumatoid arthritis; OA, osteoarthritis; HBD, hydrogen bond donor; HBA, hydrogen bond acceptor.

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Table 1
Induction or overexpression of COX-2 in the various types of organs, tissues, and cells in the reported studies.

Induction or overexpression site	Condition[a]	Reference[b]
Liver or hepatocytes	A and B	[13,14,26]
Lung	A	[13,14]
Macrophages	A, B, C, and D	[11,25–27]
Monocytes	A, B, D, and E	[3,11,25,26,28]
Bloods	A and B	[3,26]
Perivascular areas and lymphoid aggregates	D	[11]
Endothelial cells	C, D, and E	[11,27,28]
Vascular smooth muscle cells	F	[29]
Synovial tissues, synoviocytes or joint	D, G, H, and I	[11,30,31]
Paw	J	[23]
Cartilage	K	[32]
Articular chondrocytes	L	[33]
Neutrophils	M	[34]
Peripheral nerves	N	[35]
Spinal cord	C, O, and P	[23,27,36]
Fibroblasts	B and Q	[26,37]
Precancer lesions or tumor cells	R and S	[38,39]
Surgical peripheral tissues	T	[40]

[a] A: bacterial lipopolysaccharide (LPS) [3,13,14,25]; B: virus(es) [26]; C: interleukin-1β (IL-1β) [27]; D: human rheumatoid arthritis (RA) [11]; E: LPS or phorbol 12-myristate 13-acetate (PMA, a tumor promoter) [28]; F: IL-1β or a mixture of IL-1β, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and LPS [29]; G: rat adjuvant-induced arthritis [11]; H: human ankylosing spondylitis (AS), human psoriatic arthritis (PsA) or human RA [30]; I: activated T lymphocytes, IL-1β, IL-17 or TNF-α [31]; J: rat adjuvant-induced edema [23]; K: human osteoarthritis (OA) [32]; L: IL-1β TNF-α, IL-6, leukemia inhibitory factor (LIF) or LPS [33]; M: LPS, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF) or PMA [34]; N: human classic Guillain–Barré syndrome (GBS), human chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) or human vasculitic neuropathy (VN) [35]; O: local adjuvant-injection in rats [23]; P: local carrageenan-injection in rats [36]; Q: LPS, IL-1β, TNF-α or PMA [37]; R: epidermal growth factor receptor (EFGR) or type-α transforming growth factor (TGF-α) [38]; S: IL-1β or IL-1β-induced activation of nuclear factor κB (NF-κB) and p38 [39]; T: oral surgery in human [40].

[b] Reference number cited in the text.

considered as a significant target of drug candidates for the treatment of pyrexia, inflammation, and pain [5].

Historically, non-steroidal anti-inflammatory drugs (NSAIDs), for example, aspirin (acetylsalicylic acid), indomethacin, ibuprofen, and naproxen, have been used clinically for the treatment of pyrexia, inflammation that includes rheumatoid arthritis (RA) and osteoarthritis (OA), and pain, by virtue of their suppression of the effects on COX activity [1,5,7,9,11–18]; however, these agents have exhibited side-effect issues such as gastrointestinal (GI) tract bleeding, ulcer, and perforation [19–21]. The detailed mechanisms of both the anti-inflammation effects and the side-effects are associated with the existence of two COX isoforms, namely, COX-1 (EC 1.14.99.1) and COX-2 (EC 1.14.99.1). Thus, (i) COX-1 is predominantly expressed ubiquitously and constitutively, and it serves a housekeeping role in processes such as GI mucosa protection; whereas inhibition of COX-1 activity causes GI tract damage. By contrast, (ii) COX-2 is absent or exhibits a low level of expression in most tissues, but it is constitutively expressed in some tissues such as spinal cord and dorsal root ganglions (DRGs) [22], and is inducible and upregulated by inflammatory processes [3,5,9,13,23,24] related to bacterial or viral infection, or specifically, inflammatory or tissue-injury stimuli/signals such as interleukin-1β (IL-1β), IL-6, IL-17, tumor necrosis factor-α (TNF-α), bacterial lipopolysaccharide (LPS) [3,13,14,25], viruses [26], activated T lymphocytes, and growth factors. Actually, COX-2 induction or overexpression occurs in the various types of organs, tissues, and cells [3,11,13,14,23,25–40] as summarized in Table 1. COX-2 contribution to PGE_2 and PGI_2 production evokes and sustains systemic or peripheral inflammatory disease but it is not involved in the COX-1-mediated GI tract events [19–21]. Indeed, the major issues of the nonselective COX inhibitors (as well as COX-1 selective inhibitors) in the gastrointestinal tract are due to their COX-1 inhibitory activities [19–21,41]. Significant clinical concerns due to upregulation of COX-2 under inflammatory conditions result from stimulation or increases in pro-mitogenic factors, carcinogenesis, tumor angiogenesis, and lymphangiogenesis by COX-2-derived mediators including PGs and growth factors and/or by DNA oxidative damage resulting from COX-2 activity [37–39,42–44]. Therefore, the suppressive and preventive effects on various types of cancers or tumors such as colon, lung, gastric, and intestinal cancers by inhibition of COX-2 activity have been widely studied [37,42–48]. On the other hand, it has been reported that COX-2 selective inhibition improves vascular endothelial function and reduces oxidative stress in coronary artery disease, and attenuates LPS-induced cardiovascular failure or liver injury [49,50]; although rofecoxib, a potent and selective COX-2 inhibitor, had cardiovascular risk possibly due to its structural issue [51], also it was reported that some traditional NSAIDs and COX-2 inhibitors showed respective agent-dependent potential cardiovascular risk in long-term study with attention to permanent blockade against COX-2 activity [52]. In addition, the inhibitory actions of aspirin or of some other NSAIDs against COX-1 can be crucial problems in clinical pharmacotherapy [53]. Taken together, highly selective COX-2 inhibitors have been needed for the treatment of inflammatory diseases because of safety issues related to potential COX-1 inhibition [11,41,54].

The aim of the present study is to investigate and identify an orally active novel COX-2 selective inhibitor as a new-class antipyretic and anti-inflammatory drug, with drug design and synthesis around [2-{[(4-substituted)-pyridin-2-yl]carbonyl}-(6- or 5-substituted)-1*H*-indol-3-yl]acetic acid analogues. The significant findings of novel acid-type COX-2 inhibitors are reported herein.

2. Materials and methods

2.1. Synthesis

2.1.1. General

In general, reagents, solvents, and other chemicals were used as purchased without further purification. All reactions with air- or moisture-sensitive reactants and solvents were carried out under nitrogen atmosphere unless noted otherwise. Flash column chromatography (medium pressure liquid chromatography) purifications were carried out using Merck silica gel 60 (230–400 mesh ASTM) (Merck KGaA, Darmstadt, Germany). The structures of all isolated compounds were ensured by NMR, IR, MS or elementary analysis. ^{1}H nuclear magnetic resonance (^{1}H NMR) data were determined at 270 MHz on a JNM-LA 270 (JEOL Ltd., Akishima, Tokyo, Japan) spectrometer. Chemical shifts are expressed in δ (ppm). ^{1}H NMR chemical shifts were determined relative to tetramethylsilane (TMS) as internal standard. NMR data are reported as follows: chemical shift, number of atoms, multiplicities (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, double doublet; br, broadened), and coupling constants. Infrared spectra were measured by an IR-470 (Shimadzu Co., Kyoto, Japan) infrared spectrometer. Low-resolution mass spectral data (EI) were obtained on an Automass 120 (JEOL Ltd., Akishima, Tokyo, Japan) mass spectrometer. Melting points were obtained using an Exstar 6000 (Seiko Instruments Inc., Chiba, Japan) and were uncorrected. All final compounds **1**–**3** and synthetic intermediates were synthesized by us at Pfizer Global Research & Development, Nagoya Laboratories (Aichi, Japan). The suppliers and their locations for general reagents and solvents including dry or anhydrous solvents used in this study are shown