Synthesis and Biological Evaluation of Salicylic Acid Analogues of Celecoxib as a New Class of Selective Cyclooxygenase-1 Inhibitor

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Received December 8, 2020; accepted June 16, 2021

A series of salicylic acid analogues of celecoxib where the phenylsulfonamide moiety in the structure of celecoxib is replaced by salicylic acid moiety was synthesized and tested for *in vitro* cyclooxygenase (COX)-1 and COX-2 enzyme inhibition. Among the series, 5-substituted-2-hydroxy-benzoic acid analogues (7a–7h) generally showed better inhibitory activities on both enzymes than 4-substituted-2-hydroxy-benzoic acid analogues (12a–12h). In particular, the chloro analogue 7f which had the highest inhibitory effect (IC₅₀ = 0.0057 μ M) to COX-1 with excellent COX-1 selectivity (SI = 768) can be classified as a new potent and selective COX-1 inhibitor. The high inhibitory potency of 7f was rationalized through the docking simulation of this analogue in the active site of COX-1 enzyme.

Key words celecoxib salicylic acid analogue; selective cyclooxygenase-1 inhibitor; docking

INTRODUCTION

Cyclooxygenase (COX) is one of the key enzymes that catalyzes the rate limiting step in the formation of prostaglandins which are important biological mediators derived from arachidonic acid.^{1–5)} There were two isoforms of COX identified,⁶⁾ where COX-1 is constitutively expressed and cytoprotective in many tissues such as kidney, platelets, and stomach, while COX-2 is inducible in response to a variety of inflammatory stimuli and mainly expressed in inflammatory cells giving rise to pain, swelling, and stiffness.⁷⁾

Traditional nonselective non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen and indomethacin are effective in the treatment of inflammation through inhibition of both COX-1 and COX-2.⁸⁾ However, long-term therapy with NSAIDs usually was accompanied with some side effects such as gastrointestinal ulcers and bleeding, which are mainly due to the inhibition of COX-1.⁹⁾ Therefore, the development of selective COX-2 inhibitors could be a potential therapeutic approach to reduce side effects of NSAIDs such as dyspepsia and ulceration. A large number of COX-2 inhibitors have been discovered such as Celecoxib, Rofecoxib and Valdecoxib (Fig. 1). Celecoxib is currently the representative compound in this class as a highly selective COX-2 inhibitor, other selective COX-2 inhibitors were withdrawn from the market due to their adverse cardiovascular effects.¹⁰⁻¹²

Over the last two decades, despite the widespread belief that the inhibition of COX-1 enzyme is the main cause of gastric ulcer, recent studies showed that the inhibition of COX-1 alone is not sufficient to cause any gastric damage and COX-2 expression was up-regulated in the small intestine after administration of selective COX-1 inhibitor.^{13,14} In addition, experimental and clinical results have suggested a possible involvement of COX-1 in the early stages of neurodegenerative diseases, pain process and cancer development such as ovarian cancer.^{15,16} Although COX-1 has represented a potential therapeutic target for the design of potent analgesic agents, only mofezolac is clinically used as an analgesic drug in Japan and other selective COX-1 inhibitors such as SC-560 failed in clinical trials due to their poor pharmacokinetic properties^{17,18} (Fig. 1).

In the course of developing a new class of anti-inflammatory agent, we have investigated a series of salicylic acid analogues of current COX-2 inhibitors to determine if COX selectivity and potency could be dramatically improved by structural modification (Fig. 2). Surprisingly, when we replaced the phenylsulfonamide moiety in the structure of celecoxib with the salicylic acid moiety, it resulted in dramatic improvement of COX-1 selectivity and potency. Here, we report the synthesis and biological evaluation of a series of salicylic acid analogues of celecoxib as a new class of selective COX-1 inhibitor.

MATERIALS AND METHODS

General Information All materials and solvents were obtained from commercial suppliers and used as provided. Thin-layer chromatography was carried out on Merck 60 F254 250μ M silica gel plates (Merck, Burlington, MA, U.S.A.). Melting points were determined using a Fisher–Johns melting point apparatus and are uncorrected. ¹H- and ¹³C-NMR spectra were recorded on a Varian Gemini 400 spectrometer and a Jeol ECZ600R 600 MHz spectrometer (Jeol, Tokyo, Japan). Chemical shifts are reported in parts per million relatives

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Fig. 2. Schematic Design of Celecoxib Analogues

to internal tetramethylsilane (TMS). Electrospray ionization (ESI)-MS spectra were obtained by Shimadzu LCMS-2010EV (Shimadzu, Kyoto, Japan). Elemental analyses (C, H, and N) were made on a Euroea 3000 analyzer. HPLC analysis was carried out using a ZORBAX Eclipse C-18 column (5μ m particle size, 150×4.6 mm i.d.; Agilent Corporation, Santa Clara, CA, U.S.A.).

Chemistry

5-Amino-2-hydroxy-benzoic Acid Methyl Ester (2)

To a solution of 5-aminosalicylic acid (10.0 g, 65.3 mmol) in MeOH (160 mL) was added sulfuric acid (16 mL) dropwisely. After stirring at reflux temperature for 5 h, the reaction solution was cooled and concentrated *in vacuo*. The residue was poured into ice at 0 °C, basified with 1 M NaOH solution, and extracted with ethyl acetate. The organic phase was washed with brine, dried over Na₂SO₄, filtered and concentrated. The formed precipitation was filtered and washed with hexane to obtain the title compound as brown solid (9.87 g, 90.5%). mp 91 °C; ¹H-NMR (600 MHz, CDCl₃) δ : 10.19 (s, 1H), 7.15 (d, J=2.8Hz, 1H), 6.81–6.88 (m, 2H), 3.92 (s, 3H), 3.43 (s, 2H); ¹³C-NMR (151 MHz, CDCl₃) δ : 170.33, 154.74, 138.29, 124.26, 118.10, 114.66, 112.14, 52.13. The NMR data are consistent with the literature.¹⁹

4-Amino-2-hydroxy-benzoic Acid Methyl Ester (9)

4-Aminosalicylic acid (10g, 65.3 mmol) was subjected to the same reaction described for the synthesis of **2** as white solid. (8.40g, 76.1%) mp 117 °C; ¹H-NMR (600 MHz, CDCl₃) δ : 10.93 (s, 1H), 7.61 (d, J=8.3 Hz, 1H), 6.13–6.16 (m, 2H), 4.12 (s, 1H), 3.87 (s, 3H); ¹³C-NMR (151 MHz, CDCl₃) δ : 170.43, 163.53, 153.35, 131.59, 106.78, 102.96, 100.67, 51.65. The NMR data are consistent with the literature.¹⁹ 2-(4-Hydroxy-3-(methoxycarbonyl)phenyl)hydrazin-1-ium Chloride (**3**)

To a solution of NaNO₂ (11.4 g, 0.17 mmol) in water (80 mL) at 0 °C was added dropwisely a solution of **2** (25.0 g, 150 mmol) in concentrated HCl solution (300 mL). The diazonium solution was added to solution of SnCl₂ dihydrate (68.0 g) in concentrated HCl solution (140 mL) at 0 °C. After completion of dropping, the mixture was stirred at room temperature for 1h. The formed precipitate was filtered, and washed with diethyl ether to give the title compound as dark brown solid (25.9 g, 79.0%). ¹H-NMR (600 MHz, dimethyl sulfoxide (DMSO)-*d*₆) δ : 10.20 (s, 3H), 7.47 (d, *J* = 2.8 Hz, 1H), 7.29 (m, 1H), 6.96 (d, *J* = 9.0 Hz, 1H), 3.89 (s, 3H); ¹³C-NMR (151 MHz, DMSO-*d*₆) δ : 168.81, 155.35, 137.64, 124.48, 118.09, 116.03, 112.81, 52.51. The NMR data are consistent with the literature.²⁰

2-(3-Hydroxy-4-(methoxycarbonyl)phenyl)hydrazin-1-ium Chloride (10)

4-Amino-2-hydroxy-benzoic acid methyl ester (35.0 g, 209 mmol) was subjected to the same reaction described for the synthesis of **3** (36.4 g, 79.0%). ¹H-NMR (600 MHz, DMSO- d_6) δ : 11.10–10.28 (m, 4H), 9.13 (s, 1H), 7.65 (d, J = 8.3 Hz, 1H), 6.55–6.44 (m, 2H), 3.84 (s, 3H); ¹³C-NMR (151 MHz, DMSO- d_6) δ : 169.45, 161.97, 152.12, 130.89, 105.98, 104.60, 99.96, 52.16.

General Procedure for Preparation of 1,3-Dicarbonyl Compound (5a-5h)

To a solution of acetophenone (1 equivalent (equiv)) and NaH (1.8 equiv) in tetrahydrofuran (THF) at 0 °C for 10 min was added ethyl trifluoroacetate (1.3 equiv). The reaction mixture was stirred at room temperature for 3 h. The solution was poured into water, acidified with 1 M HCl solution, and extracted with ethyl acetate. The organic phase was washed with brine, dried over Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography to obtain the title compound. These compounds were synthesized by published method.²¹

(Z)-1,1,1-Trifluoro-4-hydroxy-4-phenylbut-3-en-2-one (5a)

White solid (10.5 g, 58.4%); mp 144 °C; ¹H-NMR (600 MHz, CDCl₃) δ : 7.92 (d, J = 7.6 Hz, 2H), 7.60 (t, J = 7.2 Hz, 1H), 7.48 (t, J = 7.6 Hz, 2H), 6.55 (s, 1H); ¹³C-NMR (151 MHz, CDCl₃) δ : 186.21, 177.23 (J_{C-F} = 36.2 Hz), 134.06, 132.81, 128.97, 127.57, 117.17 (J_{C-F} = 282.4 Hz), 92.23.

(Z)-1,1,1-Trifluoro-4-hydroxy-4-(*p*-tolyl)but-3-en-2-one (**5b**) White solid (8.20 g, 48.0%); mp 40 °C; ¹H-NMR (600 MHz, CDC1₃) δ : 7.82 (d, J = 8.3 Hz, 2H), 7.28 (d, J = 8.3 Hz, 2H), 6.52 (s, 1H), 2.42 (s, 3H); ¹³C-NMR (151 MHz, CDC1₃) δ :

186.37, 176.75 ($J_{C-F} = 34.7$ Hz), 145.42, 130.12, 129.72, 127.69, 117.26 ($J_{C-F} = 283.9$ Hz), 91.88, 21.66.

(Z)-1,1,1-Trifluoro-4-hydroxy-4-(4-methoxyphenyl)but-3en-2-one (**5c**)

White solid (2.0 g, 40.7%); ¹H-NMR (600 MHz, CDCl₃) δ : 7.87 (d, J = 9.0 Hz, 2H), 6.92 (d, J = 9.0 Hz, 2H), 6.45 (s, 1H), 3.84 (s, 3H); ¹³C-NMR (151 MHz, CDCl₃) δ : 186.16, 175.62 (J_{C-F} = 36.2 Hz), 164.64, 129.95, 125.16, 117.40 (J_{C-F} = 282.4 Hz), 114.29, 91.36, 55.40.

(*Z*)-1,1,1-Trifluoro-4-(4-(dimethylamino)phenyl)-4-hydroxybut-3-en-2-one (**5d**)

White solid (500 mg, 10.5%); ¹H-NMR (600 MHz, CDCl₃) δ : 7.84 (d, J = 9.0 Hz, 2H), 6.68 (d, J = 9.0 Hz, 2H), 6.42 (s, 1H), 3.10 (s, 6H); ¹³C-NMR (151 MHz, CDCl₃) δ : 185.99, 174.46 (J_{C-F} = 36.2 Hz), 154.28, 130.11, 119.38, 117.70 $(J_{C-F} = 283.9 \text{ Hz}), 111.19, 90.43, 40.02.$

(*Z*)-1,1,1-Trifluoro-4-(4-fluorophenyl)-4-hydroxybut-3-en-2-one (**5**e)

White solid (8.62 g, 50.9%); mp 135–137 °C; ¹H-NMR (600 MHz, CDCl₃) δ : 7.96–7.99 (m, 2H), 7.17–7.20 (m, 2H), 6.53 (s, 1H); ¹³C-NMR (CDCl₃) δ : 185.29, 176.60 (J_{C-F} = 36.2 Hz), 166.34 (J_{C-F} = 255.2 Hz), 130.27, 129.19, 117.17 (J_{C-F} = 282.4 Hz), 116.30 (J_{C-F} = 21.1 Hz), 92.13.

(*Z*)-1,1,1-Trifluoro-4-(4-chlorophenyl)-4-hydroxy-but-3-en-2-one (**5f**)

White solid (14.2 g, 87.7%); mp 54 °C; ¹H-NMR (600 MHz, CDCl₃) δ : 7.87 (d, J = 8.3 Hz, 2H), 7.47 (d, J = 8.3 Hz, 2H), 6.53 (s, 1H); ¹³C-NMR (151 MHz, CDCl₃) δ : 184.86, 177.29 ($J_{C-F} = 36.2$ Hz), 140.61, 131.26, 129.36, 128.89, 117.06 ($J_{C-F} = 283.9$ Hz), 92.25.

(Z)-1,1,1-Trifluoro-4-hydroxy-4-(4-(trifluoromethyl)phenyl)but-3-en-2-one (5g)

Colorless oil (700 mg, 15.5%); ¹H-NMR (600 MHz, CDCl₃) δ : 8.04 (d, J = 8.3 Hz, 2H), 7.76 (d, J = 8.3 Hz, 2H), 6.60 (s, 1H); ¹³C-NMR (151 MHz, CDCl₃) δ : 183.93, 178.50 ($J_{C-F} = 37.8$ Hz), 136.01, 135.21 ($J_{C-F} = 31.7$ Hz), 127.89, 125.99, 123.46 ($J_{C-F} = 273.3$ Hz), 116.98 ($J_{C-F} = 282.4$ Hz), 92.88.

(Z)-1,1,1-Trifluoro-4-hydroxy-4-(4-nitrophenyl)but-3-en-2one (**5h**)

Yellow solid (500 mg, 10.5%); mp 104 °C; ¹H-NMR (600 MHz, CDCl₃) δ : 8.36 (d, J = 9.0 Hz, 2H), 8.13 (d, J = 9.0 Hz, 2H), 6.64 (s, 1H); ¹³C-NMR (151 MHz, CDCl₃) δ : 182.37, 178.91 (J_{C-F} = 33.2 Hz), 150.60, 138.24, 128.53, 124.03, 116.78 (J_{C-F} = 285.4 Hz), 93.37.

General Procedure for Preparation of Intermediates 6a-6h and 11a-11h

To a solution of 1,3-dicarbonyl analogue (5a-5h) (1 equiv) and hydrazine analogue (2 or 9) (1.1 equiv) in MeOH (30 mL) were stirred at reflux temperature for 18h. After cooling to room temperature, the solvent was removed by rotary evaporator. The formed precipitation was filtered, washed with hexane and dried to give the title compound.

Methyl 2-Hydroxy-5-(5-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoate (**6a**)

White solid (1.78 g, 73.6%); mp 93 °C; ¹H-NMR (600 MHz, DMSO- d_6) δ : 10.76 (s, 1H), 7.75 (d, J = 2.8 Hz, 1H), 7.39–7.30 (m, 6H), 7.17 (s, 1H), 7.05 (d, J = 9.0 Hz, 1H), 3.83 (s, 3H); ¹³C-NMR (151 MHz, DMSO- d_6) δ : 167.36, 159.35, 144.88, 141.41 ($J_{C-F} = 37.8$ Hz), 132.25, 130.34, 129.13, 128.73, 128.70, 128.31, 127.47, 121.37 ($J_{C-F} = 268.8$ Hz), 118.20, 114.45, 105.40, 52.47.

Methyl 2-Hydroxy-5-(5-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoate (**6b**)

White solid (3.87 g, 59.4%); mp 252 °C; ¹H-NMR (600 MHz, DMSO- d_6) δ : 10.75 (s, 1H), 7.76 (d, J = 2.8 Hz, 1H), 7.35 (dd, J = 9.0, 2.8 Hz, 1H), 7.18 (s, 4H), 7.11 (s, 1H), 7.05 (d, J = 9.0 Hz, 1H), 3.84 (s, 3H), 2.29 (s, 3H); ¹³C-NMR (151 MHz, DMSO- d_6) δ : 167.32, 159.29, 144.92, 141.32 (J_{C-F} = 37.8 Hz), 138.72, 132.22, 130.39, 129.23, 128.53, 127.43, 125.39, 121.35 (J_{C-F} = 270.3 Hz), 118.15, 114.46, 105.04, 52.43, 20.68.

Methyl 2-Hydroxy-5-(5-(4-methoxyphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoate (**6c**)

White solid (200 mg, 55.7%); mp 196 °C; ¹H-NMR (400 MHz, DMSO- d_6) δ : 10.77 (s, 1H), 7.74 (d, J = 2.8 Hz, 1H), 7.33 (dd, J = 6.4, 2.8 Hz, 1H), 7.19 (d, J = 8.8 Hz, 2H), 7.04 (m, 2H), 6.90 (d, J = 8.8 Hz, 2H), 3.82 (s, 3H), 3.72 (s, 3H);

¹³C-NMR (100 MHz, DMSO- d_6) δ : 188.78, 167.22, 159.46, 159.14, 144.56, 141.13 (J_{C-F} = 37.2 Hz), 132.18, 130.33, 129.93, 127.30, 121.27 (J_{C-F} = 266.2 Hz), 120.32, 118.06, 114.00, 104.65, 55.16, 52.50.

Methyl 5-(5-(4-(Dimethylamino)phenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)-2-hydroxybenzoate (**6d**)

Yellow oil (250 mg, 67.4%); ¹H-NMR (600 MHz, CDCl₃) δ : 10.88 (s, 1H), 8.01 (d, J=2.8Hz, 1H), 7.26 (dd, J=8.6, 3.1Hz, 2H), 7.06 (dd, J=6.9, 2.1Hz, 1H), 6.92 (d, J=9.0Hz, 1H), 6.60–6.63 (m, 3H), 3.94 (s, 3H), 2.97 (s, 6H); ¹³C-NMR (151 MHz, CDCl₃) δ : 169.85, 161.22, 150.51, 145.46, 143.36 (J_{C-F} =37.9Hz), 133.02, 131.55, 129.58, 127.18, 123.38 (J_{C-F} =268.8Hz), 118.10, 116.04, 112.61, 111.85, 104.05, 52.55, 40.13.

Methyl 5-(5-(4-Fluorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)-2-hydroxybenzoate (**6e**)

White solid (3.46 g, 49.8%); mp 116 °C; ¹H-NMR (600 MHz,CDCl₃) δ : 7.83 (d, J = 9.0 Hz, 1H), 7.23–7.25 (m, 2H), 7.06 (t, J = 8.3 Hz, 2H), 6.95 (d, J = 2.1 Hz, 1H), 6.81 (dd, J = 9.0, 2.1 Hz, 1H), 6.73 (s, 1H), 3.96 (s, 3H); ¹³C-NMR (151 MHz, CDCl₃) δ : 169.59, 163.00 (J_{C-F} = 249.2 Hz), 161.46, 143.72, 143.16 (J_{C-F} = 39.3 Hz), 132.69, 130.72, 130.68, 127.07, 125.01, 121.13 (J_{C-F} = 268.8 Hz), 118.41, 116.02 (J_{C-F} = 21.2 Hz), 112.63, 105.35, 52.64.

Methyl 5-(5-(4-Chlorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)-2-hydroxybenzoate (**6f**)

White solid (4.08 g, 64.3%); mp 96 °C; ¹H-NMR (600 MHz, CDCl₃) δ : 10.90 (s, 1H), 7.91 (d, J = 2.8 Hz, 1H), 7.33–7. 25 (m, 3H), 7.16 (d, J = 8.3 Hz, 2H), 6.95 (d, J = 9.0 Hz, 1H), 6.74 (s, 1H), 3.93 (s, 3H); ¹³C-NMR (151 MHz, CDCl₃) δ : 169.57, 161.53, 143.54, 143.23 ($J_{C-F} = 39.3$ Hz), 135.37, 132.69, 130.65, 129.98, 129.13, 127.30, 127.08, 121.09 ($J_{C-F} = 268.8$ Hz), 118.47, 112.68, 105.47, 52.66.

Methyl 2-Hydroxy-5-(3-(trifluoromethyl)-5-(4-(trifluoromethyl)phenyl)-1*H*-pyrazol-1-yl)benzoate (**6g**)

Colorless oil (175 mg, 44.5%); ¹H-NMR (600 MHz, CDCl₃) δ : 10.91 (s, 1H), 7.88 (d, J = 2.8Hz, 1H), 7.61 (d, J = 8.3Hz, 2H), 7.36 (d, J = 8.3Hz, 2H), 7.29 (dd, J = 9.0, 2.8Hz, 1H), 6.96 (d, J = 9.0Hz, 1H), 6.81 (s, 1H), 3.92 (s, 3H); ¹³C-NMR (151 MHz, CDCl₃) δ : 169.50, 161.64, 143.40 (J_{C-F} = 37.8Hz), 143.15, 132.61, 131.12 (J_{C-F} = 31.7Hz), 130.48, 129.07, 127.04, 126.00, 125.82, 123.66 (J_{C-F} = 271.8Hz), 121.03 (J_{C-F} = 268.8Hz), 118.62, 112.70, 105.97, 52.63.

Methyl 2-Hydroxy-5-(5-(4-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoate (**6h**)

Colorless oil (210 mg, 56.4%); ¹H-NMR (600 MHz, CDCl₃) δ : 10.94 (s, 1H), 8.21 (dd, J = 6.9, 2.1 Hz, 2H), 7.90 (d, J = 2.8 Hz, 1H), 7.42 (dd, J = 6.9, 2.1 Hz, 2H), 7.26–7.29 (m, 1H), 6.99 (d, J = 9.0 Hz, 1H), 6.88 (s, 1H), 3.93 (s, 3H); ¹³C-NMR (151 MHz, CDCl₃) δ : 169.39, 161.87, 147.86, 143.56 ($J_{C-F} = 37.8$ Hz), 142.32, 134.94, 132.64, 130.25, 129.48, 127.18, 124.08, 120.89 ($J_{C-F} = 270.3$ Hz), 118.82, 112.87, 106.43, 52.76.

Methyl 2-Hydroxy-4-(5-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoate (**11a**)

White solid (1.70 g, 49.6%); mp 93 °C; ¹H-NMR (600 MHz, DMSO- d_6) δ : 10.70 (s, 1H), 7.78 (d, J = 8.3 Hz, 1H), 7.43–7.40 (m, 3H), 7.36–7.33 (m, 2H), 7.21 (s, 1H), 6.98 (s, 1H), 6.86 (dd, J = 8.3, 2.1 Hz, 1H), 3.87 (s, 3H); ¹³C-NMR (100 MHz, DMSO- d_6) δ : 167.49, 159.45, 144.83, 143.27, 141.91 ($J_{C-F} = 37.9$ Hz), 131.09, 129.20, 128.63, 128.60, 128.10, 121.17 ($J_{C-F} = 266.2$ Hz), 115.95, 114.02, 113.85, 106.31, 52.50.

Methyl 2-Hydroxy-4-(5-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoate (**11b**)

White solid (1.67 g, 46.6%); mp 169–171 °C; ¹H-NMR (600 MHz, CDCl₃) δ : 10.86 (s, 1H), 7.82 (d, J=9.0 Hz, 1H), 7.15 (m, 4H), 6.97 (d, J=2.1 Hz, 1H), 6.85 (dd, J=7.9, 1.7 Hz, 1H), 6.71 (s, 1H), 3.96 (s, 3H), 2.37 (s, 3H); ¹³C-NMR (100 MHz, CDCl₃) δ : 169.62, 161.70, 144.90, 144.60, 142.96 (J_{C-F} =30.4 Hz), 139.35, 130.59, 129.42, 128.48, 125.76, 121.15 (J_{C-F} =266.2 Hz), 115.81, 113.93, 111.80, 106.02, 52.63, 21.47.

Methyl 2-Hydroxy-4-(5-(4-methoxyphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoate (**11c**)

White solid (200 mg, 55.7%); mp 169–171 °C; ¹H-NMR (400 MHz, DMSO- d_6) δ : 7.76 (d, J = 8.8 Hz, 1H), 7.22 (d, J = 8.8 Hz, 2H), 7.08 (s, 1H), 6.93 (m, 3H), 6.82 (m, 1H) 3.86 (s, 3H), 3.75 (s, 3H); ¹³C-NMR (DMSO- d_6) δ : 188.78, 167.67, 159.68, 159.58, 144.74, 143.56, 141.89 (J_{C-F} = 37.2 Hz), 131.05, 130.01, 125.35 (J_{C-F} = 289.7 Hz), 120.27, 115.94, 114.08, 113.76, 105.72, 55.19, 52.51.

Methyl 4-(5-(4-(Dimethylamino)phenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)-2-hydroxybenzoate (**11d**)

Pale yellow oil (180 mg, 48.5%); ¹H-NMR (600 MHz, CDCl₃) δ : 10.84 (s, 1H), 7.81 (d, J=9.0Hz, 1H), 7.03–7.09 (m, 3H), 6.87 (dd, J=8.3, 2.1Hz, 1H), 6.62–6.63 (m, 3H), 3.95 (s, 3H), 2.98 (s, 6H); ¹³C-NMR (151 MHz, CDCl₃) δ : 169.92, 161.99, 150.69, 145.77, 145.30, 143.65 (J_{C-F} =37.8Hz), 130.60, 129.61, 121.26 (J_{C-F} =270.3Hz), 116.00, 115.91, 114.04, 111.85, 111.68, 105.11, 52.46, 40.09.

Methyl 4-(5-(4-Fluorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)-2-hydroxybenzoate (**11e**)

White solid (1.96 g, 52.0%); mp 139 °C; ¹H-NMR (CDCl₃) δ : 10.88 (s, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.23–7.26 (m, 2H), 7.06 (t, J = 8.4 Hz, 2H), 6.95 (d, J = 2.1 Hz, 1H), 6.81 (dd, J = 9.0, 2.1 Hz, 1H), 6.73 (s, 1H), 3.96 (s, 3H); ¹³C-NMR (151 MHz, CDCl₃) δ : 169.84, 163.20 ($J_{C-F} = 247.6$ Hz), 162.03, 151.26, 144.54, 133.82 ($J_{C-F} = 40.5$ Hz), 130.90, 127.66, 127.57, 119.54 ($J_{C-F} = 268.8$ Hz), 115.88 ($J_{C-F} = 37.8$ Hz), 115.85, 114.21, 112.73, 106.98, 52.63.

Methyl 4-(5-(4-Chlorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)-2-hydroxybenzoate (**11f**)

White solid (0.46 g, 28.8%); mp 98 °C; ¹H-NMR (600 MHz, CDCl₃) δ : 10.89 (s, 1H), 7.83 (d, J = 8.3 Hz, 1H), 7.18–7.35 (m, 4H), 6.97 (d, J = 2.1 Hz, 1H), 6.74–6.81 (m, 2H), 3.96 (s, 3H); ¹³C-NMR (151 MHz, CDCl₃) δ : 169.73, 162.02, 144.37, 144.12 (J_{C-F} = 37. 8 Hz), 143.73, 135.62, 130.89, 129.98, 129.18, 127.27, 120.96 (J_{C-F} = 268.8 Hz), 115.82, 114.08, 112.22, 106.44, 52.57.

Methyl 2-Hydroxy-4-(3-(trifluoromethyl)-5-(4-(trifluoromethyl)phenyl)-1*H*-pyrazol-1-yl)benzoate (**11g**)

Pale yellow oil (175 mg, 44.5%); ¹H-NMR (600 MHz, CDCl₃) δ : 9.10 (s, 1H), 7.85 (d, J = 8.3 Hz, 1H), 7.63 (d, J = 7.6 Hz, 2H), 7.39 (d, J = 8.3 Hz, 2H), 6.99 (d, J = 2.1 Hz, 1H), 6.78–6.82 (m, 2H), 3.97 (s, 3H); ¹³C-NMR (151 MHz, CDCl₃) δ : 169.70, 162.11, 144.21, 144.02 ($J_{C-F} = 39.3$ Hz), 143.35, 132.34, 131.34 ($J_{C-F} = 33.2$ Hz), 131.01, 129.06, 125.88, 123.64 ($J_{C-F} = 273.3$ Hz), 120.89 ($J_{C-F} = 268.8$ Hz), 115.85, 114.21, 112.45, 106.94, 52.61.

Methyl 2-Hydroxy-4-(5-(4-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoate (**11h**)

Yellow oil (145 mg, 38.9%); ¹H-NMR (600 MHz, $CDCl_3$) δ : 8.23 (dd, J = 6.9, 2.1 Hz, 2H), 7.87 (d, J = 8.3 Hz, 1H), 7.44–7.46 (m, 2H), 6.96 (d, J = 2.1 Hz, 1H), 6.88 (s, 1H), 6.80 (dd, J = 8.6, 2.4 Hz, 1H), 3.98 (s, 3H); ¹³C-NMR (151 MHz, CDCl₃) δ : 169.62, 162.16, 148.04, 144.20 ($J_{C-F} = 39.3 \text{ Hz}$), 143.95, 142.47, 134.94, 131.19, 129.57, 124.13, 120.77 ($J_{C-F} = 270.3 \text{ Hz}$), 115.84, 114.26, 112.70, 107.37, 52.69.

General Procedure for Preparation of Final Products 7a-7h and 12a-12h

To a solution of methyl ester analogues (6a-6h and 11a-11h) (1 equiv) in THF was added 2.5M NaOH solution (2.5 equiv) and water. The resulting mixture was refluxed for 2h. After the solution was cooled to room temperature, the solution was acidified by 1 M HCl solution. The solution was extracted with ethyl acetate, washed with brine, dry over Na₂SO₄, filtered and concentrated to give the title compound.

2-Hydroxy-5-(5-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoic Acid (7a)

White solid (392 mg, 81.5, 99% purity (according to HPLC)); mp 189 °C; ¹H-NMR (600 MHz, DMSO- d_6) δ : 7.74 (d, J = 2.1 Hz, 1H), 7.43 (dd, J = 9.0, 2.1 Hz, 1H), 7.39 (m, 3H), 7.31 (m, 2H), 7.16 (s, 1H), 7.00 (d, J = 9.0 Hz, 1H); ¹³C-NMR (151 MHz, DMSO- d_6) δ : 170.73, 160.83, 144.86, 141.41 ($J_{C-F} = 37.8$ Hz), 132.73, 130.32, 129.15, 128.79, 128.73, 128.37, 127.36, 121.40 ($J_{C-F} = 267.2$ Hz), 117.99, 113.44, 105.49; m/z 347 [M–H]⁻.

2-Hydroxy-5-(5-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoic Acid (7b)

White solid (286 mg, 84.9, 99% purity (according to HPLC)); mp 222–224 °C; ¹H-NMR (600 MHz, DMSO- d_6) δ : 7.75 (d, J=2.1 Hz, 1H), 7.41 (dd, J=9.0, 2.8 Hz, 1H), 7.19 (m, 5H), 7.00 (d, J=9.0 Hz, 1H), 2.29 (s, 3H); ¹³C-NMR (151 MHz, DMSO- d_6) δ : 170.72, 160.82, 144.95, 141.36 (J_{C-F} = 37.8 Hz), 138.79, 132.78, 130.40, 129.31, 128.63, 127.38, 125.47, 121.92 (J_{C-F} = 268.8 Hz), 117.98, 113.49, 105.18, 20.78; m/z 361 [M–H]⁻.

2-Hydroxy-5-(5-(4-methoxyphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoic Acid (7c)

White solid (50 mg, 51.9, 98% purity (according to HPLC)); mp 190–194 °C; ¹H-NMR (400 MHz, DMSO- d_6) δ : 7.75 (s, 1H), 7.40 (d, J = 8.8 Hz, 1H), 7.21 (d, J = 8.4 Hz, 2H), 7.05 (m, 2H), 6.91 (d, J = 8.4 Hz, 2H), 3.73 (s, 3H); ¹³C-NMR (100 MHz, DMSO- d_6) δ : 170.60, 160.62, 159.51, 144.57, 141.20 (J_{C-F} = 37.2 Hz), 132.61, 130.33, 129.99, 127.22, 121.30 (J_{C-F} = 266.2 Hz), 120.41, 117.87, 114.04, 113.28, 104.75, 55.18; m/z 377 [M–H]⁻.

5-(5-(4-(Dimethylamino)phenyl)-3-(trifluoromethyl)-1*H*pyrazol-1-yl)-2-hydroxybenzoic Acid (**7d**)

White solid (65 mg, 67.33, 98% purity (according to HPLC)); mp >300 °C; ¹H-NMR (600 MHz, CD₃OD) δ : 7.79 (d, J = 2.8 Hz, 1H), 7.67 (d, J = 8.3 Hz, 2H), 7.45–7.54 (m, 3H), 7.00–7.03 (m, 2H), 3.28 (s, 6H); ¹³C-NMR (151 MHz, CD₃OD) δ : 171.30, 163.03, 152.87, 148.14, 144.45 ($J_{C-F} = 39.3$ Hz), 134.82, 133.21, 131.26, 128.97, 123.38 ($J_{C-F} = 267.3$ Hz), 119.78, 117.60, 114.38, 113.59, 105.34, 40.83.

5-(5-(4-Fluorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1yl)-2-hydroxybenzoic Acid (7e)

White solid (432 mg, 90.6, 99% purity (according to HPLC)); mp 228–231 °C; ¹H-NMR (600 MHz, DMSO- d_6) δ : 7.83 (d, J = 9.0 Hz, 1H), 7.39 (m, 2H), 7.20–7.28 (m, 3H), 6.91–6.86 (m, 2H); ¹³C-NMR (151 MHz, DMSO- d_6) δ : 170.97, 162.55 ($J_{C-F} = 249.2$ Hz), 161.22, 144.12, 143.66, 142.10 ($J_{C-F} = 39.3$ Hz), 131.43, 131.27, 124.87, 121.26 ($J_{C-F} = 268.8$ Hz), 115.96, 115.95 ($J_{C-F} = 39.3$ Hz), 113.80, 113.53, 106.49; m/z 365 [M–H]⁻.

5-(5-(4-Chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)-

2-hydroxybenzoic Acid (7f)

White solid (413 mg, 85.7, 100% purity (according to HPLC)); mp 226 °C; IR (KBr) cm⁻¹ 3065, 1681; ¹H-NMR (600 MHz, DMSO- d_6) δ : 7.77 (d, J = 2.8 Hz, 1H), 7.43–7.47 (m, 3H), 7.33 (d, J = 9.0 Hz, 2H), 7.20 (s, 1H), 7.01 (d, J = 9.0 Hz, 1H); ¹³C-NMR (151 MHz, DMSO- d_6) δ : 170.71, 160.94, 143.67, 141.46 ($J_{C-F} = 37.8$ Hz), 134.03, 132.74, 130.60, 130.10, 128.80, 127.44, 127.24, 121.34 ($J_{C-F} = 268.8$ Hz), 118.08, 113.53, 105.76; m/z 381 [M–H]⁻; *Anal.* Calcd for C₁₇H₁₀ClF₃N₂O₃: C, 53.35; H, 2.63; N, 7.32. Found: C, 53.52; H, 2.73; N, 7.45. heteronuclear multiple bond connectivity (HMBC), heteronuclear single quantum coherence (HSQC) in supplementary material.

2-Hydroxy-5-(3-(trifluoromethyl)-5-(4-(trifluoromethyl)phenyl)-1*H*-pyrazol-1-yl)benzoic Acid (7g)

White solid (45 mg, 46.5, 99% purity (according to HPLC)); mp 258 °C; ¹H-NMR (600 MHz, CD₃OD) δ : 7.90 (d, J = 8.3 Hz, 1H), 7.69 (d, J = 8.3 Hz, 2H), 7.51 (d, J = 8.3 Hz, 2H), 7.04 (s, 1H), 6.81–6.91 (m, 2H); ¹³C-NMR (151 MHz, CD₃OD) δ : 172.58, 163.62, 145.46, 145.19, 144.89 (J_{C-F} = 39.3 Hz), 134.00, 132.76, 132.25 (J_{C-F} = 33.2 Hz), 130.71, 126.83, 125.28 (J_{C-F} = 271.8 Hz), 122.55 (J_{C-F} = 267.3 Hz), 117.08, 115.11, 114.40, 107.88; m/z 415 [M–H]⁻.

2-Hydroxy-5-(5-(4-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoic Acid (7**h**)

Pale yellow solid (40 mg, 41.4, 99% purity (according to HPLC)); mp 243 °C; ¹H-NMR (600 MHz, CD₃OD) δ : 8.22 (dd, J = 6.9, 2.1 Hz, 2H), 7.82 (d, J = 2.8 Hz, 1H), 7.55 (dd, J = 6.9, 2.1 Hz, 2H), 7.44 (dd, J = 9.0, 2.8 Hz, 1H), 7.10 (s, 1H), 6.92–7.01 (m, 1H); ¹³C-NMR (151 MHz, CD₃OD) δ : 172.28, 163.54, 149.35, 144.44, 144.36 ($J_{C-F} = 39.3$ Hz), 136.32, 133.92, 131.50, 131.17, 128.90, 124.89, 122.59 ($J_{C-F} = 268.8$ Hz), 119.48, 114.41, 107.44; m/z 392 [M–H]⁻.

2-Hydroxy-4-(5-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-1yl)benzoic Acid (**12a**)

White solid (243 mg, 84.7, 99% purity (according to HPLC)); mp 159 °C; ¹H-NMR (600 MHz, DMSO- d_6) δ : 7.79 (d, J = 8.3 Hz, 1H), 7.39–7.38 (m, 3H), 7.30 (dd, J = 7.6, 2.1 Hz, 2H), 7.17 (s, 1H), 6.85 (td, J = 8.6, 2.0 Hz, 2H); ¹³C-NMR (151 MHz, DMSO- d_6) δ : 170.88, 161.09, 145.07, 143.79, 142.08 ($J_{C-F} = 37.8$ Hz), 131.33, 129.36, 128.78, 128.31, 121.23 ($J_{C-F} = 268.8$ Hz), 120.34, 116.05, 113.70, 113.35, 106.38; m/z 347 [M–H]⁻.

2-Hydroxy-4-(5-(*p*-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1yl)benzoic Acid (**12b**)

White solid (172 mg, 89.6, 99% purity (according to HPLC)); mp 172 °C; ¹H-NMR (600 MHz, DMSO- d_6) δ : 7.83 (d, J = 8.3 Hz, 1H), 7.22 (s, 4H), 7.15 (s, 1H), 6.90-6.86 (m, 2H), 2.31 (s, 3H); ¹³C-NMR (DMSO- d_6) δ : 170.93, 161.11, 145.14, 143.91, 142.07 (J_{C-F} = 39.3 Hz), 139.04, 131.34, 129.35, 128.64, 125.42, 121.27 (J_{C-F} = 268.8 Hz), 116.08, 113.70, 113.30, 106.08, 20.77; m/z 361 [M-H]⁻.

2-Hydroxy-4-(5-(4-methoxyphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoic Acid (**12c**)

White solid (60 mg, 62.2, 99% purity (according to HPLC)); mp 189 °C; ¹H-NMR (400 MHz, DMSO- d_6) δ : 7.82 (d, J = 8.0 Hz, 1H), 7.24 (d, J = 7.2 Hz, 2H), 7.10 (s, 1H), 6.90 (m, 4H), 3.76 (s, 3H); ¹³C-NMR (151 MHz, DMSO- d_6) δ : 170.81, 160.97, 159.70, 144.80, 144.83, 141.87 (J_{C-F} = 37.2 Hz), 131.22, 130.08, 121.18 (J_{C-F} = 266.2 Hz), 120.33, 115.97, 114.13, 113.59, 113.08, 105.75, 55.24; m/z 377 [M–H]⁻.

4-(5-(4-(Dimethylamino)phenyl)-3-(trifluoromethyl)-1H-

pyrazol-1-yl)-2-hydroxybenzoic Acid (12d)

White solid (55 mg, 57.0, 98% purity (according to HPLC)); mp >300 °C; ¹H-NMR (600 MHz, CD₃OD) δ : 7.86 (d, J = 8.3 Hz, 1H), 7.09 (d, J = 9.0 Hz, 2H), 6.90–6.92 (m, 1H), 6.83 (dd, J = 8.3, 2.1 Hz, 1H), 6.75 (s, 1H), 6.70 (d, J = 9.0 Hz, 2H), 2.95 (s, 6H); ¹³C-NMR (151 MHz, CD₃OD) δ : 172.75, 163.52, 152.41, 147.67, 146.28, 144.55 ($J_{C-F} = 39.3$ Hz), 132.43, 130.75, 126.12, 122.79 ($J_{C-F} = 267.3$ Hz), 117.18, 117.09, 114.94, 113.16, 105.69, 40.36; m/z 390 [M–H]⁻.

4-(5-(4-Fluorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)-2-hydroxybenzoic Acid (**12e**)

White solid (179 mg, 92.7, 99% purity (according to HPLC)); mp 162 °C; ¹H-NMR (DMSO- d_6) &: 7.97–8.01 (m, 3H), 7.76 (s, 1H), 7.31 (t, J = 8.6 Hz, 2H), 7.16–7.19 (m, 2H); ¹³C-NMR (DMSO- d_6) &: 170.88, 162.51 ($J_{C-F} = 267.2$ Hz), 161.18, 150.61, 143.51, 132.65 ($J_{C-F} = 37.8$ Hz), 131.52, 127.85, 127.51, 121.30 ($J_{C-F} = 268.8$ Hz), 115.92, 115.85 ($J_{C-F} = 22.7$ Hz), 114.17, 113.63, 107.83; m/z 365 [M–H]⁻.

4-(5-(4-Chlorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1yl)-2-hydroxybenzoic Acid (**12f**)

White solid (174 mg, 91.1, 99% purity (according to HPLC)); mp 226 °C; ¹H-NMR (600 MHz, DMSO- d_6) δ : 7.84 (d, J = 9.0 Hz, 1H), 7.50 (dd, J = 6.9, 2.1 Hz, 2H), 7.36 (dd, J = 6.5, 1.7 Hz, 2H), 7.26 (s, 1H), 6.93 (d, J = 2.1 Hz, 1H), 6.87 (dd, J = 9.0, 2.1 Hz, 1H); ¹³C-NMR (151 MHz, DMSO- d_6) δ : 170.88, 161.14, 143.88, 143.58, 142.11 (J_{C-F} = 39.3 Hz), 134.24, 131.47, 130.65, 128.87, 127.16, 121.19 (J_{C-F} = 268.8 Hz), 116.14, 113.83, 113.55, 106.66; m/z 381 [M–H]⁻.

2-Hydroxy-4-(3-(trifluoromethyl)-5-(4-(trifluoromethyl)phenyl)-1*H*-pyrazol-1-yl)benzoic Acid (**12**g)

White solid (35 mg, 36.2, 99% purity (according to HPLC)); mp 218 °C; ¹H-NMR (600 MHz, CD₃OD) δ : 7.77 (d, J = 2.8 Hz, 1H), 7.64 (d, J = 8.3 Hz, 2H), 7.36–7.46 (m, 3H), 6.94–6.99 (m, 2H); ¹³C-NMR (151 MHz, CD₃OD) δ : 172.28, 163.35, 145.02, 144.24 (J_{C-F} = 36.2 Hz), 133.94, 133.78, 132.02 (J_{C-F} = 33.2 Hz), 131.53, 130.64, 128.81, 126.73, 125.22 (J_{C-F} = 271.8 Hz), 122.60 (J_{C-F} = 267.3 Hz), 119.32, 114.28, 106.98; m/z 415 [M–H]⁻.

2-Hydroxy-4-(5-(4-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzoic Acid (**12h**)

Yellow solid (35 mg, 36.2, 98% purity (according to HPLC)); mp 232 °C; ¹H-NMR (600 MHz, CD₃OD) δ : 7.86 (d, J= 8.3 Hz, 1H), 7.09 (d, J= 9.0 Hz, 2H), 6.90 (d, J= 2.1 Hz, 1H), 6.82 (dd, J= 8.3, 2.1 Hz, 1H), 6.74 (s, 1H), 6.69 (d, J= 9.0 Hz, 2H), 2.94 (s, 6H); ¹³C-NMR (151 MHz, CD₃OD) δ : 170.58, 161.35, 149.55, 146.53 (J_{C-F} = 38.4 Hz), 145.12, 142.88, 134.81, 132.66, 129.98, 123.28, 120.54 (J_{C-F} = 268.9 Hz), 116.02, 114.85, 111.22, 108.91; m/z 392 [M–H]⁻.

Biology

In Vitro COX Inhibition Assay

Inhibitory actions of celecoxib analogues toward COX-1 and COX-2 activities were individually determined using a fluorometric COX inhibitor screening assay kit as recommended by the manufacturer (catalog no. K548-100 for COX-1, and K547-100 for COX-2, Biovision, Milpitas, CA, U.S.A.).²²⁾ Briefly, the assay was performed using the reaction master mixture (COX Assay Buffer, COX Probe, Diluted COX Cofactor and COX-1). The test samples were dissolved in DMSO and evaluated at various concentrations. Then arachidonic acid in NaOH solution added to initiate the reaction and incubated at 37 °C for 20 min. The assay directly detects fluorometric PGG2 generated by the COX enzyme at Ex/Em = 535/587 nm using spectramax M2/spectraMax L (Molecular Devices, San Jose, CA, U.S.A.). SC-560 and celecoxib were used as positive controls for the COX-1 and the COX-2 assay, respectively. The average fluorescence was calculated for all samples (n = 3) to determine percent inhibition.

Molecular Docking

Molecular docking is used to generate a score for every compound considering the interaction between a target protein and the ligand.²³⁾ The molecular modeling studies of the selected compound **7f** was performed in Autodock Vina. The X-ray crystallographic structure of COX-1 (PDB ID: 3KK6) and COX-2 (PDB ID: 3LN1) was downloaded from the protein data bank website.²⁴⁾ In this study, COX-1 (PDB ID: 3KK6) and COX-2 (PDB ID: 3LN1) cocrystalized with celecoxib were chosen for molecular docking. The docking images were analyzed by UCSF Chimera.

RESULTS AND DISCUSSION

 NH_2

óн

1

COOH

Chemistry A series of salicylic acid analogues of celecoxib where the phenylsulfonamide moiety is replaced with salicylic acid moiety were prepared from 5-aminosalicylic acid and 4-aminosalicylic acid as illustrated in Chart 1. In order to prepare hydrazino-2-hydroxybenzoic acid methyl esters (**3** and **10**), the carboxylic acid group of aminosalicylic acid (**1** and **8**) was first protected, and then the resulting aminosalicylic acid methyl esters (**2** and **9**) were converted into the hydrazine compounds by the reduction of the diazonium salts which were formed as an intermediate. The corresponding 1,3-dicarbonyl analogues (**5a**–**5h**) were prepared from Claisen condensation of an acetophenone analogues with ethyl trifluoroacetate. According to the reported method for the synthesis of trifluoromethyl-substituted pyrazoles,²⁵ 1,3-dicarbonyl analogues (**5a–5h**) were reacted with hydrazino-2-hydroxybenzoic

óн

2

COOMe

acid methyl ester in refluxing methanol followed by chromatographic separation to produce the cyclized 1,5-diarylpyrazoles (6a-6h and 11a-11h). Finally, saponification of methyl ester of 1,5-diarylpyrazole in basic condition produced the desired salicylic acid analogues (7a-7h and 12a-12h).

Biology All compounds described herein were evaluated for their ability to block ovine COX-1 and human recombinant COX-2.^{26–28)} Inhibition of the COX-1 and COX-2 (IC₅₀ values, μ M) was determined using a COX fluorometric inhibitor screening assay kit (catalog number K547 and K548, Biovision) according to the previously reported method.²⁹⁾

It has been well known that a *p*-methylsulfone or sulfonamide on one of the phenyl groups in the diaryl heterocyclic class of COX-2 inhibitors is essential for good COX-2 potency and selectivity.³⁰⁾ In addition, a pyrazole ring has been attributed to potent anti-inflammatory effects and low toxicity.³¹⁾ Based on these observations, we investigated the substituent effects of the salicylic acid moiety in place of the sulfonamide moiety in the structure of celecoxib as well as the substituent effects on one of the aryl rings by introducing various groups without changing the diaryl pyrazole ring system. The potencies of the synthesized compounds (7a–7h, 12a–12h) compared to SC-560 and celecoxib were measured using the reported *in vitro* COX inhibition assay. Selectivity index (SI) value was calculated from IC₅₀ (COX-2)/IC₅₀ (COX-1). The values of IC₅₀ and SI were presented in Table 1.

As shown in Table 1, 5-substituted-2-hydroxy-benzoic acid analogues (7a-7h) generally showed better inhibitory activities on both COX-1 and COX-2 enzymes than 4-substituted-2-hydroxy-benzoic acid analogues (12a-12h). First, we compared the COX-2 inhibitory activity of methyl analogue 7b derived from 5-aminosalicylic acid and the corresponding methyl analogue 12b derived from 4-aminosalicylic acid, and found that 7b showed much higher activity than 12b with IC₅₀

HOOC

HO

 $R_1 = H$

 $R_1 = Me$

 $R_1 = OMe$



MeOOC

HO

6a-6h

NHNH₂HCI

ÓН

3

COOMe

Reagents and conditions: (a) MeOH, H_2SO_4 , reflux, 5h; (b) NaNO₂, SnCl₂, HCl solution, 0°C to r.t., 1h; (c) ethyl trifluoroacetate, NaH, THF, 0°C to r.t., 3h; (d) MeOH, reflux, 18h; (e) 2.5 M NaOH, THF, H₂O, reflux, 2h.

CF₃

7a

7b

7c

values of 5.53 and $62.5\,\mu$ M, respectively. **7b** also revealed an unexpected potent inhibition of COX-1 enzymes with IC₅₀ values of 0.0147 μ M while **12b** had no effect on the COX-1 enzyme (IC₅₀ = 12099 μ M).

Based on this result, we then investigated the substituent effects on one of the aryl rings in a series of 5-substituted-2-hydroxy-benzoic acid analogues (7a-7h). The COX-1 inhibitory effect of the hydrogen analogue 7a where the methyl group was removed from methyl analogue revealed lower activity than that of 7b with the IC_{50} value of $0.36 \,\mu$ M. Replacement of the methyl group in 7b with either a methoxy group (7c) or dimethylamino group (7d) also reduced the COX-1 inhibitory activities significantly with IC₅₀ values of 1.74 and $8.39\,\mu$ M, respectively. Similarly, the analogues which contained an electron-withdrawing group, such as the fluoro analogue 7e, the trifluoromethyl analogue 7g and the nitro analogue 7h, revealed much weaker COX-1 inhibitory activities than the methyl analogue **7b** with 5.99, 1.46 and 7.03 μ M, respectively. However, to our surprise, when the methyl group in 7b was replaced with chloro group, the chloro analogue 7f showed about three times higher inhibition (IC₅₀ = $0.0059 \,\mu$ M) than 7b even though this potency is slightly lower activity compared to the reference COX-1 selective inhibitor SC-560 $(IC_{50} = 0.0038 \,\mu M)$. These results indicated that electron density in the C-5 phenyl of pyrazole ring is not important for

Table 1. In Vitro COX-1 and COX-2 Inhibitory Activities for 1,5-Diarylpyrazole Compounds from 5-Aminosalicylic Acid and 4-Aminosalicylic Acid

R ₁		R ₁		
HOOC.				
но		HOOC		
7a-7h		12a-12h		
Compound	R ₁ -	IC ₅₀ (µM) ^{a)}		- COX 1 SI ^b
		COX-1	COX-2	COA-1 51 /
7a	Н	0.36	31.90	88.6
7b	Me	0.0147	5.53	376.2
7c	OMe	1.74	8.02	4.6
7d	NMe ₂	8.39	6.67	0.8
7e	F	5.99	5.53	0.9
7f	Cl	0.0059	4.54	768.2
7g	CF ₃	1.46	0.26	0.2
7h	NO_2	7.03	5.90	0.8
12a	Н	13750	933	0.07
12b	Me	12099	62.5	0.005
12c	OMe	1.58	1.31	0.8
12d	NMe ₂	4376	912	0.2
12e	F	1759	8.02	0.005
12f	Cl	14413	466.9	0.03
12g	CF ₃	54.6	396	7.3
12h	NO ₂	121.89	92	0.8
SC-560		0.0038	(74.9^{c})	(10700^{c})
		(0.007^{c})		
Celecoxib		(6.7^{c})	0.26	(0.13^{c})
			(0.87^{c})	

a) The concentration required for 50% inhibition of *in vitro* COX-1 and COX-2 enzyme test. *b*) Selectivity index (SI) = COX-2 IC₅₀ / COX-1 IC₅₀. *c*) Data taken from the literature for inhibition of COX-1 and COX-2.³⁷)

retaining the COX-1 activity, but the size of substituent seems to play a crucial role for COX-1 inhibitory activity. Our finding is completely different from the previous structure–activity relationship (SAR) study of the sulfonamide-possessing 1,5-diarylpyrazole class of COX inhibitors since the reported analogues containing an electron-withdrawing group such as fluoro or chloro instead of methyl group had poor COX-1 activity and similar COX-2 activity.²¹⁾

Regarding the COX-2 inhibitions of the analogues (7a–7h), most of analogues exhibited weak COX-2 inhibitory activity $(IC_{50} = 4.54-39.8 \,\mu\text{M})$ compared to celecoxib $(IC_{50} = 0.26 \,\mu\text{M})$ except the trifluoromethyl analogue 7g $(IC_{50} = 0.26 \,\mu\text{M})$ which was equipotent to celecoxib. Of note, because analogues 7b $(IC_{50} = 5.53 \,\mu\text{M})$ and 7d $(IC_{50} = 4.54 \,\mu\text{M})$ displayed almost 17-fold lower activity than celecoxib $(IC_{50} = 0.26 \,\mu\text{M})$ on the COX-2 inhibition and potent COX-1 inhibition, both analogues 7b (SI = 376) and 7d (SI = 768) can be classified as selective COX-1 inhibitors in view of the calculated SI values. Moreover, we noticed that the trifluoromethyl analogue 7g had an equipotent COX-2 activity compared to celecoxib even though it's COX-2 selectivity (SI = 0.2) was lower than celecoxib (SI = 0.13).

Since the salicylic moiety at the meta position of carboxylic acid and the hydroxy group at the para position in the compounds (7a-7h) dramatically enhanced the inhibitory COX-1 activity, we then investigated the effect of switching the position of the salicylic moiety from the para position of the hydroxy group to the meta position on COX-1/COX-2 inhibitory activity. Interestingly, in a series of the 4-substituted-2-hydroxy-benzoic acid analogues (12a-12h), most analogues showed no activity on COX-1 (IC₅₀ = 54.6-14473 μ M) or COX-2 enzymes (IC₅₀ = $62.5-912 \mu$ M) compared to SC-560 (COX-1 $IC_{50} = 0.0039 \,\mu\text{M}$) and celecoxib (COX-2 $IC_{50} = 0.26 \,\mu M$) except the methoxy analogue 12c which exhibited weak COX-1($IC_{50} = 1.58$) and COX-2 ($IC_{50} = 1.31$) activities. This result was not surprising to us because a previous report described that the analogue of celecoxib containing carboxylic acid at the para position instead of sulfonamide showed greater reduction of COX-1 inhibition than COX-2 compared to celecoxib.³²⁾ Similarly, bioisosteric replacement of the sulfonamide group with the carbothiomide moiety also resulted in lower COX-1 and COX-2 inhibitory activities.33) Therefore, our findings are consistent with these reports although there is no previous report regarding the effect of hydroxy group at the meta position on COX inhibition.

All together, our results revealed that the *meta* position of carboxylic acid and the *para* position of the hydroxy group in the salicylate group are essential for excellent COX-1 selectivity as well as the inhibitory effect on COX-2 in this series. In order to prove the structures of these analogues for selective inhibition of COX enzymes, we performed a molecular docking study with analogue **7f** to explain its excellent selectivity on COX-1.

Molecular Docking Studies The docking studies were performed to explore how the potent and selective COX-1 and COX-2 inhibitors bind in the active sites of these enzymes. Autodock Vina was used for docking calculations and UCSF Chimera was used for molecular graphics and analyses.^{34,35} 5-Aminosalicylic acid analogue **7f** is a potent and selective COX-1 inhibitor over COX-2 with SI = 768. The docking study showed the similar binding modes of **7f** in the active sites of



Fig. 3. Molecular Docking Models of COX-1 (A) and COX-2 (B) Complexed with 7f For this study, COX-1 (PDB ID: 3KK6) and COX-2 (PDB ID: 3LN1) cocrystalized with celecoxib were chosen for molecular docking. The docking study was performed in Autodock Vina and images were generated using Chimera.

COX-1 and COX-2 due to high homology. A CF₃ group of the analogue 7f is closely contacted with hydrophobic residues (Leu359, Met113, Ile345, Leu531, Val349 and Val116) in the binding pockets of both COXs.³⁶⁾ The para-substituted chloride is occupied into another binding pocket surrounded with Phe518, Leu352, Ile523 (Val523 in COX-2) and Ser353 (Fig. 3). This hydrophobic binding pocket is only big enough to interact with methyl and chloride group, which may explain the most active inhibitory effects of analogues 7b and 7f. Interestingly, the docking studies of analogue 7f showed that one of carboxylic oxygen (OH) forms hydrogen binding with a hydroxy group of Ser530 (distance = 2.23 Å) in the active site of COX-1 whereas no hydrogen bonding is observed in the active site of COX-2 (Fig. 3). The bigger Ile523 in COX-1 is likely to sterically force the chlorophenyl moiety of 7f to move, thereby positioning the salicylic moiety closer to Ser530 while the smaller val523 allows access to a hydrophobic pocket in the active site of COX-2. These docking calculations would provide not only possible explanations for the inhibitory effects of salicylic acid analogues, but also better strategies to design more potent and selective COX-1 and COX-2 inhibitors.

CONCLUSION

A novel series of salicylic acid analogues of celecoxib where the sulfonamide group is replaced by salicylic acid group were designed, synthesized and biologically evaluated. The *in vitro* enzyme inhibition study showed that the 1,5-diarylpyrazole analogues (**7a**–**7h**) derived from 5-amino-salicylic acid showed good inhibition of COX-1 and COX-2 with high COX-1 selectivity. However, the 1,5-diarylpyrazole analogues (**12a**–**12h**) derived from 4-aminosalicylic acid showed no inhibitory activity on COX-1 and COX-2 enzymes. Among the series of the compounds in this study, the chloro analogue **7f** has the highest inhibition potency on COX-1 (IC₅₀ = 0.0059 μ M) and COX-2 (IC₅₀ = 4.54 μ M) with excellent COX-1 selectivity (SI = 768). Therefore, analogue **7d** can be classified as a new selective COX-1 inhibitor in view of the calculated SI values.

Acknowledgments This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) Grant funded by the Ministry of Education [NRF-2020R1A2B5B02002032 (to S.-H. Yoon and D.-K. Choi) and (NRF-2020R111A1A01062002 and 2019R1A6A1A11051471 (to J.-Y. Park)].

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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