Research Article

Synthesis, Biological Activity, and Molecular Modeling Studies of Pyrazole and Triazole Derivatives as Selective COX-2 Inhibitors

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Series of diaryl-based pyrazole and triazole derivatives were designed and synthesized in a facile synthetic approach in order to produce selective COX-2 inhibitor. These series of derivatives were synthesized by different reactions like Vilsmeier–Haack reaction and click reaction. In vitro COX-1 and COX-2 inhibition studies showed that five compounds were potent and selective inhibitors of the COX-2 isozyme with IC_{50} values in $0.551–0.002\,\mu M$ range. In the diarylpyrazole derivatives, compound **4b** showed the best inhibitory activity against COX-2 with $IC_{50} = 0.017\,\mu M$ as one of the *N*-aromatic rings was substituted with sulfonamide and the other aromatic ring was unsubstituted. However, when the *N*-aromatic ring was substituted with sulfonamide and the other aromatic ring was substituted with sulfone (compound **4d**), best COX-2 selectivity was achieved ($IC_{50} = 0.098\,\mu M$, SI = 54.847). In the diaryltriazole derivatives, compound **15a** showed the best inhibitory activity in comparison to all synthesized compounds including the reference celecoxib with $IC_{50} = 0.002\,\mu M$ and SI = 162.5 as it could better fit the extra hydrophobic pocket which is present in the COX-2 enzyme. Moreover, the docking study supports the obtained SAR data and binding similarities and differences on both isozymes.

1. Introduction

Cyclooxygenase (COX) enzymes catalyze the biosynthesis of prostaglandin H_2 from arachidonic acid which was released by the action of phospholipase A_2 [1]. Prostaglandin H_2 is the precursor for the formation of other prostaglandins, prostacyclin, and thromboxane that play major roles in various important physiological and pathological responses [2, 3]. COX are membrane-bound enzymes with the existence of two major isoforms: COX-1 and COX-2 [4]. COX-1 is a constitutive enzyme that is involved in the production of essential prostaglandins which maintains the regular functions in the body especially gastrointestinal and cardiovascular systems [5], whereas COX-2 is an inducible enzyme that was overexpressed in various pathophysiological

conditions such as inflammation, hyperalgesia, and cancer [6, 7]. In early 1990, the structures of both COX enzymes were defined and they revealed that both isoforms are 67% identical in amino acid sequences. However, the most substantial difference between both isoforms is the presence of valine (Val523) in COX-2 instead of isoleucine (Ile523) in COX-1 which allows 25% greater available space binding region in COX-2 in comparison to COX-1 [8].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most prescribed medications for the relief of pain and inflammation [9]. Their pharmacological mechanism is the inhibition of both COX enzymes which decreases prostaglandin synthesis to exhibit their anti-inflammatory activity [10]. Since they inhibit the constitutive COX-1 enzyme beside the COX-2, their chronic use will lead to

unwanted side effects such as gastric ulcer, liver, and kidney toxicities [11]. On the other hand, the selective inhibition of the inducible COX-2 isozyme will provide a useful therapeutic approach with less produced side effects in comparison to the classical NSAIDs. In this context, various selective COX-2 inhibitors (COXIBs) were developed and marketed such as rofecoxib [12], celecoxib [13], valdecoxib [14], and etoricoxib [15]. These drugs were used extensively for the treatment of various inflammation conditions with less gastric ulceration such as rheumatoid arthritis and osteoarthritis [16]. However, different studies reported that the chronic treatment of selective COX-2 inhibitors showed potential cardiovascular side effects due to the decrease in the production of the protective antiaggregatory prostacyclin (PGI_2) while leaving the biosynthesis of prothrombotic thromboxane A2 unaffected [17]. Therefore, various COXIBs were withdrawn from the market, which increased the demand for the synthesis of novel COX-2 inhibitors. Studying the structures of most selective COX-2 inhibitors revealed the presence of diaryl substitution on carbocyclic or heterocyclic core (Figure 1). Moreover, structure-activity relationship (SAR) studies of COXIBs showed that a COX-2 selectivity raised due to a hydrophobic interaction with an extra hydrophobic region and polar interactions with a secondary polar pocket that are found in COX-2 isozyme [18]. Moreover, the presence of electron withdrawing group in one of the aryl substitutions in the para position is more preferable than corresponding electron donating group [18]. Various previous studies were implemented to synthesize selective COX-2 inhibitors by using different methods [18–22]. The reported syntheses are commonly complex which makes the production costs more expensive and beyond the reach of an ordinary person. Therefore, there is a need for new, effective, selective, cheaper, and safer COX-2 inhibitors. Herein, we aim to use a facile synthesis of series of COX-2 inhibitors based on pyrazole or triazole core (Figure 1). Moreover, pyrazole and triazole heterocycles showed interesting biological activity especially as anti-inflammatory behavior and they have been used to synthesize novel compounds as selective COX-1 inhibitors [23, 24]. In addition, they could synthesize easily with a high yield in one step reaction through Vilsmeier–Haack reaction or click reaction, respectively [25–27]. Then, the in vitro inhibitory COX-1 and COX-2 were investigated for the synthesized derivatives.

2. Materials and Methods

2.1. General.
NMR analysis was measured by Bruker Avance 500 spectrometer at Jordan University. TMS was used as internal standard and chemical shifts were expressed in ppm on δ scale and coupling constant (J) was measured in Hz. Unilab microplate reader 6000 was used to read the plate for COX (human) Inhibitor Screening Assay Kit. High-resolution mass spectra data (HRMS) were collected using a Shimadzu LCMS-IT-TOF using ESI (+) method at Doping and Narcotics Analysis Laboratory of the faculty of pharmacy, Anadolu University, Turkey. To determine the purity of the all synthesized COX-2 inhibitors, reverse phase

chromatography was used. The used column was XTERRA® MS C18, $5\,\mu$m, 4.6×250 mm cartridge column, and the mobile phase was acetonitrile : water (80 : 20). Chromatographic separation and determination of % purity of the eluted peaks for the tested compounds were performed using high-performance liquid chromatography (Waters-1525, Singapore) instrument with a binary HPLC pump and Waters-2298 Photodiode Array Detector.

2.2. General Synthesis of Hydrazone Derivatives.
An amount of acetic acid glacial was added to a mixture of acetophenone and phenyl hydrazine derivatives in 20 ml ethanol (EtOH), and the reaction was refluxed overnight at 70°C. Ethanol was removed under reduced pressure and the produced solid product was used without further purification for pyrazole synthesis.

2.3. General Synthesis of Pyrazole Derivatives.
Vilsmeier–Haack reagent was prepared by adding phosphorus oxychloride ($POCl_3$) dropwise at 0°C to a stirred dimethylformamide (DMF). Then, the synthesized hydrazone solution in 1 ml DMF was added dropwise to Vilsmeier–Haack reagent. The reaction was refluxed for 5-6 h at 70°C. Later, the reaction was poured on cold distilled water (DW) mixed with concentrated solution of sodium bicarbonate. Then, the solution was filtered by suction filtration. The precipitate was taken and purified by flash chromatography.

2.3.1. Synthesis of 3-(4-(Methylsulfonyl)Phenyl)-1-Phenyl-1H-Pyrazole-4-Carbaldehyde (4a).
To synthesize hydrazone 3a: $4'$-(methyl sulfonyl)acetophenone (198.2 mg, 1.0 mmol) with phenyl hydrazine (113 μl, 1.15 mmol) and glacial acetic acid (86 μl, 1.5 mmol) was used to obtain a pure orange product (Yield 97%, 280 mg, 0.97 mmol). R_f: 0.3 (Hexane : EtOAc 1 : 1). To synthesize pyrazole 4a: hydrazone 3a (0.3 g, 0.92 mmol) with $POCl_3$ (1.5 ml, 15.8 mmol) in DMF (813 μl, 10.50 mmol) was used to obtain a pure white product (Yield 80%, 240 mg, 0.73 mmol). R_f: 0.3 (Hexane : EtOAc 1 : 1). ^{1}H NMR (500 MHz, CDCl$_3$): δ 10.05 (s, 1H, CHO), 8.56 (s, 1H, CH pyrazole), 8.15 (d, 2H, $J = 9.0$ Hz, Ph'H-2, Ph'H-6), 8.04 (d, 2H, $J = 9.1$ Hz, Ph'H-3, Ph'H-5), 7.78 (d, 2H, $J = 7.5$ Hz, Ph H-2, Ph H-6), 7.51 (t, 2H, $J = 7.5$ Hz, Ph H-3, Ph H-5), 7.41 (t, 1H, $J = 7.5$ Hz, Ph H-4), 3.08 (s, 3H, SO$_2$CH$_3$). ^{13}C NMR (125 MHz, CDCl$_3$): δ 184.9, 151.0, 141.5, 138.9, 136.6, 136.5, 130.2, 129.9, 128.4, 127.7, 122.9, 119.8, 43.9. HRMS (ESI, m/z): calcd. for C$_{17}$H$_{14}$N$_2$O$_3$S [M + H]$^+$ 327.0803, found 327.0798. % purity of 99% (by HPLC).

2.3.2. Synthesis of 4-(4-Formyl-3-Phenyl-1H-Pyrazol-1-yl) Benzenesulfonamide (4b).
To synthesize hydrazone 3b: acetophenone (272.1 μl, 2.33 mmol) with 4-sulfonamide phenyl hydrazine HCl (600 mg, 2.68 mmol) and glacial acetic acid (116 μl, 2.03 mmol) was used to obtain a pure orange product (Yield 96%, 650 mg, 2.25 mmol). R_f: 0.3 (Hexane : EtOAc 1 : 1). To synthesize pyrazole 4b: hydrazone 3b (1.0 g, 3.46 mmol) with $POCl_3$ (2.77 ml, 29.63 mmol) in DMF (1.53 ml, 19.75 mmol) was used to obtain a white product
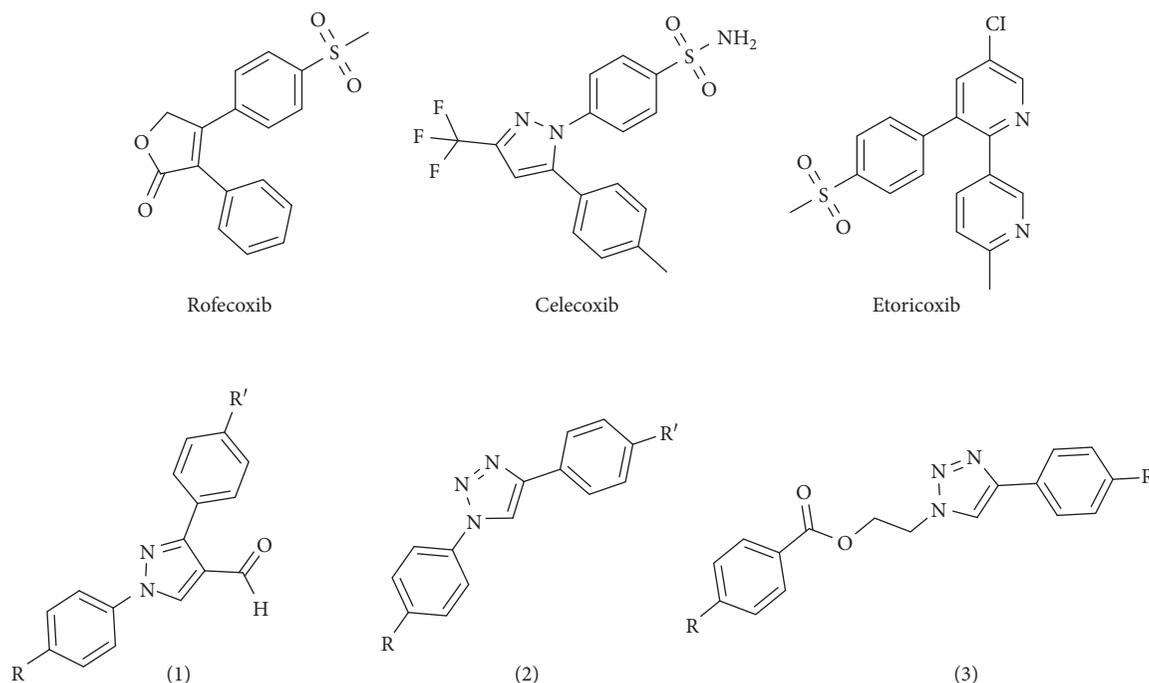
Figure 1: Structure of some selective COX-2 inhibitors and target compounds (**1–3**).

(Yield 94%, 1.07 g, 3.27 mmol). R_f: 0.3 (Hexane : EtOAc 1 : 1). ^{1}H NMR (500 MHz, CDCl₃): δ 10.02 (s, 1H, CHO), 8.59 (s, 1H, CH pyrazole), 8.00 (d, 2H, J = 8.3 Hz, Ph H-2, Ph H-6), 7.89 (d, 2H, J = 8.4 Hz, Ph H-3, Ph H-5), 7.78 (d, 2H, J = 6.0 Hz, Ph' H-2, Ph' H-6), 7.49–7.43 (m, 5H, J = 7.5 Hz, Ph' H-3, Ph' H-5, Ph'H-4, SO₂NH₂). ^{13}C NMR (125 MHz, CDCl₃): δ 185.0, 159.9, 141.6, 141.2, 132.3, 131.8, 131.0, 130.6, 129.8, 129.6, 129.0, 128.9, 128.7, 128.2, 123.0, 119.9, 119.5. HRMS (ESI, m/z): calcd. for C₁₆H₁₃N₃O₃S [M + H]⁺ 328.0756, found 328.0750. % purity of 97% (by HPLC).

2.3.3. Synthesis of 4-(3-(4-Bromophenyl)-4-Formyl-1H-Pyrazol-1yl)Benzenesulfonamide (**4c**). To synthesize hydrazone **3c**: 4-bromoacetophenone (464 mg, 2.33 mmol) with 4-sulfonamide phenyl hydrazine HCl (600 mg, 2.68 mmol) and glacial acetic acid (116 µl, 2.03 mmol) was used to obtain a pure red product (Yield 85%, 730 mg, 1.9 mmol). R_f: 0.6 (Hexane : EtOAc 1 : 2). To synthesize pyrazole **4c**: hydrazone **3c** (0.6 g, 1.6 mmol) with POCl₃ (1.28 ml, 13.7 mmol) in DMF (708 µl, 9.14 mmol) was used to obtain a pure white product (Yield 92%, 600 mg, 1.48 mmol). R_f: 0.6 (Hexane : EtOAc 1 : 2). ^{1}H NMR (500 MHz, DMSO): δ 9.32 (s, 1H, CHO), 7.56 (s, 1H, CH pyrazole), 7.45–7.42 (m, 2H, Ph H-2, Ph H-6), 7.27–7.16 (m, 6H, Ph H-3, Ph H-5, Ph'H-2, Ph'H-3, Ph'H-5, Ph'H-6), 6.97–6.94 (m, 2H, SO₂NH₂). ^{13}C NMR (125 MHz, DMSO): δ 184.9, 160.4, 152.1, 142.3, 140.9, 136.8, 132.0, 131.1, 130.7, 128.2, 127.5, 123.4, 123.1, 119.9, 119.0. HRMS (ESI, m/z): calcd. for C₁₆H₁₂N₃O₃SBr [M + H]⁺ 405.9861, found 405.9855. % purity of 97% (by HPLC).

2.3.4. Synthesis of 4-(4-Formyl-3-(4-(Methylsulfonyl)Phenyl)-1H-Pyrazol-1-yl)Benzenesulfonamide (**4d**). To synthesize

hydrazone **3d**: 4′-(methyl sulfonyl) acetophenone (308 mg, 1.55 mmol) with 4-sulfonamide phenyl hydrazine HCl (400 mg, 1.79 mmol) and glacial acetic acid (133 µl, 2.33 mmol) was used to obtain a pure orange product (Yield 88%, 500 mg, 1.36 mmol). R_f: 0.6 (Hexane : EtOAc 1 : 2). To synthesize pyrazole **4d**: hydrazone **3d** (0.6 mg, 1.48 mmol) with POCl₃ (1.16 ml, 12.68 mmol) in DMF (655 µl, 8.46 mmol) was used to get a pure white product (Yield 43%, 260 mg, 0.64 mmol). R_f: 0.6 (Hexane : EtOAc 1 : 2). ^{1}H NMR (500 MHz, DMSO): δ 10.05 (s, 1H, CHO), 8.62 (s, 1H, CH pyrazole), 8.12–7.89 (m, 8H, Ph – Ph'), 7.23 (s, 2H, SO₂NH₂), 3.08 (s, 3H, SO₂CH₃). ^{13}C NMR (125 MHz, DMSO): δ 184.9, 160.4, 151.5, 151.0, 148.1, 142.5, 141.7, 140.9, 138.7, 137.1, 136.5, 130.0, 128.2, 127.7, 127.5, 123.3, 123.0, 120.0, 119.1, 43.9. HRMS (ESI, m/z): calcd. for C₁₇H₁₅N₃O₅S₂ [M + H]⁺ 406.0531, found 406.0526. % purity of 96% (by HPLC).

2.3.5. Synthesis of 4-(4-Formyl-1-Phenyl-1H-Pyrazol-3-yl)Benzenesulfonamide (**4e**). To synthesize hydrazone **3e**: 4-acetyl benzene sulfonamide (400 mg, 2.0 mmol) with phenyl hydrazine (226 µl, 2.3 mmol) and glacial acetic acid (172 µl, 3.0 mmol) was used to get a pure red product (Yield 95%, 550 mg, 1.9 mmol). R_f: 0.5 (Hexane : EtOAc 1 : 1). To synthesize pyrazole **4e**: hydrazone **3e** (0.5 g, 1.9 mmol) with POCl₃ (1.52 ml, 16.3 mmol) in DMF (841 µl, 10.86 mmol) was used to obtain a white product (Yield 32%, 200 mg, 0.61 mmol). R_f: 0.5 (Hexane : EtOAc 1 : 1). ^{1}H NMR (500 MHz, DMSO): δ 9.95 (s, 1H, CHO), 9.27 (s, 1H, CH pyrazole), 7.95 (d, 2H, J = 8.6 Hz, Ph' H-2, Ph' H-6), 7.87 (d, 2H, J = 7.9 Hz, Ph' H-3, Ph' H-5), 7.72 (d, 2H, J = 7.9 Hz, Ph H-2, Ph H-6), 7.52 (t, 3H, J = 7.6 Hz, Ph H-3, Ph H-5, Ph H-4), 7.39 (s, 2H, SO₂NH₂). ^{13}C NMR (125 MHz, DMSO): δ 185.1, 160.4, 152.6, 148.9, 139.0, 136.3, 135.4, 131.4, 130.2,

129.6, 128.2, 126.6, 126.2, 122.7, 119.7. HRMS (ESI, m/z): calcd. for $C_{16}H_{13}N_3O_3S$ $[M + H]^+$ 328.0756, found 328.0750. % purity of 96% (by HPLC).

2.3.6. Synthesis of 1-(4-Bromophenyl)-3-(4-(Methylsulfonyl) Phenyl)-1H-Pyrazole-4-Carbaldehyde (4f).

To synthesize hydrazone **3f**: 4′-(methyl sulfonyl) acetophenone (400 mg, 2.0 mmol) with 4-bromo phenyl hydrazinum chloride (514 mg, 2.3 mmol) and glacial acetic acid (172 μl, 3.0 mmol) was used to get a pure brown product (Yield 91%, 670 mg, 1.8 mmol). R_f: 0.3 (Hexane : EtOAc 1 : 1). To synthesize pyrazole **4f**: hydrazone **3f** (0.6 g, 1.6 mmol) with POCl₃ (1.31 ml, 14.0 mmol) in DMF (720 μl, 9.33 mmol) was used to have a pure yellow product (Yield 55%, 360 mg, 0.88 mmol). R_f: 0.3 (Hexane : EtOAc 1 : 1). ^{1}H NMR (500 MHz, DMSO): δ 9.96 (s, 1H, CHO), 9.38 (s, 1H, CH pyrazole), 8.17 (d, 2H, $J = 7.9$ Hz, Ph' H-2, Ph' H-6), 8.01 (d, 2H, $J = 7.6$ Hz, Ph' H-3, Ph' H-5), 7.93 (d, 2H, $J = 10.1$ Hz, Ph H-2, Ph H-6), 7.73 (d, 2H, $J = 8.6$ Hz, Ph H-3, Ph H-5), 3.22 (s, 3H, SO₂CH₃). ^{13}C NMR (125 MHz, DMSO): δ 189.1, 155.8, 146.2, 142.8, 141.4, 137.7, 134.5, 132.3, 127.9, 126.3, 125.7, 48.8. HRMS (ESI, m/z): calcd. For $C_{17}H_{13}N_2O_3SBr$ $[M + H]^+$ 404.9909, found 404.9903. % purity of >99% (by HPLC).

2.3.7. Synthesis of 3-(4-(Methylsulfonyl)Phenyl)-1-(4-Nitrophenyl)-1H-Pyrazole-4-Carbaldehyde (4g).

To synthesize hydrazone **3g**: 4′-(methyl sulfonyl)acetophenone (400 mg, 2.0 mmol) with 4-Nitro phenyl hydrazine (352 mg, 2.3 mmol) and glacial acetic acid (172 μl, 3.0 mmol) was used to have a pure orange product (Yield 88%, 590 mg, 1.76 mmol). R_f: 0.3 (Hexane : EtOAc 1 : 1). To synthesize pyrazole **4g**: hydrazone **3g** (0.6 mg, 1.8 mmol) with POCl₃ (1.45 ml, 15.4 mmol in DMF (794 μl, 10.28 mmol) was used to have a pure brown product (Yield 99%, 663 mg, 1.8 mmol). R_f: 0.3 (Hexane : EtOAc 1 : 1). ^{1}H NMR (500 MHz, DMSO): δ 10.03 (s, 1H, CHO), 9.57 (s, 1H, CH pyrazole), 8.37 (d, 2H, $J = 6.7$ Hz, Ph H-3, Ph H-5), 8.26 (d, 2H, $J = 7.9$ Hz, Ph H-2, Ph H-6), 8.20 (d, 2H, $J = 8.5$ Hz, Ph' H-2, Ph' H-6), 8.02 (d, 2H, $J = 7.9$ Hz, Ph' H-3, Ph' H5), 3.16 (s, 3H, SO₂NH₂). ^{13}C NMR (125 MHz, DMSO): δ 184.9, 151.9, 146.5, 143.2, 141.8, 137.6, 136.1, 130.0, 127.7, 125.9, 123.7, 120.3, 43.9. HRMS (ESI, m/z): calcd. for $C_{17}H_{13}N_3O_5S$ $[M + H]^+$ 372.0654, found 372.0649. % purity of 96% (by HPLC).

2.4. Synthesis of Triazole Derivatives

2.4.1. General Synthesis of Azide Derivatives.

A solution of the amine (1 eq.) is dissolved in 4 M HCl (5 ml) at 0°C, 5 M sodium nitrite (NaNO₂) (1.2 eq.). Then, the solution was added dropwise and stirred for about 10 min. Then, 5 M solution of sodium azide (NaN₃) (1.5 eq.) was added to reaction dropwise. The reaction was stirred for 30 min at RT. A solid product was obtained after evaporation and was used without further purification.

(1) Synthesis of 4-Azidobenzenesulfonamide (**6a**). Following the general synthesis of azide derivatives, compound **6a** was obtained (yield 85.3%, 490 mg, 2.47 mmol). The characterization of **6a** was adequate to the literature [28, 29].

(2) Synthesis of 1-Azido-4(Methylsulfonyl) Benzene (**6b**). Following the general synthesis of azide derivatives, compound **6b** was obtained (yield 88%, 420 mg, 2.13 mmol). The characterization of **6b** was adequate to the literature [30].

2.4.2. General Synthesis of Triazoles (Click Reaction).

A mixture of sodium ascorbate (1 eq.) and CuSO₄ anhydrous (1 eq.) in 4 ml distilled was added to a mixture of azide derivative (1 eq.) and 4-ethynyl-α,α,α-triflurotoluene **7** (1.2 eq) in 4 ml DCM, and the reaction was left on stirrer overnight. After that, the reaction was diluted with 70 ml DCM and washed with 50 ml DW. The solvent was removed under reduced pressure and the remaining crude was purified by flash chromatography on silica gel.

(1) Synthesis of 4-(4-(4-(Trifluoromethyl)Phenyl-1H-1,2,3-Triazol-1-yl)Benzenesulfonamide (**8a**). Following the general procedure of click reaction, 4-azidobenzenesulfonamide **6a** (150 mg, 0.76 mmol), 4-ethynyl-α,α,α-trifluorotoluene **7** (149.0 μl, 0.91 mmol), sodium ascorbate (150.6 mg, 0.76 mmol), and CuSO₄ anhydrous (121.3 mg, 0.76 mmol) were used. The crude was purified by flash chromatography on silica gel, eluting with Hexane : EtOAc (2 : 1), and a pure white solid product was obtained (Yield 36%, 100 mg, 0.27 mmol). R_f: 0.3 (Hexane/EtOAc 2 : 1). ^{1}H NMR (500 MHz, DMSO): δ 9.61 (s, 1H, CH triazole), 8.18 (dd, 4H, $J = 5.8$ Hz, $J = 2.8$ Hz, Ph H-2, Ph H-6, Ph H-3, Ph H-5), 8.09 (d, 2H, $J = 8.5$ Hz, Ph' H-3, Ph' H-5), 7.91 (d, 2H, $J = 8.2$ Hz, Ph' H-2, Ph' H-6), 7.55 (s, 2H, SO₂NH₂). ^{13}C NMR (125 MHz, DMSO): δ 146.7, 144.6, 139.0, 134.5, 129.2, 128.9, 128.1, 126.5, 126.4, 125.8, 123.6, 121.6, 120.8. HRMS (ESI, m/z): calcd. for $C_{15}H_{11}N_4O_2F_3S$ $[M + H]^+$ 369.0633, found 369.0628. % purity of 99% (by HPLC).

(2) Synthesis of 1-(4-(Methylsulfonyl)Phenyl)-4-(4-(Trifluoromethyl)Phenyl)-1H-1,2,3-Triazole (**8b**). Following the general procedure of click reaction, 1-azido-4(methyl sulfonyl) benzene **6b** (150 mg, 0.76 mmol), 4-ethynyl-α,α,α-trifluorotoluene **7** (149.0 μl, 0.91 mmol), sodium ascorbate (150.6 mg, 0.76 mmol), and CuSO₄ anhydrous (121.3 mg, 0.76 mmol) were used. The crude was purified by flash chromatography on silica gel, eluting with Hexane : EtOAc (3 : 2). A pure white solid product was obtained (Yield 88%, 420 mg, 2.13 mmol). R_f: 0.4 (Hexane/EtoAc 3 : 2). ^{1}H NMR (500 MHz, DMSO): δ 9.62 (s, 1H, CH triazole), 8.23 (d, 2H, $J = 8.9$ Hz, Ph' H-3, Ph'H-5), 8.18 (dd, 4H, $J = 8.5$ Hz, $J = 8.2$ Hz, Ph H-2, Ph H-6, Ph H-3, Ph H-5), 8.87 (d, 2H, $J = 8.2$ Hz, Ph' H-2, Ph' H-6), 3.28 (s, 3H, SO₂CH₃). ^{13}C NMR (125 MHz, DMSO): δ 146.8, 141.1, 140.3, 134.4, 129.6, 129.2, 128.9, 126.5, 126.4, 125.7, 121.7, 121.0, 44.0. HRMS (ESI, m/z): calcd. for $C_{16}H_{12}N_3O_2F_3S$ $[M + H]^+$ 368.0681, found 368.0675. % purity of >99% (by HPLC).

2.4.3. Synthesis of 2-Hydroxyethyl 4-Methylbenzenesulfonate (11).

A mixture of ethylene glycol **9** (5 ml, 89.72 mmol) and triethylamine (6.25 ml, 44.86 mmol) in 6 ml DCM was stirred for 30 min. Then, tosyl chloride **10** (8.55 g, 44.80 mmol) was added dropwise for about 30 min. The

reaction was stirred overnight. The reaction was diluted with 70 ml DCM and washed with 1 M HCl (50 ml). The solvent was removed under reduced pressure and semisolid product was obtained. The crude was purified by flash chromatography using Hexane : EtOAc (1 : 1). An oily pale yellow product was obtained (Yield 8.5%, 1.65 g, 7.63 mmol). R_f: 0.4 (Hexane/EtOAc 1 : 1). ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.79 (d, 2H, $J = 8.4$ Hz, H$_2$OT$_s$), 7.34 (d, 2H, $J = 7.9$ Hz, H$_2$OT$_s$), 4.11 (t, 2H, $J = 4.3$ Hz, CH$_2$O), 3.79 (t, 2H, $J = 4.6$ Hz, CH$_2$O), 2.42 (s, 3H, CH$_3$), 2.00 (bs, 1H, OH). ^{13}C NMR (125 MHz, CDCl$_3$): δ 145.1, 132.6, 130.0, 128.0, 71.7, 60.7, 21.7.

2.4.4. Synthesis of 2-Azidoethanol (12).
Sodium azide (0.3 g, 4.65 mmol) was added to a solution of monotosylated ethylene glycol **11** (1 g, 4.2 mmol) in 5 ml ethanol, and the reaction was refluxed overnight on 70°C. Then, the reaction was diluted with diethyl ether and washed with water three times. Then, the organic layers were collected and a pure pale yellow oily product was obtained after evaporation (Yield 96%, 350 mg, 4.02 mmol). R_f: 0.6 (DCM/MeOH 20 : 1). ^{1}H NMR (500 MHz, CDCl$_3$): δ 3.73 (t, 2H, $J = 4.1$ Hz, CH$_2$OH), 3.40 (t, 2H, $J = 4.7$ Hz, CH$_2$N$_3$), 2.25 (bs, 1H, OH). ^{13}C NMR (125 MHz, CDCl$_3$): δ 61.8, 53.5.

2.4.5. General Procedure of Esterification Reaction.
A mixture of acid derivative (1 eq.), 2-azidoethanol **12** (1.5 eq.), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (2 eq.), and dimethylamino pyridine (DMAP) (1.5 eq.) in 6 ml of DCM was put on stirrer under argon and the reaction was left overnight. Then, the reaction was diluted with 70 ml DCM and washed with 50 ml 1 M HCl. The organic layer was collected and evaporated under reduced pressure. The remaining crude was purified by flash chromatography on silica gel.

(1) Synthesis of 2-Azidoethyl 4-(Methylsulfonyl)Benzoate **(14a)**. Following the general procedure for esterification reaction, 4-(methylsulfonyl) benzoicacid **13a** (150 mg, 0.75 mmol), 2-azidoethanol **12** (98 mg, 1.125 mmol), EDC (287.55 mg, 1.5 mmol), and DMAP (138.0 mg, 1.125 mmol) in 6 ml DCM were used. Then, the crude product was purified using flash chromatography with a mobile phase of Hexane : EtOAc (1 : 1). A pure white solid product was obtained (Yield 69%, 140 mg, 0.52 mmol). R_f: 0.4 (Hexane/EtOAc 1 : 1). ^{1}H NMR (500 MHz, DMSO): δ 8.17 (d, 2H, $J = 7.9$ Hz, Ph H-2, Ph H-6), 8.07 (d, 2H, $J = 8.2$ Hz, Ph H-3, Ph H-5), 4.47 (t, 2H, $J = 4.9$ Hz, CH$_2$O), 3.67 (t, 2H, $J = 5.2$ Hz, CH$_2$N$_3$), 3.24 (s, 3H, SO$_2$CH$_3$). ^{13}C NMR (125 MHz, DMSO): δ 164.9, 145.4, 134.1, 130.6, 128.0, 64.8, 49.8, 43.7.

(2) Synthesis of 2-Azidoethyl 4-Sulfamoylbenzoate **(14b)**. Following the general procedure for esterification reaction, 4-sulfamoylbenzoicacid **13b** (150 mg, 0.75 mmol), 2-azidoethanol **12** (97.4 mg, 1.12 mmol), EDC (287.6 mg, 1.5 mmol), and DMAP (138.0 mg, 1.125 mmol) in 6 ml DCM were used. The crude product was purified using flash chromatography with a mobile phase of DCM : MeOH (9 : 1). A pure white solid product was obtained (Yield 49%, 100 mg, 0.37 mmol). R_f: 0.4 (DCM : MeOH 9 : 1). ^{1}H NMR

(500 MHz, DMSO): δ 8.12 (d, 2H, $J = 8.5$ Hz, Ph H-2, Ph H-6), 7.94 (d, 2H, $J = 8.5$ Hz, Ph H-3, Ph H-5), 7.53 (s, 2H, SO$_2$NH$_2$), 4.45 (t, 2H, $J = 4.9$ Hz, CH$_2$O), 3.66 (t, 2H, $J = 4.9$ Hz, CHN$_3$). ^{13}C NMR (125 MHz, DMSO): δ 165.0, 148.7, 132.6, 130.4, 127.7, 126.6, 125.5, 64.6, 49.8.

2.4.6. Synthesis of 2-(4-(4-(Trifluoromethyl)Phenyl)-1H-1,2,3-Triazol-1-yl)Ethyl 4-(Methylsulfonyl)Benzoate (15a).
Following the general procedure of click reaction, 2-azidoethyl 4-(methylsulfonyl)benzoate **14a** (240 mg, 0.89 mmol), 4-ethynyl-α,α,α-triflurotoluene **7** (174.5 μl, 1.1 mmol), sodium ascorbate (176.3 mg, 0.89 mmol), and CuSO$_4$ anhydrous (142 mg, 0.89 mmol) were used. The crude was purified by flash chromatography on silica gel, eluting with DCM : MeOH (20 : 1). A pure white solid product was obtained (Yield 36%, 140 mg, 0.32 mmol). R_f: 0.4 (DCM/MeOH 20 : 1). ^{1}H NMR (500 MHz, DMSO): δ 8.84 (s, 1H, CH triazole), 8.10 (d, 2H, $J = 8.6$ Hz, Ph' H-3, Ph' H-5), 8.02 (dd, 4H, $J = 6.4$ Hz, $J = 1.8$ Hz, Ph H-2, Ph H-6, Ph H-3, Ph H-5), 7.78 (d, 2H, $J = 8.2$ Hz, Ph' H-2, Ph' H-6), 8.86 (t, 2H, $J = 4.6$ Hz, CH$_2$O), 4.74 (t, 2H, $J = 4.9$ Hz, CH$_2$N), 3.28 (s, 3H, SO$_2$CH$_3$). ^{13}CNMR (125 MHz, DMSO): δ 164.7, 145.6, 135.1, 134.0, 130.6, 127.9, 126.1, 123.6, 64.2, 49.3, 43.6. HRMS (ESI, m/z): calcd. for C$_{19}$H$_{16}$N$_3$O$_4$F$_3$S [M + H]$^+$ 440.0892, found 440.0885. % purity of 98% (by HPLC).

2.4.7. Synthesis of 2-(4-(4-(Trifluoromethyl)Phenyl)-1H-1,2,3-Triazol-1-yl)Ethyl 4-Sulfamoylbenzoate (15b).
Following the general procedure of click reaction, 2-azidoethyl 4-sulfamoylbenzoate **14b** (280 mg, 1.04 mmol), 4-ethynyl-α,α,α-triflurotoluene **7** (203.57 μl, 1.24 mmol), sodium ascorbate (206 mg, 1.04 mmol), and CuSO$_4$ anhydrous (166 mg, 1.04 mmol) were used. The crude was purified by flash chromatography on silica gel, eluting with Hexane : EtOAc (1 : 1), and a pure white solid product was obtained (Yield 48%, 220 mg, 0.5 mmol). R_f: 0.4 (Hexane/EtOAc 1 : 1). ^{1}H NMR (500 MHz, DMSO): δ 8.86 (s, 1H, CH triazole), 8.07-8.03 (m, 4H, Ph H-2, Ph H-6, Ph H-3, Ph H-5), 7.92-7.90 (m, 2H, Ph' H-3, Ph' H-5), 7.80–7.77 (m, 2H, Ph' H-2, Ph' H-6), 7.52 (bs, 2H, SO$_2$NH$_2$), 4.86 (t, 2H, $J = 4.0$ Hz, CH$_2$O), 4.73 (t, 2H, $J = 3.9$ Hz, CH$_2$N). ^{13}C NMR (125 MHz, DMSO): δ 164.9, 148.7, 145.5, 135.1, 132.4, 130.4, 128.6, 126.5, 123.7, 64.0, 49.4. HRMS (ESI, m/z): calcd. for C$_{18}$H$_{15}$N$_4$O$_4$F$_3$S [M + H]$^+$ 441.0844, found 441.0839. % purity of 98% (by HPLC).

2.5. In Vitro COX-1 and COX-2 Inhibition Assay.
The COX-1 and COX-2 inhibitory activities were tested on COX (human) Inhibitor Screening Assay Kit (supplied by Cayman chemicals, Ann Arbor, MI, USA). The preparation of the reagents and the testing procedure were performed according to the manufacturer recommendations. In brief, various concentrations of the inhibitors and celecoxib (concentration range 100 μM–0.001 μM) dissolved in a minimum quantity of dimethylsulfoxide (DMSO) were incubated with a mixture of COX-1 or COX-2 enzyme, and heme dissolved in the reaction buffer. The reaction was initiated by adding 50 μl of

arachidonic acid followed by incubation at 37°C for exactly 30 seconds. Then, the reaction was stopped by adding 30 μl of stannous chloride solution to each reaction tube followed by incubation for 5 min at room temperature. The produced PGF2a in the samples by COX reactions was quantified via enzyme-linked immunosorbent assay (ELISA). The 96-well plate was covered with plastic film and incubated for 18 h at room temperature on an orbital shaker. After incubation, the plate was rinsed five times with the washed buffer followed by the addition of Ellman's reagent (200 μl) and incubated for about 60–90 min at room temperature until the absorbance of Bo well is in the range 0.3–0.8 at 405 nm. The plate was then read by an ELISA plate reader Unilab microplate reader 6000. The inhibitory percentage was measured for the different tested concentrations against the control. IC_{50} was calculated from the concentration inhibition response curve and the selectivity index (SI) was calculated by dividing the IC_{50} COX-1 on the IC_{50} COX-2. Celecoxib was used as positive standard drug in the study. The COX testing procedure was done in duplicate.

2.6. Molecular Modeling Studies. The predicted protein-ligand interactions were identified by doing molecular docking with human COX-1 and human COX-2 crystal structures (PDB codes 3KK6 [31] and 5KIR [32], respectively). While building the docking grids, crystal structure of COX-1 was chosen from a highly close protein-ligand complex of celecoxib, 3KK6 [31]. Crystal structure for COX-2 was chosen by analyzing crystal evaluation. Results were analyzed for 5KIR and compared to a highly similar COXIB member containing crystal structure, 6COX [33] (e.g., resolutions were 2.7 Å and 2.8 Å, respectively). Both structures were used for docking since the active site residues (Figures S1–S3) were conserved within crystal source organisms. Generated docking results with 5KIR and 6COX were highly close (RMSD < 1 Å) based on the heavy atoms of the ligands. We decided to report the docking results obtained with 5KIR for COX-2 because the binding orientations were more relevant due to shared carbonyl moiety. The docking results of pyrazole and triazole derivatives were visualized at the COX-1 and COX-2 active sites. The structures were drawn by using *Maestro 11.1* Graphical User Interface [34]. The ligands were prepared by *LigPrep* [35] routine (pH 7.0 ± 2.0) to prepare the ligands and parameterize the atom types and the protonation states with OPLS2005 force field. The same force field was used for proteins, and predicted positions of the missing side chains were added with *Protein Preparation Wizard* [36]. The molecular docking studies were done with *Glide 7.4* [37] to predict the protein-ligand interactions. The grids were generated, and docking simulations were done in single-precision mode (GlideScore SP). The binding modes were evaluated with *PLIP 1.4.4* [38], and the resulting poses were visualized with *PyMOL 2.3* [39].

3. Results and Discussion

3.1. Chemistry

3.1.1. Synthesis of Pyrazole Derivatives. Pyrazole derivatives (**4a–4g**) were synthesized in two steps. Firstly, hydrazone compounds (**3a–3g**) were generated by reacting compounds of acetophenone derivatives with different phenyl hydrazine derivatives in ethanol (EtOH) using acetic acid glacial as an acid catalyst. After that, hydrazones were reacted with Vilsmeier–Haack reagent which was produced by reacting dimethyformamide (DMF) with phosphorus oxychloride ($POCl_3$) [40]. Different pyrazoles were produced as shown in Scheme 1. All synthesized pyrazoles were purified by flash chromatography. The structures of these compounds were confirmed by high-resolution mass spectrometry (HRMS), and ^1H NMR, ^{13}C NMR spectral data, and % purity of the synthesized compounds were determined by HPLC.

3.1.2. Synthesis of Triazole Derivatives. Click chemistry was introduced in 2001 by Sharpless and co-workers. It comprises a series of nearly perfect chemical reactions that were involved in many synthetic approaches with a variety of starting materials and reagents [26, 27, 41]. The synthesis of triazole compounds (**8a** and **8b**) was achieved by converting the amino group of sulfanilamide or 4-(methyl sulfonyl) aniline to their corresponding azide. This conversion was achieved by reacting the amino group with HCl, $NaNO_2$, and NaN_3 to synthesize compounds **6a** or **6b**, respectively, as shown in Scheme 2. These prepared azides were subjected to cycloaddition with 4-Ethynyl-α,α,α-trifluorotoluene (**7**) using anhydrous copper sulfate in the presence of sodium ascorbate in DCM/H_2O (1:1) providing the corresponding triazoles (**8a** and **8b**) as shown in Scheme 2. All synthesized compounds were purified by flash chromatography. The chemical structure and % purity of each compound were confirmed by ^1H NMR, ^{13}C NMR, HRMS, and HPLC.

The synthesis of the triazole derivatives with spacer was achieved firstly through the synthesis of bifunctional spacer **12** by selective tosylation of ethylene glycol followed by a nucleophilic substitution using sodium azide to obtain the corresponding spacer **12** as shown in Scheme 3. The synthesized spacer **12** was esterified with 4-(methyl sulfonyl) benzoic acid (**13a**) or 4-sulfamoyl benzoic acid (**13b**) using EDC as a coupling agent and DMAP as a catalyst to synthesize **14a** or **14b,** respectively, as shown in Scheme 4. In a final step, a click reaction was performed with the synthesized azide derivatives and 4-ethynyl-α,α,α-trifluorotoluene (**7**) in the presence of anhydrous copper sulfate and sodium ascorbate in $DCM:H_2O$ (1:1) as shown in Scheme 4, obtaining the final triazole derivatives **15a** and **15b**. All synthesized compounds were purified by flash chromatography. The chemical structure and % purity of each compound were confirmed by ^1H NMR, ^{13}C NMR, HRMS, and HPLC.

3.2. In Vitro COX-1 and COX-2 Inhibition Assay. All synthesized compounds were tested for inhibition assay on COX-1 and COX-2 enzymes using the COX-1 (human) Inhibitor Screening Assay Kit and COX-2 (human) Inhibitor Screening Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA). Serial concentrations in the range (100 μM–0.001 μM) were used for each compound in the

SCHEME 1: Synthesis of pyrazole derivatives (**4a–4g**). Reagents and conditions: (a) acetic acid, ethanol, and reflux; (b) dimethyl formamide, POCl₃, and reflux.

SCHEME 2: Synthesis of triazole derivatives (**8a** and **8b**). Reagents and conditions: (a) HCl, NaNO₂, and NaN₃; (b) 4-ethynyl-α,α,α-trifluorotoluene **7**, CuSO₄, sodium ascorbate, and DCM : H₂O (1 : 1).

SCHEME 3: Synthesis of spacer **12**. Reagents and conditions: (a) tosyl chloride **10**, triethylamine, and DCM; (b) sodium azide, ethanol, and reflux.

SCHEME 4: Synthesis of triazole compounds (**15a** and **15b**). Reagents and conditions: (a) EDC, DMAP, and DCM; (b) 4-ethynyl-α,α,α-trifluorotoluene **7,** CuSO₄, sodium ascorbate, and DCM : H₂O (1 : 1).

assay kit to determine the half-maximal inhibitory concentration (IC_{50}) and compared with the IC_{50} of celecoxib. Moreover, the selectivity index (SI) values were measured as IC_{50} (COX-1)/IC_{50} (COX-2). The results are shown in Table 1.

The compounds **4a–4g** with a core structure of heterocyclic pyrazole showed COX-2 inhibitory activity with IC_{50} value 1.902–0.017 μM. It was noticed that compounds in this series with sulfonamide substitution (**4b–4e**) at either the *N*-aromatic ring or the aromatic ring at position 4 showed better COX-2 selectivity in compression with compounds that did not have sulfonamide substituent. However, the compound **4b** with sulfonamide substituent on the *N*-aromatic ring and the other aromatic ring which was unsubstituted had better COX-2 inhibition activity ($IC_{50} = 0.017$ μM) compared with compounds **4c** and **4d** in which the other ring was substituted by sulfone or bromide. However, when the *N*-aromatic ring was substituted with sulfonamide and the other aromatic ring was substituted with sulfone (compound **4d**), they showed better COX-2 selectivity among the synthesized pyrazole library (SI = 54.847). In addition, this compound showed a high COX-2 inhibition activity ($IC_{50} = 0.098$ μM). It was noticed that nitro substitution on the *N*-aromatic ring showed more inhibition towards COX-1 (compound **4g**).

In the case of triazole derivatives (compounds **8a**, **8b**, **15a**, and **15b**), triazole compounds have *N*-aromatic ring either directly attached to the core or through a spacer. The results showed that the presence of a spacer improved the selectivity of inhibition towards COX-2 (compounds **15a** and **15b**). The presence of a spacer in the synthesized compounds makes it better fit the extra hydrophobic pocket which is present in the COX-2 enzyme. This is a possible explanation for the increased inhibition activity of these compounds compared to compounds without spacer. Moreover, results showed that sulfone substitution on the aromatic ring had a better COX-2 inhibition activity than compounds with sulfonamide substituted either in both spacer and without spacer compounds (Compounds **8b** and **15a**). Compound (**15a**) which has a spacer and sulfone substitution on one ring and CF_3 on the other ring showed the best COX-2 inhibition activity among the whole synthesized library ($IC_{50} = 0.002$ μM). Moreover, this compound had a two-fold increase in inhibition activity compared to celecoxib. The results also showed that compounds without spacer were more selective towards COX-1 (compounds **8a** and **8b**). A possible explanation is that these compounds fit better to COX-1 enzyme.

3.3. Molecular Modeling Study.

COX-2 isozyme has an additional secondary binding pocket, which does not exist in COX-1 isozyme and thought to be related with selective COX-2 inhibition. The formation of that pocket is related with the conformational differentiations in both isozymes, caused by the replacement of ILE-532 amino acid in COX-1 with VAL-532 in COX-2 [42]. Compounds **4d** and **15a** showed higher COX-2 selectivity index (IC_{50}COX-1/ IC_{50}COX-2), compared to the other molecules in the series.

Table 1: In vitro activity on COX-1 and COX-2 with selectivity index.

Compound	IC_{50} COX-1 (μM)	IC_{50} COX-2 (μM)	Selectivity index
4a	17.507 ± 1.050	1.386 ± 0.083	12.631
4b	0.263 ± 0.016	0.017 ± 0.001	15.471
4c	18.646 ± 1.119	1.305 ± 0.078	14.288
4d	5.375 ± 0.323	0.098 ± 0.006	54.847
4e	21.306 ± 1.278	0.551 ± 0.033	38.668
4f	3.438 ± 0.206	1.902 ± 0.114	1.902
4g	0.012 ± 0.001	1.460 ± 0.088	0.008
8a	0.145 ± 0.009	3.324 ± 0.199	0.044
8b	0.075 ± 0.005	0.226 ± 0.014	0.332
15a	0.325 ± 0.020	0.002 ± 0.000	162.500
15b	0.273 ± 0.016	0.131 ± 0.008	2.084
Celecoxib	1.479 ± 0.089	0.004 ± 0.000	369.750

Structure-activity relationship (SAR) data were supported with predicted binding data, retrieved by molecular docking studies with human COX-1 (PDB code 3KK6 [31]) and human COX-2 (PDB code 5KIR [32]). The findings obtained by molecular modeling study support the SAR data and binding similarities and differences on both isozymes.

Results of COX-1 binding analysis show shared interaction pattern with amino acids located at the active site. van der Waals (vdW) interactions with amino acids were observed with ILE-89 LEU-93, VAL-116, and TYR-355 amino acids and the substituted or unsubstituted phenyl groups located at the right hydrophobic pocket of the active site. Additional vdW interactions were observed at the left binding pocket, LEU-352, and ILE-523 amino acids. π-π interactions were also seen with TYR-355 amino acid and central core of the ligands. Hydrogen bonds were identified with pyrazole or triazole central cores of the ligands and side chain of ARG-120 amino acid. The existence of the sulfone fragment on the molecules supports the hydrogen bonds with SER-530 of COX-1. Most of the pyrazole derivatives are having undesired steric limitations for having optimal binding and resulting with very limited IC_{50} values. The position of the aldehyde group of **4b** is shifted compared to **4a** and **4e,** and this showed better inhibition profile compared to both ligands, which is related with an undesired interaction between ALA-527 amino acid and aldehyde group of both ligands. Replacing hydrogen at the para position of **4b** with a larger bromine atom also resulted with a decrease in COX-1 inhibitory profile, but **4f** was affected less due to the formation of halogen bond with guanidino group of ARG-83 amino acid. Another hydrophilic interaction—a hydrogen bond—was seen between sulfonamide group of **4d** and guanidino group of ARG-86 amino acid. The most active pyrazole containing COX-1 inhibitor was **4g** due to having smaller volume and more suitable positioning of the negatively charged nitro group in the same location. The same trend can also be observed with the triazole series and pyrazole series with an ester spacer. Aldehyde group on the heterocyclic group was cut out and trifluoromethyl containing para-substituted phenyl groups of **8a**, **8b**, **15a,** and **15b** form a more favored positioning at the same location. This showed increased potency against COX-1
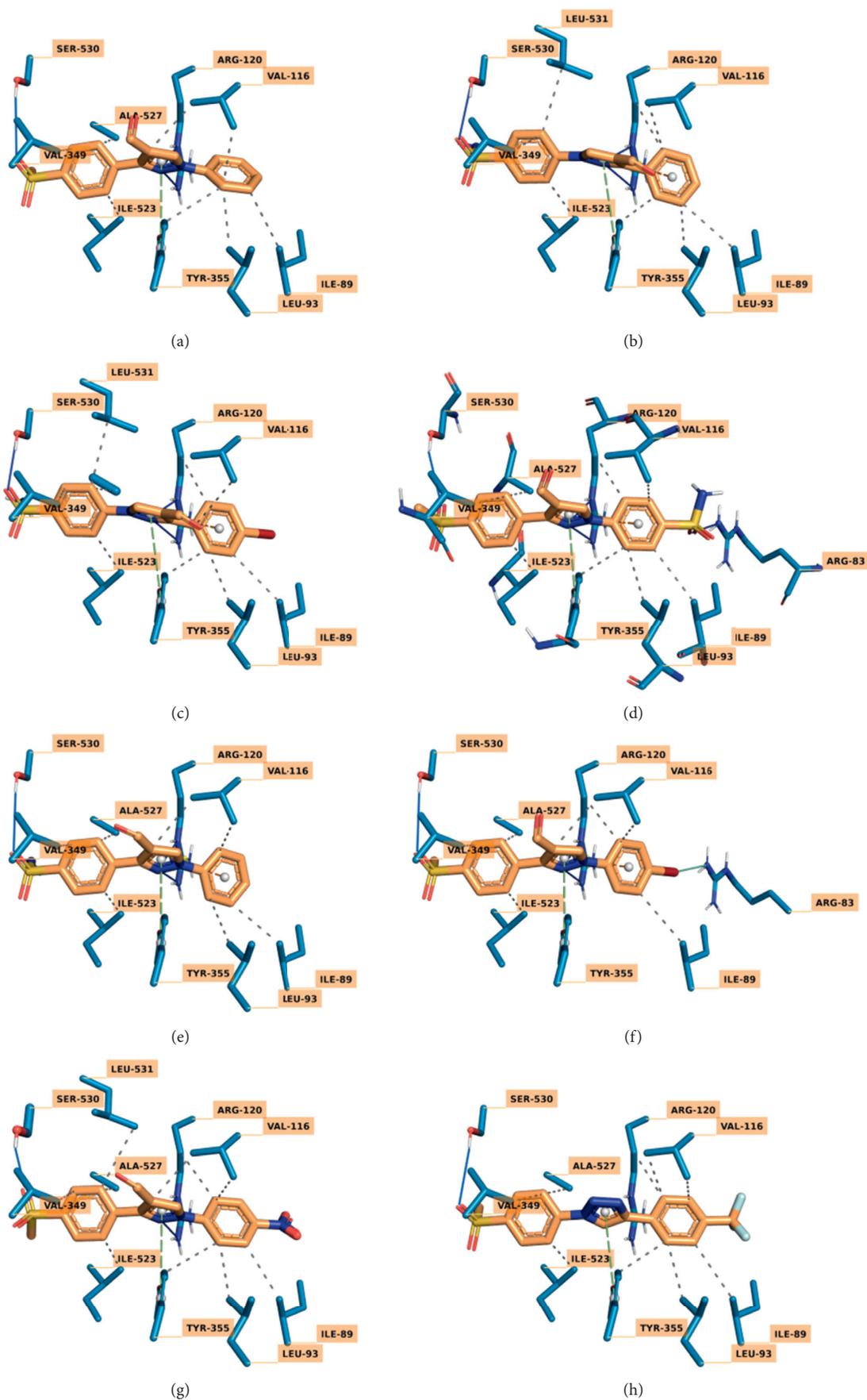
(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)

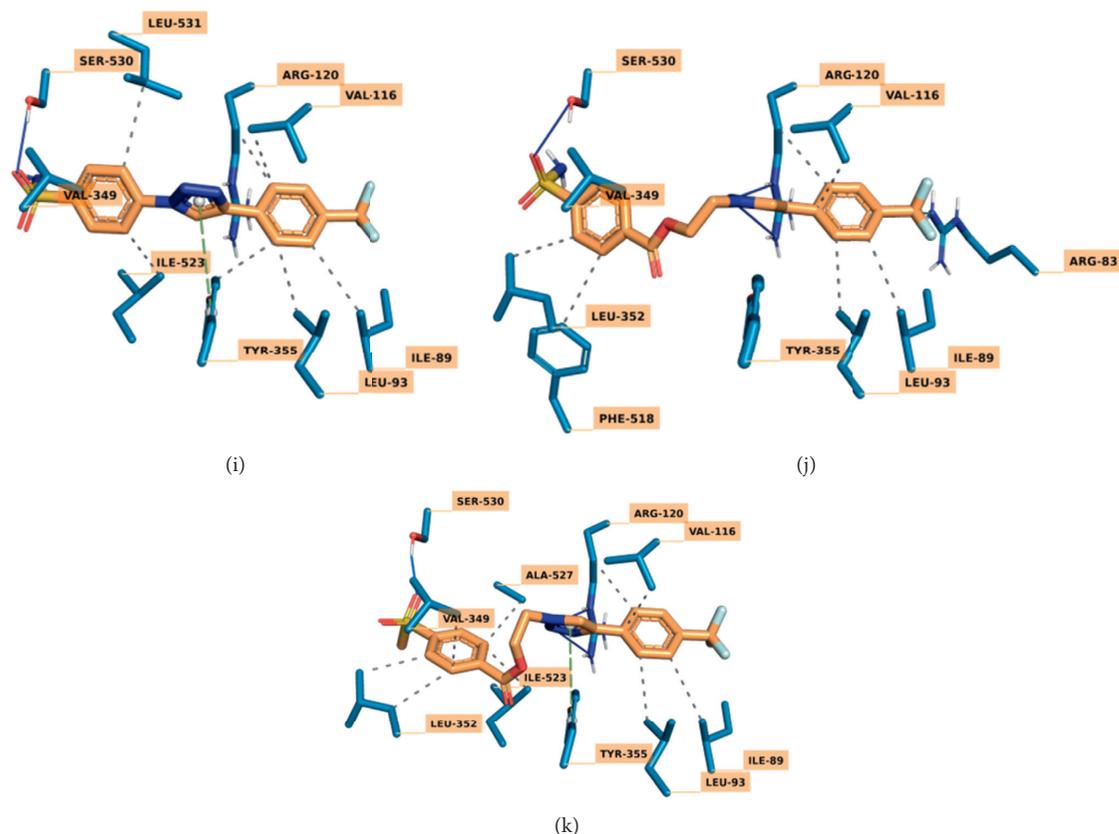Figure 2: Continued.

(i)

(j)

(k)

FIGURE 2: Predicted binding orientations of (a) **4a**, (b) **4b**, (c) **4c**, (d) **4d**, (e) **4e**, (f) **4f**, (g) **4g**, (h) **8a**, (i) **8b**, (j) **15a**, and (k) **15b** visualized in COX-1 active site (PDB code 3KK6 [31]); ligands are shown in orange and amino acids are shown in blue. Only the interacting residues and their interaction patterns are shown.

isozyme, where the whole molecule fits in a better orientation for triazole derivatives, **8a** and **8b**, but ester spacer carrying triazole derivatives show differed binding mode in COX-1 active site due to the size and flexibility of the spacer, which showed slight decrease in COX-1 inhibitory effect. Interacting residues and interacting locations are visualized in Figure 2.

Celecoxib [43] and SC-558 [33] were chosen as reference ligands to identify the COX-2 binding mode of the synthesized compounds. As can be seen, all synthesized compounds bind to the secondary pocket of COX-2. The phenyl group of each compound is forming vdW interactions with the surrounding amino acids, LEU-252, PHE-518, and VAL-523. COX-2 inhibition data showed that all molecules bind to the COX-2 enzyme with inhibition values in the range of micro or nanomolar. Both methylsulfonyl and aminosulfonyl functional groups fall in this region and help positioning the ligands in that binding domain of COX-2. Both groups make hydrogen bonds with the imidazole group of HIS-90 and guanidino group of ARG-513 amino acids. Additionally, aminosulfonyls form hydrogen bonds with backbone atoms of ILE-517 and PHE-518 amino acids, which is in agreement with previously published COX-2 binding modes of coxibs, such as celecoxib, SC-558, and rofecoxib.

The fragments causing steric issues and unfavored binding orientation have resulted in a decreased COX-2 inhibitory profile. When the bromine atom is taken out from **4c** and **4e**, which causes unfavored contacts with TYR-385, the resulting compound **4b** shows increased binding affinity for COX-2. Other structurally close analogues of **4b** in the molecule series are **4a** and **4e** which showed less favored interactions. This is due to the positional change of the aldehyde group which caused less steric issues and unfavored contact with ALA-527. Nitro group of **4g** was noticed to form undesired contact with the phenyl group of TYR-385. Compound **4d** showed better inhibitory profile because of the hydrogen bond formed with SER-530 amino acid. Triazole derivatives also showed similar binding pattern to pyrazoles, but due to the absence of a polar group on the heterocyclic group, they have not showed better binding profile compared to the pyrazoles. Ester spacer containing triazole derivatives changed the binding mode of **15a** and **15b** and resulted in the most active compound in the series, **15b**. The strong change in the inhibition profile is due to having favored binding in the hydrophobic pocket between LEU-96 and VAL-116 amino acids, without having hydrogen bond restrictions with the polar backbone atoms of ILE-517 and PHE-518. All acquired binding modes of COX-2 inhibitors: celecoxib, rofecoxib, pyrazole derivatives, and triazole derivatives, are shown in Figure 3.
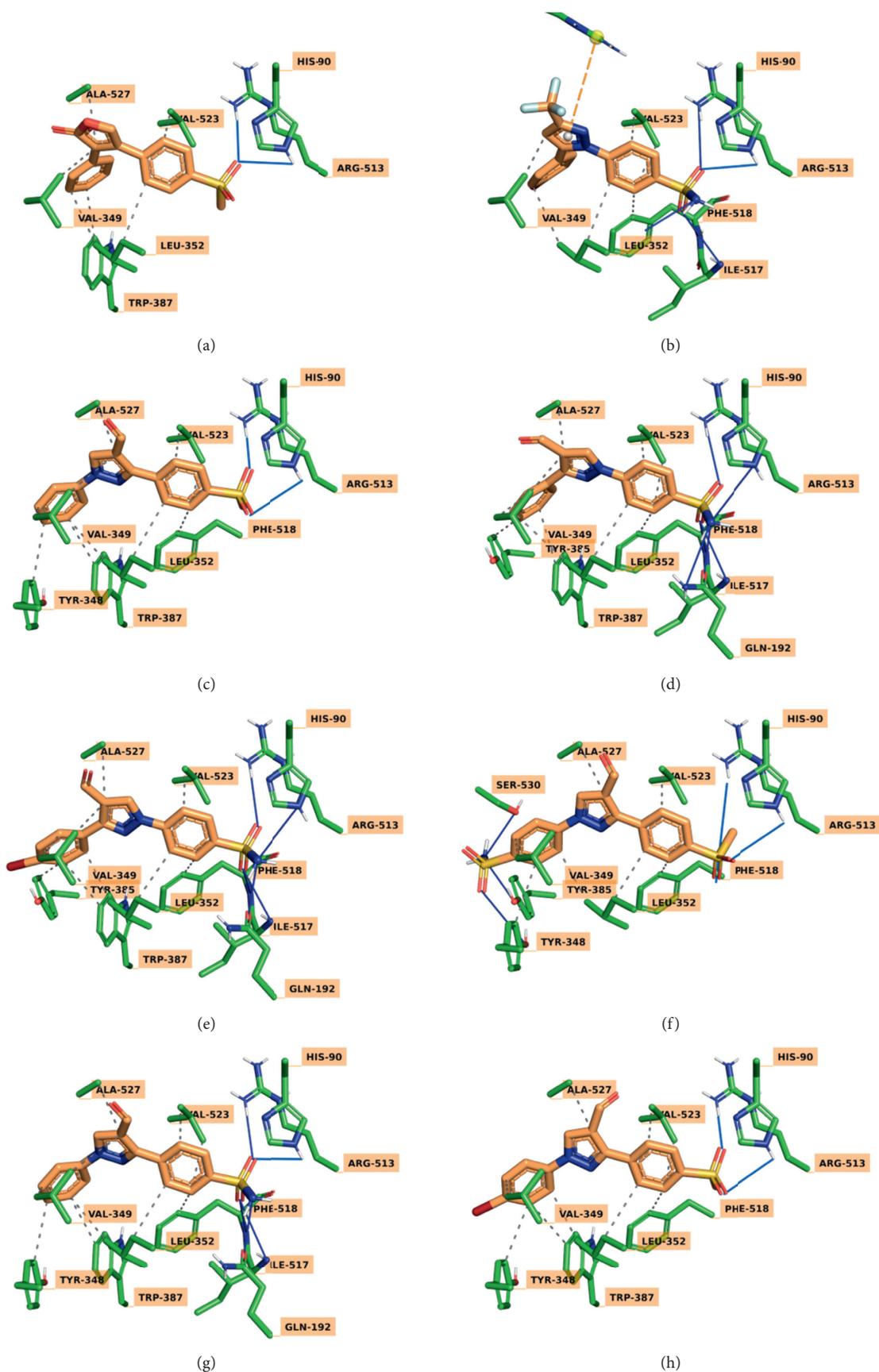
(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)

Figure 3: Continued.
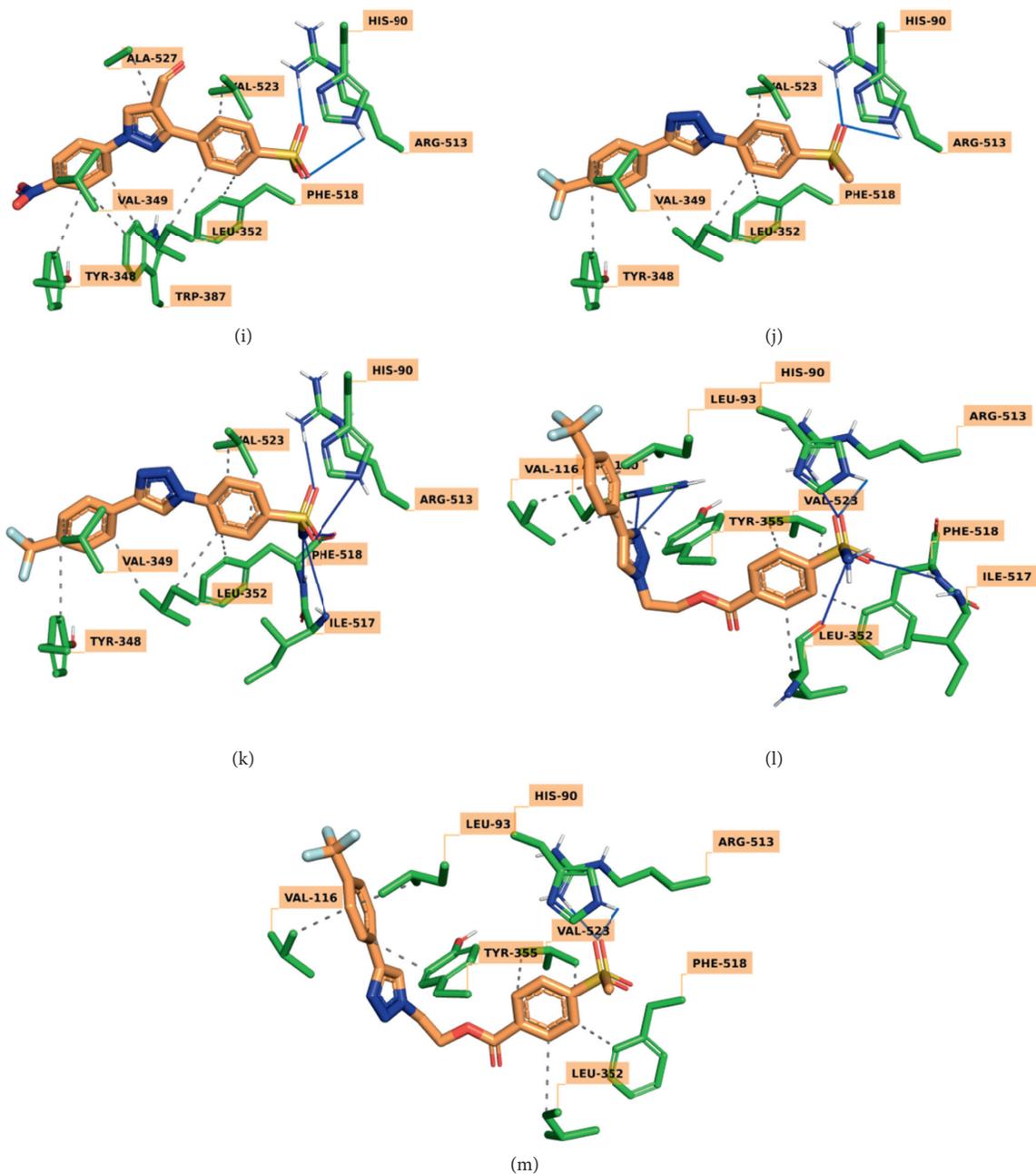
(i)

(j)

(k)

(l)

(m)

FIGURE 3: Crystal binding mode of (a) rofecoxib, and predicted binding orientations of (b) celecoxib, (c) **4a**, (d) **4b**, (e) **4c**, (f) **4d**, (g) **4e**, (h) **4f**, (i) **4g**, (j) **8a**, (k) **8b**, (l) **15a**, and (m) **15b** visualized in COX-2 active site (PDB code 5KIR [32]); ligands are shown in orange and amino acids are shown in green. Only the interacting residues and their interaction patterns are shown.

4. Conclusion

A facile synthesis was achieved to synthesize a variety of COX-2 inhibitors based on pyrazole and triazole rings. Pyrazole derivatives were successfully synthesized using Vilsmeier–Haack reaction, whereas triazole derivatives without or with spacer were successfully synthesized mediating click reaction. The pyrazole derivatives **4b** and **4d** showed potent and good COX-2 selective inhibitory activity with IC_{50} of 0.017 and 0.098 μM, respectively. Regarding the triazole derivatives, compound **15a** showed the most potent

inhibitory activity of COX-2 in comparison to celecoxib with IC_{50} of 0.002 μM. Furthermore, SAR and docking studies showed the importance of the spacer availability in the inhibitor structure because the spacer placed the aromatic ring in the favored position for hydrophobic binding in the COX-2 enzyme. More interestingly, the two aryl groups on the heterocyclic cores (pyrazole or triazole) are not vicinal in contrast to the most known COX-2 inhibitors. Further study is needed as a continuation of this work to synthesize a variety of pyrazole and triazole compounds with a different spacer length and variety of substitutions to evaluate their

role in COX-2 inhibitory activity and selectivity. Moreover, in vivo anti-inflammatory and cardiotoxicity studies will be managed to support the obtained in vitro results.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Supplementary Materials

The supplementary material is supplementary figures regarding the molecular modeling study. Figure S1: pharmacophore features of SC-558 and rofecoxib. Figure S2: binding orientation of SC-558 (cyan) in 6COX and the docking result of SC-558 (orange) in 5KIR. Figure S3: sequence alignment results of COX-2 sequence in *Homo sapiens* (human) and *Mus musculus* (mouse). (*Supplementary Materials*)

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