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Research Article

Synthetic strategies and biological screening of novel pyrazolines as COX inhibitors

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ABSTRACT

In this study, a novel series of pyrazolines was afforded by the reaction of 2-hydrazinyl-*N*-(4-aminosulfonylphenyl)acetamide (**2**) and chalcone intermediates. The structures of target compounds were confirmed by IR, 1H NMR, ^{13}C NMR and HRMS. The synthesized compounds were further evaluated for their *in vitro* COX inhibitory activity in order to discover new, effective and safer anti-inflammatory agents. Most of the compounds were more selective for COX-2 isozyme than COX-1. All compounds were weak inhibitors when compared to standard drugs ibuprofen, celecoxib and nimesulide. Among them, compounds **4a**, **4e** and **4f** were the most potent inhibitors of COX-2. These findings suggest that these compounds could be lead compounds for further development as selective COX-2 inhibitors.

Keywords: Anti-inflammatory, COX-1, COX-2, pyrazoline

1. INTRODUCTION

Cyclooxygenases (COXs) are heme-containing enzymes that catalyze the biosynthesis of prostaglandins from arachidonic acid, playing a role in the inflammation process [1, 2]. Cyclooxygenase-1 (COX-1) is widely expressed in most tissues, including the gastric mucosa, platelets, uterine epithelium, and kidney and is described as a "housekeeping enzyme". COX-2, on the other hand, is inducible in inflammation by a variety of inflammatory factors [3-5]. Conventional non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory effects via blocking both the constitutive COX-1 and the inducible COX-2 isozymes. As a result, the reduced cytoprotective effect of COX-1 leads to gastrointestinal mucosal damage, bleeding, intolerance and renal toxicity [6-8]. Subsequently, the development of novel anti-inflammatory drugs with improved safety profiles is still a great need.

Selective COX-2 inhibitors (known as coxibs, e.g. celecoxib, valdecoxib, rofecoxib) were developed as promising therapeutic avenues for inflammation management to retain the potency of conventional NSAIDs without gastrointestinal and renal toxicities. However, prolonged usage of coxibs has been reported to be associated with certain cardiovascular side effects, resulting in the withdrawal of some selective COX-2 inhibitors as rofecoxib and valdecoxib [9-12]. Among various selective COX-2 inhibitors, celecoxib is the only Food and Drug Administration (FDA)-approved selective COX-2 inhibitor that is effectively used as an anti-inflammatory and analgesic drug [13].

Various selective COX-2 inhibitors possess a tri-substituted planar five-membered heterocyclic ring, such as pyrazole, furanone, or isoxazole, as a core structure [14-17]. 3,5-Diaryl-4,5-dihydro-1*H*-pyrazole, specifically, representative of the 2-pyrazoline class, is one of the substantial rings with steric and electronic properties of the substituents at the N-1, C-3 and C-5 positions. Moreover, a variety of research outlines the anti-inflammatory activity and analgesic activity of 2-pyrazoline derivatives [18-27].

In considering the aforementioned facts, we designed and synthesized a novel series of pyrazoline derivatives by incorporating the substituted sulfonyl group, such as methylsulfonyl or aminosulfonyl, which are considered pharmacophoric moieties responsible for selectivity towards COX-2 (**Figure 1**) [14-17]. The synthesized compounds were evaluated for their ability to inhibit COX1/2 *in vitro*.

2. MATERIALS AND METHODS

2.1. Chemistry

All chemicals used in the synthesis of the compounds were purchased from Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA) or Merck Chemicals (Merck KGaA, Darmstadt, Germany). Melting points of the synthesized compounds were detected by using MP90 digital melting point apparatus (Mettler Toledo, OH, USA) and were uncorrected. NMR spectra (^{1}H NMR and ^{13}C NMR) of the obtained compounds were obtained by a Bruker 300 MHz and 75 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO d_6, respectively. Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet in the NMR spectra. Coupling constants (J) were reported in Hertz. Mass spectra of the compounds were recorded on an LCMS-IT-

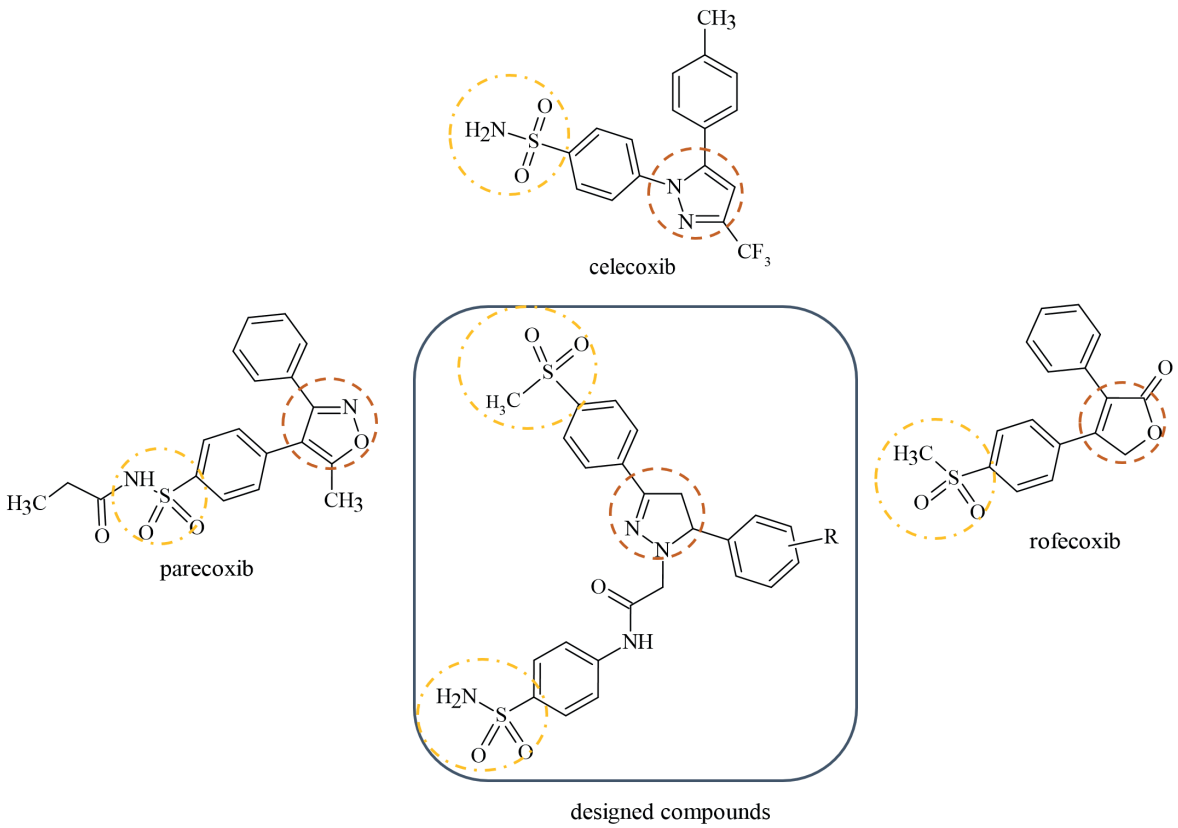


Figure 1. The structure of some selective COX-2 inhibitors and the designed compounds.

TOF (Shimadzu, Kyoto, Japan) by means of the ESI method. All reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany).

2.1.1. Synthesis of 2-chloro-*N*-(4-aminosulfonylphenyl)acetamide (1)

4-Aminobenzenesulfonamide (10 g, 0.06 mol) was dissolved in DMF (20 mL) and cooled in an ice bath. The solution of chloroacetyl chloride (4.77 mL, 0.072 mol) in DMF (5 mL) was added dropwise to the ice bath solution of 4-aminobenzenesulfonamide with constant stirring. The mixture was stirred for an additional 4 hours. Following that, the mixture was poured into iced water. The precipitated compound was filtered and crystallized from ethanol.

2.1.2. Synthesis of 2-hydrazinyl-*N*-(4-aminosulfonylphenyl)acetamide (2)

Compound **1** (11.8 g, 0.048 mol) was dissolved in ethanol (100 mL) and stirred at room temperature. Hydrazine hydrate (4.48 mL, 0.14 mol) was dissolved in ethanol (10 mL) and added dropwise to the initial solution with stirring. After an additional 5 hours of stirring, the precipitated compound was filtered and washed with ethanol to remove the excess hydrazine hydrate. The resulting product was crystallized from ethanol.

2.1.3. General procedure for the synthesis of 1-[4'-(methylsulfonyl)phenyl]-3-substitutedphenylprop-2-en-1-one derivatives (3a-3i) [28]

To the solution of 4'-(methylsulfonyl)acetophenone (1.4 g, 0.007 mol) in methanol (50 ml), sodium hydroxide (0.34 g, 0.008 mol) and substituted aldehyde derivative (0.07 mol) were added and the mixture was allowed to stir at room temperature for 12 hours. Upon completion of the reaction, confirmed by TLC, the resulting compound was filtered and washed with water to remove sodium hydroxide and crystallized from ethanol.

2.1.4. General synthesis of 2-[3-(4-(methylsulfonyl)phenyl)-5-substitutedphenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-*N*-(4-aminosulfonylphenyl)acetamides (4a-4i)

Compound **2** (0.003 mol) and 1-[4'-(methylsulfonyl)phenyl]-3-substitutedphenylprop-2-en-1-one derivative (**3a-3i**) (0.003 mol) were refluxed in ethanol (40 mL) in the presence of sodium hydroxide (0.004 mol) for 12-18 hours. After the completion of the reaction, the precipitated compound was removed by filtration and washed with water. The product was crystallized from ethanol.

2-[3-(4-(Methylsulfonyl)phenyl]-5-(2-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-yl]-*N*-(4-aminosulfonylphenyl)acetamide (4a)

Yield: % 83%, m.p. 158.7-161.3 °C. IR ν_{maks} (cm^{-1}): 3342.64, 3296.35 (N-H stretching bands), 3068.75 (aromatic C-H stretching band), 2974.23, 2893.22 (aliphatic C-H stretching bands), 1683.86 (amide C=O stretching band), 1593.20, 1521.84, 1508.33 (N-H bending, C=N, C=C stretching bands), 1307.74 (SO_2 asymmetric stretching band), 1147.65 (SO_2 stretching band).

^{1}H NMR (300 MHz, DMSO-*d6*, ppm) δ 3.06 (1H, dd, *JAB* = 16.6 Hz, *JAX* = 12.8 Hz, C_4-H_A pyrazoline), 3.23 (3H, s, -SO_2CH_3), 3.79 (1H, dd, *JBA* = 16.6 Hz, *JBX* = 11.2 Hz, C_4-H_B pyrazoline), 3.85 (1H, d, *J*= 16.3 Hz, CO-CH-H_1), 4.13 (1H, d, *J*= 16.3 Hz, CO-CH-H_2), 5.20 (1H, t, *J*= 12.1 Hz, C_5-H_X pyrazoline), 7.20-7.42 (4H, m, phenyl-H, NH_2), 7.65-7.70 (2H, m, phenyl-H), 7.74 (4H, s, phenyl-H), 7.86 (2H, d, *J*= 8.6 Hz, phenyl-H), 7.93 (2H, d, *J*= 8.7 Hz, phenyl-H), 10.33 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-*d6*, ppm) δ 41.01 (CH_2, pyrazoline-C_4), 43.99 (CH_3, -SO_2CH_3), 55.50 (CH, pyrazoline C_5), 62.75 (CH_2, CO-CH_2), 116.05 (CH, d, *J*= 21.2 Hz, phenyl), 119.31 (2CH, phenyl), 125.34 (CH, d, *J*= 3.5 Hz, phenyl), 126.57 (2CH, phenyl), 127.08 (2CH, phenyl), 127.54 (CH, d, *J*= 12.6 Hz, phenyl), 127.80 (2CH, phenyl), 129.32 (CH, d, *J*=

3.7 Hz, phenyl), 130.19 (C, d, *J*= 7.7 Hz, phenyl), 137.59 (C, phenyl), 138.97 (C, phenyl), 140.34 (C, phenyl), 141.98 (C, phenyl), 147.55 (C, pyrazoline C_3), 160.98 (C, d, *J*= 243.7 Hz, phenyl), 168.57 (C, C=O).

HRMS (m/z): $[M+H]^+$ calcd for $C_{24}H_{23}FN_4O_5S_2$: 531.1167; found: 531.1146.

2-[3-(4-(Methylsulfonyl)phenyl]-5-(3-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-yl]-*N*-(4-aminosulfonylphenyl)acetamide (4b)

Yield: % 77%, m.p. 170.7-173.4 °C. IR ν_{maks} (cm^{-1}): 3325.28, 3296.35 (N-H stretching bands), 3068.75 (aromatic C-H stretching band), 2972.31, 2895.15 (aliphatic C-H stretching bands), 1683.86 (amide C=O stretching band), 1593.20, 1521.84 (N-H bending, C=N, C=C stretching bands), 1307.74 (SO_2 asymmetric stretching band), 1145.72 (SO_2 symmetric stretching bands).

^{1}H NMR (300 MHz, DMSO-*d6*, ppm) δ 3.05 (1H, dd, *JAB* = 16.6 Hz, *JAX* = 13.5 Hz, C_4-H_A pyrazoline), 3.23 (3H, s, -SO_2CH_3), 3.74 (1H, dd, *JBA* = 16.6 Hz, *JBX* = 10.8 Hz, C_4-H_B pyrazoline), 3.85 (1H, d, *J*= 16.2 Hz, CO-CH-H_1), 4.05 (1H, d, *J*= 16.2 Hz, CO-CH-H_2), 4.94 (1H, dd, J_{MX} = 12.9 Hz, J_{AX} = 11.2 Hz, C_5-H_X pyrazoline), 7.11-7.18 (1H, m, phenyl-H), 7.27 (2H, s, NH_2), 7.34-7.47 (3H, m, phenyl-H), 7.74 (4H, s, phenyl-H), 7.86 (2H, d, *J*= 8.6 Hz, phenyl-H), 7.94 (2H, d, *J*= 8.8 Hz, phenyl-H), 10.25 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-*d6*, ppm) δ 42.36 (CH_3, -SO_2CH_3), 43.98 (CH_2, pyrazoline-C_4), 55.74 (CH, pyrazoline C_5), 69.34 (CH_2, CO-CH_2), 114.76 (CH, d, *J*= 21.8 Hz, phenyl), 115.19 (CH, d, *J*= 21.1 Hz, phenyl), 119.36 (2CH, phenyl), 124.29 (CH, d, *J*= 2.2 Hz, phenyl), 126.57 (2CH, phenyl), 127.06 (2CH, phenyl), 127.83 (2CH, phenyl), 131.13 (CH, d, *J*= 8.4 Hz, phenyl), 137.58 (C, phenyl), 138.95 (C, phenyl), 140.38 (C, phenyl), 141.94 (C, phenyl), 143.65 (C, d, *J*= 7.0 Hz, phenyl), 147.75 (C, pyrazoline C_3), 162.78 (C, d, *J*= 242.2 Hz, phenyl), 168.55 (C, C=O).

HRMS (m/z): $[M+H]^+$ calcd for $C_{24}H_{23}FN_4O_5S_2$: 531.1167; found: 531.1151.

2-[3-(4-(Methylsulfonyl)phenyl]-5-(3-phenoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-yl]-*N*-(4-aminosulfonylphenyl)acetamide (4c)

Yield: % 75%, m.p. 207.0-208.4 °C. IR ν_{maks} (cm^{-1}): 3338.93, 3325.28, 3290.56 (N-H stretching bands), 3057.17 (aromatic C-H stretching band), 2974.23, 2895.15 (aliphatic C-H stretching bands), 1670.35 (amide C=O stretching band), 1683.56, 1506.41, 1489.05 (N-H bending, C=N, C=C stretching bands), 1338.60 (SO_2 asymmetric stretching band), 1157.29 (SO_2 symmetric stretching band).

^{1}H NMR (300 MHz, DMSO-*d6*, ppm) δ 3.04 (1H, dd, *JAB* = 16.5 Hz, *JAX* = 13.3 Hz, C_4-H_A pyrazoline), 3.22 (3H, s, -SO_2CH_3), 3.72 (1H, dd, *JBA* = 16.6 Hz, *JBX* = 10.9 Hz, C_4-H_B pyrazoline), 3.86 (1H, d, *J*= 16.2 Hz, CO-CH-H_1), 4.04 (1H, d, *J*= 16.2 Hz, CO-CH-H_2), 4.89 (1H, dd, J_{MX} = 13.1 Hz, J_{AX} = 11.0 Hz, C_5-H_X pyrazoline), 6.89-6.93 (1H, m, phenyl-H), 6.97-7.00 (2H, m, phenyl-H), 7.09-7.15 (1H, m, phenyl-H), 7.19-7.20 (1H, m, phenyl-H), 7.28 (2H, s, NH_2), 7.31-7.42 (4H, m, phenyl-H), 7.73 (4H, s, phenyl-H), 7.84 (2H, d, *J*= 8.7 Hz, phenyl-H), 7.92 (2H, d, *J*= 8.6 Hz, phenyl-H), 10.23 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-*d6*, ppm) δ 42.33 (CH_3, -SO_2CH_3), 43.98 (CH_2, pyrazoline C_4), 55.81 (CH, pyrazoline C_5), 69.69 (CH_2, CO-CH_2), 118.12 (CH, phenyl), 118.36 (CH, phenyl), 119.10 (2CH, phenyl), 119.37 (CH, phenyl), 123.15 (CH, phenyl), 124.00 (2CH, phenyl), 126.53 (2CH, phenyl), 127.04 (2CH, phenyl), 127.81 (2CH, phenyl), 130.53 (2CH, phenyl), 130.80 (CH, phenyl), 137.62 (C, phenyl), 138.94 (C, phenyl), 140.32 (C, phenyl), 141.95 (C, phenyl), 142.87 (C, phenyl), 147.69 (C, pyrazoline C_3), 156.87 (C, phenyl), 157.33 (C, phenyl), 168.52 (C, C=O).

HRMS (m/z): $[M+H]^+$ calcd for $C_{30}H_{28}N_4O_6S_2$: 605.1523; found: 605.1511.

2-[3-(4-(Methylsulfonyl)phenyl]-5-(4-ethoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-yl]-*N*-(4-aminosulfonylphenyl)acetamide (4d)

Yield: % 73%, m.p. 188.8-193.1 °C. IR ν_{maks} (cm^{-1}): 3354.21, 3309.85 (N-H stretching bands), 3080.32

(aromatic C-H stretching band), 2974.23, 2897.08 (aliphatic C-H stretching band), 1683.86 (amide C=O stretching band), 1653.94, 1548.84, 1506.41 (N-H bending, C=N, C=C stretching bands), 1311.59 (SO_2 asymmetric stretching band), 1149.57 (SO_2 symmetric stretching bands).

^{1}H NMR (300 MHz, DMSO-d*6*, ppm) δ 1.30 (3H, t, *J*= 6.9 Hz, OCH_2-CH_3), 3.02 (1H, dd, *JAB* = 16.4 Hz, *JAX* = 13.7 Hz, C_4-H_A pyrazoline), 3.22 (3H, s, -SO_2CH_3), 3.64 (1H, dd, *JBA* = 16.5 Hz, *JBX* = 10.7 Hz, C_4-H_B pyrazoline), 3.78 (1H, d, *J*= 16.3 Hz, CO-CH-H_1), 3.95-4.02 (3H, m, CO-CH-H_2, OCH_2-CH_3), 4.82 (1H, dd, J_{MX} = 13.2 Hz, J_{AX} = 10.8 Hz, C_5-H_X pyrazoline), 6.91 (2H, d, *J*= 8.7 Hz, phenyl-H), 7.26 (2H, s, NH_2), 7.42 (2H, d, *J*= 8.6 Hz, phenyl-H), 7.73 (4H, s, phenyl-H), 7.85 (2H, d, *J*= 8.7 Hz, phenyl-H), 7.93 (2H, d, *J*= 8.7 Hz, phenyl-H), 10.17 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-d*6*, ppm) δ 15.12 (OCH_2CH_3), 41.99 (CH_3, -SO_2CH_3), 44.00 (CH_2, pyrazoline C_4), 55.38 (CH, pyrazoline C_5), 63.46 (OCH_2CH_3), 69.57 (CH_2, CO-CH_2), 114.96 (2CH, phenyl), 119.38 (2CH, phenyl), 126.45 (2CH, phenyl), 127.04 (2CH, phenyl), 127.81 (2CH, phenyl), 129.39 (2CH, phenyl), 131.74 (C, phenyl), 137.84 (C, phenyl), 138.92 (C, phenyl), 140.21 (C, phenyl), 141.94 (C, phenyl), 147.63 (C, pyrazoline C_3), 158.73 (C, phenyl), 168.61 (C, C=O).

HRMS (m/z): $[M+H]^+$ calcd for $C_{26}H_{28}N_4O_6S_2$: 557.1523; found: 557.1499.

2-[3-(4-(Methylsulfonyl)phenyl]-5-(2,3-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-yl]-*N*-(4-aminosulfonylphenyl)acetamide (4e)

Yield: % 78%, m.p. 234.2-235.7 °C. IR ν_{maks} (cm^{-1}): 3329.14, 3263.56 (N-H stretching bands), 3057.17 (aromatic C-H stretching band), 2899.01 (aliphatic C-H asymmetric stretching bands), 1683.86 (amide C=O stretching band), 1593.20, 1541.12 (N-H bending, C=N, C=C stretching bands), 1305.81 (SO_2 asymmetric stretching bands), 1147.65 (SO_2 symmetric stretching band).

^{1}H NMR (300 MHz, DMSO-*d6*, ppm) δ 2.92 (1H, dd, *JAB* = 16.9 Hz, *JAX* = 12.6 Hz, C_4-H_A pyrazoline), 3.23 (3H, s, -SO_2CH_3), 3.89 (1H, d, *J*= 16.3 Hz, CO-CH-H_1), 3.94 (1H, dd, *JBA* = 17.1 Hz, *JBX* = 11.6 Hz, C_4-H_B pyrazoline), 4.21 (1H, d, *J*= 16.3 Hz, CO-CH-H_2), 5.37 (1H, t, *J*= 12.1 Hz, C_5-H_X pyrazoline), 7.27 (2H, s, NH_2), 7.44 (1H, t, *J*= 7.9 Hz, phenyl-H), 7.62 (1H, d, *J1*= 1.5 Hz, *J2*= 8.0 Hz, phenyl-H), 7.72-7.74 (5H, m, phenyl-H), 7.85 (2H, d, *J*= 8.7 Hz, phenyl-H), 7.92 (2H, d, *J*= 8.7 Hz, phenyl-H), 10.36 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-*d6*, ppm) δ 41.05 (CH_2, pyrazoline-C_4), 43.97 (CH_3, -SO_2CH_3), 55.50 (CH, pyrazoline C_5), 66.47 (CH_2, CO-CH_2), 119.29 (2CH, phenyl), 126.63 (2CH, phenyl), 127.14 (2CH, phenyl), 127.26 (CH, phenyl), 127.80 (2CH, phenyl), 129.23 (CH, phenyl), 130.12 (CH, phenyl), 130.88 (C, phenyl), 132.49 (C, phenyl), 137.41 (C, phenyl), 138.95 (C, phenyl), 140.41 (C, phenyl), 141.77 (C, phenyl), 141.97 (C, phenyl), 147.12 (C, pyrazoline C_3), 168.65 (C, C=O).

HRMS (m/z): $[M+H]^+$ calcd for $C_{24}H_{22}Cl_2N_4O_5S_2$: 581.0481; found: 581.0469.

2-[3-(4-(Methylsulfonyl)phenyl]-5-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-yl]-*N*-(4-aminosulfonylphenyl)acetamide (4f)

Yield: % 88%, m.p. 229.1-230.9 °C. IR ν_{maks} (cm^{-1}): 3319.49 3259.70 (N-H stretching bands), 3066.82 (aromatic C-H stretching band), 2974.23 (aliphatic C-H stretching band), 1683.86 (amide C=O stretching band), 1589.34, 1521.84 (N-H bending, C=N, C=C stretching bands), 1307.74 (SO_2 asymmetric stretching band), 1143.79 (SO_2 symmetric stretching band).

^{1}H NMR (300 MHz, DMSO-*d6*, ppm) δ 2.92 (1H, dd, *JAB* = 16.7 Hz, *JAX* = 12.7 Hz, C_4-H_A pyrazoline), 3.22 (3H, s, -SO_2CH_3), 3.84-3.94 (2H, m, CO-CH-H_1, C_4-H_B pyrazoline), 4.18 (1H, d, *J*= 16.2 Hz, CO-CH-H_2), 5.30 (1H, t, *J*= 12.1 Hz, C_5-H_X pyrazoline), 7.27 (2H, s, NH_2), 7.51 (1H, dd, *J1*= 2.2 Hz, *J2*= 8.4 Hz, phenyl-H), 7.67-7.80 (6H, m, phenyl-H), 7.85 (2H, d, *J*= 8.6 Hz, phenyl-H), 7.92 (2H, d, *J*= 8.7 Hz, phenyl-H), 10.37 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-*d6*, ppm) δ 41.03 (CH_2, pyrazoline-C_4), 43.97 (CH_3, -SO_2CH_3), 55.44 (CH, pyrazoline C_5), 65.47 (CH_2, CO-CH_2), 119.29 (2CH, phenyl), 126.64 (2CH, phenyl), 127.13 (2CH, phenyl), 127.80 (2CH, phenyl), 128.45 (CH, phenyl),

129.44 (CH, phenyl), 130.25 (C, phenyl), 133.36 (C, phenyl), 133.95 (C, phenyl), 137.41 (C, phenyl), 137.99 (C, phenyl), 139.02 (C, phenyl), 140.42 (C, phenyl), 141.94 (C, phenyl), 147.26 (C, pyrazoline C_3), 168.60 (C, C=O).

HRMS (m/z): $[M+H]^+$ calcd for $C_{24}H_{22}Cl_2N_4O_5S_2$: 581.0481; found: 581.0466.

2-[3-(4-(Methylsulfonyl)phenyl]-5-(2,4-difluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-yl]-*N*-(4-aminosulfonylphenyl)acetamide (4g)

Yield: % 69%, m.p. 175.3-177.6 °C. IR ν_{maks} (cm^{-1}): 3354.21, 3334.92, 3296.35 (N-H stretching bands), 3080.32 (aromatic C-H stretching bands), 2974.23, 2895.15 (aliphatic C-H stretching bands), 1683.86 (amide C=O stretching band), 1593.20, 1521.84, 1506.41 (N-H bending, C=N, C=C stretching bands), 1309.67 (SO_2 asymmetric stretching band), 1145.72 (SO_2 symmetric stretching band).

^{1}H NMR (300 MHz, DMSO-*d6*, ppm) δ 3.05 (1H, dd, *JAB* = 16.6 Hz, *JAX* = 12.8 Hz, C_4-H_A pyrazoline), 3.23 (3H, s, -SO_2CH_3), 3.72-3.88 (2H, m, C_4-H_B pyrazoline, CO-CH-H_1), 4.11 (1H, d, *J*= 16.2 Hz, CO-CH-H_2), 5.16 (1H, t, *J*= 12.0 Hz, C_5-H_X pyrazoline), 7.13 (1H, td, *J1*= 2.1 Hz, *J2*= 8.5 Hz, phenyl-H), 7.25-7.32 (1H, m, phenyl-H), 7.70-7.73 (5H, m, phenyl-H), 7.85 (2H, d, *J*= 8.6 Hz, phenyl-H), 7.93 (2H, d, *J*= 8.7 Hz, phenyl-H).

^{13}C NMR (75 MHz, DMSO-*d6*, ppm) δ 40.92 (CH_3, -SO_2CH_3), 43.99 (CH_2, pyrazoline-C_4), 55.58 (CH, pyrazoline C_5), 62.44 (CH_2, CO-CH_2), 104.57 (CH, t, *J*= 26.1 Hz, phenyl), 112.36 (CH, dd, *J1*= 2.9 Hz, *J2*= 20.9 Hz, phenyl), 119.31 (2CH, phenyl), 123.96 (C, dd, *J1*= 3.7 Hz, *J2*= 12.9 Hz, phenyl), 126.60 (2CH, phenyl), 127.08 (2CH, phenyl), 127.81 (2CH, phenyl), 130.72 (CH, dd, *J1*= 9.4 Hz, *J2*= 5.6 Hz, phenyl), 137.52 (C, phenyl), 139.12 (C, phenyl), 140.40 (C, phenyl), 141.91 (C, phenyl), 147.70 (C, pyrazoline C_3), 161.03 (C, dd, *J1*= 12.1 Hz, *J2*= 246.7 Hz, phenyl), 162.26 (C, dd, *J1*= 12.5 Hz, *J2*= 244.8 Hz, phenyl), 168.53 (C, C=O).

HRMS (m/z): $[M+H]^+$ calcd for $C_{24}H_{22}F_2N_4O_5S_2$: 549.1072; found: 549.1071.

2-[3-(4-(Methylsulfonyl)phenyl]-5-(2-chloro-6-fluorophenyl)-4,5-dihydro-1*H*-pyrazole-1-yl]-*N*-(4-aminosulfonylphenyl)acetamide (4h)

Yield: % 74%, m.p. 136.7-139.4 °C**.** IR ν_{maks} (cm^{-1}): 3365.78, 3334.49, 3311.78 (N-H stretching bands), 3066.82 (aromatic C-H stretching bands), 2974.23, 2895.15 (aliphatic C-H stretching bands), 1683.86 (amide C=O stretching band), 1593.20, 1521.84 (N-H bending, C=N, C=C stretching bands), 1311.59 (SO_2 asymmetric stretching band), 1147.65 (SO_2 symmetric stretching band).

^{1}H NMR (300 MHz, DMSO-*d6*, ppm) δ 3.23 (3H, s, -SO_2CH_3), 3.56-3.76 (2H, m, C_4-H_A pyrazoline, C_4-H_B pyrazoline), 3.83 (1H, d, *J*= 16.4 Hz, CO-CH-H_1), 4.20 (1H, d, *J*= 16.4 Hz, CO-CH-H_2), 5.57 (1H, t, *J*= 12.1 Hz, C_5-H_X pyrazoline), 7.27 (2H, s, NH_2), 7.33-7.39 (1H, m, phenyl-H), 7.68 (2H, d, *J*= 8.9 Hz, phenyl-H), 7.74 (2H, d, *J*= 9.0 Hz, phenyl-H), 7.85 (2H, d, *J*= 8.6 Hz, phenyl-H), 7.94 (2H, d, *J*= 8.6 Hz, phenyl-H), 8.07 (1H, d, *J*= 8.5 Hz, phenyl-H), 8.18 (1H, d, *J*= 8.5 Hz, phenyl-H), 10.29 (1H, s, NH).

^{13}C NMR (75 MHz, DMSO-*d6*, ppm) δ 42.78 (CH_2, pyrazoline-C_4), 44.02 (CH_3, -SO_2CH_3), 55.26 (CH, pyrazoline C_5), 64.15 (CH_2, CO-CH_2), 116.07 (CH, d, *J*= 24.3 Hz, phenyl), 119.18 (2CH, phenyl), 126.42 (CH, phenyl), 127.14 (2CH, phenyl), 127.85 (2CH, phenyl), 129.36 (2CH, phenyl), 131.12 (CH, d, *J*= 9.6 Hz, phenyl), 134.63 (C, d, *J*= 6.0 Hz, phenyl), 137.63 (CH, phenyl), 138.93 (C, phenyl), 140.26 (C, d, *J*= 13.9 Hz, phenyl), 141.89 (C, phenyl), 144.82 (C, phenyl), 146.44 (C, pyrazoline C_3), 162.15 (C, d, *J*= 233.2 Hz, C-F), 168.47 (C, C=O).

HRMS (m/z): $[M+H]^+$ calcd for $C_{24}H_{22}ClFN_4O_5S_2$: 565.0777; found: 565.0779.

2-[3-(4-(Methylsulfonyl)phenyl]-5-(3,4-methylenedioxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-yl]-*N*-(4-aminosulfonylphenyl)acetamide (4i)

Yield: % 82%, m.p. 253-254 °C. IR ν_{maks} (cm^{-1}): 3360.00, 3284.77 3255.84 (N-H stretching bands), 3030.17 (aromatic C-H stretching band), 2974.23, 2891.30 (aliphatic C-H asymmetric stretching bands), 1664.57 (amide C=O stretching band), 1525.69,

1521.84 (N-H bending, C=N, C=C stretching bands), 1305.81 (SO_2 asymmetric stretching band), 1145.72 (SO_2 symmetric stretching band).

^{1}H NMR (300 MHz, DMSO-*d6*, ppm) δ 3.02 (1H, dd, *JAB* = 16.4 Hz, *JAX* = 13.7 Hz, C_4-H_A pyrazoline), 3.23 (3H, s, -SO_2CH_3), 3.63 (1H, dd, *JBA* = 16.5 Hz, *JBX* = 10.6 Hz, C_4-H_B pyrazoline), 3.83 (1H, d, *J*= 16.1 Hz, CO-CH-H_1), 3.98 (1H, d, *J*= 16.2 Hz, CO-CH-H_2), 4.79 (1H, dd, J_{BX} = 13.4 Hz, J_{AX} = 10.9 Hz, C_5-H_X pyrazoline), 5.94 (1H, d, *J*= 0.9 Hz, O-CH_2), 5.99 (1H, d, *J*= 0.9 Hz, O-CH_2), 6.88 (1H, d, *J*= 8.0 Hz, phenyl-H), 6.94 (1H, dd, *J1*= 1.6 Hz, *J2*= 8.1 Hz, phenyl-H), 7.16 (1H, d, *J*= 1.5 Hz, phenyl-H), 7.72 (4H, s, phenyl-H), 7.85 (2H, d, *J*= 8.7 Hz, phenyl-H), 7.93 (2H, d, *J*= 8.7 Hz, phenyl-H).

^{13}C NMR (75 MHz, DMSO-*d6*, ppm) δ 42.04 (CH_3, -SO_2CH_3), 43.99 (CH_2, pyrazoline C_4), 55.65 (CH, pyrazoline C_5), 70.05 (CH_2, CO-CH_2), 101.48 (CH_2), 108.05 (CH, phenyl), 108.60 (CH, phenyl), 119.32 (2CH, phenyl), 121.95 (CH, phenyl), 126.48 (2CH, phenyl), 126.92 (2CH, phenyl), 127.81 (2CH, phenyl), 133.95 (C, phenyl), 137.76 (C, phenyl), 139.62 (C, phenyl), 140.25 (C, phenyl), 141.72 (C, phenyl), 147.42 (C, phenyl), 147.74 (C, phenyl), 148.01 (C, pyrazoline C_3), 168.57 (C, C=O).

HRMS (m/z): $[M+H]^+$ calcd for $C_{25}H_{24}N_4O_7S_2$: 557.1159; found: 557.1136.

2.2. Biological Evaluation

Fluorometric COX-1 and COX-2 inhibitor screening kits (Biovision) were used to evaluate the *in vitro* inhibitory potency of the compounds against COX-1/COX-2, in accordance with the manufacturer's instructions [29, 30]. The intermediate product produced by the COX enzyme, prostaglandin G_2, is detected fluorometrically as the basis for the assay. Each kit contains COX-1, COX-2 enzymes, COX assay buffer, COX probe (in DMSO), COX cofactor (in DMSO), arachidonic acid, NaOH and their selective inhibitors SC560 and celecoxib. All compounds were dissolved in 2% DMSO. For all compounds, the initial *in vitro* assay was conducted at two concentrations (10^{-3} and 10^{-4} M). To prepare the diluted COX cofactor, 398 µl of COX-1/COX-2 assay buffer and 2 µl of COX-1 cofactor were mixed. 5 µl of arachidonic acid was mixed with 5 µl of NaOH to prepare a dilute arachidonic acid/NaOH solution, which was subsequently diluted with 90 µl of ddH_2O. COX-1/COX-2 assay buffer (76 µl), COX probe (1 µl), diluted COX cofactor (2 µl), and COX-1/2 solution (1 µl) were placed in a 96-well plate. To the solution above, 10 µl of the test compounds were added. All the pipetting in the assay was performed by Biotek Precision robotic system (BioTek Instruments, Inc.). The assay mixture was incubated at 25°C for 5−10 min. Additionally, to stop the reaction following incubation, 10 µl of diluted arachidonic acid/NaOH solution was applied to each well. Fluorescence (Ex/Em = 535/587 nm) of the samples was kinetically measured by BioTek-Synergy H1 multimode microplate reader (BioTek Instruments, Inc.) for 5-minute intervals. The results were displayed as mean ± standard deviation.

2.3. ADME Study

The ADME features of the pyrazoline derivatives (**4a-4i**) were assessed using the online tool SwissADME [31], and relevant findings are described in **Table 2**.

3. RESULTS AND DISCUSSION

3.1. Chemistry

In the current study, target pyrazoline derivatives were synthesized via a four-step synthetic procedure, as shown in **Scheme 1**. Initially, 2-chloro-*N*-(4-aminosulfonylphenyl)acetamide (**1**) was synthesized *via* the reaction of 4-aminobenzenesulfonamide with chloroacetyl chloride in dimethylformamide. In the second step, compound **1** was treated with hydrazine hydrate in ethanol at room temperature to gain 2-hydrazinyl-*N*-(4-aminosulfonylphenyl) acetamide (**2**). The Claisen−Schmidt condensation of 4′-(methylsulfonyl)acetophenone with substituted aromatic aldehydes in ethanol, in the presence of sodium hydroxide afforded the corresponding chalcones (**3a-3i**). Finally, the target compounds **4a-4i** were acquired by the reaction of compound **2** and substituted chalcones **3a-3i** in the presence of sodium hydroxide in ethanol, under reflux conditions. All the synthesized compounds have been characterized by IR, ^{1}H NMR, ^{13}C NMR, and HRMS.

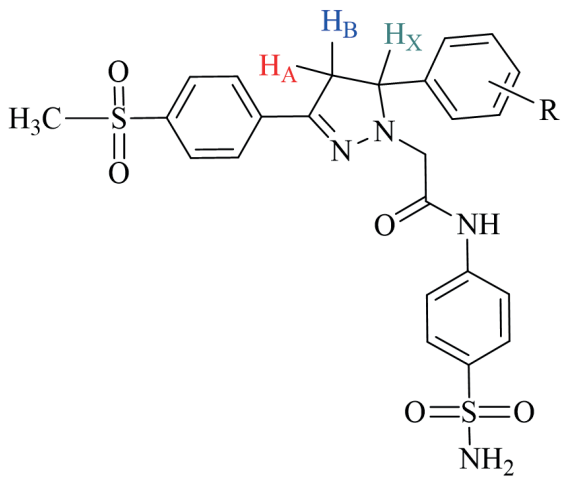
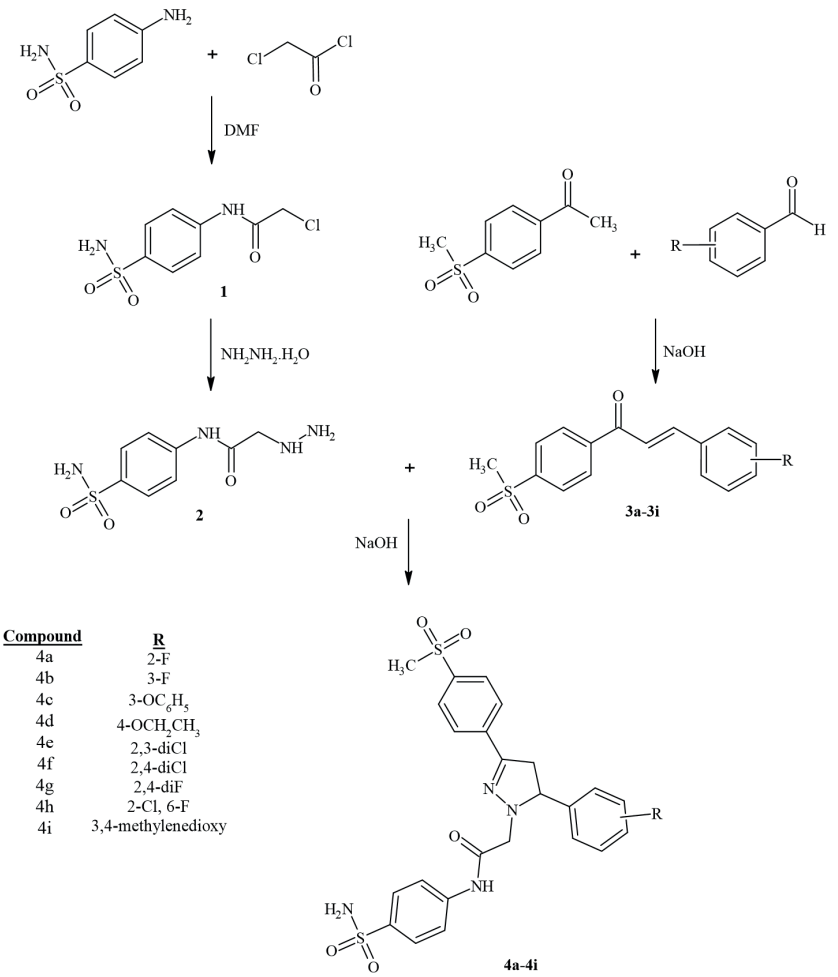


Figure 2. ABX spin system in the pyrazoline ring.

In the IR spectra of the compounds, characteristic N-H and C=O stretching bands were detected at about 3365.78-3255.84 cm^{-1} and 1683.86-1662.64 cm^{-1} regions, respectively.

In the ^1H NMR spectra of the compounds, the three protons present at fourth (H_A and H_B) and fifth (H_X) positions of the pyrazoline ring exhibited the splitting pattern characteristic of ABX system. (**Figure 2**) The proton belonging to the fourth position of the pyrazoline ring (H_A), resonated as a doublet of doublets (J_{AB} = 14.0-16.9 Hz, J_{AX} = 7.0-13.7 Hz) or a multiplet at 2.92-3.56 ppm. The other fourth proton H_B was observed as a doublet of doublets (J_{BA} = 16.5-17.1 Hz, J_{BX} = 10.5-11.6 Hz) or a multiplet at 3.59-



Compound	R
4a	2-F
4b	3-F
4c	3-OC_6H_5
4d	4-OCH_2CH_3
4e	2,3-diCl
4f	2,4-diCl
4g	2,4-diF
4h	2-Cl, 6-F
4i	3,4-methylenedioxy

Scheme 1. The synthetic procedure of the compounds **(4a-4i)**.

3.94 ppm. The fifth position proton (H_X) appeared as a doublet of doublets (J_{AX} = 10.7-12.0 Hz, J_{BX} = 12.9-13.4 Hz) at 4.79-4.94 ppm, or triplets (J= 12.0-12.1 Hz) at 5.20-5.57 ppm. The protons belonging to the methylsulfonyl group (-SO_2CH_3) were recorded as a singlet at 3.22-3.23 ppm. The protons on the aromatic ring were assigned between 6.91 and 8.18 ppm. NH_2 protons were determined as a singlet or broad singlet at 7.26-7.28 ppm, while NH protons were observed at 10.15-10.37 ppm as a singlet or broad singlet.

In the ^{13}C NMR spectra of compounds **4a-4i**, the peaks belonging to C_3, C_4 and C_5 carbon of the pyrazoline ring were observed at 145.67-148.01 ppm, 38.83-44.02 ppm and 55.08-55.81 ppm, respectively. The peak of methylsulfonyl carbon was recorded at 40.92-44.04 ppm. The peak due to the carbonyl carbon was detected at 168.47-168.67 ppm. Aromatic carbon peaks were recorded at 104.57-162.26 ppm. Moreover, HRMS analysis of all synthesized compounds was consistent with their molecular formulas.

3.2. Biological Evaluation

Compounds 4a-4i were evaluated for their *in vitro* anti-inflammatory activity by COX-1 and COX-2 inhibition via fluorometric COX inhibitory screening assay kits (Biovision). The results were summarized in **Table 1**. According to data, the tested compounds had weak inhibitory activities against both COX isoforms when compared with celecoxib, ibuprofen, and nimesulide. The COX-1 inhibitory activity of compounds was in the 22.02-45.26% and 14.52-29.59% ranges at COX-1 inhibition concentrations of 10^{-3} and 10^{-4}, respectively. Most of the compounds exhibited higher inhibitory potency toward COX-2 than COX-1. At 10^{-3} and 10^{-4} M concentrations, compounds showed 28,12-59.10% and 15.19-47.82% inhibitory activity against COX-2, respectively. Compounds **4a**, **4e** and **4f** were recorded as the most active inhibitors against COX-2 with 53.58%, 54.84% and 59.10% inhibition rates, respectively, at 10^{-3} concentration. The presence of 2-fluoro (**4a**), 2,3-dichloro (**4e**) and 2,4-dichloro (**4f**) substitutions on the C-5 phenyl ring resulted in the highest inhibitory activity against COX-2. The higher COX-2 inhibitory activity of the compounds may be attributed to the presence of methylsulfonyl (-SO_2CH_3) and sulfonamide (-SO_2NH_2) groups.

3.3. ADME Study

The studies on the absorption, distribution, metabolism, and excretion characteristics of the compounds are critical in the drug development process. **Table 2** summarizes the predicted ADME properties for the most potent compounds (**4a**, **4e** and **4f**) and references (ibuprofen, celecoxib and nimesulide).

Table 1. *In vitro* % inhibition of compounds **4a-4i** and references against the COX enzymes at concentrations of 10^{-3} and 10^{-4} M

Compound	COX-1 Inhibition (%)		COX-2 Inhibition (%)	
	10^{-3} M	10^{-4} M	10^{-3} M	10^{-4} M
4a	22.02 ± 0.42	14.52 ± 0.37	53.58 ± 0.61	30.46 ± 0.58
4b	23.09 ± 0.60	17.14 ± 0.50	40.19 ± 0.61	37.45 ± 0.42
4c	45.26 ± 0.62	29.59 ± 0.69	31.47 ± 0.65	28.57 ± 0.51
4d	33.85 ± 0.45	19.18 ± 0.57	49.88 ± 0.98	21.07 ± 0.62
4e	28.64 ± 0.71	15.56 ± 0.51	54.84 ± 0.92	45.63 ± 0.85
4f	44.16 ± 0.82	17.46 ± 0.53	59.10 ± 0.86	47.82 ± 0.63
4g	38.76 ± 0.77	25.19 ± 0.41	37.33 ± 0.51	15.19 ± 0.686
4h	29.75 ± 0.71	20.42 ± 0.42	40.13 ± 0.55	33.27 ± 0.39
4i	24.16 ± 0.75	20.65 ± 0.75	28.12 ± 0.69	19.48 ± 0.61
Ibuprofen	98.15 ± 1.05	89.36 ± 1.24	98.234 ± 1.21	88.16 ± 1.35
Celecoxib			89.09 ± 1.12	80.15 ± 1.09
Nimesulide			97.821 ± 1.21	89.58 ± 1.05

Table 2. Predicted ADME of the most potent compounds (**4a**, **4e** and **4f**), ibuprofen, celecoxib and nimesulide

	4a	4e	4f	Optimal Range	Ibuprofen	Celecoxib	Nimesulide
Physicochemical Properties							
Molecular weight (g/mol)	530.59	581.49	581.49	150 - 500	206.28	381.37	308.31
Num. heavy atoms	36	37	37	-	15	26	21
Num. aromatic heavy atoms	18	18	18	-	6	17	12
Insaturation	0.17	0.17	0.17	0.25 - 1.0	0.46	0.12	0.08
Num. rotatable bonds	8	8	8	0 - 9	4	4	5
Num. H-bond acceptors	8	7	7	-	2	7	5
Num. H-bond donors	2	2	2	-	1	1	1
Molar Refractivity	139.90	149.97	149.97	-	62.18	89.96	80.05
TPSA (Å²)	155.76	155.76	155.76	20 - 130	37.30	86.36	109.60
Lipophilicity & Water Solubility							
Lipophilicity (consensus)	2.47	3.17	3.28	-0.7 to 5.0	3.00	3.40	1.66
Water Solubility (ESOL)	-4.40	-5.44	-5.44	-6.0 to 0	-3.36	-4.57	-3.48
Pharmacokinetics							
GI absorption	low	low	low	-	high	high	high
BBB permeant	no	no	no	-	yes	no	no
Pgp substrate	no	no	no	-	no	no	no
Skin permeability (cm/s)	-7.92	-7.41	-7.41	-	-5.07	-6.21	-6.33
Violations							
Lipinski Druglikeness	1	1	1	-	0	0	0
Leadlikeness	2	2	2	-	1	1	0

The Molecular Weight (MW) of the compounds varied from 530.59 to 581.49 g/mol, indicating that they are above the normal range for drug-like molecules. The topological polar surface area (TPSA) values of the compounds are notably high compared to reference drugs, limiting their oral bioavailability. None of the compounds demonstrated significant permeability across the blood-brain barrier (BBB). The high rotatable bonds metric of the compounds indicated a high level of molecular flexibility, which is frequently linked to favorable drug-like activity. The consensus logP values (iLOGP, XLOGP3, WLOGP, and MLOGP) showed that the lipophilicity varied from 2.47 to 3.28, implying a moderate level of hydrophobicity, which is in line with characteristics of a medication. The water solubility investigation, as shown by the ESOL values, revealed that the log S values of the most potent compounds range from -5.44 to -4.40, which are within the optimal range. The skin permeability of the compounds, as indicated by the skin permeability coefficient log Kp (cm/s), is lower for the analyzed derivatives compared to control drugs. Based on the criteria established by Lipinski, the analyzed compounds each have one violation, suggesting that they have favorable pharmacokinetic characteristics.

4. CONCLUSION

In conclusion, a new series of pyrazolines was synthesized and assessed for their potential inhibitory activities against the COX-1 and COX-2 enzymes. All compounds were proven to be weak inhibitors compared with the references. Compounds **4a**, **4e** and **4f** were found to be the most active COX-2 inhibitors in the series, with 53.58%, 54.84% and 59.10% inhibition rates, respectively, at 10^{-3} concentration. The presence of 2-fluoro (**4a**), 2,3-dichloro (**4e**) and

2,4-dichloro (**4f**) substitutions on the C-5 phenyl ring resulted in the highest inhibitory activity against COX-2. Finally, the theoretical ADME properties of the compounds **4a-4i** were analyzed by calculations. Our study provided insights into *in vitro* COX inhibitory activities of pyrazoline derivatives. These compounds could be further optimized and explored as lead molecules for developing novel and effective pyrazoline-based COX-2 inhibitors.

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Ethical approval

Ethics committee approval is not required as there are no *in vivo* or clinical studies.

Author contribution

Conceptualization, B.K., Y.Ö. and Z.A.K.; Methodology, B.K. and B.M.S.Ö.; Writing-original draft preparation, B.K. and B.N.S.Ö.; Writing-review and editing, B.K., B.N.S.Ö., Y.Ö. and Z.A.K.; Supervision, Y.Ö. and Z.A.K. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declared that there is no conflict of interest.

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