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Synthesis, characterization and preliminary screening of regioisomeric 1-(3-pyridazinyl)-3-arylpyrazole and 1-(3-pyridazinyl)-5-arylpyrazole derivatives towards cyclooxygenase inhibition

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Abstract

In a search for novel compounds with analgesic and anti-inflammatory activity, a series of regioisomeric 1-(3-pyridazinyl)-3-arylpyrazole (**5a**–**f**, **6a**–**f**) and 1-(3-pyridazinyl)-5-arylpyrazole (**7a**–**f**, **8a**–**f**) derivatives were synthesized. The structure of these regioisomers was confirmed by spectral techniques. The compounds were preliminarily screened at 8 μM concentration for their inhibitory activity against cyclooxygenase enzymes, COX-1 and COX-2, using a human whole blood test. The tested derivatives showed inhibitory activity for both enzymes and are worthy of further investigation for developing better leads.

Keywords: *COX-1, COX-2, pyrazole, pyridazine, pyridazinone, cyclooxygenase*

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Introduction

The discovery of two distinct cyclooxygenase (COX) isoforms, namely COX-1 and COX-2, made it possible to separate the pharmacological effects from the general side effects of traditional non-steroidal anti-inflammatory drugs (NSAIDs) [1,2]. Hence, this discovery suggested that the inhibition of COX-2, but not COX-1, was of importance for designing compounds that lack the gastrointestinal and renal side effects of currently used NSAIDs [2–4]. Clinical data also indicated that selective inhibition of COX-2 produced efficacy equivalent to traditional NSAIDs in the treatment of inflammatory disorders with increased gastric safety profile [1–5]. However, the occurrence of newer side-effects, most notably on the cardiovascular system, caused a hot debate on the usefulness of the selective COX-2 inhibition concept [6–10]. During this time, the availability and widespread use of COX-2 selective inhibitors resulted in a better understanding of the roles of COX-1 and COX-2 in health and disease such as pain perception and the progression of cancers [2,11]. Apart from inflammation, a few COX-2 inhibitors are also being studied for treating other ailments like cancer [12–14] and Alzheimer's diseases [15]. Although what is now known about the efficacy and safety of COX-2 inhibitors and their potential use in various pathologies including inflammation and cancer are under debate, we are still in need of better tolerated and different classes of COX-2 selective agents to extend our understanding on this drug class.

Pyridazine derivatives have recently received much attention due to their analgesic and anti-inflammatory activities [16–18]. Various pyridazinone derivatives endowed with analgesic/anti-inflammatory effects have been reported and among them, emorfazone was launched in Japan as an anti-inflammatory drug [19,20]. In addition, 3-*O*-substituted benzyl pyridazinone derivatives [21] and 4,5-diarylsubstituted pyridazinones [22] were recently shown to exhibit *in vivo* potent anti-inflammatory activity using the carrageenan-induced rat paw oedema assay through the mechanism involving selective COX-2 inhibition.

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With the aim to discover new analgesic and anti-inflammatory drugs, our research group has long been interested in the chemistry and pharmacology of different lactamic heterocyclic systems and recently prepared distinct pyridazinone derivatives and found that they showed good analgesic and anti-inflammatory activities [23–28].

The objective of this study was the replacement of one of the aryl groups in the celecoxib template, a known COX-2 inhibitor drug, with pyridazine to evaluate the significance of the aryl substituents of the central pyrazole ring. For this purpose and based on the fact that pyridazinone derivatives bear potential analgesic/anti-inflammatory properties, we evaluated the biological consequences of incorporation of a pyridazine/pyridazinone ring as one of the aryl substituents, and also the effect of a 1,3- or 1,5-diarylsubstitution pattern around the pyrazole ring on the *in vitro* COX inhibitory potency of the resulting derivatives. Herein, we describe the synthesis and the *in vitro* preliminary screening of the first series of compounds to evaluate their COX-1/COX-2 inhibitory activity.

Materials and methods

Materials and instruments

3,6-Dichloropyridazine, hydrazine hydrate, ethyl trifluoroacetate, 4,4,4-trifluoro-1-phenyl-1,3-butanedione (**2a**) and substituted acetophenones were obtained from Sigma Chemicals. 3-Chloro-6-hydrazinopyridazine (**3**) was synthesized from the reaction of 3,6-dichloropyridazine with hydrazine hydrate in an ice bath according to the previously published procedure [29] with modifications. Previously reported β-diketones [30,31] were synthesized by the condensation of the appropriate acetophenone or acetonaphthone derivatives with ethyl trifluoroacetate in the presence of NaH. All other chemicals were obtained from commercial sources. The yields reported are unoptimized. 1D NMR (^{1}H: 500 MHz and ^{13}C: 125 MHz) and 2D NMR spectra were measured with a JEOL JNM A500 and Bruker AVANCE500 NMR spectrometer at 30°C. All chemical shifts were reported as δ (ppm) values using TMS as an internal standard in DMSO-d_6. Two dimensional phase-sensitive NOESY spectra were measured with field gradient mode in mixing time 1800 or 2000 msec. Inverse proton detected HMBC spectra were carried out with field gradient mode applying a delay optimized for long-range coupling constants of 8 Hz. Elemental analyses were performed with Leco-932 (C,H,N,S,O-Elemental Analyser) at the Scientific and Technical Research Council of Turkey, Instrumental Analysis Center (Ankara, Turkey). High resolution mass spectra (HRMS) was measured with a JEOL AX-505 spectrometer.

*General procedure for the synthesis of 4,4,4-trifluoro-1-(4-substitutedphenyl)-1,3-butandiones (**2b–d**)*

Appropriate *p*-substituted acetophenone (6′-Methoxy-2′-acetonaphtenone for **2f**) (30 mmol) was dissolved in hexane (30 mL) and cooled to −10°C in an ice–salt bath. To the cooled solution, a 60% suspension of NaH in parafin oil (30 mmol) was added and stirred for 10 min. Then, a solution of ethyl trifluoroacetate (33 mmol) in hexane (20 mL) was added dropwise over 30 min. After the addition was complete, the mixture was stirred at this temperature for 30 min and at room temperature for a further 1 h (in the case of **2e**, it was refluxed for 6 h with vigorously stirring). The hexane layer was evaporated to half volume under reduced pressure, and the precipitate was suction-filtered, washed with cold hexane, dried and suspended in water (200 mL). The suspension was acidified with concentrated HCl and the precipitate was filtered off, dried and recrystallized from the appropriate solvent.

*4,4,4-trifluoro-1-(4-chlorophenyl)-1,3-butandione (**2b**)*. Recrystallized from petroleum ether (40–60°C). Yield: 69%, mp 63–64°C (lit. mp 61–62°C [30,31]). Anal. Calcd. for $C_{10}H_6ClF_3O_2$: C, 47.93; H 2.41. Found: C, 48.35; H 2.11%.

*4,4,4-trifluoro-1-(4-methylphenyl)-1,3-butandione (**2c**)*. Recrystallized from petroleum ether (40–60°C). Yield: 70%, mp 47–48°C (lit. mp 43–44°C [30,31]). Anal. Calcd. for $C_{11}H_9F_3O_2$: C, 57.40; H, 3.94. Found: C, 57.32; H, 3.54%.

*4,4,4-trifluoro-1-(4-methoxyphenyl)-1,3-butandione (**2d**)*. Recrystallized from petroleum ether (40–60°C). Yield: 67%, mp 58–59°C (lit. mp 57–58°C [30,31]). Anal. Calcd. for $C_{11}H_9F_3O_3$: C, 53.67; H, 3.68. Found: C, 53.50; H, 3.28%.

*4,4,4-trifluoro-1-(6-methoxy-2-naphthyl)-1,3-butandione (**2f**)*. Recrystallized from petreloum ether (40–60°C). Yield: 63%, mp 90–91°C. Anal. Calcd. for $C_{15}H_{11}F_3O_3$: C, 60.82; H, 3.74. Found: C, 60.90; H, 3.92%.

*General procedure for the synthesis of 1-(6-chloropyridazine-3-yl)-3-aryl-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazoles (**4a–f**)*

The solution of benzoylacetone or the appropriate 4,4,4-trifluoro-1-phenyl-1,3-butanedione derivative (15 mmol) and 6-chloro-3-hydrazinopyridazine (15 mmol) in absolute ethanol (30 mL) was refluxed

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for 8 h, and cooled to room temperature. After addition of a sufficient amount of water for complete precipitation, the precipitate was filtered off, dried and recrystallized from the appropriate solvent.

*1-(6-chloropyridazine-3-yl)-3-phenyl-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazole (**4a**)*. Recrystallized from methanol. Yield, 66%; mp 142–143°C; ^{1}H NMR (DMSO-d_6) δ: 3.72(1H, d, J = 18.9 Hz, H-4α), 4.02(1H, d, J = 18.9 Hz, H-4β), 7.48(3H, m, H-3″, 4″, 5″), 7.79(1H, d, J = 9.5 Hz, H-5′), 7.84(2H, m, H-2″, 6″), 7.85 (1H, d, J = 9.5 Hz, H-4′), 8.14(1H, s, 5-OH). ^{13}C NMR (DMSO-d_6) δ: 44.81(C4), 92.93(C5, ^2J_F = 33 Hz), 120.08(C4′), 123.44 (CF_3, ^1J_F = −286 Hz), 126.38 (C2″, 6″), 128.75(C3″, 5″), 130.00 (C5′), 130.28(C4″), 130.34(C1″), 149.62(C6′), 151.40(C3), 156.62 (C3′). Anal. Calcd. for $C_{14}H_{10}ClF_3N_4O$: C, 49.12; H, 2.95; N, 16.38. Found: C, 49.54; H, 2.7; N, 16.36%.

*1-(6-chloropyridazine-3-yl)-3-(4-chlorophenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazole (**4b**)*. Recrystallized from acetonitrile. Yield, 64%; mp 207–208; ^{1}H NMR (DMSO-d_6) δ: 3.70(1H, d, J = 19.2 Hz, H-4α), 4.03(1H, d, J = 19.2 Hz, H-4β), 7.55(2H, dd, J = 8.9, 2.4 Hz, H-3″, 5″), 7.79(1H, d, J = 9.5 Hz, H-5′), 7.85(1H, d, J = 9.5 Hz, H-4′), 7.86(2H, dd, J = 8.9, 2.4 Hz, H-2″, 6″), 8.17(1H, s, 5-OH). ^{13}C NMR (DMSO-d_6) δ: 44.70(C4), 93.13(C5, ^2J_F = 33 Hz), 120.13(C4′), 123.38(CF_3, ^1J_F = −288 Hz), 128.14(C2″, 6″), 128.81(C3″, 5″), 129.27(C1″), 130.03(C5′), 134.85(C4″), 149.77(C6′), 150.43(C3), 156.53(C3′). Anal. Calcd. for $C_{14}H_9Cl_2F_3N_4O$: C, 44.68; H, 2.41; N, 14.90. Found: C, 45.01; H, 2.05; N, 15.18%.

*1-(6-chloropyridazine-3-yl)-3-(4-methylphenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazole (**4c**)*. Recrystallized from ethanol. Yield, 62%; mp 177–178°C; ^{1}H NMR (DMSO-d_6) δ: 2.36(3H, s, CH_3), 3.68(1H, d, J = 18.9 Hz, H-4α), 3.98(1H, d, J = 18.9 Hz, H-4β), 7.29(2H, d, J = 8.6 Hz, H-3″, 5″), 7.73(2H, d, J = 8.6 Hz, H-2″, 6″), 7.77(1H, d, J = 9.5 Hz, H-5′), 7.83(1H, d, J = 9.5 Hz, H-4′), 8.1(1H, s, 5-OH). ^{13}C NMR (DMSO-d_6) δ: 20.96(CH_3), 44.88(C4), 92.80(C5, ^2J_F = 33 Hz), 120.01(C4′), 123.47(CF_3, ^1J_F = −286 Hz), 126.36(C2″, 6″), 127.61(C1″), 129.32(C3″, 5″), 129.98(C5′), 140.17 (C4″), 149.49(C6′), 151.42(C3), 156.63(C3′). Anal. Calcd. for $C_{15}H_{12}ClF_3N_4O$: C, 50.55; H, 3.40; N, 15.73. Found: C, 50.84; H, 3.02; N, 16.14%.

*1-(6-chloropyridazine-3-yl)-3-(4-methoxyphenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazole (**4d**)*. Recrystallized from ethanol. Yield, 61%; mp 172–173°C; ^{1}H NMR (DMSO-d_6) δ: 3.68(1H, d, J = 18.9 Hz, H-4α), 3.81(3H, s, 4″-OCH_3), 3.98(1H, d, J = 18.9 Hz, H-4β), 7.03(2H, d, J = 8.9 Hz, H-3″, 5″), 7.73(1H, d, J = 9.5 Hz, H-5′), 7.78(2H, d, J = 8.6 Hz, H-2″, 6″), 7.82(1H, d, J = 9.5 Hz, H-4′), 8.09(1H, s, 5-OH). ^{13}C NMR (DMSO-d_6) δ: 45.00(C4), 55.33(OCH_3), 92.74(C5, ^2J_F = 33 Hz), 114.22(C3″. 5″), 119.95 (C4′), 122.86(C1″), 123.51(CF_3, ^1J_F = −288 Hz), 128.11(C2″, 6″), 129.97(C5′), 149.33(C6′), 151.25(C3), 156.68 (C3′), 160.94(C4″). Anal. Calcd. for $C_{15}H_{12}ClF_3N_4O_2$: C, 48.38; H, 3.25; N, 15.05. Found: C, 47.99; H, 3.15; N, 14.9%.

*1-(6-chloropyridazin-3-yl)-3-(4-fluorophenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazole (**4e**)*. Recrystallized from ethanol to give colorless prisms. Yield, 64%; mp 179–180°C; ^{1}H NMR δ: 3.71(1H, d, J = 19.0 Hz, H-4α), 4.05(1H, d, J = 19.1 Hz, H-4β), 7.32(2H, dd, J = 8.9 Hz, H-3″, 5″), 7.78(1H, d, J = 9.4 Hz, H-4′), 7.85(1H, d, J = 9.4 Hz, H-5′), 7.90 (2H, dd, J = 8.9, 5.5 Hz, H-2″, 6″), 8.15(1H, s, 5-OH). ^{13}C NMR δ: 44.90(C4), 93.05(q, ^2J_F = 33.2 Hz, C5), 115.84(d, ^2J_F = 22.0 Hz, C3″, 5″), 120.10(C5′), 123.44(q, ^1J_F = −286.1 Hz, CF3), 127.01(d, ^4J_F = 2.8 Hz, C1″), 128.83(d, ^3J_F = 8.2 Hz, C2″, 6″), 130.04(C4′), 149.63(C3′), 150.58(C3), 156.62(C6′), 163.22(d, ^1J_F = −248.4 Hz, C4″).Anal. Calcd. for $C_{14}H_9ClF_4N_4O$: C, 46.62; H, 2.52; N, 15.53. Found: C, 46.63; H, 2.74; N, 15.45%.

*1-(6-chloropyridazine-3-yl)-3-(6-methoxynapht-2-yl)-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazole (**4f**)*. Recrystallized from ethanol. Yield, 71%; mp 169–170°C; ^{1}H NMR (DMSO-d_6) δ: 3.81(1H, d, J = 18.9 Hz, H-4α), 3.90(3H, s, 6″-OCH_3), 4.11(1H, d, J = 18.9 Hz, H-4β), 7.23(1H, dd, J = 8.9, 2.7 Hz, H-7″), 7.38(1H, d, J = 2.4 Hz, H-5″), 7.79(1H, d, J = 9.5 Hz, H-5′), 7.88(1H, d, J = 8.6 Hz, H-4″), 7.89(1H, d, J = 9.5 Hz, H-4′), 7.90(2H, d, J = 8.9 Hz, H-8″), 8.03(1H, dd, J = 8.6, 1.8 Hz, H-3″), 8.16(1H, s, 5-OH), 8.21(1H, d, J = 1.8 Hz, H-1″). ^{13}C NMR (DMSO-d_6) δ: 44.88(C4), 55.28(OCH_3), 92.90(C5, ^2J_F = 33 Hz), 106.33(C5″), 119.22(C7″), 120.03 (C5′), 123.29(C3″), 123.52(CF_3, ^1J_F = −286 Hz), 125.56(C2″), 127.09(C1″), 127.19(C4″), 127.94(C8a″), 130.00(C8″, 5′), 135.15(C4″a), 149.51(C6′), 151.58(C3), 156.63(C3′), 158.36(C6″). Anal. Calcd. for $C_{19}H_{14}ClF_3N_4O_2$: C, 54.02; H, 3.34; N, 13.27. Found: C, 54.37; H, 3.13; N, 13.3%.

*General procedure for the synthesis of 1-[3(2H)-pyridazinone-6-yl]-3-aryl-5-trifluoromethylpyrazoles (**5a**–**f**)*

The appropriate 1-(6-chloropyridazine-3-yl)-3-aryl-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazole derivative (10 mmol) and sodium acetate trihydrate

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(15 mmol) in glacial acetic acid (30 mL) were refluxed for 15 h and poured into ice-water mixture (150 mL). The precipitate formed was filtered off, washed with sodium carbonate solution (2.5%, w/v) and then with water, dried and recrystallized from the appropriate solvent.

*1-[3(2H)-pyridazinone-6-yl]-3-phenyl-5-trifluoromethylpyrazole (**5a**)*. Recrystallized from toluene. Yield, 71.9%; mp 225–226°C; ^{1}H NMR (DMSO-d_6) δ: 7.16(1H, d, J = 10.1 Hz, H-4′), 7.43(1H, ddd, J = 7.3, 1.2 Hz, H-4″), 7.49(2H, ddd, J = 7.3, 1.2 Hz, H-3″, 5″), 7.78(1H, s, H-4), 7.96(2H, dd, J = 7.3, 1.2 Hz, H-2″, 6″), 7.98(1H, d, J = 10.1 Hz, H-5′), 13.29(1H, s, N2′—H). ^{13}C NMR (DMSO-d_6) δ: 108.79(C4), 119.35(CF_3, ^1J_F = −269 Hz), 125.77 (C2″, 6″), 128.90(C3″, 5″), 129.19(C4″), 129.65 (C5′), 130.52(C1″), 132.22(C5, ^2J_F = 39 Hz), 132.52(C4′), 140.20(C6′), 151.80(C3), 159.89(3′-CO). Anal. Calcd. for $C_{14}H_9F_3N_4O$: C, 54.91; H, 2.96; N, 18.29. Found: C, 54.87; H, 2.58; N, 18.08%.

*1-[3(2H)-pyridazinone-6-yl]-3-(4-chlorophenyl)-5-trifluoromethylpyrazole (**5b**)*. Recrystallized from ethanol. Yield, 79%; mp 247–248°C; ^{1}H NMR (DMSO-d_6) δ: 7.15(1H, d, J = 10.0 Hz, H-4′), 7.55(2H, dd, J = 8.6, 1.8 Hz, H-3″, 5″), 7.82(1H, s, H-4), 7.97(1H, d, J = 10.0 Hz, H-5′), 7.99(2H, dd, J = 8.6, 1.8 Hz, H-2″, 6″), 13.3(1H, s, N2′—H). ^{13}C NMR (DMSO-d_6) δ: 108.96(C4), 119.29(CF_3, ^1J_F = −269 Hz), 127.51(C2″, 6″), 128.96 (C3″, 5″), 129.42(C1″), 129.62(C5′), 132.34(C5, ^2J_F = 41 Hz), 132.53(C4′), 133.83(C4″), 140.13(C6′), 150.68(C3), 159.87(3′-CO). Anal. Calcd. for $C_{14}H_8ClF_3N_4O$: C, 49.36; H, 2.37; N, 16.45. Found: C, 49.53; H, 1.98; N, 16.48%.

*1-[3(2H)-pyridazinone-6-yl]-3-(4-methylphenyl)-5-trifluoromethylpyrazole (**5c**)*. Recrystallized from ethanol. Yield, 91%; mp 265–266°C; ^{1}H NMR (DMSO-d_6) δ: 2.35(3H, s, CH_3), 7.14(1H, d, J = 10.0 Hz, H-4′), 7.29(2H, d, J = 8.2 Hz, H-3″, 5″), 7.73(1H, s, H-4), 7.85(2H, d, J = 8.2 Hz, H-2″, 6″), 7.96(1H, d, J = 10.0 Hz, H-5′), 13.3(1H, s, N2′-H). ^{13}C NMR (DMSO-d_6) δ: 20.81(4″-CH_3), 119.37 (CF_3, ^1J_F = −269 Hz), 125.69(C2″, 6″), 127.76 (C1″), 129.44(C3″, 5″), 129.64(C5′), 132.11(C5, ^2J_F = 39 Hz), 132.58(C4′), 138.75(C4″), 140.23 (C6′), 151.83(C3), 159.87(3′-CO). Anal. Calcd. for $C_{15}H_{11}F_3N_4O$: C, 56.25; H, 3.46; N, 17.49. Found: C, 56.62; H, 3.06; N, 17.63%.

*1-[3(2H)-pyridazinone-6-yl]-3-(4-methoxyphenyl)-5-trifluoromethylpyrazole (**5d**)*. Recrystallized from toluene. Yield, 84%; mp 233–234°C; ^{1}H NMR (DMSO-d_6) δ: 3.8(3H, s, OCH_3), 7.03(2H, dd, J = 8.9, 2.4 Hz, H-3″, 5″), 7.14(1H, d, J = 10.0 Hz, H-4′), 7.69(1H, s, H-4), 7.89(2H, dd, J = 8.9, 2.4 Hz, H-2″, 6″), 7.96(1H, d, J = 10.0 Hz, H-5′), 13.26(1H, s, N2′-H). ^{13}C NMR (DMSO-d_6) δ: 55.19(4″-OCH_3), 108.35(C4), 114.29(C3″, 5″), 119.40(CF_3, ^1J_F = −269 Hz), 123.06(C1″), 127.22(C2″, 6″), 129.64(C5′), 132.05(C5, ^2J_F = 39 Hz), 132.45(C4′), 140.25(C6′), 151.70(C3), 159.89(3′-CO), 160.04(C4″). Anal. Calcd. for $C_{15}H_{11}F_3N_4O_2$: C, 53.58; H, 3.30; N, 16.66. Found: C, 53.98; H, 2.89; N, 16.59%.

*1-(3(2H)-pyridazinon-6-yl)-3-(4-fluorophenyl)-5-trifluoromethylpyrazole (**5e**)*. Recrystallized from ethanol to give colorless fine needles. Yield, 75%; mp 237.5–238°C; ^{1}H NMR δ: 7.16(1H, d, J = 10.0 Hz, H-4′), 7.32(2H, dd, J = 8.8 Hz, H-3″, 5″), 7.79(1H, s, H-4), 7.97 (1H, d, J = 10.0 Hz, H-5′), 8.01(2H, dd, J = 8.8, 5.5 Hz, H-2″, 6″), 13.29(1H, s, N—H). ^{13}C NMR δ: 108.81(C4), 115.90(d, ^2J_F = 22.0 Hz, C3″, 5″), 119.34(q, ^1J_F = −268.9 Hz, CF3), 127.14(d, ^4J_F = 2.7 Hz, C1′), 127.14(d, ^3J_F = 8.2 Hz, C2″, 6″), 129.65(C-5′), 132.27(q, ^2J_F = 40.3 Hz, C5), 132.56(C4′), 140.19(C6′), 150.92(C3), 159.91 (3-CO), 162.66(d, ^1J_F = −246.5 Hz, C4″). Anal. Calcd. for $C_{14}H_8F_4N_4O$: C, 51.86; H, 2.49; N, 17.28. Found: C, 51.78; H, 2.70; N, 17.27%.

*1-[3(2H)-pyridazinone-6-yl]-3-(6-methoxynapht-2-yl)-5-trifluoromethylpyrazole (**5f**)*. Recrystallized from ethanol. Yield, 88%; mp 257–258°C; ^{1}H NMR (DMSO-d_6) δ: 3.89(3H, s, OCH_3), 7.17(1H, d, J = 10.1 Hz, H-4′), 7.21(1H, dd, J = 8.9, 2.4 Hz, H-7″), 7.36(1H, d, J = 2.4 Hz, H-5″), 7.86(1H, s, H-4), 7.89 (1H, d, J = 8.9 Hz, H-8″), 7.91(1H, d, J = 8.9 Hz, H-4″), 8.02(1H, d, J = 10.1 Hz, H-5′), 8.05(1H, dd, J = 8.9, 1.5 Hz, H-3″), 8.46(1H, d, J = 1.5 Hz, H-1″), 13,30(1H, s, N2′—H). ^{13}C NMR (DMSO-d_6) δ: 55.23(6″-OCH_3), 106.05(C7″), 108.84(C4), 119.26 (C7″), 119.40(CF_3, ^1J_F = −269 Hz), 123.83(C3″), 124.93(C1″), 125.69(C2″), 127.35(C4″), 128.29 (C8a″), 129.67(C5′), 129.72(C8″), 132.22(C5, ^2J_F = 39 Hz), 132.52(C4′), 134.57(C4a″), 140.26 (C6′), 151,98(C3), 157.90(C6″), 159.89(3′-CO). Anal. Calcd. for $C_{19}H_{13}F_3N_4O_2$: C, 59.07; H, 3.39; N, 14.50. Found: C, 58.90; H, 3.32; N, 14.43%.

*General procedure for the synthesis of 1-(6-chloropyridazine-3-yl)-3-aryl-5-trifluoromethylpyrazoles (**6a–f**)*

The appropriate 1-[3(2H)-pyridazinone-6-yl]-3-aryl-5-trifluoromethylpyrazole (0.001 mol) was refluxed in $POCl_3$ (10 mL) for 1 h, and poured into crushed-ice (150 g) and stirred for a further 30 min. The mixture was subsequently neutralized by K_2CO_3 and the

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precipitate was filtered off, washed with water, dried and recrystallized from the appropriate solvent.

*1-(6-chloropyridazine-3-yl)-3-phenyl-5-trifluoromethylpyrazole (**6a**)*. Recrystallized from isopropanol. Yield, 78%; mp 133–135°C; ^{1}H NMR (DMSO-d_6) δ: 7.51 (3H, m, H-3″, 4″, 5″), 7.96(1H, s, H-4), 8.06(2H, d, J = 8.43, H-2″, 6″), 8.23(1H, d, J = 9.2 Hz, H-5′), 8.44(1H, d, J = 9.2 Hz, H-4′). Anal. Calcd. for $C_{14}H_8ClF_3N_4$: C, 51.79; H, 2.48; N, 17.26. Found: C, 51.94; H, 2.19; N, 16.93%.

*1-(6-chloropyridazine-3-yl)-3-(4-chlorophenyl)-5-trifluoromethylpyrazole (**6b**)*. Recrystallized from petroleum ether. Yield, 92%; mp 183–184°C; ^{1}H NMR (DMSO-d_6) δ: 7.56(2H, dd, J = 8.6, 2.1 Hz, H-3″, 5″), 7.95(1H, s, H-4), 8.04(2H, dd, J = 8.6, 2.1 Hz, H-2″, 6″), 8.20(1H, d, J = 9.2 Hz, H-5′), 8.39(1H, d, J = 9.2 Hz, H-4′). ^{13}C NMR (DMSO-d_6) δ: 110.90(C4), 119.40(CF_3, ^1J_F = −269 Hz), 124.42 (C4′), 127.70(C2″, 6″), 129.01(C3″, 5″), 129.14 (C1″), 132.00(C5′), 132.95(C5, ^2J_F = 39 Hz), 134.18(C4″), 151.73(C3), 153.93(C6′), 155.70 (C3′). Anal. Calcd. for $C_{14}H_7Cl_2F_3N_4$: C, 46.82; H, 1.96; N, 15.60. Found: C, 47.20; H, 2.30; N, 15.60%.

*1-(6-chloropyridazine-3-yl)-3-(4-methylphenyl)-5-trifluoromethylpyrazole (**6c**)*. Recrystallized from isopropanol. Yield, 95%; mp 169–171°C; ^{1}H NMR (DMSO-d_6) δ: 7.34(2H, d, J = 8.04 Hz, H-3″, 5″), 7.91(1H, s, H-4), 7.94(2H, d, J = 8.14 Hz, H-2″, 6″), 8.22(1H, d, J = 9.2 Hz, H-5′), 8.43(1H, d, J = 9.2 Hz, H-4′), 2.36 (3H, s, CH_3). Anal. Calcd. for $C_{15}H_{10}ClF_3N_4$: C, 53.19; H, 2.98; N, 16.54. Found: C, 53.28; H, 2.27; N, 16.14%.

*1-(6-chloropyridazine-3-yl)-3-(6-methoxyphenyl)-5-trifluoromethylpyrazole (**6d**)*. Recrystallized from isopropanol. Yield, 94%; mp 159–160°C; ^{1}H NMR (DMSO-d_6) δ: 7.08(2H, d, J = 8.8 Hz, H-3″, 5″), 7.87(1H, s, H-4), 7.96(2H, d, J = 8.8 Hz, H-2″, 6″), 8.22(1H, d, J = 9.2 Hz, H-4′), 8.41(1H, d, J = 9.2 Hz, H-5′), 3.83 (3H, s, OCH_3). Anal. Calcd. for $C_{15}H_{10}ClF_3N_4O$: C, 50.79; H, 2.84; N, 15.79. Found: C, 50.93; H, 2.39; N, 15.47%.

*1-(6-chloropyridazin-3-yl)-3-(4-fluorophenyl)-5-trifluoromethylpyrazole (**6e**)*. Recrystallized from n-hexane to give colorless fine bar. Yield, 90%; mp 176.5–177°C; ^{1}H NMR δ: 7.35(2H, dd, J = 8.9 Hz, H-3″, 5″), 7.93(1H, s, H-4), 8.08(2H, d, J = 8.9, 5.5 Hz, H-2″, 6″), 8.22(1H, d, J = 9.2 Hz, H-5′), 8.40 (1H, d, J = 9.2 Hz, H-4′). ^{13}C NMR δ: 110.79(C4), 115.96(d, ^2J_F = 22.0 Hz, C3″, 5″), 119.46(q, ^1J_F = −268.6 Hz, *C*F3), 124.39(C5′), 126.85(d, ^4J_F = 2.8 Hz C1″), 128.24(d, ^2J_F = 8.2 Hz, C2″, 6″), 132.02 (C4′), 132.89(q, ^2J_F = 40.7 Hz, C3), 151.95(C5), 153.96(C6′), 155.64(C3′), 162.84(d, ^1J_F = −246.6 Hz, C4″). Anal. Calcd. for $C_{14}H_7ClF_4N_4$: C, 49.07; H, 2.06; N, 16.35. Found: C, 49.10; H, 2.25; N, 16.30%.

*1-(6-chloropyridazin-3-yl)-3-(6-methoxynapht-2-yl)-5-trifluoromethylpyrazole (**6f**)*. Recrystallized from isopropanol. Yield, 95%; mp 185–187°C; ^{1}H NMR (DMSO-d_6) δ: 3.91(3H, s, OCH_3), 7.24(1H, dd, J = 8.9, 2.5 Hz, H-7″), 7.4(1H, dd, J = 8.5, 1.6 Hz, H-3″), 7.94(2H, m, H-4″, 5″), 8.04(1H, s, H-4), 8.14 (1H, d, J = 10 Hz, H-8″), 8.24 (1H, d, J = 9.1 Hz, H-5′), 8.46(1H, d, J = 9.1 Hz, H-4′) 8.55(1H, s, H-1″). Anal. Calcd. for $C_{19}H_{12}ClF_3N_4O$: C, 56.38; H, 2.99; N, 13.84. Found: C, 56.91; H, 2.55; N, 13.55%.

*General procedure for the synthesis of 1-(6-chloropyridazin-3-yl)-5-aryl-3-trifluoromethylpyrazoles (7**a**–**f**)*

The solution of the appropriate 4,4,4-trifluoro-1-phenyl-1,3-butanedione derivative (0.001 mol) and the hydrochloride salt of 6-chloro-3-hydrazinopyridazine (0.001 mol) in diglyme (30 mL) was refluxed for 5 h, and cooled to room temperature. Concentrated hydrochloric acid was dropped over concentrated sulfuric acid for liberation of gaseous HCl which was passed through the reaction medium to prepare the salt of 6-chloro-3-hydrazinopyridazine. After addition of a sufficient amount of water for complete precipitation, the precipitate was filtered off, dried and recrystallized from the appropriate solvent.

*1-(6-chloropyridazin-3-yl)-5-phenyl-3-trifluoromethylpyrazole (7**a**)*. Recrystallized from n-hexane. Yield, 65%; mp 131–133°C; ^{1}H NMR (DMSO-d_6) δ: 7.34 (1H, s, H-4), 7.35–7.41(5H, m, H—Ph), 8.22(2H, s, H-4′, 5′). ^{13}C NMR δ: 107.28(C4), 120.94(q, ^1J_F = −269.2 Hz, CF3), 127.31(C5′), 128.43(C1″), 128.56 (C2″, 6″), 128.70(C3″, 5″), 129.22(C4″), 131.85 (C4′), 143.44(q, ^2J_F = 38.3 Hz, C3), 146.35(C5), 154.89(C6′), 156.11(C3′). EI-HRMS: 324.0388, Calcd. for 324.0396.

*1-(6-chloropyridazin-3-yl)-5-(4-chlorophenyl)-3-trifluoromethylpyrazole (7**b**)*. Recrystallized from n-hexane. Yield, 56%; mp 143–146°C; ^{1}H NMR (DMSO-d_6) δ: 7.38(1H, s, H-4), 7.42(2H, d, J = 8.4 Hz, H-2″, 6″), 7.48(2H, d, J = 8.4 Hz, H-3″, 5″), 8.22(1H, d, J = 9.1 Hz, H-5′), 8.24(1H, d, J = 9.1 Hz, H-4′). ^{13}C NMR δ: 107.75(C4), 120.87(q, ^1J_F = −269.3 Hz, CF3), 127.10(C5′), 127.49(C1″),

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128.58(C3″, 5″), 130.59(C2″, 6″), 131.92(C4′), 134.11(C4″), 143.48(q, ^2J_F = 38.2 Hz, C3), 145.07 (C5), 154.75(C6′), 156.08(C3′). EI-HRMS: 357.9972, Calcd. for 358.0000.

*1-(6-chloropyridazin-3-yl)-5-(4-methoxyphenyl)-3-trifluoromethylpyrazole (7**c**)*. Recrystallized from n-hexane. Yield, 48%; mp 159–160°C; ^{1}H NMR (DMSO-d_6) δ: 3.77(3H, s, OCH3), 6.95(2H, d, J = 8.8 Hz, H-3″, 5″), 7.25(1H, s, H-4), 7.28(2H, d, J = 8.8 Hz, H-2″, 6″), 8.18(1H, s, H-5′), 8.20(1H, s, H-4′). ^{13}C NMR δ: 55.22(OCH3), 106.57(C4), 114.06(C3″, 5″), 120.55(C1″), 121.01(q, ^1J_F = −268.3 Hz, CF3), 127.47(C5′), 130.19(C2″, 6″), 131.79(C4′), 143.36(q, ^2J_F = 36.2 Hz, C3), 146.34 (C5), 155.06(C6′), 156.09(C3′), 159.92(C4″). EI-HRMS: 354.0485, Calcd. for 354.0495.

*1-(6-chloropyridazin-3-yl)-5-(4-methylphenyl)-3-trifluoromethylpyrazole (7**d**)*. Recrystallized from cyclohexane. Yield, 50%; mp 136–137°C; ^{1}H NMR (DMSO-d_6) δ: 2.31(3H, s, CH3), 7.20(2H, d, J = 8.1 Hz, H-3″, 5″), 7.24(2H, d, J = 8.1 Hz, H-2″, 6″), 7.29(1H, s, H-4), 8.20(2H, s, H-4′, 5′). ^{13}C NMR δ: 20.75(CH3), 106.88(C4), 120.97(q, ^1J_F = −269.2 Hz, CF3), 125.50(C1″), 127.40(C5′), 128.57 (C2″, 6″), 129.15(C3″, 5″), 131.82(C4′), 138.93 (C4″), 143.40(q, ^2J_F = 38.1 Hz, C3), 146.46(C5), 154.96(C6′), 156.10(C3″). EI-HRMS: 338.0554, Calcd. for 338.0546.

*1-(6-chloropyridazin-3-yl)-5-(4-fluorophenyl)-3-trifluoromethylpyrazole (7**e**)*. Recrystallized from n-hexane to give colorless needles. Yield, 51%; mp 136–137°C; ^{1}H NMR (DMSO-d_6) δ: 7.25(2H, d, J = 8.8 Hz, H-3″, 5″), 7.34(1H, s, H-4), 7.44(2H, d, J = 8.7, 5.4 Hz, H-2″, 6″), 8.22(1H, d, J = 9.1 Hz, H-4′), 8.22(1H, d, J = 9.1 Hz, H-5′). ^{13}C NMR δ: 108.06(C4), 116.10(d, ^2J_F = 22.0 Hz, C3″, 5″), 121.44(q, ^1J_F = −269.3 Hz, CF3), 125.59(d, ^4J_F = 3.2 Hz, C1″), 127.72(C5′), 131.70(d, ^2J_F = 8.2 Hz, C2″, 6″), 132.40(C4′), 143.93(q, ^2J_F = 38.3 Hz, C3), 145.84(C5), 155.34(C6′), 156.58(C3′), 162.99(d, ^1J_F = −247.5 Hz, C4″). Anal. Calcd. for $C_{14}H_7ClF_4N_4$: C, 49.07; H, 2.06; N, 16.35. Found: C, 49.15; H, 2.29; N, 16.28%.

*1-(6-chloropyridazin-3-yl)-5-(6-methoxy-2-naphthyl)-3-trifluoromethylpyrazole (7**f**)*. Recrystallized from cyclohexane. Yield, 67%; mp 152.5–154°C; ^{1}H NMR (DMSO-d_6) δ: 3.88(3H, s, OCH3), 7.21(1H, dd, J = 8.9, 2.5 Hz, H-7″), 7.31(1H, dd, J = 8.5, 1.6 Hz, H-3″), 7.34(1H, d, J = 2.4 Hz, H-5″), 7.40(1H, s, H-4), 7.78(1H, d, J = 8.6 Hz, H-4″), 7.82(1H, d, J = 9.0 Hz, H-8″), 7.96(1H, s, H-1″), 8.21 (1H, d, J = 9.1 Hz, H-4′), 8.24(1H, d, J = 9.1 Hz, H-5′). ^{13}C NMR δ: 55.27(OCH3), 105.89(C5″), 107.27(C4), 119.33(C7″), 120.99(q, ^1J_F = −269.1 Hz, CF3), 123.52(C2″), 126.38(C3″), 126.81(C4″), 127.14 (C5′), 127.83(C8a″), 128.06(C1″), 129.76(C8″), 131.82(C4′), 134.24(C4a″), 143.52(q, ^2J_F = 37.8 Hz, C3), 146.60(C5), 154.94(C6′), 156.00(C3′), 158.21(C6″). EI-HRMS: 404.0652, Calcd. for 404.0652.

*General procedure for the synthesis of 1-[3(2H)-pyridazinone-6-yl]-5-aryl-3-trifluoromethylpyrazoles (**8a**–**f**)*

The appropriate 1-(6-chloropyridazine-3-yl)-5-aryl-3-trifluoromethylpyrazole derivative (10 mmol) and sodium acetate trihydrate (15 mmol) in glacial acetic acid (30 mL) were refluxed for 15 h and poured into an ice-water mixture (150 mL). The precipitate formed was filtered off, washed with sodium carbonate solution (2.5%, w/v) and then with water, dried and recrystallized from the appropriate solvent.

*1-(3(2H)-pyridazinon-6-yl)-5-phenyl-3-trifluoromethylpyrazole (**8a**)*. Recrystallized from toluene. Yield, 74%; mp 189–190°C; ^{1}H NMR (DMSO-d_6) δ: 7.10 (1H, d, J = 9.9 Hz, H-4′), 7.25(1H, s, H-4), 7.40–7.45(5H, m, H—Ph), 7.76(1H, d, J = 9.9 Hz, H-5′), 13.12(1H, brs, N—H). ^{13}C NMR δ: 106.07(C4), 121.02(q, ^1J_F = −268.9 Hz, CF3), 128.08(C1″), 128.49(C2″, 6″), 128.76(C3″, 5″), 129.28(C4″), 131.30(C5′), 132.18(C4′), 140.47(C6′), 142.47(q, ^2J_F = 36.8 Hz, C3), 145.73(C5), 159.86(C3′). EI-HRMS: 306.0709 Calcd. For 306.0728.

*1-(3(2H)-pyridazinon-6-yl)-5-(4-chlorophenyl)-3-trifluoromethylpyrazole (**8b**)*. Crude product washed with hot cyclohexane. Yield, 71%; mp 209–210°C; ^{1}H NMR (DMSO-d_6) δ: 7.11(1H, d, J = 9.9 Hz, H-4′), 7.30(1H, s, H-4), 7.45(2H, d, J = 8.6 Hz, H-2″, 6″), 7.53(2H, d, J = 8.5 Hz, H-3″, 5″), 7.78(1H, d, J = 10.0 Hz, H-5′), 13.10(1H, s, N—H). ^{13}C NMR δ: 106.52(C4), 120.93(q, ^1J_F = −269.9 Hz, CF3), 127.08(C1″), 128.78(C3″, 5″), 130.37(C2″, 6″), 131.04(C5′), 132.28(C4′), 134.13(C4″), 140.32 (C6′), 142.46(q, ^2J_F = 37.9 Hz, C3), 144.41(C5), 159.81(C3′). EI-HRMS: 340.0331, Calcd. for 340.0339.

*1-(3(2H)-pyridazinon-6-yl)-5-(4-methoxyphenyl)-3-trifluoromethylpyrazole (**8c**)*. Crude product washed with hot cyclohexane. Yield, 94%; mp 169–170°C; ^{1}H NMR (DMSO-d_6) δ: 3.77(3H, s, OCH3), 6.99 (2H, d, J = 8.7 Hz, H-3″, 5″), 7.03(1H, d, J = 9.9 Hz, H-4′), 7.15(1H, s, H-4), 7.32(2H, d, J = 8.7 Hz,

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H-2″, 6″), 7.67(1H, d, $\mathcal{J} = 9.9$ Hz, H-5′), 13.11(1H, br, N—H). ^{13}C NMR δ: 55.23(O*C*H3), 105.22(C4), 114.21(C3″, 5″), 120.30(C1″), 121.09(q, $^{1}\mathcal{J}_F = -269.2$ Hz, CF3), 129.92(C2″, 6″), 131.08(C5′), 131.41(C4′), 140.64(C6″), 142.26(q, $^{2}\mathcal{J}_F = 37.7$ Hz, C3), 145.64(C5), 159.90(C4″), 160.53(C3′). EI-HRMS: 336.0837, Calcd. for 336.0834.

*1-(3(2H)-pyridazinon-6-yl)-5-(4-methylphenyl)-3-trifluoromethylpyrazole (**8d**)*. Crude product washed with hot cyclohexane. Yield, 91%; mp 214.5–216.5°C; ^{1}H NMR (DMSO-d_6) δ: 2.31(3H, s, CH3), 7.08(1H, d, $\mathcal{J} = 9.9$ Hz, H-4′), 7.20(1H, s, H-4), 7.24(2H, d, $\mathcal{J} = 8.2$ Hz, H-3″, 5″), 7.28(2H, d, $\mathcal{J} = 8.2$ Hz, H-2″, 6″), 7.21(1H, d, $\mathcal{J} = 9.9$ Hz, H-5′), 13.11(1H, brs, N—H). ^{13}C NMR δ: 20.74(CH3), 105.67(C4), 121.04(q, $^{1}\mathcal{J}_F = -269.1$ Hz, CF3), 125.20(C1″), 128.36(C2″, 6″), 129.33(C3″, 5″), 131.31(C5′), 132.03(C4′), 138.99(C4″), 140.53(C6′), 142.42(q, $^{2}\mathcal{J}_F = 37.8$ Hz, C3), 145.82(C5), 159.95(C3′). EI-HRMS: 320.0887, Calcd. for 320.0885.

*1-(3(2H)-pyridazinon-6-yl)-5- (4-fluorophenyl)-3-trifluoromethylpyrazole (**8e**)*. Recrystallized from ethanol to give colorless fibers. Yield, 90%; mp 197–198°C; ^{1}H NMR (DMSO-d_6) δ: 7.20(1H, d, $\mathcal{J} = 9.9$ Hz, H-4′), 7.25(1H, s, H-4), 7.30 (2H, dd, $\mathcal{J} = 8.9$ Hz, H-3″, 5″), 7.48(2H, d, $\mathcal{J} = 8.8$, 5.4 Hz, H-2″, 6″), 7.76(1H, d, $\mathcal{J} = 9.9$ Hz, H-5′), 13.10(1H, s, N—H). ^{13}C NMR δ: 106.30(C4), 115.80(d, $^{2}\mathcal{J}_F = 22.0$ Hz, C3″, 5″), 121.00(q, $^{1}\mathcal{J}_F = -268.9$ Hz, CF3), 124.71 (d, $^{4}\mathcal{J}_F = 3.9$ Hz, C1″), 130.97(d, $^{3}\mathcal{J}_F = 8.2$ Hz, C2″, 6″), 131.19(C5′), 132.23(C4′), 140.40(C6′), 142.41 (q, $^{2}\mathcal{J}_F = 37.9$ Hz, C3), 144.69(C5), 159.86(3-CO), 162.49(d, $^{1}\mathcal{J}_F = -248.0$ Hz, C4″). Anal. Calcd. for $C_{14}H_8F_4N_4O$: C, 51.86; H, 2.49; N, 17.28. Found: C, 51.75; H, 2.64; N, 17.23%.

*1-(3(2H)-pyridazinon-6-yl)-5-(6-methoxy-2-naphthyl) 3-trifluoromethylpyrazole (**8f**)*. Crude product washed with hot cyclohexane. Yield, 93%; mp 158–159°C; ^{1}H NMR (DMSO-d_6) δ: 3.87(3H, s, OCH3), 6.70 (1H, d, $\mathcal{J} = 9.7$ Hz, H-4′), 7.19(1H, dd, $\mathcal{J} = 8.9$, 2.3 Hz, H-7″), 7.25(1H, s, H-4), 7.33(1H, s, H-5″), 7.34(1H, dd, $\mathcal{J} = 10.2$, 1.3 Hz, H-3″), 7.41(1H, d, $\mathcal{J} = 9.7$ Hz, H-5′), 7.80(2H, d, $\mathcal{J} = 8.7$ Hz, H-4″, 8″), 7.93(1H, s, H-5″), N—H was not observed. ^{13}C NMR δ: 55.27(OCH3), 105.30(C4), 105.84(C5″), 119.37 (C7″), 121.28(q, $^{1}\mathcal{J}_F = -268.5$ Hz, CF3), 123.53 (C2″), 126.07(C3″), 126.92(C1″, 4″), 127.79(C4′), 127.89(C8a″), 128.72 (C5′), 129.73(C8″), 129.89 (C1″), 134.09(C4a″), 141.02(C6′), 141.78(q, $^{2}\mathcal{J}_F = 37.6$ Hz, C3), 145.61(C5), 158.12(C6″), 164.82 (C3′). EI-HRMS: 386.0996, Calcd. for 386.0991.

Effect of compounds on human whole blood COX-1 and COX-2 activities

COX-1 assay. Fresh blood from healthy volunteers who had not taken NSAIDs in the previous week was collected into vacutainers containing no anticoagulants. Aliquots of 0.5 mL were immediately transferred into tubes containing vehicle or the test compound. Each drug was evaluated at a final 8 μM concentration in duplicate determinations. The samples were incubated at 37°C with gentle shaking for 60 min to allow the blood to clot. At the end of incubation, the reaction was stopped by submerging the tubes in a cold bath and centrifuging at 13 000 rpm for 10 min at 4 °C. Levels of TXB_2 in the serum were determined by using an enzyme immunoassay kit (Amersham, TXB_2 Biotrak Assay, #RPN 220).

COX-2 assay. The blood was collected into heparinized (20 U/mL) tubes and distributed in 0.5 aliquots in tubes containing 10 μg/mL of LPS (Sigma, St. Louis, MO, #L-2630 from E. coli serotype 0111:B4, 100 mg/mL final concentration, diluted in phosphate-buffered saline) together with vehicle or test compound at a final concentration of 8 μM. Each drug was evaluated in duplicate determinations. The samples were incubated in a bath at 37 °C for 24 h; during this time COX-2 was induced in mononuclear cells. The reaction was stopped by submerging the tubes in a cold bath and centrifuging at 13,000 rpm for 10 min at 4 °C. Levels of PGE_2 in the supernatant were determined by using an enzyme immunoassay kit (Amersham, PGE_2 Biotrak Assay, #RPN 222).

Results

The general method employed for the preparation of regioisomeric 1-(3-pyridazinyl)-5-arylpyrazole and 1-(3-pyridazinyl)-3-arylpyrazole derivatives is illustrated in Scheme 1. As a starting point, Claisen condensation of an appropriate acetophenone derivative (**1a**–**f**) with ethyl trifluoroacetate provided the expected 1,3-dicarbonyl adduct (**2a**–**f**) in good yield. As might be expected that the reaction of these unsymmetrical β-diketones with a monoaryl-substituted hydrazine derivative might lead to the formation of a mixture of pyrazole isomers, depending on the reaction conditions which affect the site of initial nucleophilic attack in a regioselective process. As seen in Scheme 1, when we condensed trifluoromethyl-substituted β-diketones (**2a**–**f**) with 3-chloro-6-hydrazinopyridazine (**3**) in refluxing ethanol without any acid or base catalyst, stable 1,3-diaryl-5-hydroxy-4,5-dihydropyrazole derivatives (**4a**–**f**) were isolated while we were expecting ring closure followed by dehydration to obtain the pyrazole. The 1,3-diaryl-5-hydroxy-4,5-dihydropyrazoles were only converted to the aromatic pyrazoles on treatment with acetic acid at elevated

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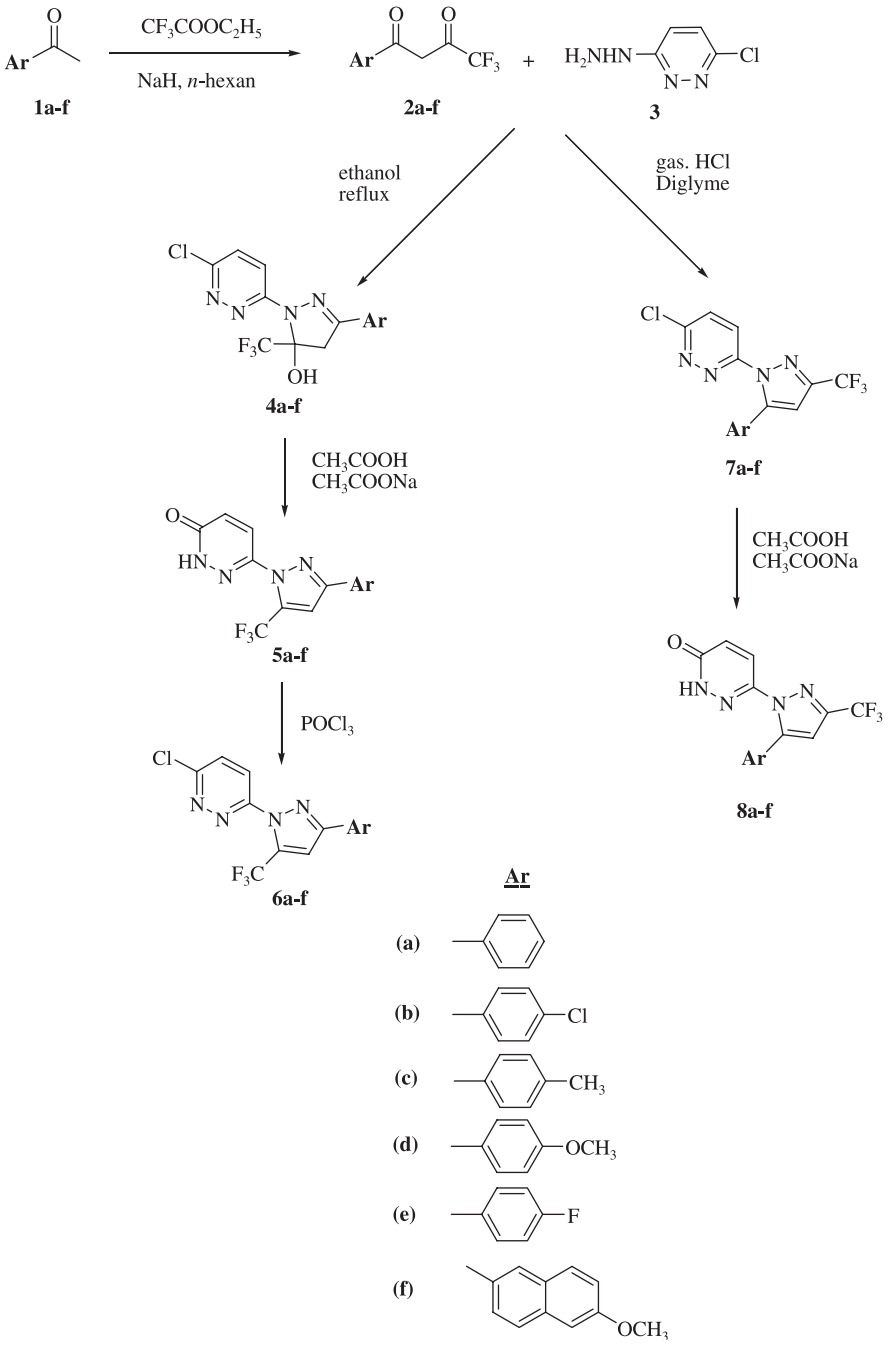

Scheme 1. The synthetic methodology used in the preparation of title compounds.

temperature and regioisomeric 1-(pyridazin-3(2H)-on-6-yl)-3-arylpyrazole derivatives (**5a**–**f**) were obtained, which were subsequently converted to 1-(6-chloro-3-pyridazinyl)-3-arylpyrazole derivatives (**6a**–**f**) by reaction with phosphorus oxychloride. The other regioisomers, 1-(3-pyridazinyl)-5-arylpyrazoles (**7a**–**f**), were generated almost exclusively by carrying out the condensation in the presence of the hydrochloride salt of the 3-chloro-6-hydrazinopyridazine in diglyme. These were subsequently converted to the corresponding 3(2H)-pyridazinone derivatives (**8a**–**f**) by refluxing in glacial acetic acid.

The structures of synthesized 1,3- and 1,5-isomers were fully elucidated using ^{1}H- and ^{13}C-NMR, DEPT and HMBC experiments. To avoid repetition in describing the structural verification between the 1,3- and 1,5-regioisomers, only the structure elucidations of **6e** and **7e** as representative examples will be discussed here.

For both compounds **6e** and **7e**, elemental analysis and mass spectral data (m/z 342 M^{+}) established their molecular formula as $C_{14}H_7ClF_4N_4$, suggesting that they are possibly the 1,3- and 1,5-diarypyrazole regioisomers. The summary of spectral properties

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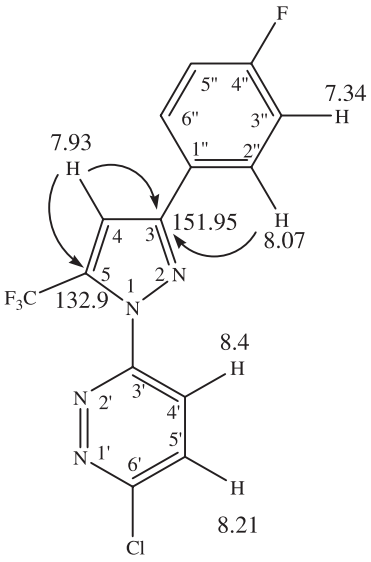
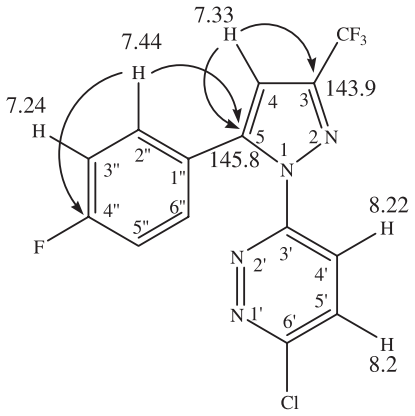


6e



7e

Observed HMBC Correlations	
^{1}H	**^{13}C**
H4 ($^{2}J_{CH}$)	C3, C5
H4' ($^{3}J_{CH}$,$^{2}J_{CH}$)	C6', C5'
H5' ($^{3}J_{CH}$,$^{2}J_{CH}$)	C3', C4'
H2'',6'' ($^{3}J_{CH}$)	C3, C4''
H3'',5'' ($^{3}J_{CH}$,$^{2}J_{CH}$)	C1'', C4''

Observed HMBC Correlations	
^{1}H	**^{13}C**
H4 ($^{2}J_{CH}$)	C3, C5
H4' ($^{3}J_{CH}$)	C6'
H5' ($^{3}J_{CH}$)	C3'
H2'',6'' ($^{3}J_{CH}$)	C5, C4''
H3'',5'' ($^{3}J_{CH}$,$^{2}J_{CH}$)	C1'', C4''

Figure 1. Summary of spectral differences between compound **6e** and **7e**.

of the two regioisomeric structures is shown in Figure 1. First of all, the ^{1}H-NMR spectrum of compound **6e** indicated very simple analysis of their structures showing a singlet proton signal at δ 7.93 (H-4) and two sets of aromatic proton signals attached to the phenyl and pyridazine rings, respectively. ^{13}C-NMR and its DEPT experiment indicated the presence of a quaternary sp^{2} carbon signal at 151.95 ppm which showed a remarkable $^{2}\mathcal{J}$-correlation with two proton signals at δ 7.93 ppm (s, pyrazole H-4) and δ 8.07 ppm (dd, H-2″, 6″). Moreover, two quartet C signals at δ 119.4 ppm (CF_3, $\mathcal{J} = 269$ Hz) and δ 132.9 ppm (pyrazole C-5, $\mathcal{J} = 40$ Hz) showing $\mathcal{J}_{CH}$ long-range correlation with the proton signal at δ 7.93 ppm (pyrazole-H4) were observed. All of the obtained correlations unambiguously confirmed that the ^{13}C signal at δ 151.95 ppm belongs to the imino carbon (C-3) indicating that ring closure had occurred to put the phenyl substituent at 3-position of the central pyrazole. In the case of the 1,5-isomer (**7e**), the olefinic carbon signal (C-5) was observed at about δ 145.8 ppm as a singlet, and C-3 was observed at 143.9 as a quartet ($\mathcal{J} = 38.3$ Hz). In conclusion, the structures of compound **6e** and **7e** were unambiguously elucidated as 1,3- and 1,5-diaryl substituted regioisomers, respectively, as shown in Figure 1.

After comparing the physical and spectral data of the 1,3- and 1,5-regioisomers, it was discovered that melting points of all the 1,5-regioisomers (i.e. **7e**, mp 136°C) were relatively lower than those of the corresponding 1,3-regioisomers (i.e. **6e**, mp 176°C), and that the similar characteristic spectral patterns were observed for the same type of regioisomers. Firstly, the ^{1}H-NMR spectra of all the 1,5-regioisomers showed an overlapping couple of doublet signals at about 8.2 and 8.22 ppm belonging to pyridazine-H4′ and H5′ protons, respectively. In contrast, these proton peaks were observed as clearly separated two doublets at about 8.4 ppm (H4′) and 8.2 ppm (H5′) in the 1,3-regioisomers indicating a chemical shift of pyridazine-H4′ to lower field. Secondly, in the ^{1}H NMR spectra of all the 1,3-regioisomers, the signal of phenyl-H2″ and 6″ appeared at low field (8.07 ppm in **6e**) as compared to the signal of these phenyl protons in the 1,5-regioisomer which occurred at higher field (7.44 ppm in **7e**). Therefore, we can indicate another slight distinction between 1,3- and 1,5-regioisomers in the aromatic region indicating that any phenyl proton signals lower than 7.5 ppm did not appear in the 1,5-type derivatives, which were characteristic low field phenyl proton signals corresponding to the 1,3-type (Figure 1). It is worthwhile mentioning that our

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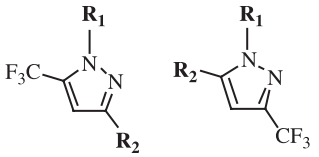
Table I. % Inhibition of compounds against COX-1 and COX-2 at 8 μM concentration



5a-f, 6a-f **7a-f, 8a-f**

Compound	R_1	R_2	COX-1 inh. (%)	COX-2 inh. (%)	Selectivity ratio COX-2/COX-1
5a	3(2H)-pyridazinone	ph	64	48	0.75
5b	3(2H)-pyridazinone	4-Cl—ph	48	28	0.58
5c	3(2H)-pyridazinone	4-CH_3—ph	65	22	0.34
5d	3(2H)-pyridazinone	4-OCH_3—ph	62	54	0.87
5e	3(2H)-pyridazinone	4-F—ph	73	58	0.79
5f	3(2H)-pyridazinone	6-OCH_3-naphthyl	47	65	1.38
6a	6-Cl-pyridazin	ph	78	44	0.56
6b	6-Cl-pyridazin	4-Cl—ph	60	58	0.97
6c	6-Cl-pyridazin	4-CH_3—ph	48	56	1.17
6d	6-Cl-pyridazin	4-OCH_3—ph	61	48	0.79
6e	6-Cl-pyridazin	4-F—ph	74	56	0.76
6f	6-Cl-pyridazin	6-OCH_3-naphthyl	74	58	0.78
7a	6-Cl-pyridazin	Ph	100	58	0.58
7b	6-Cl-pyridazin	4-Cl—ph	100	56	0.56
7c	6-Cl-pyridazin	4-CH_3—ph	100	78	0.78
7d	6-Cl-pyridazin	4-OCH_3—ph	100	76	0.76
7e	6-Cl-pyridazin	4-F—ph	100	72	0.72
7f	6-Cl-pyridazin	6-OCH_3-naphthyl	70	67	0.96
8a	3(2H)-pyridazinone	ph	68	63	0.93
8b	3(2H)-pyridazinone	4-Cl—ph	82	60	0.73
8c	3(2H)-pyridazinone	4-CH_3—ph	97	56	0.58
8d	3(2H)-pyridazinone	4-OCH_3—ph	93	65	0.70
8e	3(2H)-pyridazinone	4-F—ph	77	33	0.43
8f	3(2H)-pyridazinone	6-OCH_3-naphthyl	40	52	1.30
DuP-697			NT	100	
SC-650			100	NT	

NT = not tested.

observations of the difference in mp and NMR spectra between the above regioisomers can be used as a valuable reference when dealing with structure verification of related compounds.

The title compounds at a concentration of 8 μM were tested for their inhibitory potency against COX-1 and COX-2 in a human whole blood assay which is considered to be the more biologically relevant way to assess the inhibition of the cyclooxygenase isoenzymes, COX-1 and COX-2, by a test compound [32,33]. The results of the % inhibitory activity on COX-1 and COX-2 enzymes are shown in Table I.

Discussion

The *in vitro* potencies of regioisomeric 1-pyridazinyl-3(5)-aryl-5(3)-trifuoromethylpyrazoles are shown in Table I. The compounds illustrated here were tested at an initial dose of 8 μM for preliminary screening of inhibitory potency. As is evident from Table I, all compounds with both a 1,3- and 1,5-diarylsubstitution pattern did not show an appreciable selectivity on the inhibition of COX enzymes as expected. The inhibitory activities at this high dose level were not very marked except for compounds **7a**–**f**, **8c** and **8d**, which showed comparable activity to the reference compound SC-560, giving 100% inhibition toward COX-1, but with low selectivity (selectivity ratios of 0.58–0.96). Most of the compounds showed preference for the COX-1 isoform to some extent, and this was particularly noted when the phenyl and pyridazine groups were adjacent to each other about a central pyrazole template as in compounds **7a**–**f** (1,5-diaryl substitution pattern). The replacement of pyridazine with pyridazinone in this particular series resulted in compounds (**8a**–**f**) with reduced inhibitory potency and selectivity against COX-1. As also seen from Table I, a range of substituents on the phenyl is tolerated to the same extent and did not contribute to the selectivity as much at this high test concentration. The replacement of the phenyl substituent with a larger naphthalene moiety usually caused a small decrease in the inhibitory potency in the series.

In conclusion, the results of our preliminary screening of the novel compounds incorporating the

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pyridazine nucleus as one of the aryl substituents about the central pyrazole ring showed promising leads for developing newer COX inhibitors. Although the spatial orientation of the two aromatic rings of the tricyclic inhibitor class (coxibs) was known to be critical for COX inhibition, our initial results indicated that the synthesized 1-pyridazinyl-3-phenyl-5-trifuoromethylpyrazoles, showing the 1,3-diarylsubstitution pattern, also resulted in inhibition of both COX isoforms, although the inhibitory potency was not very pronounced at the 8 μM screening dose used.

In addition, the possibility of easy functionalization(s) at various ring positions of pyridazinones, the electron withdrawing ability of the pyridazinone moiety, their stability and moderate solubility in organic solvents make this ring system interesting for synthetic applications to obtain biologically active distinct derivatives [34]. Further studies for developing better candidates are under ongoing investigation in our laboratory.

Acknowledgements

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