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Synthesis and Anti-inflammatory Activity of Some New Thiadiazole Linked Pyrazole Benzene Sulphonamides as Cyclooxygenase Inhibitors

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ABSTRACT

A new series of thiadiazole linked pyrazole benzenesulfonamide derivatives were synthesized by the condensation of aldehydic pyrazole with aryl substituted thiadiazole amine followed by Schiff base reaction. The synthesized compounds (**6a-o**) were characterized by IR, NMR, and Mass spectral data, further evaluated their *in-vivo* anti-inflammatory, analgesic and *in-vitro* COX-II inhibition assay. The compounds **6b** and **6m** showed most significant *in-vivo* anti-inflammatory with 72.33 &71.17% inhibition along analgesic activity having 67.89% and 71.37 % respectively. Their selectivity against COX-II enzyme with selectivity index 67.81 and 66.38 was established for **6b** and **6m,** which is compared with Celecoxib. During the gastric ulceration study, selected compounds couldn't observed any ulcerogenic effect on gastric mucosa.The *in-silico* pharmacokinetic profile and molecular docking study exposed very good binding affinity towards the Cyclooxygenase (COX-II) enzyme (PDB Id: 3PGH), therefore the compounds **6b** and **6m** are used as promising lead candidates for the support of drug development.

Key words: Benzenesulfonamide, Cyclooxygenase, Prostanoids, Traumatic infections.

INTRODUCTION

Tissues in response to traumatic infections, post-ischaemia, toxicity and autoimmune injury cause inflammation and pain. The pathophysiological conditions such as evolution of persistent tissue damage of leucocytes, lymphocytes and collagen, the defensive process normally lead to recovery from noxious stimulus[1]. According to WHO report approximately 90% of the illness are associated with inflammation and pain[2]. The inflammatory mediators of eicosanoids are activated by the nociceptors which leads to hyperalgesia[3]. The key focus of medicine is to reduce the pain and classical inflammation such as fever, redness and swelling, which are mediated by pro-inflammatory eicosanoids[4]. During eicosanoids pathway the cellular enzymatic activity of arachidonic acid

produces the prostanoids(a pain inducer) which is initiated by cyclooxygenase[5]. Cyclooxygenase (EC 1.14.99.1) enzyme is an inter-convertible form of Cyclooxygenases I & II (COX-I, COX-II), both of this enzyme hassimilar molecular weight, approximately 70 and 72 kDa.The COX-I is responsible in physiological function and COX-IIis responsible for inflammation and pain[6]. For the treatment of pain and inflammation in rheumatoid arthritis and inflammatory diseases have been the most widely used are non-steroidal anti-inflammatory drugs (NSAIDs)[7].Theconventional and most frequent use of NSAIDs causes severe side effects such as irritation of the gastric mucosa and damage to gastrointestinal tract. This new era of research, our main aim is to investigate a newer and safe anti-inflammatory agents. The COX-II inhibitors are a main target for management of pain and inflammation[8].Some of the marketed drug such as celecoxib and etoricoxib are very selective COX-II inhibitors which showed little gastric irritation and also decreases the risk of peptic ulceration. But still some of the adverse effects of selective COX-II inhibitors are observed during clinical study[9]. Hence our aim is to find a new safer and potent compound having as analgesic and anti-inflammatory as selective COX-II inhibitors along with less gastric toxicities.

The thiadiazole and pyrazole derivatives represent a class of organic compounds of great importance in biological chemistry. For instance, thiadiazole and pyrazole derivatives shows anticancer[10], antibacterial[11], antiviral[12], antitubercular[13], antifungal[14], anti-inflammatory[15], antiprotozoal[16], Cardioprotective[17], antidepressant properties[18], analgesic activities[19].Searching these compounds we have found thiadiazole linked pyrazole benzene sulphonamides are one of the moieties on which studied have been focused (Figure-1). In our laboratory, we have designed (Figure-2) and synthesized some 4-(5-chloro-3-methyl-4-(((5-phenyl-1,3,4-thiadiazol-2-yl)imino)methyl)-1H-pyrazol-1-yl)benzenesulfonamide derivatives (**6a-o**)in search for new compound with expected biological activities. We hereby report the thiadiazole linked pyrazole benzene sulphonamides and their characterization by IR, NMR & Mass spectrometry techniques. Newly synthesized compounds were also screened for their anti-inflammatory, Analgesic, Ulcerogenic and in-vitro COX-II inhibitory assay.

MATERIALS AND METHOD

Chemistry

Melting points were determined by the open capillary method with electrical melting point apparatus and are uncorrected. IR spectra were recorded as KBr (pellet) on Bio Rad FT-IR spectrophotometer and ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker DPX 300 MHz spectrophotometer using DMSO-d_6 or $CDCl_3$ as NMR solvent. Mass spectra were recorded on JEOL SX102/DA-6000 mass spectrometer using *m*-nitrobenzylalcohol as a matrix and elemental analysis on Vario-EL III CHNOS-Elemental analyzer. Thin Layer Chromatography (TLC) was performed to monitor progress of the reaction and purity of the compounds, the spot being located under iodine vapours or UV-light.

Synthesis of 4-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide (3)

3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**3**) was synthesized by heating equimolar mixture of 4-hydrazinylbenzenesulfonamide (**1**) and ethyl acetoacetate (**2**) at 110-120 °C for 4 h. The reaction mixture was cooled and ether (20 ml) is added to it. The mixture was stirred to give a solid product. It was filtered, washed with ether and recrystallized from ethanol to give the desired product as a pale yellow crystalline compound[20]. Yield 75%, mp 122-124 °C

Synthesis of 4-(5-chloro-4-formyl-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (4)

Compound (**3)** in 100ml round bottom flask were dissolved in dry DMF and it was cooled to 0°C and treated dropwise with phosphorous oxychloride ($POCl_3$), maintaining the temperature between 10-15 °C. The reaction mixture was heated on a steam bath for 1 h, cooled and poured into crushed ice with stirring. The separated product was filtered and washed with water to obtained 4-(5-chloro-4-formyl-3-methyl-1H-pyrazol-1-yl) benzenesulfonamide(**4**). It was recrystallized from ethanol and obtained as yellow needles[20]. Yield 74%, mp 147°C;IR (KBr) cm^{-1}: 3167 (SO_2NH_2), 3079(C-H of CHO), 1667 (C=O), 1595 (C=N), 1327, 1159 (SO_2), 1012 (C-N).^{1}H-NMR

(600 MHz, $CDCl_3$): δ 2.53 (s, 3H, Me),6.73 (bs, 2H, SO_2NH_2 , D_2O exchangeable), 7.53 (d, 2H, Ph H-2,6, *J*=7.4 Hz), 7.51 (d, 2H, Ph H-3,5, *J*=7.8), 10.32 (s, 1H, CHO);^{13}C-NMR (75 MHz, $CDCl_3$): δ183.81 (CHO), 151.7 (C-3), 136.9 (Ph C-1), 133.4 (C-5), 129.2 (Ph C-3,5), 129.1 (Ph C-4), 125.1 (Ph C- 2,6), 117.3 (C-4,CHO), 13.8 (Me);ESI-MS: m/z 301.08, (M+2).Anal. calcd for $C_{11}H_{10}ClN_3O_3S$:C, 44.08; H, 3.36; N, 14.04;Found: C, 44.25; H, 3.26; N, 14.78;%. Substituted-5-phenyl-1, 3, 4-thiadiazol-2-amine **(5)** was synthesized by according to reported literature[21].Equimolar mixture of substituted benzoic acid and semicarbazide in 100ml round bottom flask, $POCl_3$ (13 ml) were added to it and heated at 75 ^{0}C for half an hour. After cooling down to room temperature then add water. The reaction mixture was reflux for 4 hr, after cooling the mixture was basified to PH-8 by drop wise addition of 50 % NaOH solution under the stirring. The precipitate was filtered and recrystallized from ethanol to obtained pure yield of compounds. **5-phenyl-1,3,4-thiadiazol-2-amine(5a);**Yield: 61%; m.p.: 222-224°C; IR (KBr) cm^{-1}: 3283, 3117 (NH_2), 1642 (C=N), 696 (C=S); ^{1}H-NMR (300 MHz, DMSO-d_6); δ 7.42 (s, 2H), 7.47-7.52 (dd, *J*= 6.4 Hz, *J*=14.00 Hz, 2H), 7.83 (d, 2H), ESI-MS: m/z [M+H] 178.31; Anal. calcd for $C_8H_7N_3S$: C, 54.22; H, 3.98; N, 23.71; Found: C, 54.25; H, 3.97; N, 23.73; %.

Synthesis of (E)-4-(5-chloro-3-methyl-4-(((5-substituted-phenyl-1,3,4-thiadiazol-2-yl)methylene)amino)-1H-pyrazol-1-yl)benzenesulfonamide derivatives (6a-6o)

In a 100ml of round-bottom flask, a equimolar mixture compound **(4)** were dissolve in 30 ml of absolute ethanol, catalytic amount of Glacial acidic acid (0.3ml) added to it. Equimolar amount of compound **(5)** was added and the mixture was refluxed for 6 hr. The reaction was monitored by TLC until the disappearance of starting materials, precipitates come out and filtered, washed with Ethanol, dried and recrystallized from ethanol to obtained white solid product. Progress of the reaction was checked by TLC using ethyl acetate: hexane (8:2) as solvent system.

(E)-4-(5-chloro-3-methyl-4-(((5-phenyl-1,3,4-thiadiazol-2-yl)methylene)amino)-1H-pyrazol-1-yl)benzenesulfonamide (6a)

Yield: 58%; m.p.: 174-176°C; IR (KBr) cm^{-1}: 3373(SO_2NH_2), 1634 (C=N), 1550 (C=C), 1343(SO_2NH_2), 1012 (C-N).^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.39 (s, 3H, Pyrazole-CH_3), 3.43 (bs, 2H, SO_2NH_2 , D_2O exchangeable),4.61 (bs, H, HC=N), 7.33 (s, 1H, Ar-H), 7.57(d, 2H, Ar-H *J* = 7.5 Hz), 7.77 (d, 2H, Ar-H J = 7.1 Hz),7.93 (d, 2H, Ar-H *J* = 7.6 Hz), 8.11 (d, 2H, Ar-H *J* = 7.8 Hz).^{13}C-NMR (75 MHz, DMSO-d_6); δ 14.39, 114.23, 123.27, 128.11, 130.16, 135.72, 141.51, 150.09(C-Pyrazolo), 161.91(HC=N), 173.34(C-thiadiazole). ESI-MS: m/z 460.13, (M+2).Anal. calcd for $C_{19}H_{15}ClN_6O_2S_2$:C, 49.72; H, 3.29; N, 18.31; Found: C, 49.77; H, 3.28; N, 18.32; %.

(E)-4-(5-chloro-4-(((5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl)methylene)amino)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6b)

Yield: 63%; m.p.: 176-178°C; IR (KBr) cm^{-1}: 3376($SO_2\underline{NH_2}$), 3154 (N-H), 1638 (C=N), 1555 (C=C), 1346($\underline{SO_2}NH_2$) 1014 (C-N), 754(C-Cl).^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.42 (s, 3H, Pyrazole-CH_3), 3.45 (bs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 4.65 (bs, H, HC=N), 7.33 (s, 1H, Ar-H), 7.57(d, 1H, Ar-H *J* = 7.5 Hz), 7.77 (d, 2H, Ar-H *J* = 7.1 Hz),7.96 (d, 2H, Ar-H *J* = 7.6 Hz), 8.03 (d, 2H, Ar-H *J* = 7.8 Hz).^{13}C-NMR (75 MHz, DMSO-d_6); δ 14.41, 114.25, 123.29, 128.08, 130.18, 135.71, 141.53, 150.12(C-Pyrazolo), 161.94(HC=N), 173.30(C-thiadiazole). ESI-MS: m/z 494.13, (M+2).Anal. calcd for $C_{19}H_{14}Cl_2N_6O_2S_2$: C, 46.25; H, 2.86; N, 17.03; Found: C, 46.39; H, 2.78; N, 17.11; %.

(E)-4-(5-chloro-4-(((5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6c)

Yield: 65%; m.p.: 167-169°C; IR (KBr) cm^{-1}: 3375 ($SO_2\underline{NH_2}$), 3152 (N-H), 1633 (C=N), 1556 (C=C), 1344 ($\underline{SO_2}NH_2$), 1010 (C-N), 750 (C-Cl).^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.41 (s, 3H, Pyrazole-CH_3), 3.48 (bs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 4.63 (bs, H, HC=N), 7.54 (d, 2H, Ar-H, *J* = 7.3 Hz), 7.79 (d, 2H, Ar-H *J* = 7.2 Hz), 7.90 (d, 2H, Ar-H *J* = 7.6 Hz), 8.03 (d, 2H, Ar-H *J* = 7.8 Hz). ^{13}C-NMR (75 MHz, DMSO-d_6); δ 14.43, 114.24, 123.32, 128.07, 130.17, 135.74, 141.51, 150.14 (C-Pyrazolo), 161.93 (HC=N), 173.31(C-thiadiazole). ESI-MS: m/z 494.19, (M+2). Anal. calcd for$C_{19}H_{14}Cl_2N_6O_2S_2$: C, 46.25; H, 2.86; N, 17.03; Found: C, 46.37; H, 2.77 ; N, 17.12; %.

(E)-4-(5-chloro-4-(((5-(2,4-dichlorophenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6d)

Yield: 68%; m.p.: 174-176°C; IR (KBr) cm^{-1}: 3373 ($SO_2\underline{NH_2}$), 3151 (N-H), 1628 (C=N), 1553 (C=C), 1343 ($\underline{SO_2}NH_2$), 1015 (C-N), 767 (C-Cl). ^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.48 (s, 3H, Pyrazole-CH_3), 3.43 (bs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 4.60 (bs, H, HC=N), 7.54 (d, H, Ar-H, *J* =6.8 Hz), 7.61 (d, H, Ar-H), 7.79 (d, H, Ar-H *J* = 7.2 Hz), 7.90 (d, 2H, Ar-H *J* = 7.6 Hz), 8.03 (d, 2H, Ar-H *J* = 7.8 Hz).^{13}C-NMR (75 MHz, DMSO-d_6); δ 14.42, 114.27, 123.30, 128.11, 130.14, 135.71, 141.49, 150.11 (C-Pyrazolo), 161.89 (HC=N), 173.32 (C-thiadiazole). ESI-MS: m/z 525.39, [M-2]. Anal. calcd for $C_{19}H_{13}Cl_3N_6O_2S_2$: C, 43.23; H, 2.48; N, 15.92; Found: C, 43.33; H, 2.43; N, 15.86; %.

(E)-4-(4-(((5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)-5-chloro-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide(6e)

Yield: 62%; m.p.: 178-179°C; IR (KBr) cm^{-1}: 3374 ($SO_2\underline{NH_2}$), 3147 (N-H), 1626 (C=N), 1547 (C=C), 1347 ($\underline{SO_2}NH_2$), 1016 (C-N), 821 (C-Br).^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.45 (s, 3H, Pyrazole-CH_3), 3.43 (bs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 4.67 (bs, H, HC=N), 7.54 (d, 2H, Ar-H, *J* = 7.3 Hz), 7.79 (d, 2H, Ar-H *J* = 7.2 Hz), 7.90 (d, 2H, Ar-H *J* = 7.6 Hz), 8.03 (d, 2H, Ar-H *J* = 7.8 Hz).^{13}C-NMR (75 MHz, DMSO-d_6); δ 14.45, 114.22, 123.29, 128.06, 130.12, 135.71, 141.49, 150.11 (C-Pyrazolo), 161.96 (HC=N), 173.37(C-thiadiazole). ESI-MS: m/z 537.71, (M+2). Anal. Calcd for $C_{19}H_{14}BrClN_6O_2S_2$: C, 42.43; H, 2.62; N, 15.63; Found: C, 42.39; H, 2.67; N, 15.72; %.

(E)-4-(5-chloro-3-methyl-4-(((5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)-1H-pyrazol-1-yl)benzenesulfonamide (6f)

Yield: 74%; m.p.: 169-171°C; IR (KBr) cm^{-1}: 3346 ($SO_2\underline{NH_2}$), 33247 (N-H), 1632 (C=N), 1579 (C=C), 1538 (N=O), 1332 ($\underline{SO_2}NH_2$), 1013 (C-N).^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.44 (s, 3H, Pyrazole-CH_3), 3.57 (bs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 4.76 (bs, H, HC=N), 7.81 (d, 2H, Ar-H, *J* = 7.3 Hz), 7.93 (d, 2H, Ar-H *J* = 7.2 Hz), 8.07 (d, 2H, Ar-H *J* = 7.6 Hz), 8.30 (d, 2H, Ar-H *J* = 7.8 Hz).^{13}C-NMR (75 MHz, DMSO-d_6); δ 14.53, 114.21, 123.39, 128.05, 130.11, 135.61, 141.69, 150.09 (C-Pyrazolo), 161.89 (HC=N), 173.35 (C-thiadiazole). ESI-MS: m/z 503.49, (M+). Anal. Calcd for $C_{19}H_{14}ClN_7O_2S_2$: C, 45.28; H, 2.80; N, 19.46; Found: C, 45.33; H, 2.75; N, 19.52; %.

(E)-4-(5-chloro-4-(2-(5-(2, 4-dinitrophenyl)-1, 3, 4-thiadiazol-2-yl) vinyl)-3-methyl-1H-pyrazol-1-yl) benzenesulfonamide (6g)

Yield: 73%; m. p.: 180-182°C; IR (KBr) cm^{-1}: 3343 ($SO_2\underline{NH_2}$), 3168 (N-H), 1633 (C=N), 1540 (C=C), 1542 (N=O), 1331 ($\underline{SO_2}NH_2$), 1012 (C-N).^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.48 (s, 3H, Pyrazole-CH_3), 3.54 (bs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 4.73 (bs, H, HC=N), 7.79 (d, 2H, Ar-H, *J* = 7.3 Hz), 7.87 (d, 2H, Ar-H *J* = 7.2 Hz), 8.31 (d, H, Ar-H), 8.69 (d, 2H, Ar-H);Anal. Calcd for $C_{19}H_{13}ClN_8O_6S_2$: C, 41.57; H, 2.39; N, 20.41; Found: C, 41.48; H, 2.37; N, 20.49; %.

(E)-4-(5-chloro-3-methyl-4-(((5-(o-tolyl)-1,3,4-thiadiazol-2-yl)imino)methyl)-1H-pyrazol-1-yl)benzenesulfonamide (6h)

Yield: 70%; m.p.: 175-177°C; IR (KBr) cm^{-1}: 3375 ($SO_2\underline{NH_2}$), 3158 (N-H), 1632 (C=N), 1547 (C=C), 1347 ($\underline{SO_2}NH_2$), 1013 (C-N). ^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.58 (s, 3H, Phenyl-CH_3), 2.69 (s, 3H, Pyrazole-CH_3), 3.58 (bs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 4.54 (bs, H, HC=N), 7.33 (s, 1H, Ar-H), 7.57(d, 2H, Ar-H *J* = 7.5 Hz), 7.77 (d, H, Ar-H *J* = 7.1 Hz),7.93 (d, 2H, Ar-H *J* = 7.6 Hz), 8.11 (d, 2H, Ar-H *J* = 7.8 Hz).^{13}C-NMR (75 MHz, DMSO-d_6); δ 13.39, 18.64, 114.43, 123.28, 128.12, 130.18, 135.70, 141.56, 150.08(C-Pyrazolo), 161.93(HC=N), 173.32 (C-thiadiazole). Anal. calcd for $C_{20}H_{17}ClN_6O_2S_2$: C, 50.79; H, 3.62; N, 17.77; Found: C, 50.83; H, 3.57; N, 17.85; %

(E)-4-(5-chloro-3-methyl-4-(((5-(p-tolyl)-1,3,4-thiadiazol-2-yl)imino)methyl)-1H-pyrazol-1-yl)benzenesulfonamide (6i)

Yield: 76%; m. p.: 173-175°C; IR (KBr) cm^{-1}: 3371($SO_2\underline{NH_2}$), 3169 (N-H), 1635 (C=N), 1551 (C=C), 1342 ($\underline{SO_2}NH_2$), 1011 (C-N).^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.35 (s, 3H, Phenyl-CH_3), 2.46 (s, 3H, Pyrazole-CH_3), 3.45 (bs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable),4.60 (bs, H, HC=N), 7.33 (s, 2H, Ar-H), 7.57(d, 2H, Ar-H *J* = 7.5 Hz), 7.77 (d, 2H, Ar-H *J* = 7.1 Hz),7.93 (d, 2H, Ar-H *J* = 7.6 Hz).Anal. calcd for $C_{20}H_{17}ClN_6O_2S_2$: C, 50.79; H, 3.62; N, 17.77; Found: C, 50.83; H, 3.57; N, 17.85; %.

(E)-4-(5-chloro-4-(((5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6j)

Yield: 78%; m. p.: 167-169°C; IR (KBr) cm^{-1}: 3368 ($SO_2\underline{NH_2}$), 3180 (N-H), 1632 (C=N), 1530 (C=C), 1358 ($\underline{SO_2}NH_2$), 1546 (C-O-C), 1016 (C-N). ^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.44 (s, 3H, Pyrazole-CH_3), 3.81 (s, 3H, Phenyl-OCH_3), 3.38 (bs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 4.52 (bs, H, HC=N), 7.33 (s, 2H, Ar-H), 7.57(d, 2H, Ar-H *J* = 7.5 Hz), 7.77 (d, 2H, Ar-H *J* = 7.1 Hz),7.93 (d, 2H, Ar-H *J* = 7.6 Hz).Anal. calcd for $C_{20}H_{17}ClN_6O_3S_2$: C, 49.13; H, 3.50; N, 17.19; Found: C, 49.17; H, 3.43; N, 17.26 ; %.

(E)-4-(5-chloro-4-(((5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazol-2-yl)methylene)amino)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6k):

Yield: 74%; m. p.: 182-184°C; IR (KBr) cm^{-1}: 3378 ($SO_2\underline{NH_2}$), 3184 (N-H), 1636 (C=N), 1534 (C=C), 1354 ($\underline{SO_2}NH_2$), 1254 (C-O-C), 1015 (C-N).^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.45 (s, 3H, Pyrazole-CH_3), 3.73 (s, 6H, Phenyl- 2×OCH_3), 3.65 (bs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 4.57 (bs, H, HC=N), 7.33 (s, 2H, Ar-H), 7.57(d, 1H, Ar-H *J* = 7.5 Hz), 7.77 (d, 2H, Ar-H *J* = 7.1 Hz),7.93 (d, 2H, Ar-H *J* = 7.6 Hz).^{13}C-NMR (75 MHz, DMSO-d_6); δ 13.45, 56.87, 111.03, 112.71, 114.73, 120.60, 123.82, 126.16, 127.65, 133.61, 140.43, 149.12, 150.40, 160.04 (C=O), 174.81 (Aromatic NH-C=O); ESI-MS: m/z 504.13, [M+2]; Anal. calcd for $C_{21}H_{19}ClN_6O_4S_2$: C, 48.60; H, 3.69; N, 16.19; Found: C, 48.73; H, 3.53; N, 16.26; %.

(E)-4-(5-chloro-4-(((5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6l):

Yield: 77%; m.p.: 169-171°C; IR (KBr) cm^{-1}: 3373 ($SO_2\underline{NH_2}$), 3179 (N-H), 3430(O-H), 1631 (C=N), 1538 (C=C), 1357 ($\underline{SO_2}NH_2$), 1014 (C-N). ^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.42 (s, 3H, Pyrazole-CH_3), 3.55 (bs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 4.68 (bs, H, HC=N), 5.39 (s,1H, OH), 7.57(d, 2H, Ar-H *J* = 7.5 Hz), 7.77 (d, 2H, Ar-H *J* = 7.1 Hz),7.93 (d, 2H, Ar-H *J* = 7.6 Hz), 8.11 (d, 2H, Ar-H *J* = 7.8 Hz).^{13}C-NMR (75 MHz, DMSO-d_6); δ 13.23, 114.12, 116.04, 123.27, 126.34, 128.82, 133.13, 140.32, 149.45, 158.03 (C-Pyrazolo), 160.23 (HC=N), 174.32 (C-thiadiazole). Anal. calcd for $C_{19}H_{15}ClN_6O_3S_2$: C, 48.05; H, 3.18; N, 17.69; Found C, 48.24; H, 3.12; N, 17.72; % .

(E)-4-(5-chloro-4-(((5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6m):

Yield: 73%; m.p.: 186-188°C; IR (KBr) cm^{-1}: 3372 ($SO_2\underline{NH_2}$), 3174 (N-H), 3435(O-H), 1645 (C=N), 1533 (C=C), 1356 ($\underline{SO_2}NH_2$), 1017 (C-N). ^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.47 (s, 3H, Pyrazole-CH_3), 3.53 (bs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 4.63 (bs, H, HC=N), 5.33 (s,2H, 2×OH), 6.37 (s, H, Ar-H), 6.57(d, H, Ar-H *J* = 7.5 Hz), 7.48 (d, H, Ar-H *J* = 7.1 Hz),7.93 (d, 2H, Ar-H *J* = 7.6 Hz), 8.11 (d, 2H, Ar-H *J* = 7.8 Hz). Anal. calcd for $C_{19}H_{15}ClN_6O_4S_2$: C, 46.48; H, 3.08; N, 17.12; Found C, 46.48; H, 3.08; N, 17.12; %

(E)-4-(4-(((5-(2-amino-6-hydroxyphenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)-5-chloro-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6n):

Yield: 62 %; m. p.: 178-180°C; IR (KBr) cm^{-1}: 3372 ($SO_2\underline{NH_2}$), 3172 (N-H), 3433(O-H), 1629 (C=N), 1534 (C=C), 1359 ($\underline{SO_2}NH_2$), 1019 (C-N). ^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.43 (s, 3H, Pyrazole-CH_3), 3.54 (bs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 4.72 (bs, H, HC=N), 5.39 (s,1H, OH), 6.28 (s,2H, Ar-N-H), 6.33 (s, 1H, Ar-H), 6.46(d, 2H, Ar-H *J* = 7.5 Hz), 7.01 (d, 1H, Ar-H *J* = 7.1 Hz),7.93 (d, 2H, Ar-H *J* = 7.6 Hz), 8.11 (d, 2H, Ar-H *J* = 7.8 Hz).^{13}C-NMR (75 MHz, DMSO-d_6); δ 13.70, 107.12, 109.3, 110.03, 114.34, 123.02, 127.54, 130.23, 133.03, 140.42, 146.08, 149.20, 156.03 (C-Pyrazolo), 160.25 (HC=N), 174.34 (C-thiadiazole). Anal. calcd for $C_{19}H_{16}ClN_7O_3S_2$: C, 48.58; H, 3.29; N, 20.01; Found C, 48.38; H, 3.26; N, 20.29 % .

(E)-4-(4-(((5-(4-aminophenyl)-1,3,4-thiadiazol-2-yl)imino)methyl)-5-chloro-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6o):

Yield: 75%; m. p.: 163-165°C; IR (KBr) cm^{-1}: 3371 ($SO_2\underline{NH_2}$), 3173 (N-H), 1648 (C=N), 1535 (C=C), 1354 ($\underline{SO_2}NH_2$), 1013 (C-N). ^{1}H-NMR (300 MHz, DMSO-d_6); δ 2.43 (s, 3H, CH_3), 3.52 (bs, 2H, $SO_2\underline{NH_2}$, D_2O exchangeable), 4.46 (bs, H, HC=N), 6.23 (s,2H, NH), 6.67 (s, 2H, Ar-H), 7.57(d, 2H, Ar-H *J* = 7.5 Hz), 7.93 (d, 2H, Ar-H *J* = 7.6 Hz), 8.11 (d, 2H, Ar-H *J* = 7.8 Hz). Anal. calcd for $C_{19}H_{16}ClN_7O_2S_2$: C, 48.15; H, 3.40; N, 20.69; Found C, 48.12; H, 3.44; N, 20.78; %

Pharmacology

Anti-inflammatory activity

Carrageenan-induced rat paw oedema[22] method was used for the evaluation of *in-vivo* anti-inflammatory activity of synthesised compounds. Wistar rats were procured from Central Animal House of Jamia Hamdard, New Delhi (Registration no. 1141/CPCSEA) and were adapted to the room temperature in our laboratory. The animals were fasted overnight (12 h) of either sex weighing 150-200 g and divided into groups of six animals each. The Group- I served as control received; 0.5% w/v carboxymethyl cellulose (CMC), Group-II received standard drug celecoxib orally as a positive control at a dose level of 20mg/kg/body wt. and the test groups were administered orally with equamolar dosage of the synthesized compounds as the standard drug, After 1 hr, all animals were injected with 0.1 ml of 1% carrageenan solution (prepared in 0.9% of 0.1 ml of saline solution) in the sub plantar aponeurosis of left hind paw and the volume of paw was measured by using plethysmometer at interval of 3 h and 4 h post-carrageenan treatment.

Analgesic activity

The writhing test in mice was carried out using the method of Adeyemi [23] *et al*. The writhing effect was induced by intraperitoneal injection of 0.6% acetic acid (v/v) (80 mg/kg). Standard and tests compounds were administered orally at a dose of 20 mg/kg of body weight at an equimolar dosage to groups of six animals each, 30 min before chemical stimulus,Celecoxib was used as standard. The frequencies of muscle contractions were counted over a period of 20 min after acetic acid injection. The data represents the total number of writhes observed during 20 min and is expressed as writhing numbers.

Ulcerogenic activity

The test compounds having anti-inûammatory & analgesic activities comparable with the Celecoxib were further tested for their acute ulcerogenic risk evaluation according to the *Cioli et al* method[24].The dose of the tested and standard were used as three times of the dose used for the estimation of the anti-inflammatory activity, i.e. 60 mg/kg body weight.The control group received only 0.5% CMC. After the drug treatment, the rats were fed a normal diet for 17 h and then sacriûced. The stomach was removed and opened along the greater curvature. The tested and standard are compared with after opening of the gastric mucosa and the compounds did not cause any gastric ulceration and disruption of gastric epithelial cells at the above mentioned oral dose. Using microscope with a magnifying lens the effect of ulceration was examined. The mucosal damage in each stomach was assessed according to the following scoring system. The damage of the gastric mucosal damage was assessed according to the following scoring system: 0.5 redness, 1.0 spot ulcers, 1.5 hemorrhagic streaks, 2.0 ulcers < 3, but -5, 3.0 ulcers >5.

***In-Vitro* Cyclooxygenase (COX)InhibitionAssay**

The selected synthesised compounds were accomplished there *In-Vitro* COX-II Inhibition Assay by previously reported method using enzymeimmuno assay (EIA)kit[25]. By measuring the formation of PGH_2 during the biosynthetic process of arachidonic acid catalysed by COX-II enzymes, by the reduction of stannous chloride. The duplicate assay was performed as per the guidelines by the manufacturer. The absorption of diverse yellow colour was measured by UV-visible spectrophotometer (EI 2371**)** at λ 412 nm. The intensity of the yellow colour is depends on the enzymatic reaction which is proportional to the prostaglandin tracer bound to the well and inversely to the amount in which present in well during incubation. In comparisons of tested compounds to various controlled incubation the percentage inhibition was measured. The concentration response curve was plotted for calculation of the concentration of test compounds that gives IC_{50} µM (COX-II).

Molecular Docking

To predict binding modes of ligand to receptor and good biological activity on the basis of structures, a molecular docking studies were carried out using Glide extra precision (XP) Maestro 10.1 Schrodinger, running on Linux 64 operating system based on X-ray crystal structure ofkey enzymes that are important for inflammatory process including COX-I (PDB: 1PGG) and COX-II (PDB: 3PGH).All the structure retrieved from protein data bank (www.rcsb.org).Molecular docking studies mainly involve selection and preparation of appropriate

protein, grid generation, ligand preparation followed by docking & its analysis. The docking scoreand hydrogen bonds & pi-pi interaction formed with the surrounding amino acids were used to predict their binding affinities and proper alignment of these compounds at the active site of the enzyme.

RESULTS AND DISCUSSION

Chemistry

As per the scheme outlined in Figure (3),4-(5-chloro-3-methyl-4-(((5-phenyl-1,3,4-thiadiazol-2-yl) imino)methyl) -1H-pyrazol-1-yl) benzenesulfonamide derivatives were synthesized. Initially on refluxing equimolar mixture of phenyl hydrazine (**1**) and ethyl acetoacetate (**2**) at 110-120°C for four hour, 3-methyl-1-phenyl-1*H*-pyrazol-5(4H)-one (**3**) was synthesized and further it was formylated using dry DMF in presence of $POCl_3$, 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxaldehyde (**4**) was attained[20] (Kaushik *et al.,* 2010).In another step using equimolar amount of substituted benzoic acid and semicarbazide werestirred in presence of $POCl_3$ at 75^{0}C to get a substituted 5-phenyl-1, 3, 4-thiadiazol-2-amine[21] (Tu *et al*., 2008).Finally formation of Schiff base as final compounds were obtained by refluxing the equimolar amount of 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxaldehyde (**4**) and substituted 5-phenyl-1, 3, 4-thiadiazol-2-amine **(5)** In presence of catalytic amount of glacial acetic acid, achieved the title compound, 4-(5-chloro-3-methyl-4-(5-Substituted-1,3,4-thiadiazol-2-yl)imino)methyl)-1H-pyrazol-1-yl) benzene sulphonamide derivatives (**6a-o**). The structures of varied 4-(5-chloro-3-methyl-4-(5-Substituted-1,3,4-thiadiazol-2-yl)imino)methyl)-1H-pyrazol-1yl) benzenesulfonamide derivatives(**6a-o**) were elucidated by combined use of infrared (IR), ^{1}H-NMR,^{13}C-NMR and mass spectral (MS) data. In ^{1}H-NMR spectra the D_2O exchangeable broad singlet peak was observed around ä 7.12 ppm integrating two protons ascertained free SO_2NH_2 group in 4-formylpyrazoles.In IR spectra of **(4)** displayed two absorption bands in the region 3167cm^{-1} characteristic peak of N-H stretching signifying the

Table 1: Biological activities of (E)-4-(5-chloro-3-methyl-4-(((5-substituted-phenyl-1, 3,4-thiadiazol-2-yl)methylene)amino)-1H-pyrazol-1-yl)benzenesulfonamide derivatives 6 (a-o)

Compound	Docking Scores		Anti-inflammatory activity≠ (% inhibition ± SEM)		Analgesic activity# (% inhibition ± SEM After 4 h)	Ulcerogenic effect≠ (Severity index ± SEM)
	COX-I	COX-II	3h	4h		
6a	-5.99	—	57.21 ± 6.87	53.18 ± 8.76	49.05 ± 3.75^d	0.58 ± 0.36^b
6b	-8.26	-8.76	75.20± 4.38^b	72.33± 3.83*	67.89 ± 2.33^c	0.77 ± 0.27^b
6c	-8.72	-7.21	67.20± 4.90^b	64.48±4.19*	45.25 ± 3.87^d	0.27 ± 0.71^b
6e	-8.35	-7.40	63.98 ±6.07	60.87±7.28^b	53.45 ± 2.26^d	0.41 ± 0.10^b
6f	-5.33	-3.65	49.98 ±5.77	44.51 ± 6.19	50.73 ± 1.87^c	0.47 ± 0.36^b
6g	-8.88	-6.23	66.63± 4.94^b	61.49± 4.97^a	57.33 ± 1.87^c	0.54 ± 0.43^b
6h	-6.25	—	54.98 ± 6.47	49.87 ± 2.81	49.21 ± 1.87^c	0.59 ± 0.36^b
6j	-8.30	—	51.63 ± 6.89	48.72 ± 8.96	65.12 ± 1.20 ^{c}	0.58 ± 0.36^b
6k	-4.36	-6.01	47.05 ±6.60^b	44.31 ± 4.53	37.34 ± 4.15^d	0.50 ± 0.27^b
6l	-8.76	-5.42	58.73±3.17 ^{b}	55.57 ± 7.31	64.71 ± 2.90^d	0.39 ± 0.12^a
6m	-8.57	-9.38	74.91 ± 7.69	71.17 ± 5.23	71.37 ± 1.67^d	0.79 ± 0.40^b
Celecoxib	-	-	78.01 ± 3.75	74.59 ± 6.89	73.56 ± 1.25	0.93 ± 0.47
Control	-	-	-	-	-	0.00 ± 0.00

Relative to their respective standard and data were analyzed by ANOVA followed by Dunnett's multiple comparison test for n = 6; $^aP < 0.05$; $^bP < 0.01$.

#Relative to normal and data were analyzed by paired Student's t-test for n = 6; $^cP < 0.0001$; $^dP < 0.005$.

Table 2: *In vitro* COX-I & COX-II Enzyme Inhibition Data for compound

Compounds	IC_{50} (μM)		Selectivity index
	COX-I	COX-II	COX-I/COX-II
6b	217.10	3.20	67.81
6c	109.65	2.4	45.68
6g	129.71	5.2	24.94
6l	183.68	5.03	36.51
6m	239.73	3.6	66.38
Celecoxib	19.98	0.26	76.84

[a] Values are acquired using an ovine experiments were carried in duplicate and have less than 10% error.

presence of a free SO_2NH_2 group. In addition of IR spectra a band at 3079 cm^{-1} and 1667 cm^{-1} indicates C-H stretching of aldehydic and ketone group. The typical peak of SO_2 was observed at 1327 and 1159 cm^{-1}. A free aldehydic singlet peak was observed in ^1H NMR spectra at δ 10.02-10.06 ppm along another singlet at δ 8.99-9.50 ppm for C5-H of pyrazole ring. Existence of CHO peak was further confirmed by a signal at δ 183.81ppm observed in the ^{13}C NMR spectra. Formation of compounds **(5)** was confirmed by characteristic peak of NH_2 group around sharp bands around 3283 and 3117 cm^{-1}, stretching vibration of aromatic C-H and C-S peak were also observed at 1642 (C=N), 696 (C=S) cm^{-1}. In a titled compounds **(6a)**, disappearance of characteristics peak of NH_2 and appearance of characteristic peak of stretching vibration at 1634 cm^{-1} due to formation of Schiff bases azomethine group (-CH=N-) was observed. It confirmed the formation of **(**E)-4-(5-chloro-3-methyl- 4-(((5-phenyl-1, 3, 4-thiadiazol -2-yl) methylene) amino)-1H-pyrazol-1-yl) benzene sulphonamide **(6a).**^1H NMR spectra showed broad singlet peak of Schiff base of azomethine group (–CH=N-) at δ 4.61ppm.^{13}C NMR spectra showed disappearance of aldehyde peak and appearance of (-CH=N-) at δ 161.91 ppm and at δ 173.34 ppm of thiadiazole confirmed the titled compounds. This was further supported by a mass spectrum of compound **6a** (m/z 460.13, (M+2)).

***In-vivo* anti-inflammatory activity**

With the help of docking analysis, synthesized eleven compounds were selected for anti-inflammatory, analgesic activity and ulcerogenic activity. Anti-inflammatory activity was done on Wistar rats (body weight 150-200 g) by Winter *et al.*, method assuming rat paw edema inhibition test[22].The paw oedema in rats was measured by plethysmometer. The dose of the compounds were selected at equimolar dose of 20 mg/kg of celecoxib. All the tests and reference compounds exposed anti-inflammatory activity in percentage inhibition in the ranging from 44.51 ± 6.19% to 74.59 ± 6.89% after 4 hr (Table-1, Figure-4). The structure activity relationship of the synthesized compounds was examined on the heart of the nature of substituted thiadiazole linked pyrazole benzene sulphonamide derivatives. The nature of the substituent varied on the aryl ring which is attached with 1, 3, 4-thiadiazole ring. The presence of hydroxyl substituent on aryl ring possesses very good *in-vivo* anti-inflammatory activity along with the halogen ring. The compounds (**6c** and **6e**) with halogen substitution of chloro and bromo at para position bearing promising docking score and *in-vivo* anti-inûammatory activity (64.48±4.19 and 60.87±7.28) and inhibition of intermediaries such as COX-I and COX-II. Substitution (**6l**) of hydroxy on aryl group at para position holds better dock score and *in-vivo* anti-inflammatory activity (58.73±3.17) and inhibition of intermediaries such as COX-I and COX-II. The substitution (**6m**) of hydroxy at both ortho and para, surprisingly increases the dock score and *in-vivo* anti-inûammatory (71.17±5.23 inhibition), analgesic and also acted to inhibit potentially COX-II as well as COX-I. The chloro substitution (**6b**) on aryl group at ortho position resulted in higher *in-vivo* anti-inflammatory activity (72.33± 3.83) than para position. The compounds containing CH_3, OCH_3 and NO_2 group on aryl ring having low dock

Table 3: ADME of compounds

Comp.	[a]MW	[b]PSA	[c]Dipole	[d]SASA	[e]Donor	[f]Accpt	[g]logPo/w	[h]logS	[i]CNS	% Human Oral Absorption	[j]Rule Of Five
			1 –12.5	300 –	HB ≤ 5	HB ≤ 10	< 5	–6.5	+2	> 80 good	≤ 1
Range	< 500	7– 200		1000				– 0.5	-2	< 25 poor	
6a	458.93	119.53	6.54	746.93	2	8.5	3.01	-6.27	-2	80.12	0
6b	493.38	119.71	9.56	768.55	2	8.5	3.47	-6.90	-2	83.13	0
6c	493.38	119.53	5.61	771.04	2	8.5	3.49	-6.99	-2	82.95	0
6d	527.83	119.71	8.32	792.58	2	8.5	3.95	-7.62	-2	73.00	1
6e	548.93	208.01	10.42	820.22	2	10.5	1.71	-6.48	-2	16.38	2
6f	503.93	164.45	7.54	785.28	2	9.5	2.30	-6.41	-2	33.54	2
6g	537.83	119.54	5.70	776.08	2	8.5	3.57	-7.10	-2	70.43	1
6h	472.96	119.06	7.47	771.56	2	8.5	3.32	-6.69	-2	82.81	0
6i	472.96	119.54	6.77	779.31	2	8.5	3.30	-6.82	-2	81.86	0
6j	488.96	127.83	7.27	784.01	2	9.25	3.09	-6.47	-2	80.62	0
6k	518.99	132.71	6.00	828.79	2	10	3.25	-6.81	-2	68.58	1
6l	474.93	142.08	5.90	759.42	3	9.25	2.28	-6.05	-2	66.61	0
6m	490.93	162.19	6.82	769.81	4	10	1.66	-5.78	-2	71.47	0
6n	489.95	166.10	4.27	770.96	4.5	10.25	1.55	-5.69	-2	55.99	0
6o	473.95	145.89	8.89	762.88	3.5	9.5	2.10	-6.00	-2	64.34	0

[a]Molecular weight of the molecule; [b]Van der Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms; [c]Computed dipole moment of the molecule; [d]Total solvent accessible surface area in square angstroms using a probe with a 1.4 Å radius; [e]Estimated number of hydrogen bonds that would be donated by the compound to water molecules in an aqueous solution; [f]Estimated number of hydrogen bonds that would be accepted by the compound from water molecules in an aqueous solution; [g]Predicted octanol/water partition coefficient; [h]Predicted aqueous solubility, log S. S in mol dm^{-3} is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid; [i]CNS toxicity (+2 CNS active and -2 CNS inactive); [j]Lipinski's violations;

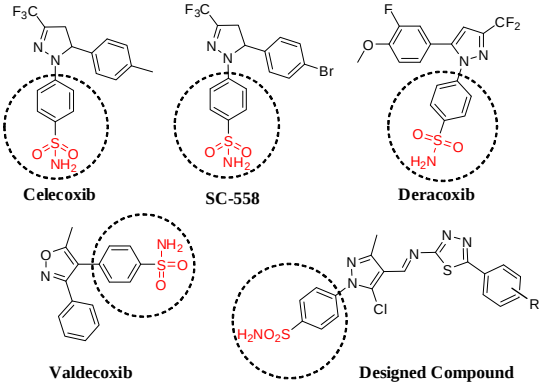

Fig. 1: Chemical Structures of reported pyrazole containing NSAIDs

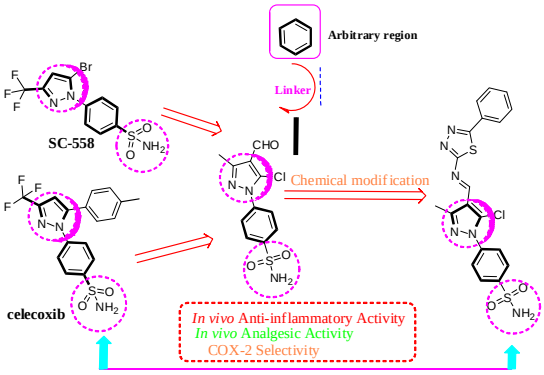

Fig. 2: The structural resemblance of Celecoxib with designed molecule is shown

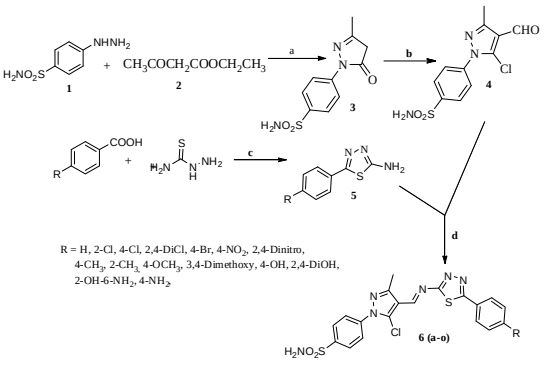

Fig. 3: Route of synthesis of various Pyrazole derivatives 6(a-o). Reagent and conditions: (a) 4-hydrazinylbenzenesulfonamide, Ethylacetoacetae, reflux (b) DMF/$POCl_3$, heating (c) Subst. benzoic acid, semicarbazide, $POCl_3$ stirring and reflux at 75^{0}C (d) Subst. thiadiazole amine, Abs. EtOH, GAA, reflux

score as well as *in-vivo* and *in-vitro* biological activity.

Analgesic activity

The tested compounds displaying significant anti-inflammatory activity in comparison with standard were tested for their analgesic activity by the writhing test method [23]. All the tested compounds exhibited analgesic activity in a range of 37.34 ± 4.15% to 69.37±1.67% inhibition, whereas standard drug celecoxib showed 73.56 ± 1.25% (Table-1, Figure-5).The compound with 2-chloro substitution **(6b)** showed very good analgesic activity (67.89 ± 2.33% inhibition) and the compound **(6m)** exhibited significant analgesic activity (71.37 ± 1.67% inhibition) as compared with reference drug celecoxib (73.56 ± 1.25% inhibition). The compound possesses potential anti-inflammatory and analgesic activities were further tested for their gastric ulceration activity according to Cioli *et al.* method at a dose of 60 mg/kg.

Ulcerogenic studies

The compounds having potential anti-inflammatory and analgesic activity was further evaluated their gastric ulceration activity Cioli *et al*[24].Once accompanying with celecoxib, compounds **6b, 6c, 6e, 6l** and **6m** did not influence any gastric ulceration and rupture of the gastric mucosal layer (Table-1).

In-vitro COX Inhibition Studies

The selected compounds with promising *in-vivo* anti-inflammatory and analgesic activity were examined their *in-vitro* COX Inhibition studies using enzymeimmunoassay (EIA) kit accordingtoa previously described method[25].The result of tested and reference drug were depicted in Table-2 figure-6. The results indicated that the compounds **6b** and **6m** exhibited significant inhibitory effect against COX-II which is compared with COX-I and it also possesses good selective index as compared to the reference drug. Selective profile of the selected compounds was calculated as ratios of (COX-I/COX-II) and it was compared with standard COX-II selective profile of celecoxib. The percentage inhibition of *in-vitro* COX inhibition was depicted in figure-6.

In-silico ADME

ADME plays a crucial role in the design,

screening and testing of the molecules for therapeutic intervention. To check the criteria of compounds for desirable pharmacokinetic properties, a QikProp study for prediction of ADME properties of the derivatives was performed using Schrodinger Maestro 10.1, running on Linux 64 operating system (QikProp. Version 3.6).[26] Lipinski's rule of five has been used to design and filter the compound that would likely to develop new clinical therapeutic agents and it is based on the observation that orally administered compounds have a MW < 500, log Po/w < 5, donor HB ≤ 5 and accpt HB ≤ 10. Compounds violating more than one of these rules may have problems with oral bioavailability of compounds. Only two compounds **6e** and **6f** were violated Lipinski's rule of five. From all these ADME parameters, it was concluded that most of the compounds followed Lipinski's rule, making them potentially promising drug candidates for the treatment of inflammation as anti-inflammatory agents summarized in Table 3.

Molecular Docking

Docking study of all the synthesised compounds **(6a-o)** were performed using Glide extra precision (XP) Maestro 10.1 Schrodinger software[27] on COX-I (PDB: 1PGG) and COX-II (PDB: 3PGH) enzyme. The docking scores of the titled compounds with the active site of COX-I and COX-II is summarized in Table 1. Both the crystal structure of COX-I and COX-II was prepared for docking with the Protein Preparation Wizard workflow of Maestro that allows addition of hydrogen atoms which were subsequently minimized with OPLS-2005 force field and optimize the protonation state. The receptor grid was generated by applying a van der Waals radii of non-polar atoms, which decreases penalties for close contacts (scaling factor =1.00 and partial charge cut off = 0.25). Before docking calculation, all the compounds were subjected to ligand preparation with the LigPrep tool. Finally the ligand docking were run using receptor grid file and LigPrep out file in the Glide tool of Application view. It was observed that the compound **6m** and **6b** has shown less selectivity for COX-I and more for COX-II. The compounds also assume favourable orientation within the COX binding site. The **6m** form a pi-pi interaction with TYR 355, while **6b** form only hydrophobic interaction at active site of COX-I as shown in docked pose Figure-7.The compound **6m** and **6b** formed strong hydrogen bonds with ARG 120 and TYR 355 respectively at active site of COX-II as

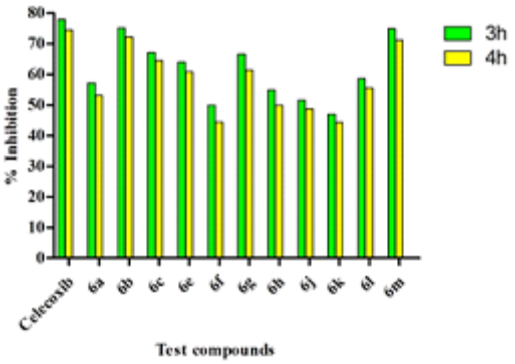

Fig. 4: Graph depicted *in-vivo* anti-inflammatory activity of the tested compounds bywinter *et al.,* method

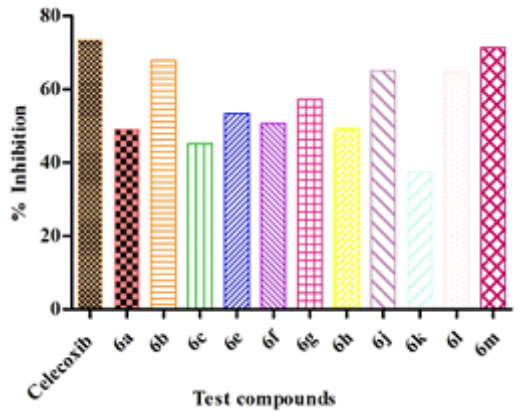

Fig. 5:Graph depicted *in-vivo* analgesic activity of the tested compounds by Writhing test method

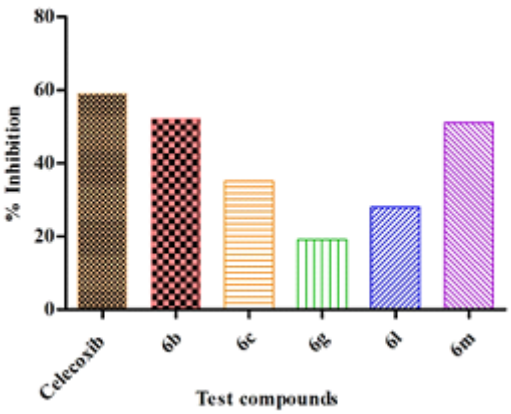

Fig. 6:*In-vitro* COX-II activity tests and standards by COX-II immunoassay kit

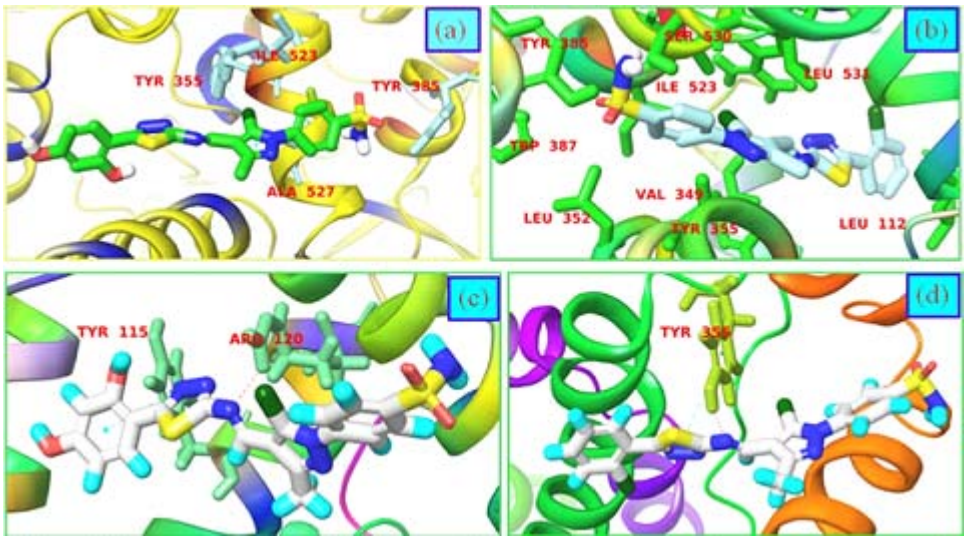

Fig. 7: Docked Pose of (a)Compound 6m,green colour(b)Compound 6b,turquoise colour, represented as tube in the binding site of COX-1 showing pi-pi interaction (turquoise dash lines) with TYR 355 and Docked Pose of (c)Compound 6m,lime green colour(d)Compound 6b,turquoise colour, represented as tube in the binding site of COX-2 showing hydrogen bond interaction (red dash lines) with ARG 120 and TYR 355 and pi-pi interaction (turquoise dash lines) with TYR 115 and TYR 355

shown in docked pose Figure-7.The docking studies of compound **6m** and **6g**also revealed that the presence of linker moiety between two heterocyclic rings are important for hydrogen bonding with ARG 120 and TYR 355 respectively at COX-II site. The binding orientation of titled compounds was found to be same as co-crystal ligand. In COX-II, the smaller size of the valine (VAL 523) side chain coupled with the conformational changes at TYR 355 opens up the hydrophobic segment of the new pocket. Because of substitution of bulkier isoleucine (ILE) of COX-I to smaller valine (VAL) at position 523 lead to inhibition of COX-II by compounds.

CONCLUSION

It can be concluded that we have synthesized thiadiazole linked pyrazole benzene sulphonamide derivatives **(6a-o)** and characterized by IR NMR and Mass spectral data and estimated their anti-inflammatory, analgesic activity and ulcerogenic activity along with *in-vitro* COX-II inhibitory activity. The compounds **6b, 6c, 6i, 6l** and **6m** exhibited significant anti-inflammatory and analgesic activity without showing any gastric ulceration. The COX-II inhibitory potential represented that the compound **6b** and **6m** exhibited very good anti-inflammatory and analgesic activity with selective index of (SI-67.81 and 66.38 respectively), which was compared with reference drug of Celecoxib (SI- 76.84). The molecular docking and *in-silico* computational study revealed that the compound **6b** and **6m** has very good binding affinity with amino acid ARG 120 and TYR 355 for COX-II and also embraces a prominent pharmacokinetic profile. Hence the compounds **6b** and **6m** were selected as promising lead candidates for further development of selective inhibition for COX-II and anti-inflammatory activity.

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Conflict of interest

The authors declares no Conflict of Interest.

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