CHAPTER 2

Synthesis of 1, 5-Diaryl-4,5-dihydro-1H-pyrazol-3-yl-substituted-heteroazoles
2.1. Section A: Synthesis of 1, 5-diaryl-4,5-dihydro-1H-pyrazol-3-yl-5-substituted-[1,3,4]-oxadiazoles.

2.1.1. Introduction

Pyrazoline derivatives are important heterocycle used extensively in medicinal and pharmaceutical chemistry. Pyrazoline nucleus and its chemistry has been the focus of attention due to versatile biological activities of pyrazoline derivatives appearing as antimicrobials\(^1\), antidiabetic\(^2\), anti-inflammatory\(^3\), anti-obesity\(^4\), antiviral\(^5\), antitubercular activity\(^6\) agents. Diaryl substituted pyrazoles have been used as nonsteroidal anti-inflammatory drugs (NSAIDs)\(^7\) (Figure 2.1.1.)

![Figure 2.1.1. Structure of marketed COX-2 inhibitors and synthesized compound](image)

Literature survey revealed that numerous pyrazoline derivatives have found their clinical application as NSAIDs. Antipyrine, 2,3-dimethyl-1-phenyl-3-pyrazolin-5-one, was the first pyrazolone derivative used in the management of pain and inflammation. Several
analogenes of pyrazolidin-3,5-diones, pyrazolin-3-ones and pyrazolin-5-ones are also available as NSAIDs; examples are felcobuzone, mefobutazone, morazone, famprofazone, and ramifenazone. Besides these, many pyrazoline derivatives are also reported in literature as having potent anti-inflammatory activity.

Carboxylic acid and its bioisoeter functionality serve a good role in development of NSAID ligands. Isosteric replacement of functional groups in a compound is a widely used approach to study receptor chemistry and to develop new drugs with optimized behavior. When this replacement affords products with broadly similar biological properties, the groups are called bioisosters. A number of clear bioisosteric relationships have been established for many functional groups, in particular for the carboxyl group, which successfully has been substituted by heterocycles such as tetrazole, 3-hydroxyisoxazole, 3-hydroxyisothiazole, 3-hydroxy-1,2,5-thiadiazole, 2-hydroxy-1,3,4-oxadiazole, 2-thiol-1,3,4-oxadiazole, 2-amino-1,3,4-oxadiazole, 2-hydroxy-1,3,4-thiadiazole, 2-thiol-1,3,4-thiadiazole, 2-amino-1,3,4-thiadiazole. Here we report synthesis of acid hetrocycles such as 1,3,4 oxadiazole, 1,3,4 thiadiazole bearing acidic or basic functionality.

Taking celecoxib, SC-558 (Figure 2.1.2.) as the basis for designing we have replaced central pyrazole ring with its bioisoeter pyrazoline and further modified the trifluoromethyl group with acid or amide hetrocycles.

\[
\begin{align*}
\text{Celecoxib (A) } & R=\text{CH}_3 \\
\text{SC-558 (B) } & R= \text{Br}
\end{align*}
\]

Where \( X = \text{H, SO}_2\text{NH}_2 \) & \( Z=\text{CH, N, O, S} \)

Figure 2.1.2.
2.1.2. Present Work:

The basic cores were synthesized by following Scheme 2.1.1. The 4-(4-chlorophenyl)-2-oxo-but-3-enoic acid ethyl ester 1 was prepared by esterification of potassium salt of 4-(4-chlorophenyl)-2-oxobut-3-enoic acid\textsuperscript{18} in ethanol using thionyl chloride. Ester on reaction with substituted phenyl hydrazine hydrochloride in ethanol and acetic acid furnished ethyl 5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-1\textsubscript{H}-pyrazole-3-carboxylate 2\textsubscript{a} and 5-(4-chloro-phenyl)-1-(4-sulfamoyl-phenyl)-4,5-dihydro-1\textsubscript{H}-pyrazole-3-carboxylic acid ethyl ester 2\textsubscript{b}. These esters were further converted into respective hydrazides 3\textsubscript{a,b} using hydrazine hydrate in ethanol at reflux. The ester 2\textsubscript{a,b} were also used to prepare amide derivative 4\textsubscript{a,b} using aq. ammonia in tetrahydrofuran. The nitrile core 5\textsubscript{a,b} were obtained by dehydrating corresponding amides using dimethyl formamide and oxallyl chloride as dehydrating agent. Nitriles were further reacted with hydroxyl amine hydrochloride in presence of Na\textsubscript{2}CO\textsubscript{3} to furnish amidoxime cores 6\textsubscript{a,b}.

\[ \text{Scheme 2.1.1. Reagents and conditions: (A) } p\text{-R-phenyl hydrazine, ethanol, acetic acid, reflux; (B) Hydrazine hydrate, ethanol, reflux; (C) 30\% Aq. ammonia, THF, 50-59^\circ \text{C}; (D) DMF, Oxallyl chloride, 0\textdegree \text{C}; (E) Hydroxyl amine HCl, Na\textsubscript{2}CO\textsubscript{3}, Methanol, 25-30\textdegree \text{C.} \]
Diaryl hydrazides 3a,b were used for synthesis of 5-substituted-1,3,4-heterodiazoles. (Scheme 2.1.2.) 2-hydroxy-1,3,4-oxadiazoles 7a,b were prepared by reaction of carbonyl diimidazole with 3a,b in tetrahydrofuran using triethyl amine as base. The 2-thiol-1,3,4-oxadiazoles 8a,b were obtained by reaction of 3a,b with carbon disulfide under basic conditions. The 2-amino-1,3,4-oxadiazoles 9a,b resulted from the reaction of cyanogen bromide and NaHCO$_3$ on 3a,b. 2-thiomethyl-1,3,4-thiadiazole 10a,b were synthesized by reaction of hydrazide with KOH and carbon disulfide followed by methylation using methyl iodide, and further cyclised in toluene using p-toluene sulphonic acid at reflux.

![Diagram of 2-hydroxy-1,3,4-oxadiazoles, 2-thiol-1,3,4-oxadiazoles, 2-amino-1,3,4-oxadiazoles, and 2-thiomethyl-1,3,4-thiadiazoles]

Where For a. R=H, b. R = SO$_2$NH$_2$

**Scheme 2.1.2. Reagents and conditions**: - (F)CDI, Et$_3$N, THF, 25-30°C; (G) CS$_2$, KOH, methanol, 60-65°C; (H) CNBr, NaHCO$_3$, Dioxane:Water, 25-30°C; (I) 1. CS$_2$, KOH, Mel, methanol, 25-30°C; 2. p-toluene sulphonilic acid, toluene, reflux.

2-alkyl-1,3,4-oxadiazoles were synthesized by general procedure, (Scheme 2.1.3.) wherein the hydrazide 3a on reaction with various acid chlorides or anhydrides in presence of triethylamine as base forms open chain diamide intermediate, which in-situ on reaction with p-toluene sulphonyl chloride and triethylamine gets cyclised to form 2-
alkyl-1,3,4-oxadiazoles 13a-16a. The esters were converted into respective acids 17a and 18a by hydrolysis with LiOH in THF: water solvent mixture.

\[ R' = CH_3 (13a), CF_3 (14a), COOEt (15a), CH_2COOEt (16a), COOH (17a), CH_2COOH (18a) \]

**Scheme 2.1.3. Reagent and conditions:** (L) 1. R'COCl, Et_3N, p-toluene sulfonyl chloride, 0-25°C; (M) LiOH, THF, Water, 25-30°C
2.1.3. Experimental & Spectral Data:

The melting points were determined on a Veego apparatus and are uncorrected. Infrared spectra were recorded on a Bruker spectrophotometer in a KBr disc, and the absorption bands are expressed in cm\(^{-1}\). \(^1\)H-NMR spectra were recorded on a Varian AS 400 MHz spectrometer in CDCl\(_3\)/DMSO-\(d_6\), chemical shifts (\(\delta\)) are in ppm relative to TMS, and coupling constants (\(J\)) are expressed in hertz (Hz). Mass spectra were taken on a Macro mass spectrometer (Waters) by electro-spray method (ES).

**4-(4-chlorophenyl)-2-oxo-but-3-enoic acid ethyl ester (I)**

To 500 mL ethanol, was added thionyl chloride (35 mL, 481 mmole) at 0-5 °C under argon. To this solution, (60 g, 240 mmole) potassium salt of 4-(4-chlorophenyl)-2-oxobut-3-enoic acid\(^{17}\) was added portion wise at 0-5 °C. Reaction mixture was stirred at 25-30 °C for 1 hour and further refluxed for 3 hours. Reaction mixture was cooled to room temperature and ethanol was removed under reduced pressure. Residue obtained was partitioned between 300 mL ethyl acetate and 200 mL 5% sodium bicarbonate solution. Organic layer was washed with brine. The organic layer was separated and dried over anhydrous Na\(_2\)SO\(_4\), and evaporated on a rotary evaporator under reduced pressure. Yellow colored semi solid product obtained (50 g, 87%). IR: (KBr) 3360, 1723, 1680 cm\(^{-1}\), \(^1\)H NMR (400MHz, CDCl\(_3\)) \(\delta\) 7.88 (d, \(J = 16\) Hz, 1H), 7.64 (d, 2H), 7.45 (d, 2H), 7.37 (d, \(J = 16\) Hz, 1H), 4.44 (q, 2H), 1.42 (t, 3H); ESI-MS (m/z): 239.1 [M+H]; Molecular Weight:- 238; Molecular Formula:- C\(_{12}\)H\(_{11}\)ClO\(_3\).

**Ethyl 5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazole-3-carboxylate (2a)**

To I (30.0 g, 126 mmol) in ethanol (200 mL) was added phenyl hydrazine hydrochloride (18.5 g, 126 mmol), and the reaction mixture was stirred at 25-30 °C over a period of 2 h under argon atmosphere. To the reaction mixture was added acetic acid (50 mL), and the mixture refluxed for 10 h. The reaction mixture was cooled, ethanol and acetic acid was removed under reduced pressure. Residue obtained was poured into water (300 mL) and extracted with ethyl acetate (3×100 mL). The combined ethyl acetate layer was washed with 5% NaHCO\(_3\) solution (100 mL) and brine (100 mL). The organic layer was separated, dried over anhydrous Na\(_2\)SO\(_4\), and solvent was evaporated on a rotatory
evaporator under reduced pressure. The residue obtained was triturated in methanol (100 mL) to afford a solid which was filtered on a Buchner funnel under suction and dried to afford 2a as a yellow solid (20 g, 49%): mp 115-117 °C; IR (KBr) 2981, 2361, 1737, 1706, 1575, 1556, 1479 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.31 (d, 2H), 7.15-7.20 (m, 4H), 7.09 (d, 2H), 6.90 (t, 1H), 5.36-5.41 (dd, J = 12 and 8 Hz, 1H), 4.31-4.36 (q, 2H), 3.67-3.74 (dd, J = 18 and 12 Hz, 1H), 2.97-3.03 (dd, J = 18 and 8 Hz, 1H), 1.38 (t, 3H); ESI-MS m/z: 329.0 [M+H]. Molecular Weight:- 328; Molecular Formula:- C₁₈H₁₇ClN₂O₂.

5-(4-chloro-phenyl)-1-(4-sulfamoyl-phenyl)-4,5-dihydro-1H-pyrazole-3-carboxylic acid ethyl ester (2b)
The compound 2b was prepared by using same method for 2a using 4-Hydrazino-benzenesulfonamide.
Yellow solid, yield 52% :mp 132-134 °C; IR (KBr) 2981, 2362, 1737, 1706, 1575, 1556, 1479 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 7.62 (d, 2H), 7.40 (d, 2H), 7.24 (d, 2H) 7.10 (d, 2H), 7.08 (bs, 2H), 5.46-5.51 (dd, J = 13 and 8 Hz, 1H), 4.21-4.26 (q, 2H), 3.74-3.80 (dd, J = 18 and 13 Hz, 1H), 2.85-3.91 (dd, J = 18.0 and 7.2Hz, 1H), 1.28 (t, 3H); ESI-MS (m/z): 408.0 [M+H]. Molecular Weight:- 407; Molecular Formula:- C₁₈H₁₈ClN₃O₄S.

5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazole-3-carboxyldrazid. (3a)
The ester 2a (10 g, 30.48 mmole) was charged into 200 mL ethanol and refluxed with 3 mL hydrazine hydrate for 4 hours. Reaction mixture was cooled to room temperature. Ethanol was removed on rotavapour under reduced pressure. Yellow solid residue obtained was stirred in 150 mL water for 30 mins. Solid precipitates were filtered under suction on Buchner funnel. Product was washed with 50 mL water twice followed by 50 mL hexane. Yellow colored powder obtained 3a (8 g, 83%): mp 134-136 °C; IR (KBr) 3497, 3313, 3063, 1648, 1597, 1570, 1494 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 9.52 (bs, 1H) 7.38 (d, 2H), 7.23 (d, 2H), 7.16 (t, 2H), 7.01 (d, 2H), 6.78 (t, 1H), 5.49-5.54 (dd, J = 4 and 12 Hz, 1H), 4.38 (bs, 2H), 3.63-3.70 (dd, J = 12 and 18 Hz, 1H), 2.79-2.85 (dd,
$J = 4 \text{ Hz and 18 Hz, 1H}; \text{ ESI-MS (m/z): 315.2 [M+H]. Molecular Weight:- 314; Molecular Formula:- C}_{16}H_{15}ClN_{4}O. $

4-[5-(4-chloro-phenyl)-3-hydrazinocarbonyl-4,5-dihydro-pyrazol-1-yl]-benzenesulfonamide. (3b). The compound 3b was prepared by using method as per 3a
Yellow solid, yield 80% :mp 155-157 °C; IR (KBr) 3490, 3310, 3073, 1648, 1597, 1570, 1494 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-d\(_6\)) (400 MHz, DMSO-d\(_6\)) \(\delta\) 9.54 (bs, 1H), 7.60 (d, 2H), 7.30 (d, 2H), 7.10 (d, 2H), 7.03 (bs, 2H), 5.48-5.53 (dd, \(J = 6\) and 12 Hz, 1H), 4.44 (bs, 2H), 3.63-3.70 (dd, \(J = 12\) and 18 Hz, 1H), 2.78-2.84 (dd, \(J = 6\) and 18 Hz, 1H) ; ESI-MS (m/z): 392.1[M-H]. Molecular Weight:- 393; Molecular Formula:- C\(_{16}\)H\(_{16}\)ClN\(_5\)O\(_3\)S.

5-(4-chloro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazole-3-carboxylic acid amide (4a).
The ester 2a (10 g, 30.48 mmole) was charged to 50 mL tetrahydrofuran and 100 mL aq. ammonia. It was stirred for 10 hours and then heated at 60 °C for 2 hrs. Reaction mixture was cooled to room temperature. THF was concentrated on rotavap under reduced pressure. Yellow solid residue obtained was stirred at 10-15 °C for 30 minutes. Solid precipitates were filtered under suction on Buchner funnel. Product was washed with 50 mL water twice followed by 50 mL hexane. Yellow colored powder obtained (7 g, 76.83%) 4a amide: mp 234-236 °C; IR (KBr) 3310, 1648, 1597, 1570, 1494 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.70 (bs, 1H), 7.44 (bs, 1H), 7.30 (d, 2H), 7.15-7.20 (m, 4H), 7.06 (d, 2H), 6.90 (t, 1H), 5.36-5.42 (dd, \(J = 12\) and 8 Hz, 1H), 3.67-3.74 (dd, \(J = 18\) and 12 Hz, 1H), 2.97-3.03 (dd, \(J = 18\) and 8 Hz, 1H); ESI-MS (m/z): 300.0[M+H]; Molecular Weight:- 299; Molecular Formula:- C\(_{16}\)H\(_{14}\)ClN\(_3\)O.

5-(4-chloro-phenyl)-1-(4-sulfamoyl-phenyl)-4,5-dihydro-1H-pyrazole-3-carboxylic acid amid (4b)
The compound 4b was synthesized using same procedure as 4a
Yellow solid, yield 71% :mp 230-232 °C; IR (KBr) 3497, 3313, 3063, 1648, 1597, 1570, 1494 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO- d\(_6\)) \(\delta\) 7.72 (bs, 1H), 7.58 (d, 2H), 7.40 (bs, 1H), 7.29 (d, 2H), 7.20 (d, 2H), 7.10(d, 2H), 7.01 (bs, 2H), 5.48-5.53 (dd, \(J = 6\) and 12 Hz,
5-(4-chloro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazole-3-carbonitrile (5a)

To 10 mL anhydrous DMF at 0 °C was added (4 mL, 4.682 mmol) of oxallyl chloride. The mixture was stirred at 0 °C for 10 min, and to this solution of a solution of amide 4a (7 g, 2.341 mmol) in 10 mL DMF was added to the vigorously stirring solution over 5 min. After 15 min, pyridine (7.5 mL, 9.364 mmol) was added to quench the reaction. The mixture was poured into 1 N HCl (100 mL) and extracted with ethyl acetate (2×100 mL). The combined organic extracts were washed with 1 N HCl (2×25 mL) and brine (2×50 mL), dried over Na₂SO₄, filtered, and concentrated in vacuum to obtain 5.5 g (83 %) of 5a as a yellow solid: mp 142-144 °C; IR (KBr) 3497, 3323, 3080, 1648, 1597, 1570, 1494 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 7.35 (d, 2H), 7.23 (d, 2H), 7.15 (d, 2H), 7.01(d, 2H), 6.94 (t, 1H), 5.40-5.45 (dd, J = 8 and 12 Hz, 1H), 3.66-3.73 (dd, J = 12 and 18 Hz, 1H), 2.94-3.00 (dd, J = 8 and 18 Hz, 1H); ESI-MS (m/z): 282.1 [M+H]; Molecular Weight:- 281; Molecular Formula:- C₁₆H₁₂ClN₃.

4-[5-(4-chloro-phenyl)-3-cyano-4,5-dihydro-pyrazol-1-yl]-benzenesulfonamide (5b)

The compound 5b was synthesized as per the procedure for 5a

Yellow solid, yield 80% :mp 185-186 °C; IR (KBr) 3490, 3300, 3083, 1648, 1597, 1570, 1494 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 7.61 (d, 2H), 7.40 (d, 2H), 7.27 (d, 2H), 7.00-7.03 (m, 4H), 5.70-5.75 (dd, J = 8 and 12 Hz, 1H), 3.91-3.98 (dd, J = 12 and 18 Hz, 1H), 3.02-3.07 (dd, J = 8 and 18 Hz, 1H). ESI-MS 361 [M+H]; Molecular Weight:- 360; Molecular Formula:- C₁₆H₁₃ClN₄O₂S.

5-(4-chloro-phenyl)-N-hydroxy-1-phenyl-4,5-dihydro-1H-pyrazole-3-carboxamidine (6a)

To a solution of 5a (5 g, 17mmol) in methanol (35 mL) was added sodium carbonate (3.68 g, 35 mmol) and hydroxylamine hydrochloride (2.3 g, 35 mmol), and the mixture was stirred at 25-30 °C for 6 h. The reaction mixture was concentrated under reduced pressure and residue obtained was stirred in water (50 mL), the solid precipitated was filtered on Buchner funnel and washed with water. Dried in oven at 50 °C for 5 hrs to
obtain yellow solid. (4.5g, 80%) :mp 155-157 °C; IR (KBr) 3322, 3053, 1648, 1597, 1570, 1494 cm\(^{-1}\); \(^1^H\) NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 9.97 (bs, 1H), 7.38 (d, 2H), 7.24 (d, 2H), 7.14 (d, 2H), 6.97 (d, 2H), 6.71 (t, 1H), 5.53 (bs, 2H), 5.37-5.42 (dd, \(J = 8\) and 12 Hz, 1H), 3.58-3.65 (dd, \(J = 12\) and 18 Hz, 1H), 2.70-2.76 (dd, \(J = 8\) and 18 Hz, 1H); ESI-MS (\(m/z\)): 315.0 [M+H]; Molecular Weight:- 314; Molecular Formula:- C\(_{16}\)H\(_{15}\)ClN\(_4\)O.

5-(4-chloro-phenyl)-N-hydroxy-1-(4-sulfamoyl-phenyl)-4,5-dihydro-1H-pyrazole-3-carboxamidine (6b)

The compound 6b was synthesized using method for 6a

Yellow solid, yield 78% :mp 188-190 °C; IR (KBr) 3497, 3313, 3063, 1648, 1597, 1570, 1494 cm\(^{-1}\); \(^1^H\) NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 9.90 (bs, 1H), 7.40 (d, 2H), 7.28 (d, 2H), 7.18 (d, 2H), 7.04-7.00 (m, 4H), 5.56-5.60 (dd, \(J = 8\) and 12 Hz, 1H), 5.52 (bs, 2H), 3.60-3.68 (dd, \(J = 12\) and 18 Hz, 1H), 2.72-2.78 (dd, \(J = 8\) and 18 Hz, 1H); ESI-MS (\(m/z\)): 394.1 [M+H]; Molecular Weight:- 393; Molecular Formula:- C\(_{16}\)H\(_{16}\)ClN\(_3\)O\(_5\)S.

5-(5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-3-yl)-1,3,4-oxadiazol-2-ol (7a)

1,l'-carbonyldiimidazole (95 mg, 1.59 mmol) was added to a 0 °C solution of 3a (250 mg, 0.796 mmol) and triethylamine (332 µL, 2.38 mmol) in 10 mL of tetrahydrofuran. After the reaction mixture was stirred at 0 °C for 2 h, and then allowed to warm to room temperature for overnight. The volatiles were removed under reduced pressure and the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed consecutively with 1N hydrochloric acid, saturated sodium bicarbonate, and brine. The organic layer was separated, dried over sodium sulfate, filtered and concentrated in vacuum. Yellow solid 7a was obtained. (168 mg, 62 %): mp 115-116°C; IR (KBr) 3310, 1597, 1570, 1496 cm\(^{-1}\); \(^1^H\) NMR (400 MHz, DMSO- \(d_6\)) \(\delta\) 7.39 (d, 2H), 7.27 (d, 2H), 7.19 (d, 2H) 6.98 (d, 2H), 6.81(t, 1H), 5.61-5.66 (dd, \(J = 8\) and 12 Hz, 1H), 3.70-3.78 (dd, \(J = 12\) and 18 Hz, 1H), 2.88-2.94 (dd, \(J = 8\) and 18 Hz, 1H); ESI-MS (\(m/z\)): 339.0 [M-H]; Molecular Weight:- 340; Molecular Formula:- C\(_{17}\)H\(_{13}\)ClN\(_4\)O\(_2\).
4-[5-(4-chloro-phenyl)-3-(5-hydroxy-[1,3,4]oxadiazol-2-yl)-4,5-dihydro-pyrazol-1-yl]-benzenesulfonamide(7b)

The compound 7b was synthesized using same procedure for 7a

Yellow solid, yield 57% : mp 195-197 °C; IR (KBr) 3300, 1598, 1570, 1494 cm⁻¹; ¹H NMR (400 MHz, DMSO- d₆) δ 7.73 (d, 2H), 7.41 (d, 2H), 7.21 (t, 2H), 7.09 (d, 2H), 7.03 (bs, 2H), 5.75-5.80 (dd, J = 8 and 12 Hz, 1H), 3.79-3.86 (dd, J = 12 and 18 Hz, 1H), 2.91-2.96 (dd, J = 8 Hz and 18 Hz, 1H); ESI-MS (m/z): 418.1 [M-H]; Molecular Weight:- 419; Molecular Formula:- C₁₇H₁₄ClN₅O₄S.

5-(5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-3-yl)-1,3,4-oxadiazol-2-thiol (8a)

The hydrazide 3a (250 mg, 0.796 mmol) was dissolved in 20 mL of methanol, and the solution was cooled to 0 °C. Carbon disulfide (124µL, 1.59 mmol) was added, followed by potassium hydroxide (58 mg, 0.87 mmol). The solution was stirred at 25-30 °C for 1 hr and heated at reflux for 4 hr. Heating was stopped and reaction mixture was allowed to stir at room temperature for overnight. The solution was concentrated in vacuum and the residue dissolved in water. The aqueous solution was acidified with 1N hydrochloric acid, and the resulting solids were extracted by ethyl acetate. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuum provided yellow solid 8a (220, mg 80%): mp 120-122 °C; IR (KBr) 3760, 3484, 2362, 1649, 1596, 1496 cm⁻¹; ¹H NMR (400 MHz, DMSO- d₆) δ 7.39 (d, 2H), 7.30 (d, 2H), 7.21 (t, 2H), 7.03 (d, 2H), 6.85 (t, 1H), 5.71-5.76 (dd, J = 6 and 12 Hz, 1H), 3.82-3.90 (dd, J = 12 and 18 Hz, 1H), 2.95-3.01 (dd, J = 6 and 18 Hz, 1H); ESI-MS (m/z): 355.1 [M-H]; Molecular Weight:- 356; Molecular Formula:- C₁₇H₁₃ClN₄OS.

4-[5-(4-chlorophenyl)-3-(5-mercapto-[1,3,4]oxadiazol-2-yl)-4,5-dihydro-pyrazol-1-yl]-benzenesulfonamide(8b)

The compound 8b was prepared using same procedure for 8a

Yellow solid, yield 73% : mp 157-159 °C; IR (KBr) 3219, 3115, 1663, 1594, 1475 cm⁻¹; ¹H NMR (400 MHz, DMSO- d₆) δ 7.63 (d, 2H), 7.40 (d, 2H), 7.29 (d, 2H), 7.14 (d, 2H), 7.06 (bs 2H), 5.81-5.86 (dd, J = 6 and 12Hz, 1H), 3.88-3.96 (dd, J = 12 and 18 Hz,
5-(5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-3-yl)-2-amino-1,3,4-oxadiazol (9a)
Sodium bicarbonate (134 mg, 1.59 mmol) in 15 mL water was added to a solution of 3a (250 mg, 0.796 mmol) in 20 mL of dioxan at room temperature. The mixture was stirred at room temperature for 5 min and cyanogen bromide (126 mg, 1.19 mmol) was added. After 3 h, the volatiles were removed in vacuum. Residue obtained was stirred in 10 mL water and precipitate was collected by filtration to provide 9a as yellow solid (210 mg 77%): mp 240-245 °C; IR (KBr) 3310, 3060, 1597, 1570, 1496 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 7.43 (bs, 2H) 7.39 (d, 2H), 7.28 (d, 2H), 7.11 (t, 2H), 6.98 (d, 2H), 6.78 (t, 1H), 5.58-5.63 (dd, \(J = 6\) and 12 Hz, 1H), 3.84-3.92 (dd, \(J = 12\) and 18 Hz, 1H), 2.96-3.02 (dd, \(J = 6\) and 18 Hz, 1H); ESI-MS (\(m/z\)): 340.1 [M+H]; Molecular Weight:- 339.1; Molecular Formula:- C\(_{17}\)H\(_{14}\)ClN\(_5\)O.

4-[3-(5-Amino-[1,3,4]oxadiazol-2-yl)-5-(4-chloro-phenyl)-4,5-dihydro-pyrazol-1-yl]-benzenesulfonamide (9b)
The compound 9b was synthesized using same procedure for 9a. Yellow solid yield 74% mp 185-187 °C; IR (KBr) 3309, 2362, 1655, 1590, 1501, 1415 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 7.58 (d, 2H), 7.40 (d, 2H), 7.21 (t, 2H), 7.11 (d, 2H), 6.98 (d, 2H), 6.78 (t, 1H), 5.58-5.63 (dd, \(J = 6\) and 12 Hz, 1H), 3.84-3.92 (dd, \(J = 12\) and 18 Hz, 1H), 2.96-3.02 (dd, \(J = 6\) and 18 Hz, 1H); ESI-MS (\(m/z\)): 417.0 [M-H]; Molecular Weight:- 418; Molecular Formula:- C\(_{17}\)H\(_{15}\)ClN\(_6\)O\(_3\)S.

2-(5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-3-yl)-5-(methylthio)-1,3,4-thiadiazole (10a)
Carbon disulfide (70µL, 0.875mmol) was added to a solution of 3a (250 mg, 0.796 mmol) in 25 mL of methanol at 0 °C. Potassium hydroxide (58 mg, 0.875 mmol) was added, and the reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. Iodomethane (135 mg, 0.955 mmol) was added and stirring
continued overnight. The solution was concentrated in vacuum and crude methyl thiocarbamate intermediate and \(p\)-toluene sulfonic acid (145 mg, 0.796 mmol) taken in toluene and heated to reflux for 6 hrs. Toluene was concentrated and crude product purified by column chromatography eluted in ethyl aceate: hexane (1:1), (105 mg, 34 %): mp: 77-78 °C; IR (KBr) 3310, 1603, 1565, 1494 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO- \(d_6\)) \(\delta\) 7.39 (d, 2H), 7.30 (d, 2H), 7.21 (t, 2H), 7.03 (d, 2H), 6.84 (t, 1H), 5.71-5.76 (dd, \(J = 6\) and 12 Hz, 1H), 3.91-3.99 (dd, \(J = 12\) and 18 Hz, 1H), 3.04-3.10 (dd, \(J = 6\) and 18 Hz, 1H), 2.74 (s, 3H); ESI-MS (\(m/z\)): 385.1 [M-H]; Molecular Weight:- 386; Molecular Formula:- C\(_{18}\)H\(_{15}\)ClN\(_4\)O\(_2\)S\(_2\).

4-\([5-(4\text{-chloro-phenyl})-3-(5\text{-methylsulfanyl-}[1,3,4]\text{thiadiazol-2-yl})-4,5\text{-dihydro-pyrazol-1-yl}]\text{-benzenesulfonamide (10b)}\)

The compound 10b was prepared by using same procedure as 10a

Yellow solid, yield 32% mp: 117-118 °C; IR (KBr) 3310, 1603, 1565, 1494 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO- \(d_6\)) \(\delta\) 7.69 (d, 2H), 7.41 (d, 2H), 7.26 (t, 2H), 7.10 (d, 2H), 7.04 (bs, 2H), 5.81-5.86 (dd, \(J = 8\) and 12 Hz, 1H), 3.91-3.99 (dd, \(J = 12\) and 18 Hz, 1H), 3.04-3.10 (dd, \(J = 8\) and 18 Hz, 1H), 2.78 (s, 3H); ESI-MS (\(m/z\)): 464.0 [M-H]; Molecular Weight:- 465; Molecular Formula:- C\(_{18}\)H\(_{16}\)ClN\(_5\)O\(_2\)S\(_3\).

**General procedure for synthesis of alkyl-1,3,4 oxadiazole (13 a-16a):**

To a solution of 3a (1 mole. eq) in dichloromethane 25 mL was added triethyl amine (4 mole. eq) followed by respective acid chloride or anhydride (1mole. eq) viz. acetic anhydride, trifluoroacetic anhydride, ethyl chloro oxalate and ethyl 2-(chlorocarbonyl)acetate at 0 °C under argon. Reaction mixture was allowed to stir at 25-30 °C for 3 hours. Solution of \(p\)-toluenesulfonyl chloride (1 mole. eq) in dichloromethane was added slowly to the reaction mixture at 0 °C. Reaction mixture was stirred at 25-30 °C for 3 hours. Reaction was quenched by addition of 10 mL 5% sodium bicarbonate solution. Organic layer was washed by brine and dried over sodium sulfate. Volatiles were removed under reduced pressure to get 13a-16a.
5-(5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-3-yl)-methyl-1,3,4-oxadiazol (13a).
Yellow solid, yield 78% mp: 190-192; IR (KBr) 3315, 1595, 1570, 1493 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.39 (d, 2H), 7.33 (d, 2H), 7.17 (t, 2H), 7.02 (d, 2H), 6.75 (t, 1H), 5.59-5.64 (dd, \(J = 6\) and 12 Hz, 1H), 3.73-3.80 (dd, \(J = 12\) and 18 Hz, 1H), 2.89-2.95 (dd, \(J = 6\) and 18 Hz, 1H), 2.30 (s, 3H); ESI-MS (m/z): 339.0 [M+H]; Molecular Weight:- 338; Molecular Formula:- C\(_{18}\)H\(_{15}\)ClN\(_4\)O.

5-(5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-3-yl)-trifluoromethyl-1,3,4-oxadiazol (14a).
Yellow solid, yield 68% mp: 184-185 °C; IR (KBr) 3325, 1600, 1574, 1490 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.41 (d, 2H), 7.32 (d, 2H), 7.24 (t, 2H), 7.09 (d, 2H), 6.89 (t, 1H), 5.85-5.90 (dd, \(J = 6\) and 12 Hz, 1H), 3.98-4.05 (dd, \(J = 12\) and 18 Hz, 1H); ESI-MS (m/z): 393.1 [M+H]; Molecular Weight: - 392; Molecular Formula:- C\(_{18}\)H\(_{12}\)ClF\(_3\)N\(_4\)O.

Ethyl 5-(5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-3-yl)-1,3,4-oxadiazole-2-carboxylate (15a)
Yellow solid, yield 80% mp: 125-126 °C; IR (KBr) 3305, 1600, 1575, 1494 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO- \(d_6\)) \(\delta\) 7.38 (d, 2H), 7.23 (d, 2H), 7.16 (t, 2H), 7.01 (d, 2H), 6.78 (t, 1H), 5.49-5.54 (dd, \(J = 6\) and 12 Hz, 1H), 4.36 (q, 2H), 3.63-3.70 (dd, \(J = 12\) and 18 Hz, 1H), 2.79-2.85 (dd, \(J = 6\) and 18 Hz, 1H), 1.334 (t, 3H); ESI-MS (m/z): 397.1 [M+H]; Molecular Weight: - 396; Molecular Formula:- C\(_{20}\)H\(_{17}\)ClN\(_4\)O\(_3\).

Ethyl 2-(5-(5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-3-yl)-1,3,4-oxadiazol-2-yl)acetate (16a)
Yellow solid, yield 75% mp: 130-132 °C; IR (KBr) 3305, 1600, 1575, 1494 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO- \(d_6\)) \(\delta\) 7.40 (d, 2H), 7.310 (d, 2H), 7.21 (t, 2H), 7.03 (d, 2H), 6.84 (t, 1H), 5.72-5.77 (dd, \(J = 6\) and 12 Hz, 1H), 4.27( s, 2H), 4.18 (q, 2H), 4.13-4.18 (dd, \(J = 8\) and 12 Hz, 1H), 3.07-3.13 (dd, \(J = 6\) and 18 Hz, 1H), 1.12 (t, 3H); ESI-MS (m/z): 411 [M+H]; Molecular Weight: - 410; Molecular Formula:- C\(_{21}\)H\(_{19}\)ClN\(_4\)O\(_3\).
**General procedure for synthesis of acids (17a-18a).**

**15a/16a** (1 mole. eq) with lithium hydroxide (1 mole. eq) was stirred in tetrahydrofuran and water 4:1 v/w at 25-30 °C for 18 hours. Tetrahydrofuran was removed under reduced pressure and residue was acidified to pH 4 by 1N hydrochloric acid. Yellow colored solid was filtered on Buchner funnel and dried in oven at 60 °C for 4 hours.

5-(5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-3-yl)-1,3,4-oxadiazole-2-carboxylic acid (**17a**).

Yellow solid, yield 72% mp: 135-136 °C; IR (KBr) 3305, 2900, 1605, 1570, 1494 cm⁻¹; ¹H NMR (400 MHz, DMSO- d6) δ 7.38 (d, 2H), 7.231 (d, 2H), 7.163 (t, 2H), 7.01 (d, 2H), 6.78 (t, 1H), 5.49-5.54 (dd, J = 6 and 12 Hz, 1H), 3.63-3.70 (dd, J = 12 and 18 Hz, 1H); ESI-MS (m/z): 369 [M+H]; Molecular Weight:- 368; Molecular Formula:- C₁₈H₁₃ClN₄O₃.

2-(5-(5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-3-yl)-1,3,4-oxadiazol-2-yl)acetic acid (**18a**)

Yellow solid, yield 70% mp: 140-142 °C; IR (KBr) 3324, 3268, 3084, 2362, 1657, 1622, 1599, 1567 cm⁻¹; ¹H NMR (400 MHz, DMSO- d6) δ 7.40 (d, 2H), 7.32 (d, 2H), 7.21 (t, 2H), 7.03 (d, 2H), 6.84 (t, 1H), 5.72-5.77 (dd, J = 6 and 12 Hz, 1H), 4.15 (s, 2H), 3.94-4.02 (dd, J = 12 and 18 Hz, 1H), 3.07-3.13 (dd, J = 6 and 18 Hz, 1H); ESI-MS (m/z): 383.1 [M+H]; Molecular Weight:- 382; Molecular Formula:- C₁₉H₁₅ClN₄O₃.
**$^1$H NMR (3a)**

![H NMR spectrum](image)

**MS (3a)**

![Mass spectrum](image)
$^1$H NMR (9b)

MS (9b)
IR (9b)

$^1$H NMR (18a)
MS (18a)

IR (18a)
2.2. Section B:

Synthesis of 1,5-diaryl-4,5-dihydro-1\textit{H}-pyrazol-3-yl-5-substituted-[1,2,4]-oxadiazoles and tetrazoles.

2.2.1. Present work:

For a $R = H$, for $b R = \text{SO}_2\text{NH}_2$

\textbf{Scheme 2.2.1. Reagents and condition:} - (J) 1. Ethylchloroformate, Pyridine; 2. Xylene, reflux; (K) 1. Ac\textsubscript{2}O, Et\textsubscript{3}N, 0-5\textdegree C; 2. NaH, CS\textsubscript{2}, 0-5\textdegree C

1,2,4-oxadiazoles were synthesized using carboxamidine 6a and 6b (\textbf{Scheme 2.2.1}). 5-hydroxy-1,2,4-oxadiazoles 11a\textsubscript{b} were prepared by reaction of ethyl chloroformate using pyridine as base and further cyclisation of ethyl carbamate intermediate in xylene at reflux. The 5-mercapto-1,2,4-oxadiazoles 12a\textsubscript{b} were obtained by acylation of 6a\textsubscript{b} and further reaction with carbon disulfide under basic conditions using sodium hydride in THF.

Alkyl-1,2,4-oxadiazoles were synthesized by general procedure (\textbf{Scheme 2.2.2.}) wherein carboxamidine 6a on reaction with various acid chlorides or anhydrides in pyridine as base and solvent at reflux gives 5-alkyl-1,2,4-oxadiazoles 19a-22a. The esters were converted into respective acids 23a and 24a by hydrolysis with LiOH in THF: Water solvent mixture.
5-(4-chloro-phenyl)-N-hydroxy-1-substituted-phenyl-4,5-dihydro-1\textsubscript{H}-pyrazole-3-carbonitrile 5\textsubscript{a,b} were converted into tetrazole derivatives 25\textsubscript{a,b} by reaction of azidotrimethylsilane in toluene using dibutyltin oxide as catalyst (Scheme 2.2.3.) These were further methylated to get 26\textsubscript{a,b}.

**Scheme 2.2.2. Reagent and conditions:**

R\textsuperscript{1}= R'COCI/(R'C)\textsubscript{2}O,Pyridine, heat 100\degree C; O = LiOH:H\textsubscript{2}O, THF, water, 25-30\degree C.

For a R=H and For b SO\textsubscript{2}NH\textsubscript{2}

**Scheme 2.2.3. Reagents and Conditions:**
P=1.TMSN\textsubscript{3},DBTO,Toluene, reflux; Q.MeI, CsCO\textsubscript{3}, DMF 25-30\degree C
2.2.2 Experimental & Spectral Data:

The melting points were determined on a Veego apparatus and are uncorrected. Infrared spectra were recorded on a Bruker spectrophotometer in a KBr disc, and the absorption bands are expressed in cm$^{-1}$. $^1$H-NMR spectra were recorded on a Varian AS 400 MHz spectrometer in CDCl$_3$/DMSO-d-6, chemical shifts (δ) are in ppm relative to TMS, and coupling constants ($J$) are expressed in hertz (Hz). Mass spectra were taken on a Macro mass spectrometer (Waters) by electro-spray method (ES).

3-[5-(4-chloro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-[1,2,4]oxadiazol-5-ol(11a)

Ethylchloroformate (103 mg, 0.995 mmol) was added drop wise to an ice-cooling mixture of 6a (250 mg, 0.796 mol) and pyridine (11.1 g, 1.19 mmol) in dichloromethane (100 mL). The resulting mixture was stirred at 0 °C for 30 min. It was diluted with water and extracted with ethyl acetate. The extract was washed with brine and dried over Na$_2$SO$_4$. The solvent was evaporated in vacuum, and the residue was dissolved in xylene (10 mL). The solution was heated under reflux for 6 hours. The reaction mixture was concentrated in vacuum, to get 11a after column purification using silica gel 100-200 mesh size and hexane:ethylacetate (5:5) as eluting solvents (125mg, 46%): mp: 105-106 °C; IR (KBr) 3320, 2363, 1590, 1572, 1494 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO- $d_6$) δ 7.38 (d, 2H), 7.26 (d, 2H), 7.18 (d, 2H) 6.98 (d, 2H), 6.81(t, 1H), 5.60-5.65 (dd, $J = 8$ and 12 Hz, 1H), 3.70-3.78 (dd, $J = 12$ and 18 Hz, 1H); ESI-MS (m/z): 339.0 [M-H]; Molecular Weight:- 340; Molecular Formula:- C$_{17}$H$_{13}$ClN$_4$O$_2$.

4-[5-(4-chloro-phenyl)-3-(5-hydroxy-[1,2,4]oxadiazol-3-yl)-4,5-dihydro-pyrazol-1-yl]-benzenesulfonamide(11b)

The compound 11b was prepared using same procedure of 11a

Yellow solid, yield 39% mp: 128-132°C; IR (KBr) 3234, 3093, 2362, 1662, 1617, 1413, 1328 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO- $d_6$) δ 7.72 (d, 2H), 7.42 (d, 2H), 7.22 (t, 2H), 7.10 (d, 2H), 7.02 (bs, 2H), 5.76-5.80 (dd, $J = 8$ and 12 Hz, 1H), 3.78-3.86 (dd, $J = 12$ and 18 Hz, 1H), 2.92-2.97 (dd, $J = 8$ Hz and 18 Hz, 1H); ESI-MS (m/z): 418.0 [M-H]; Molecular Weight:- 419; Molecular Formula:- C$_{17}$H$_{14}$ClN$_5$O$_4$S.
3-[(4-chloro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-[1,2,4]oxadiazol-5-thiol(12a)

A mixture of 6a (250 mg, 0.796 mmol), acetic anhydride (82 mg, 0.796 mmol), and triethylamine (221 µL, 1.59 mmol) in CH₂Cl₂ (20 mL) was stirred at room temperature for 2 h. The mixture was washed with water and dried over Na₂SO₄. The solvent was evaporated in vacuum, to give acylated product.

To an ice-cooling mixture of acylated product (230 mg, 0.644 mmol) and carbon disulfide (60 mg, 0.773 mmol) in THF (4 mL) was added sodium hydride (60% in oil, 26 mg, 0.644 mmol), and the resulting mixture was stirred at 0°C for 50 min. The reaction mixture was diluted with 1 N HCl and extracted with ethyl acetate. The extract was washed with water and dried (Na₂SO₄). The solvent was evaporated in vacuum, and purified by column chromatography using 100:200 mesh size silica gel and hexane: ethyl acetate (1:1) as eluting solvent to get 12a (120 mg, 43%): mp: 125-127 °C; IR (KBr) 3315, 2362, 1595, 1570, 1493 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 7.39 (d, 2H), 7.30 (d, 2H), 7.21 (t, 2H), 7.03(d, 2H), 6.85 (t, 1H), 5.71-5.76 (dd, J = 6 and 12 Hz, 1H), 3.82-3.90 (dd, J = 12 and 18 Hz, 1H), 2.95-3.01 (dd, J = 6 and 18 Hz, 1H); ESI-MS (m/z): 357 [M+H]; Molecular Weight:- 356; Molecular Formula:- C₁₇H₁₃ClN₄O₄.

4-[(5-(4-chloro-phenyl)-3-(5-mercapto-[1,2,4]oxadiazol-3-yl)-4,5-dihydro-pyrazol-1-yl]-benzenesulfonamide(12b)

The compound 12b was prepared using same procedure for 12a Yellow solid, yield 38% mp: 125-127 °C; IR (KBr) 3315, 2362, 1595, 1570, 1493 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 7.65 (d, 2H), 7.40 (d, 2H), 7.30 (d, 2H), 7.15(d, 2H), 7.06 (bs, 2H), 5.82-5.87 (dd, J = 8 and 16 Hz, 1H), 3.88-3.96 (dd, J = 12 and 18 Hz, 1H), 3.00-3.06 (dd, J = 8Hz and 18 Hz, 1H); ESI-MS (m/z): 434 [M-H]; Molecular Weight:- 435; Molecular Formula:- C₁₇H₁₄ClN₅O₃S₂.

General procedure for synthesis of alkyl-1,2,4 oxadiazole (19a-22a)

To a solution of 6a (1 mole. eq) in pyridine (10 v/w) was added acid chloride or anhydride (1 mole. eq) viz. acetic anhydride, trifluoroacetic anhydride, ethyl chloro oxalate and ethyl 2-(chlorocarbonyl)acetate at 0 °C under argon. Reaction mixture was
allowed to stir at 25-30 °C for 3 hours. Solution was heated at 100 °C for 2 hrs. Reaction was cooled and pyridine removed under reduced pressure and residue quenched by addition of 2N HCl solution and extracted by ethyl acetate thrice. Organic layer was washed by brine and dried over sodium sulfate. Volatiles were removed under reduced pressure to get 19a-22a

3-[5-(4-chloro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-5-methyl-[1,2,4]oxadiazole(19a)
Yellow solid, yield 88% mp: 154-156 °C; IR (KBr) 3359, 2930, 2362, 1605, 1507, 1488 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.39 (d, 2H), 7.28 (d, 2H), 7.20 (d, 2H), 7.18 (d, 2H), 6.80 (t, 1H), 5.66-5.70 (dd, J = 8 and 12 Hz, 1H), 3.87-3.94 (dd, J = 12 and 18 Hz, 1H), 2.97-3.03 (dd, J = 8 and 18 Hz, 1H), 2.62 (s, 3H); ESI-MS (m/z): 337.1 [M-H]; Molecular Weight:- 338; Molecular Formula:- C₁₈H₁₅ClN₄O.

3-[5-(4-chloro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-5-trifluoromethyl-[1,2,4]oxadiazole (20a)
Yellow solid, yield 68% mp: 148-150 °C; IR (KBr) 3310, 2361, 1603, 1576, 1492 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.41 (d, 2H), 7.32 (d, 2H), 7.24 (t, 2H), 7.09 (d, 2H), 6.89 (t, 1H), 5.85-5.89 (dd, J = 6 and 12 Hz, 1H), 3.98-4.04 (dd, J = 12 and 18 Hz, 1H), 3.12-3.18 (dd, J = 8 and 18 Hz, 1H); ESI-MS (m/z): 393.1 [M+H]; Molecular Weight:- 392; Molecular Formula:- C₁₈H₁₂ClF₃N₄O.

3-[5-(4-chloro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-[1,2,4]oxadiazole-5-carboxylic acid ethyl ester (21a)
Yellow solid, yield 78% mp: 108-110 °C; IR (KBr) 3305, 2360, 1685, 1605, 1576, 1493 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.38 (d, 2H), 7.23 (d, 2H), 7.16 (t, 2H), 7.01 (d, 2H), 6.78 (t, 1H), 5.49-5.54 (dd, J = 6 and 12 Hz, 1H), 4.36 (q, 2H), 3.63-3.70 (dd, J = 12 and 18 Hz, 1H), 2.79-2.85 (dd, J = 6 and 18 Hz, 1H), 1.33 (t, 3H); ESI-MS (m/z): 397.0 [M+H]; Molecular Weight:- 396; Molecular Formula:- C₂₆H₁₇ClN₄O₃.

{3-[5-(4-chloro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-[1,2,4]oxadiazol-5-yl}-acetic acid ethyl ester (22a)
Yellow solid, yield 71% mp: 114-115 °C; IR (KBr) 3455, 2930, 2361, 1742, 1590, 1490 cm⁻¹; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.40 (d, 2H), 7.31 (d, 2H), 7.21 (t, 2H), 7.03 (d, 2H), 6.84 (t, 1H), 5.72-5.77 (dd, \(J = 6\) and 12 Hz, 1H), 4.35 (s, 2H), 4.18 (q, 2H), 4.13-4.18 (dd, \(J = 8\) and 18 Hz, 1H), 3.07-3.13 (dd, \(J = 6\) and 18 Hz, 1H), 1.12 (t, 3H); ESI-MS (m/z): 411.1 [M+H]; Molecular Weight:- 410; Molecular Formula:- C\(_{21}\)H\(_{19}\)ClN\(_4\)O\(_3\).

**General procedure for synthesis of acids (23a-24a).**

21a/22a (1 mole. eq) with lithium hydroxide (1 mole. eq) was stirred in tetrahydrofuran and water 4:1 v/w at 25-30 °C for 18 hours. Tetrahydrofuran was evaporated under reduced pressure and residue was acidified to pH 4 by 1N hydrochloric acid. Yellow colored solid was filtered on Buchner funnel and dried in oven at 60 °C for 4 hours.

3-[5-(4-chloro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-[1,2,4]oxadiazole-5-carboxylic acid (23a).

Yellow solid, yield 78% mp: 130-134 °C; IR (KBr) 3315, 2920, 2363, 1604, 1572, 1494 cm⁻¹; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 7.38 (d, 2H), 7.23 (d, 2H), 7.16 (t, 2H), 7.01 (d, 2H), 6.78 (t, 1H), 5.49-5.54 (dd, \(J = 6\) and 12 Hz, 1H), 3.63-3.70 (dd, \(J = 12\) and 18 Hz, 1H), 2.79-2.85 (dd, \(J = 6\) and 18 Hz, 1H); ESI-MS (m/z): 369 [M+H]; Molecular Weight:- 368; Molecular Formula:- C\(_{18}\)H\(_{13}\)ClN\(_4\)O\(_3\).

{3-[5-(4-chloro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-[1,2,4]oxadiazol-5-yl}acetic acid (24a)

Yellow solid, yield 81% mp: 105-106 °C; IR (KBr) 3313, 2362, 1606, 1570, 1490 cm⁻¹; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 7.38 (d, 2H), 7.28 (d, 2H), 7.21 (d, 2H), 7.04 (d, 2H), 6.82 (t, 1H), 5.69-5.74 (dd, \(J = 8\) and 12 Hz, 1H), 4.30 (s, 2H), 3.88-3.96 (dd, \(J = 12\) and 18 Hz, 1H), 3.00-3.04 (dd, \(J = 8\) and 18 Hz, 1H); ESI-MS (m/z): 383 [M+H]; Molecular Weight:- 382; Molecular Formula:- C\(_{19}\)H\(_{15}\)ClN\(_4\)O\(_3\).

**General procedure for synthesis of Tetrazole derivatives (25a, 25b)**

5a/5b (1 mol. eq) with azidotrimethyl silane (1.5 mole. eq) and dibutyl tin oxide (0.1 mole. eq) were refluxed in toluene for 4 hours. Reaction mixture cooled to room temperature and to this 5 mL methanol was added. Volatiles removed under reduced
pressure. Residue obtained was stirred in 1N HCl. Solid precipitated was filtered and washed with 2*20 mL water dried in oven at 50 °C for 4 hours.

5-[5-(4-chloro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-2H-tetrazole (25a)
Yellow solid, yield 83% mp: 175-177 °C; IR (KBr) 3386, 2362, 2336, 1647, 1596, 1494 cm⁻¹; ¹H NMR (400 MHz, DMSO- d6) δ 7.30 (d, 2H), 7.23 (d, 2H), 7.02 (d, 2H), 6.77 (d, 2H), 6.66 (t, 1H), 5.43-5.48 (dd, J = 8 and 12 Hz, 1H), 3.86-3.93 (dd, J = 12 and 18 Hz, 1H), 2.97-3.02 (dd, J = 8 and 18 Hz, 1H); ESI-MS (m/z): 323.1 [M-H]; Molecular Weight:- 324; Molecular Formula:- C₁₆H₁₃ClN₆.

4-[5-(4-chloro-phenyl)-3-(1H-tetrazol-5-yl)-4,5-dihydro-pyrazol-1-yl]-benzenesulfonamide (25b)
Yellow solid yield 68% mp: 163-165 °C; IR (KBr) 3313, 2362, 2336, 1640, 1598, 1495 cm⁻¹; ¹H NMR (400 MHz, DMSO- d6) δ 7.59 (d, 2H), 7.40 (d, 2H), 7.31 (d, 2H), 7.09(d, 2H), 7.05 (bs, 2H), 5.73-5.77 (dd, J = 8 and 12 Hz, 1H), 4.02-4.10 (dd, J = 12 and 18 Hz, 1H), 3.15-3.21 (dd, J = 8 and 18 Hz, 1H); ESI-MS (m/z): 402.0 [M-H]; Molecular Weight:- 403; Molecular Formula:- C₁₆H₁₄ClN₇O₂S.
General procedure for synthesis of Methyl tetrazole derivatives (26a, 26b).

Iodomethane (1.5 mole. eq) was added to 25a/25b (1 mole. eq) and cesium carbonate in DMF at 5-10°C. Reaction mixture was stirred for 4 hours at 25-30°C. Reaction mixture poured in cold water stirred for 10 minutes. The solid separated was filtered on Buckner funnel, washed by water and dried in oven at 60°C. Purified by column chromatography to obtain major isomer eluted in hexane: ethyl acetate (7:3) using 100:200 mesh size silica gel.

5-\([5-(4\text{-chloro-phenyl})-1\text{-phenyl}-4,5\text{-dihydro}-1\text{H}-\text{pyrazol-3-yl}]-2\text{-methyl}-2\text{H}-\text{tetrazole}\) (26a)

Yellow solid, yield 65% mp: 175-177 °C; IR (KBr) 3313, 2361, 1656, 1570, 1490 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 7.30 (d, 2H), 7.23 (d, 2H), 7.02 (d, 2H), 6.77 (d, 2H), 6.66 (t, 1H), 5.43-5.49 (dd, \(J = 8\) and 12 Hz, 1H), 3.86-3.93 (dd, \(J = 12\) and 18 Hz, 1H), 2.97-3.02 (dd, \(J = 8\) and 18 Hz, 1H) 2.80 (s, 3H); ESI-MS (\(m/z\)): 337.1 [M-H]; Molecular Weight:- 338; Molecular Formula:- C\(_{17}\)H\(_{15}\)ClN\(_6\).

4-\([5-(4\text{-chloro-phenyl})-3\text{-}(2\text{-methyl-2H-tetrazol-5-yl})-4,5\text{-dihydro-pyrazol-1-yl}]-\text{benzenesulfonamide}\) (26b)

Yellow solid, yield 63% mp: 163-165 °C; IR (KBr) 3386, 2362, 2336, 1647, 1596, 1494 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 7.59 (d, 2H), 7.40 (d, 2H), 7.31 (d, 2H), 7.09 (d, 2H), 5.73-5.77 (dd, \(J = 8\) and 12 Hz, 1H), 4.02-4.10 (dd, \(J = 12\) and 18 Hz, 1H), 3.15-3.21 (dd, \(J = 8\) and 18 Hz, 1H), 3.10 (bs, 2H), 2.82 (s, 3H); ESI-MS (\(m/z\)): 418.0 [M+H]; Molecular Weight:- 417; Molecular Formula:- C\(_{17}\)H\(_{16}\)ClN\(_7\)O\(_2\)S.
$^{1}$H NMR (11b)

MS (11b)
IR (11b)

\[ \text{IR in KBr Pellets} \]

\[ \text{C:\OPUS\NTWEAS\MAR-2011\MMS.27} \]

\[ 3/14/1111:37:20 AM \]

\[ \text{MMS} \]

\[ \text{H NMR (19a)} \]

\[ \text{\textsuperscript{1}H NMR (19a)} \]
\(^1\)H NMR (22a)

MS (22a)
IR (22a)

\[ \text{IR spectrum showing chemical shifts and absorptions.} \]

\[ \text{Chemical structure diagram.} \]

\(^1\text{H NMR (26b)}\)

\[ \text{NMR spectrum showing proton peaks.} \]

\[ \text{Chemical structure diagram showing proton positions.} \]
MS (26b)

IR (26b)
2.3. Section C

Biological evaluation of diaryl pyrazoline compounds for anti-inflammatory activity and analgesic activity

2.3.1. Anti-inflammatory activity:

The *in-vivo* anti-inflammatory activity of the synthesized compounds 7a,b - 26a,b was evaluated by carrageenan-induced footpad edema method. Diclofenac Sodium was used as a standard drug for comparison. All the compounds were administered orally and assayed at a dose level of 100 mg/kg of body weight. The obtained pharmacological results revealed that replacement of trifluoromethyl functionality with acid heterocycles varied the anti-inflammatory property of compounds from proinflammatory to equally potent compound as compared to standard.

**a. Anti-inflammatory activity by carrageenan footpad edema:**

The *in-vivo* anti-inflammatory activity of the synthesized compounds 7a,b-26a,b was determined following the carrageenan-induced paw edema method in rat. Carrageenan solution (1% w/v) was prepared by dissolving 100 mg of carrageenan (Marine Colloidal Div., Springfield, NJ) in 10 mL of sterile saline (0.9%) solution. Male Wistar rats were orally dosed with compound (at dose of 100 mg/kg p.o. as a suspension in 5% carboxymethylcellulose) 1 h before carrageenan challenge. Diclofenac sodium given at 100 mg/kg p.o. was used as a standard drug. Foot paw oedema was induced by injecting 0.05 mL of the carrageenan solution subcutaneously into the planter portion of the right hind paw of each rat under light anesthesia. Initial foot paw volume was measured immediately by digital plethysmometry (Ugo Basile Digital Plethysmometer, Model-7140, Italy) following carrageenan challenge. Edema was measured 4 h after carrageenan administration. The swelling in each test group of animals were used to calculate the percent inhibition ± SEM of edema achieved by the compound at the test dose compared with the vehicle control group. The results are summarized in Table 2.3.1.
Table 2.3.1. Results of anti-inflammatory effect of pyrazoline derivatives against carrageenan induced footpad edema model in rats.

<table>
<thead>
<tr>
<th>Compound Entry</th>
<th>Test compounds</th>
<th>% Rise in Inflammation at 4 hours</th>
<th>% Protection at 4th hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control STD</td>
<td>control</td>
<td>84.22 ± 6</td>
<td>50.93 ± 7</td>
</tr>
<tr>
<td>5a</td>
<td>R=H</td>
<td>51.01 ± 2</td>
<td>39.43 ± 6</td>
</tr>
<tr>
<td>5b</td>
<td>R= SO₂NH₂</td>
<td>42.38 ± 1</td>
<td>49.67 ± 1</td>
</tr>
<tr>
<td>7a</td>
<td>R=H, R₁= OH</td>
<td>63.78 ± 3</td>
<td>24.26 ± 4</td>
</tr>
<tr>
<td>7b</td>
<td>R=SO₂NH₂, R₁= OH</td>
<td>54.42 ± 4</td>
<td>35.38 ± 5</td>
</tr>
<tr>
<td>8a</td>
<td>R=H, R₁= SH</td>
<td>56.64 ± 1</td>
<td>32.74 ± 1</td>
</tr>
<tr>
<td>8b</td>
<td>R=SO₂NH₂, R₁= SH</td>
<td>53.23 ± 4</td>
<td>36.76 ± 3</td>
</tr>
<tr>
<td>9a</td>
<td>R=H, R₁= NH₂</td>
<td>80.42 ± 2</td>
<td>4.50 ± 4</td>
</tr>
<tr>
<td>9b</td>
<td>R=SO₂NH₂, R₁= NH₂</td>
<td>68.74 ± 2</td>
<td>18.38 ± 2</td>
</tr>
<tr>
<td>10a</td>
<td>R=H, R₁= SCH₃</td>
<td>74.24 ± 1</td>
<td>11.84 ± 3</td>
</tr>
<tr>
<td>10b</td>
<td>R=SO₂NH₂, R₁= SCH₃</td>
<td>62.36 ± 4</td>
<td>26.12 ± 2</td>
</tr>
<tr>
<td>11a</td>
<td>R=H, R₁= OH</td>
<td>56.69 ± 3</td>
<td>32.68 ± 1</td>
</tr>
<tr>
<td>11b</td>
<td>R=SO₂NH₂, R₁= OH</td>
<td>45.32 ± 2</td>
<td>46.18 ± 3</td>
</tr>
<tr>
<td>12a</td>
<td>R=H, R₁= SH</td>
<td>50.10 ± 1</td>
<td>40.51 ± 5</td>
</tr>
<tr>
<td>12b</td>
<td>R=SO₂NH₂, R₁= SH</td>
<td>43.10 ± 3</td>
<td>48.82 ± 3</td>
</tr>
<tr>
<td>13a</td>
<td>R₁= CH₃</td>
<td>70.36 ± 7</td>
<td>16.45 ± 8</td>
</tr>
<tr>
<td>14a</td>
<td>R₁= CF₃</td>
<td>123.74 ± 7</td>
<td>-46.9 ± 4</td>
</tr>
<tr>
<td>15a</td>
<td>R₁= COOEt</td>
<td>80.43 ± 3</td>
<td>4.50 ± 4</td>
</tr>
<tr>
<td>16a</td>
<td>R₁=CH₂COOEt</td>
<td>99.05 ± 2</td>
<td>-17.6 ± 9</td>
</tr>
<tr>
<td>17a</td>
<td>R₁= COOH</td>
<td>100.47 ± 6</td>
<td>-19.3 ± 3</td>
</tr>
<tr>
<td>18a</td>
<td>R₁= CH₂COOH</td>
<td>77.93 ± 2</td>
<td>7.46 ± 2</td>
</tr>
<tr>
<td>19a</td>
<td>R₁= CH₃</td>
<td>42.75 ± 6</td>
<td>49.24 ± 1</td>
</tr>
<tr>
<td>20a</td>
<td>R₁= CF₃</td>
<td>42.57 ± 4</td>
<td>49.45 ± 7</td>
</tr>
<tr>
<td>21a</td>
<td>R₁= COOEt</td>
<td>67.08 ± 2</td>
<td>20.35 ± 2</td>
</tr>
<tr>
<td>22a</td>
<td>R₁= CH₂COOEt</td>
<td>58.16 ± 3</td>
<td>30.94 ± 2</td>
</tr>
<tr>
<td>23a</td>
<td>R₁= COOH</td>
<td>72.09 ± 1</td>
<td>14.40 ± 4</td>
</tr>
<tr>
<td>24a</td>
<td>R₁= CH₂COOH</td>
<td>78.25 ± 3</td>
<td>7.08 ± 5</td>
</tr>
<tr>
<td>25a</td>
<td>R=H</td>
<td>52.38 ± 1</td>
<td>37.80 ± 3</td>
</tr>
<tr>
<td>25b</td>
<td>R= SO₂NH₂</td>
<td>38.25 ± 4</td>
<td>54.58 ± 3</td>
</tr>
<tr>
<td>26a</td>
<td>R=H</td>
<td>55.32 ± 5</td>
<td>34.31 ± 6</td>
</tr>
<tr>
<td>26b</td>
<td>R= SO₂NH₂</td>
<td>43.30 ± 2</td>
<td>48.58 ± 4</td>
</tr>
</tbody>
</table>
A representation of % rise in paw volume after administration of drug and standard in a group of animals is shown in following Figure 2.3.1.

**Result and discussion:**

The experimental data of footpad edema have revealed very interesting result. It can be concluded from above data that, the attempt of replacing trifluoromethyl group with different heterocycle like 1,3,4-oxadiazole, 1,2,4-oxadiazole, 1,3,4-thiadiazole and tetrazole have shown variety in activity from proinflammatory effect to equipotent activity as compared to standard Diclofenac sodium.

In 1,3,4-heterodiazoles series, compounds 7a, 8a, 9a, 10a having hydroxyl, thiol, amino and thiomethyl substituent’s respectively showed very low % protection against inflammation. 1,3,4-oxadiazoles 13a, 14a, 15a, 16a, 17a, 18a having alkyl substituent’s like methyl, trifluoromethyl, ethyl ester , acetic acid ethyl ester, carboxylic acid and acetic acid carboxylic acid showed proinflammatory behavior. It was assumed that free carboxylic acid present in 17a and 18a may be responsible for the proinflammatory behavior, while positioning of trifluoromethyl group present in 1,3,4-oxadiazoles 14a may be considered responsible for this effect. The most potent compound in 1,3,4-heterodiazole series was 8a which has 32.74 % inhibition against inflammation.
Surprisingly, we have not noticed any influence of sulfonamide group introduction on 1-phenyl ring. Among the compounds 7b, 8b, 9b, 10b comprising of sulfonamide group in 1,3,4-heterodiazole series only compound 8b showed 36.76% protection.

The 1,2,4-oxadiazoles on the other hand showed much increase in anti-inflammatory activity as compared to 1,3,4-heterodiazoles. The compounds 11a, 11b, 12a, 12b were found to be less active as compared to the standard diclofenac sodium, and they have shown 32-48% protection against inflammation, wherein 12b which was equipotent to the standard. We have noticed very different observation in case of alkyl substituent on 1,2,4-oxadiazoles like 19a, 20a, 21a, 22a, 23a, 24a having methyl, trifluoromethyl, ethyl ester, acetic acid ethyl ester, carboxylic acid and acetic acid carboxylic acid respectively. Out of these compounds, 19a and 20a were equipotent to the standard showing % protection of 49.24 and 49.45 respectively. Totally reverse trend was observed as compared to proinflammatory behavior of 1,3,4-oxadiazole derivatives 13a, 14a. Acids 23a and 24a has confirmed, that the free carboxylic acid substituent on heterodiazoles was responsible for proinflammatory behavior of the compounds.

The tetrazoles 25a, 25b, 26a, 26b were equipotent to the standard diclofenac sodium, while the 4 substituted sulfonamide group on 1-phenyl ring of tetrazole analogue showed increase in anti-inflammatory activity than that of their parent unsubstituted compounds. 25b was the most potent derivative among all the derivatives showing 54.58% protection at 4th hour. Nitriles substituted compound 15a, 15b also showed good activity, 15b showing 49.67% protection.

b. COX-2 inhibition assay.\(^{19}\)

Inhibition of Cyclooxygenases and therefore prostaglandin production is the common mechanism of action of NSAIDs (Nonsteroidal anti-inflammatory drugs). COX exists as two isoforms COX-1 and COX-2. Cyclooxygenase-1 is constitutive whereas Cyclooxygenases 2 is induced by pro-inflammatory cytokines and endotoxin such as Lipopolysachharide (LPS) in cells. Arachidonic acid is stored esterified in phospholipids of cell membranes. It is released from the cell membrane upon demand via phospholipase A2. The free Arachidonic acid is then oxygenated by cyclooxygenase or lipoxygenase pathway. Thromboxane is the end product of Cyclooxygenase pathways and a measure of COX activity.
Selected Compounds were screened for its ability to inhibit Human whole blood COX-2 at the concentration of 10 µM in duplicates using Assay Designs TXB2 EIA kit (Cat. No. 900-002). These compounds were compared with Diclofenac sodium at 20 µM concentration. The results are summarized in Table 2.3.2.

**Assay Procedure**

To the heparinized blood, aspirin was added at a conc. of 12ug/mL, to inactivate COX-1 and incubated for 6h, following which blood was half diluted and treated with 10 µM concentration of test compounds 1 hour at 37 °C/5%CO2 in a 96-well plate. After preincubation with test compound, LPS was added at a final con of 10ug/mL and incubated for another 18 hrs. 250 µL of 1:1 diluted blood was added for both the tests, and 50 µL of 7X concentration of LPS and test compounds were added to make a final volume of 350µLµ. The TXB2 assay is based on the competitive binding technique in which TXB2 present in a sample competes with a fixed amount of alkaline phosphatase-labeled TXB2 for sites on a rabbit polyclonal antibody. During the incubation, the polyclonal antibody binds to the goat anti-rabbit antibody coated onto the microplate. Following a wash to remove excess conjugate and unbound sample, a substrate solution is added to the wells to determine the bound enzyme activity. The color development is stopped and the absorbance is read at 405 nm. The intensity of the color is inversely proportional to the concentration of TXB2 in the sample. The assay includes following controls:

Blank: Background absorbance caused by the substrate (p-nitrophenyl phosphate).

TA (Total Activity): Total enzymatic activity of the alkaline phosphatase conjugated to TXB2.

NSB (Non-Specific Binding): Non-immunological binding of the conjugated TXB2 to the well.

B0 (Maximum Binding): Maximum amount of TXB2 conjugate bound by the antiserum in the absence of free analyte.

Standards: A 3-fold serial dilution was done starting with highest standard (3333pg/mL) in assay buffer. Standards # 1 (3333pg/mL) serves as highest and Std # 7 (13.7pg/mL) as lowest.
2.3.2 Analgesic activity by acetic acid writhing method:

For this test, male Wistar rats were used. The drug treatment was same as stated above. The treatments were administered 1 h prior to acetic acid injection. For induction of pain, 0.25 mL of 0.6% v/v acetic acid was injected i.p. Total number of writhings were counted along the period of 30 min following acetic acid injection. Average numbers of writhings in the control group were used to calculate percentage inhibition of writhings in other groups. The results are summarized in Table 2.3.2.

Table 2.3.2. Results of analgesic effect of selected pyrazoline derivatives against acetic acid induced writhing method and % inhibition of COX-2 at 10 µM.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Test Compound</th>
<th>No. of Writhings</th>
<th>% inhibition of COX-2(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19a</td>
<td>R(^1)= CH(_3)</td>
<td>22.2 ± 1.35</td>
<td>4</td>
</tr>
<tr>
<td>20a</td>
<td>R(^1)= CF(_3)</td>
<td>24.5 ± 2.34</td>
<td>6</td>
</tr>
<tr>
<td>25b</td>
<td>R= SO(_2)NH(_2)</td>
<td>18.6 ± 1.65</td>
<td>7</td>
</tr>
<tr>
<td>26b</td>
<td>R= SO(_2)NH(_2)</td>
<td>22.2 ± 2.65</td>
<td>11</td>
</tr>
<tr>
<td>Control</td>
<td>Control</td>
<td>29.1 ± 1.21</td>
<td>0</td>
</tr>
<tr>
<td>STD</td>
<td>Diclofenac</td>
<td>6.8 ± 0.51</td>
<td>99(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Data are indicated as percentage of inhibition at 10 µM mean of 2 tests. \(^b\) Diclofenac was assayed at 20 µM for COX-2.

Effect of each compound on no. of writhings induced by acetic acid in experimental animal is represented in a graph as shown in Figure 2.3.2.
Result and Discussion:

4 selected compounds were tested for their analgesic activity by acetic acid induced writhing method. All compounds 19a, 20a, 25b and 26b showed mild analgesic activity. Out of four tested compounds, 25b showed 36.08 % decrease in writhing and all other compound were ineffective in reducing the number of writhing.

The % inhibition of COX-2 in human whole blood for the compounds 19a, 20a, 25b, 26b ranged from 4-11% at 10µM concentration.

Conclusion:

In conclusion, acid heterocycles like tetrazole and 1,2,4-oxadiazole showed better anti-inflammatory activity than amide heterocycles like 1,3,4-heterodiazole. The sulfonamide substitution on para position of 1-phenyl ring enhances the potency effectively. From the above experimental data, we can summarize that, the compound 25b which is a tetrazole derivative was found to be the most potent anti-inflammatory compound in the present series as compared to diclofenac sodium. Even the des-sulfonamide 1,2,4-oxadiazole derivatives 19a, 20a also showed good in-vivo anti-inflammatory potency. In the present series, the pyrazoline compounds being an enantiomeric mixture were found good anti-
inflammatory agents. So, it would be interesting to resolve the racemic mixture and to study the anti-inflammatory efficacy of individual isomer. Therefore, compounds 19a, 20a, 25b deserves further attention in order to develop new leads in this series.
2.4. References:

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