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Synthesis and anti-inflammatory activity of some new 4,5-dihydro-1,5-diaryl-1*H*-pyrazole-3-substituted-heteroazole derivatives

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ABSTRACT

A series of 4,5-dihydro-1,5-diaryl-1*H*-pyrazole-3-substituted-heteroazoles were designed and synthesized in order to obtain new compounds with potential anti-inflammatory activity. The title compounds were screened for in vivo anti-inflammatory activity by using Carrageenan induced rat paw edema method. Diclofenac sodium was used as a standard drug for comparison. Out of the 30 compounds tested, compound **19a**, **19b**, **25a**, **25b** exhibited significant anti-inflammatory activity. Selected compounds were also screened for in vitro COX-2 inhibition assay and analgesic activity in the acetic acid induced writhing model.

Cyclooxygenases (COXs) are key enzymes in the synthesis of prostaglandin H_2 which is a precursor for the biosynthesis of prostaglandins, thromboxanes, and prostacyclins.[1] COX enzymes exist in two isoforms: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2).[2] The COX-1 enzyme is constitutively expressed and is critical for protection of gastric mucosa, platelet aggregation, and renal blood flow whereas the COX-2 enzyme is inducible and expressed during inflammation, pain, and oncogenesis.[3] The association of COX-2 with induced inflammation has led to the hypothesis that selective inhibition of COX-2 over COX-1 might provide good anti-inflammatory agents with reduced side effects than classical NSAIDs. Therefore, selective COX-2 inhibitors (coxibs) with better safety profile have been marketed as a new generation NSAIDs.[4,5] But careful prospective examination of coxibs has revealed unexpected cardiovascular adverse effect.[6] Therefore, development of novel compounds having anti-inflammatory and analgesic activities with an improved safety profile is still a necessity. In addition, inflammation is known not only as a symptom of great deal of common diseases but also as an early phase of some life-threatening diseases such as cancer, heart vascular diseases and Alzheimer's dementia. Thus the discovery of novel anti-inflammatory agents has been attracting a lot of interests.

Pyrazoles and its derivatives are an important nitrogenous five-membered heterocyclic component of the drugs. Literature survey revealed that numerous pyrazole 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 analogues 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.[7] Besides these, many pyrazole derivatives are also reported in literature as having potent anti-inflammatory activity[8–11] (Fig. 1).

Recently Javed et al. reported synthesis and anti-inflammatory activity of 1,3,5-trisubstituted pyrazoline bearing benzene sulfonamide moiety.[12] These compounds exhibited excellent in vitro and in vivo anti-inflammatory profile.

Based on the report of Javed et al., we have considered the 1,5-diaryl pyrazoline moiety as our parent nucleus. The present work describes the synthesis of 4,5-dihydro-1,5-diaryl-1*H*-pyrazole-3-substituted-heteroazole derivatives with the aim to discover novel and potent anti-inflammatory agents. Here, we have synthesized 1,5-diaryl-pyrazoline-3-carbohydrazide, carbonitrile and carboxamidine compounds. These compounds were then converted to acid or amide heterocycles such as 1,3,4-oxadiazole, 1,3,4-thiadiazole, 1,2,4-oxadiazole, tetrazole bearing acidic, basic or neutral functionality. Keeping the 1,5-diaryl pyrazoline moiety constant, we have studied the effect of different azoles on the anti-inflammatory effect in the series.

The 4-(4-chlorophenyl)-2-oxo-but-3-enoic acid ethyl ester **1** was prepared by esterification of potassium salt of 4-(4-chloro-

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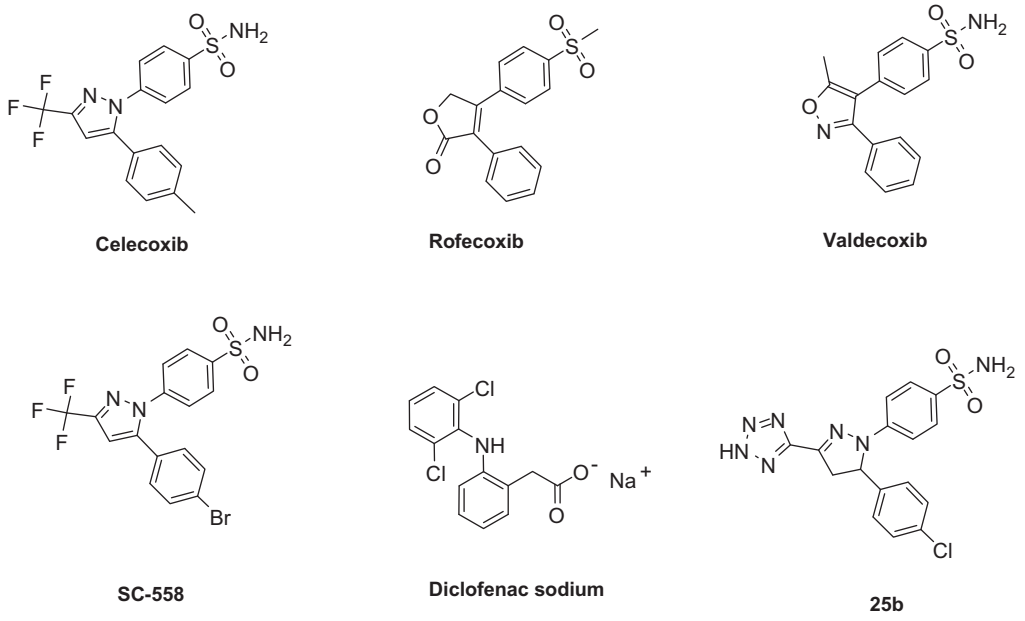
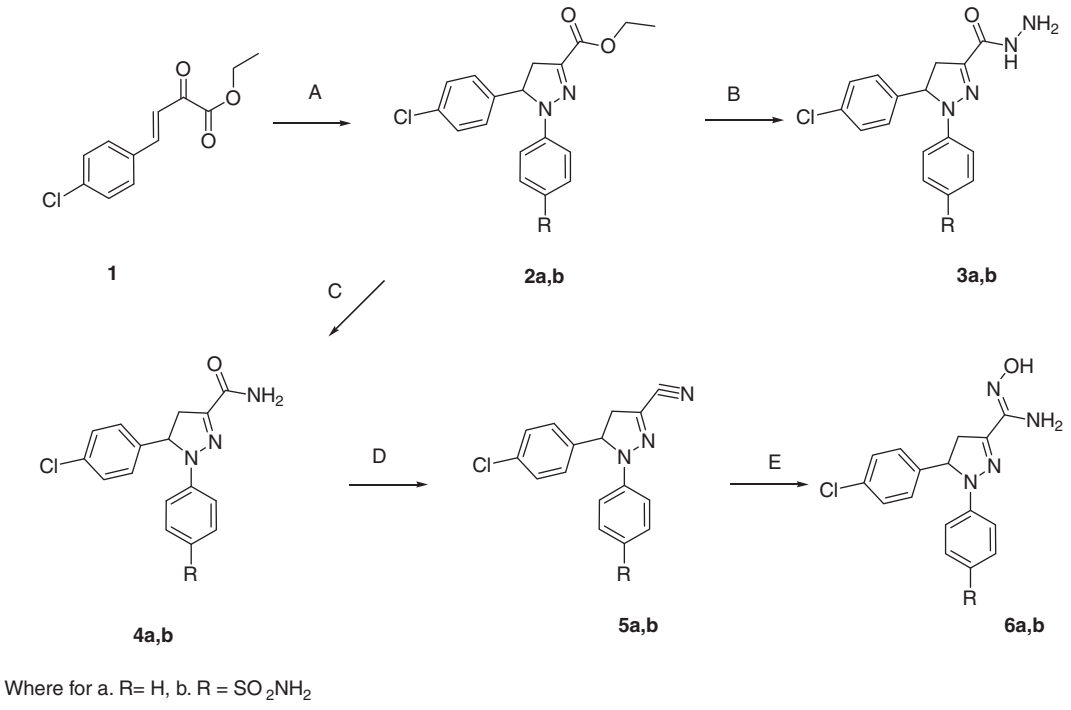


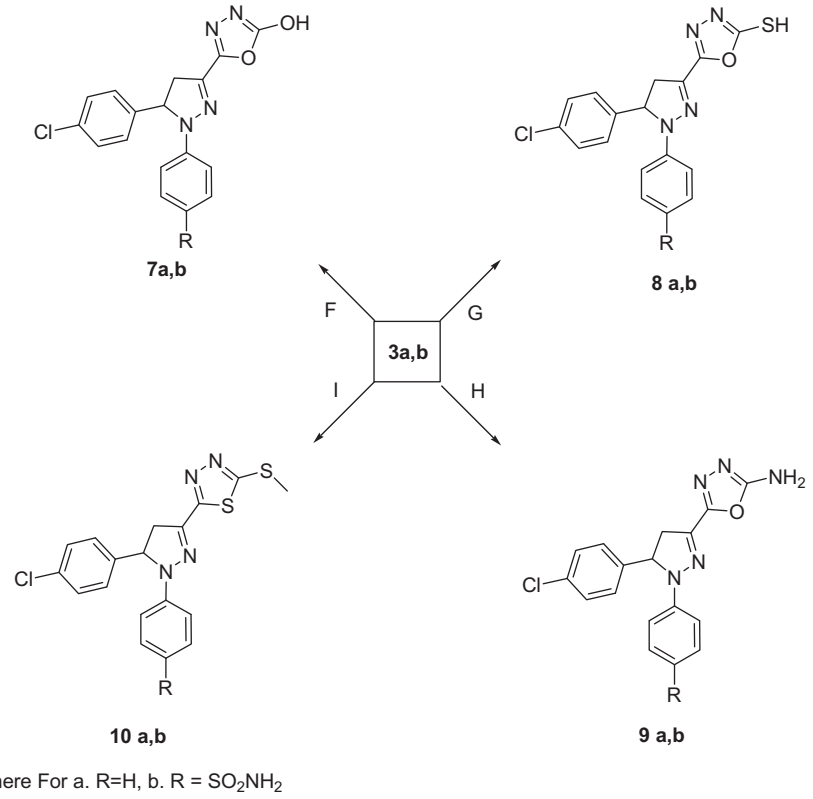
Figure 1. Structure of marketed NSAIDs and synthesized compound.



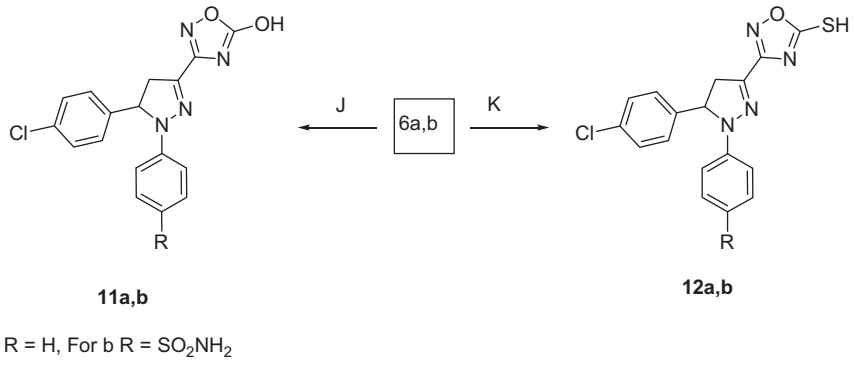
Scheme 1. Reagents and conditions: (A) *p*-*R*-phenyl hydrazine, ethanol, acetic acid, reflux; (B) hydrazine hydrate, ethanol, reflux; (C) 30% aq ammonia, THF, 50–55 °C; (D) DMF, oxallyl chloride, 0 °C; (E) hydroxyl amine HCl, Na_2CO_3, methanol, 25–30 °C.

phenyl)-2-oxobut-3-enoic acid[13] in ethanol using thionyl chloride (Scheme 1). Ester on reaction with different substituted phenyl hydrazine hydrochlorides in ethanol and acetic acid furnished ethyl 5-(4-chloro-phenyl)-4,5-dihydro-1-phenyl-1*H*-pyrazole-3-carboxylate **2a** and 5-(4-chloro-phenyl)-1-(4-sulfamoyl-phenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic acid ethyl ester **2b**. These esters were further converted to respective hydrazides **3a**,**b** using hydrazine hydrate in ethanol at reflux. The esters **2a**,**b** were also used to prepare amide derivatives **4a**,**b** using ammonium hydroxide in tetrahydrofuran. The nitrile cores **5a**,**b** were prepared by dehydrating corresponding amides using *N*,*N*′-dimethylformamide and oxalyl chloride as dehydrating agent. Nitriles were further reacted with hydroxylamine hydrochloride in presence of Na_2CO_3 to furnish amidoxime cores **6a**,**b**.

The carbohydrazides **3a,b** were converted to different amide hetrocycles (Scheme 2). The 2-hydroxy-1,3,4-oxadiazoles **7a,b** were prepared by reaction of carbonyldiimidazole with **3a,b** in tetrahydrofuran using triethylamine as base. The 2-thiol-1,3,4-oxadiazoles



Scheme 2. Reagents and conditions: (F) CDI, Et_3N, 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, MeI, methanol, 25–30 °C; (2) *p*-toluene sulphonic acid, toluene, reflux.



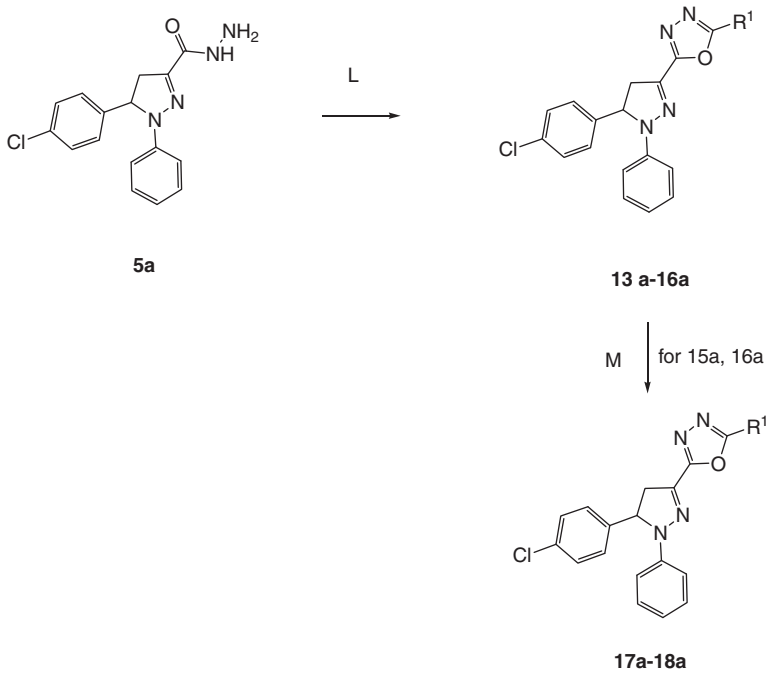
Scheme 3. Reagents and condition: (J) (1) ethylchloroformate, pyridine; (2) xylene, reflux; (K) (1) Ac_2O, Et_3N, 0–5 °C; (2) NaH, CS_2, 0–5 °C.

8a,b were obtained by reaction of **3a,b** with carbon disulfide under basic condition. The 2-amino-l,3,4-oxadiazoles **9a,b** resulted from the reaction of cyanogen bromide and $NaHCO_3$ with **3a,b**. The 2-thiomethyl-1,3,4-thiadiazoles **10a,b** were synthesized by reaction of hydrazide with KOH and carbon disulfide followed by methylation using methyl iodide. These compounds were further cyclised in refluxing toluene using *p*-toluene sulphonic acid.

The 1,2,4-oxadiazoles were synthesized using another key intermediate 5-(4-chloro-phenyl)-*N*-hydroxy-1-substituted-phenyl-4,5-dihydro-1*H*-pyrazole-3-carboxamidine **6a,b**. The carboxamidines **6a,b** were converted to different hetrocycles (Scheme 3). 5-Hydroxy-1,2,4-oxadiazoles **11a,b** were prepared by reaction of ethyl chloroformate with **6a,b** in pyridine and further their cyclization in refluxing xylene. The 2-thiol-1,2,4-oxadiazoles **12a,b** were obtained by acylation of **6a** and further cyclization with carbon disulfide in basic condition.

The 2-alkyl-1,3,4-oxadiazoles were synthesized by general procedure (Scheme 4), wherein hydrazides **3a–b** on reaction with various acid chlorides or anhydrides in presence of triethylamine as base yielded an open chain diamide intermediate, which in situ on reaction with *p*-toluene sulphonyl chloride and triethylamine underwent cyclization to give 2-alkyl-1,3,4-oxadiazoles **13a–16a**. The esters **15a**, **16a** were converted into respective acids **17a** and **18a** by hydrolysis using lithium hydroxide monohydrate in tetrahydrofuran, water mixture of solvent.

The 5-alkyl-1,2,4-oxadiazoles were synthesized by general procedure (Scheme 5). The carboxamidine **6a** on reaction with various acid chlorides or anhydrides in pyridine at reflux gives



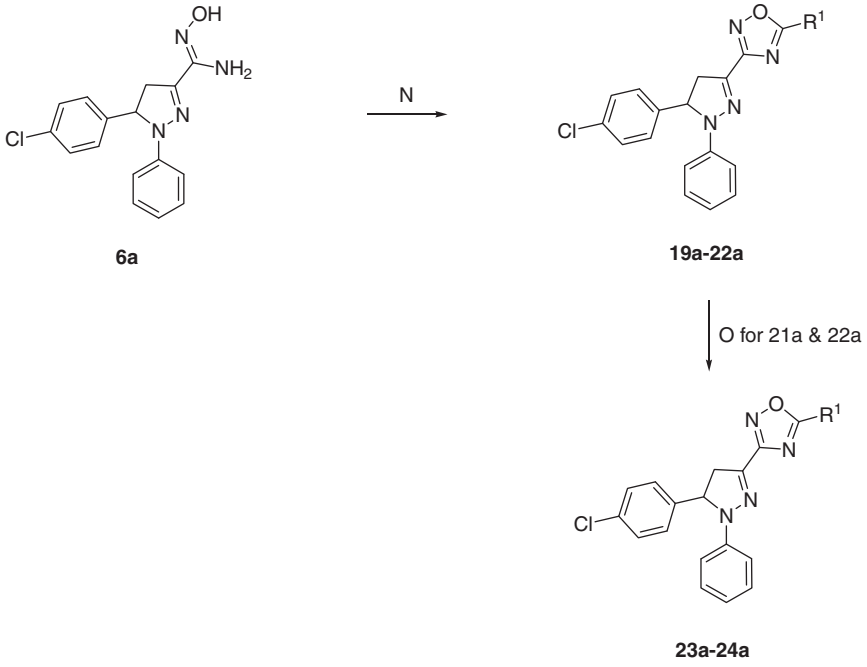
5a

13 a-16a

17a-18a

R^1 = CH_3 (13a), CF_3 (14a), COOEt (15a), CH_2COOEt (16a), COOH (17a), CH_2COOH (18a)

Scheme 4. Reagent and conditions: (L) (1) R^1COCl, Et_3N, *p*-toluene sulfonyl chloride, 0–25 °C; (M) LiOH, THF, water, 25–30 °C.



6a

19a-22a

23a-24a

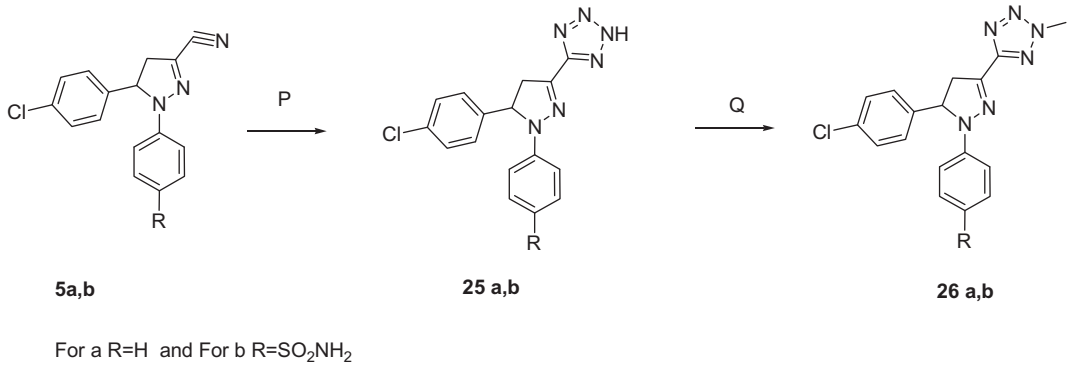
Where R^1 = CH_3 (19a), CF_3 (20a), COOEt (21a), CH_2COOEt (22a), COOH (23a), CH_2COOH (24a)

Scheme 5. Reagent and conditions: (N) $R^1COCl/(R^1CO)_2O$, pyridine, heat 100 °C; (O) LiOH–H_2O, THF, water, 25–30 °C.

cyclised 5-alkyl-1,2,4-oxadiazoles **19a–22a**. The esters were converted to respective acids **23a** and **24a** by treatment with LiOH in tetrahydrofuran, water solvent mixture.

The 5-(4-chloro-phenyl)-*N*-hydroxy-1-substituted-phenyl-4,5-dihydro-1*H*-pyrazole-3-carbonitriles **5a,b** were converted to tetrazole derivatives **25a,b** by reaction of azidotrimethylsilane in toluene using dibutyltin oxide as catalyst (Scheme 6). These were further methylated to give **26a,b**.

The experimental data of rat paw edema have revealed very interesting result.[14] It is observed form the data that, the various synthesized analogs of 1,5-diaryl pyrazoline moiety like 1,3,4-oxadiazole, 1,2,4-oxadiazole, 1,3,4-thiadiazole and tetrazole



Scheme 6. Reagents and conditions: (P) (1) $TMSN_3$, DBTO, toluene, reflux; (Q) MeI, $CsCO_3$, DMF, 25–30 °C.

have shown variety in activity from proinflammatory effect to equipotent activity as compared to standard diclofenac sodium. The results are given in Table 1

In the 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. The 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 the free carboxylic acid group present in **17a** and **18a** may be responsible for the proinflammatory behavior, while the positioning of trifluoromethyl group present in 1,3,4-oxadiazole **14a** may be considered responsible for this effect. The most potent compound in the 1,3,4-heterodiazole series was **8a** which has shown 32.74% inhibition against inflammation. Surprisingly, we have not noticed any influence of introduction of sulfonamide group on 1-phenyl ring. Among the different compounds in 1,3,4-heterodiazole series like **7b**, **8b**, **9b**, **10b** which are comprising of sulfonamide group, only the 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. They have shown 32–48% protection against inflammation, wherein **12b** which was equipotent to that of 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 49.24% and 49.45% protection against inflammation, respectively. Totally reverse trend was observed as compared to proinflammatory behavior of 1,3,4-oxadiazole derivatives like **13a** and **14a**. The acids **23a** and **24a** have confirmed that the free carboxylic acid substituent on heterodiazoles was responsible for proinflammatory behavior of the compounds.

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

The four selected compounds were tested for their analgesic activity by acetic acid induced writhing method.[15] The compounds **19a**, **20a**, **25b** and **26b** showed mild analgesic activity. Out of four tested compounds, **25b** showed 36.08% decrease in writhings and all other compound were ineffective in reducing the number of writhings. The results are summarized in Table 2.

The % inhibition of COX-2 in human whole blood[16] for the compounds **19a**, **20a**, **25b**, **26b** ranged from 4% to 11% at 10 μM concentration. The selected compounds showed insignificant COX-2 inhibition activity, these compounds might be acting by some different mechanism of action in vivo. The results are summarized in Table 2

The four selected compounds were further evaluated for their ulcerogenic potential in rats.[17] Gross observation of the isolated rat stomachs showed a normal stomach texture for all of the tested

Table 1
Results of anti-inflammatory effect of pyrazoline derivatives against carrageenan induced rat paw edema model in rats

Compound entry	Test compounds	% Rise in inflammation at 4 h[a]	% Protection at 4th h
Control	Control	84.22 ± 6.23	0
STD	Diclofenac sodium	41.32 ± 6.15	50.93 ± 7.18
5a	R = H	51.01 ± 2.35	39.43 ± 6.02
5b	R = SO_2NH_2	42.38 ± 1.12	49.67 ± 1.23
7a	R = H	63.78 ± 3.25	24.26 ± 4.10
7b	R = SO_2NH_2	54.42 ± 4.11	35.38 ± 5.56
8a	R = H, R′ = SH	56.64 ± 1.08	32.74 ± 1.85
8b	R = SO_2NH_2	53.23 ± 4.23	36.76 ± 3.12
9a	R = H	80.42 ± 2.62	4.50 ± 4.14
9b	R = SO_2NH_2	68.74 ± 2.36	18.38 ± 2.32
10a	R = H	74.24 ± 1.25	11.84 ± 3.07
10b	R = SO_2NH_2	62.36 ± 4.42	26.12 ± 2.98
11a	R = H	56.69 ± 3.65	32.68 ± 1.97
11b	R = SO_2NH_2	45.32 ± 2.59	46.18 ± 3.11
12a	R = H	50.10 ± 1.65	40.51 ± 5.23
12b	R = SO_2NH_2	43.10 ± 3.25	48.82 ± 3.36
13a	R = H, R^1 = CH_3	70.36 ± 7.25	16.45 ± 8.06
14a	R = H, R^1 = CF_3	123.74 ± 7.25	−46.9 ± 4.28
15a	R = H, R^1 = COOEt	80.43 ± 3.96	4.50 ± 4.25
16a	R = H, R^1 = CH_2COOEt	99.05 ± 2.68	−17.6 ± 9.69
17a	R = H, R^1 = COOH	100.47 ± 6.39	−19.3 ± 3.25
18a	R = H, R^1 = CH_2COOH	77.93 ± 2.18	7.46 ± 2.67
19a	R = H, R^1 = CH_3	42.75 ± 6.04	49.24 ± 1.23
20a	R = H, R^1 = CF_3	42.57 ± 4.71	49.45 ± 7.07
21a	R = H, R^1 = COOEt	67.08 ± 2.35	20.35 ± 2.68
22a	R = H, R^1 = CH_2COOEt	58.16 ± 3.75	30.94 ± 2.65
23a	R = H, R^1 = COOH	72.09 ± 1.69	14.40 ± 4.05
24a	R = H, R^1 = CH_2COOH	78.25 ± 3.56	7.08 ± 5.65
25a	R = H	52.38 ± 1.58	37.80 ± 3.23
25b	R = SO_2NH_2	38.25 ± 4.68	54.58 ± 3.26
26a	R = H	55.32 ± 5.23	34.31 ± 6.35
26b	R = SO_2NH_2	43.30 ± 2.98	48.58 ± 4.16

[a] The results are expressed as means ± SEM (n = 6) following a 100 mg/kg oral dose of the test compound.

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

Entry	Test compound	No. of writhings	% Inhibition	% Inhibition of COX-2[a]
19a	R = H, $R^1 = CH_3$	22.2 ± 1.35	23.71 ± 1.45	4
20a	R = H, $R^1 = CF_3$	24.5 ± 2.34	15.80 ± 1.98	6
25b	R = SO_2NH_2	18.6 ± 1.65	30.08 ± 1.28	7
26b	R = SO_2NH_2	22.2 ± 2.65	23.71 ± 2.35	11
Control	Control	29.1 ± 1.21	—	0
STD	Diclofenac	6.8 ± 0.51	76.63 ± 0.88	99[b]

[a] Data are indicated as percentage of inhibition at 10 μM mean of two tests.
[b] Diclofenac was assayed at 20 μM for COX-2.

compounds with no observable hyperemia indicating a superior GI safety profile (no ulceration) in the population of the test animals at an oral dose of 300 mg/kg, when administered twice at 2 h interval in fasted rats. It was found that the diclofenac sodium; the reference standard anti-inflammatory drug; was found to cause ulceration under the same experimental conditions.

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 in some compounds. 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 enantiomeric mixture and to study the anti-inflammatory efficacy of individual enantiomer. Therefore, compounds **19a**, **20a**, **25b** deserve further attention in order to develop new leads in this series.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.08.061.

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