ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF MANNICH BASES OF 1,3,4-OXADIAZOLE DERIVATIVE

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ABSTRACT

A novel series of substituted 1,3,4-oxadiazole derivative were synthesized by condensing different amine with 5-(1-(4-isobutylphenyl)ethyl)-1,3,4-oxadiazole-2(3H)-thione (III) in presence of formaldehyde. The structure of these novel synthesized compounds was characterized on the bases of physicochemical, spectral and elemental analysis. All newly synthesized compounds were tested in vivo for their anti-inflammatory activity by carrageenan induced rat paw oedema model. The compounds, which showed good anti-inflammatory activities were further screened for their analgesic, antipyretic and ulcerogenic activity. Results of acute anti-inflammatory studies showed compounds IVa, IVb, IVc and IVe showed promising activity and in analgesic studies compound IVa, IVc and IVe showed better activity as compared to standard drug ibuprofen. Compounds IVa and IVb showed good antipyretic activity and less ulcerogenic activity as compared to standard drug ibuprofen.

Keywords: 1,3,4-Oxadiazole, Mannich base, Ibuprofen, Anti Inflammatory

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are most preferred class of drugs which are used in the treatment of pain, fever, inflammatory diseases and rheumatoid arthritis [1]. The mechanism of action of NSAIDs is related to the suppression of prostaglandin biosynthesis from arachidonic acid this is by inhibiting the cyclooxygenases enzyme that is called COXs [2].

Regular use of NSAIDs, including ibuprofen gives many side effects like GI irritation, ulceration and many more. It is found that NSAIDs make GI tract damage, it having mainly two factors, first one is local irritation by the carboxylic acid moiety present in mostly NSAIDs (topical effect) and decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining the GI health and homeostasis [3]. It has been reported that the derivatization of the carboxyl function of representative NSAIDs, resulted in an increased anti-inflammatory activity with reduced ulcerogenic effect [4].

The modification in the carboxylic acid functionality of some NSAIDs, eg, flufenamic and meclofenamic acids, with heterocyclic group like tetrazole group not only increase action, but also reduced side effect [5]. Some other heterocyclic compounds, including thiazoles, midazoles, substituted oxadiazole and thiadiazole derivatives [6-8] have been studies and proved to be potent COX/5-LOX inhibitors.

Synthetic approaches based upon NSAIDs chemical modification have been taken with the aim of improving NSAID safety profile. It has been reported in literature that certain compounds bearing 1,3,4-oxadiazole nucleus possess significant anti-inflammatory activity with reduced ulcerogenicity [9, 10]. In continuation [11] of our attempt to discover new and useful agents for treatment of inflammatory diseases, we have synthesized mannich base of 1,3,4-oxadiazole derivatives, which have been found to possess an interesting profile of anti-inflammatory activity with significant reduction in their ulcerogenic effect.

2. MATERIAL AND METHODS

Chemicals used in this synthetic work were purchased from S.D. Fine Chem. Ltd. Mumbai, and Sigma Aldrich India (Merck). Solvents except laboratory reagent grade were dried and purified according to the literature when necessary.

Purity of the compounds was checked on TLC plates using silica gel G as stationary phase and iodine vapors as visualizing agent. Melting points of synthesized compounds were determined using Thermonik melting point apparatus and are uncorrected. IR spectra were recorded on Thermo Nicolet Spectrophotometer by using KBr pellets. The ^{1}HNMR was recorded on Bruker Avance II NMR 500 MHz instruments using appropriated solvent and TMS as internal standard, chemical shifts are expressed as δ values (ppm).

2.1.Synthesis and spectral studies:

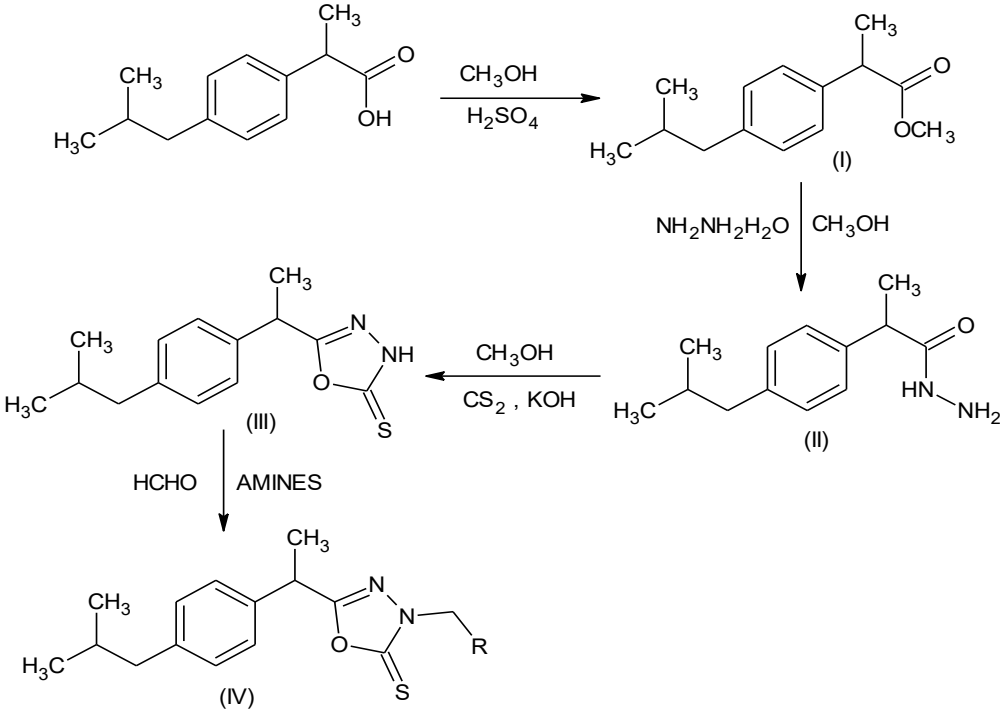
The title compounds were synthesized as given in the scheme 1.

2.1.1. Synthesis of methyl ester of Ibuprofen (methyl 2-(4-isobutylphenyl)propanoate) (I)

The methyl ester was prepared as per procedure reported in the literature [12. B.p. 95-97°C, yield: 78.43%. IR Spectra showed bands at 3088(C-H), 1745 (C=O) 1531(C=C). ^{1}H NMR chemical shift at ($CDCl_3$, δ ppm) 7.16 (s,4H, Aromatic), 3.96 (m,1H, CH), 3.45 (s,3H, CH_3), 2.55 (d,2H CH_2), 1.80-1.92 (m,1H of CH), 1.85 (d, 3H, CH_3), 1.06(d, 6H, CH_3).

2.1.2. Synthesis of 2-(4-isobutylphenyl)propane hydrazide (II)

Compound I (0.01 mol) and hydrazine hydrate (99%) (0.02 mol) were refluxed in absolute methanol (50 ml) for 20 hours (monitored by TLC). The mixture was concentrated, cooled and poured in ice cold water. The solid thus separated out was filtered, dried and recrystallized from ethanol: water (4:1). M.p. 72-74°C, yield: 75%. IR Spectra showed bands at 3425 cm^{-1} (NH-NH_2), 3258(NH_2), 3085 (C-H) 1645 (C=O) 1511(C=C). ^{1}H NMR chemical shift at ($CDCl_3$, δ ppm) 7.96 (s,4H, Aromatic), 7.15 (s, 1H, NH), 4.14 (s,2H, NH_2), 3.96 (m,1H, CH), 2.65 (d,2H CH_2), 1.88-1.96 (m,1H of CH), 1.6 (d, 3H, CH_3)1.15 (d, 6H, CH_3).



Scheme 1

2.1.3. 5-(1-(4-isobutylphenyl)ethyl)-1,3,4-oxadiazole-2(3H)-thione (III)

A mixture of II (0.005 mol) KOH (0.005mol) and carbon disulphide (5 ml) in methanol (50 ml) was refluxed on a steam bath for 12 hours (monitored by TLC). The solution was then concentrated, cooled and acidified with dil. HCl. The solid mass that separated out was filtered, washed with ethanol, dried and recrystallized from ethanol: water (4:1). Mp 116-118°C, yield: 72%. IR Spectra showed bands at N-H at 3185 (Ar-H), 3068 (ArC-H), 2955 (C-H), 1645 (C=N) 1352 (C=S), 1250 (C-O-C). ^{1}H NMR chemical shift at ($CDCl_3$, δ ppm) 9.98 (s,1H, NH), 7.66 (s,4H, Aromatic), 3.98 (m,1H, CH), 2.55 (d,2H CH_2), 1.88-1.96 (m,1H of CH), 1.68 (d, 3H, CH_3), 1.10 (d, 6H, CH_3).

2.2. General procedure for the synthesis of derivatives (IVa-h)

To a solution of III (0.01mol) in ethanol, a mixture of formaldehyde (0.015 mol) and a secondary amine (0.01 mol) in ethanol was added with stirring. After complete addition, the stirring was continued overnight at room temperature. The precipitated solids were filtered, washed with water and recrystallized from methanol.

2.2.1.5-(1-(4-isobutylphenyl)ethyl)-3-(morpholino methyl)-1,3,4-oxadiazole-2(3H)-thione (IVa)

This was obtained by reacting III, (0.01mol) and morpholine (0.015mol) as described in general procedure. IR Spectra showed bands at 3125 (Ar-H), 3008 (ArC-H), 2955 (C-H), 1605 (C=N) 1362 (C=S), 1240 (C-O-C). ^{1}H NMR chemical shift at ($CDCl_3$, δ ppm) 7.46 (s,4H, Aromatic), 5.12 (S,2H, N-CH_2-N), 4.18 (m,1H, CH), 3.84 (t, 4H, morpholine), 2.94 (t, 4H, morpholine), 2.45 (d,2H CH_2), 1.88-1.96 (m,1H of CH), 1.18 (d, 3H, CH_3), 1.02 (d, 6H, CH_3).

2.2.2.5-(1-(4-isobutylphenyl)ethyl)-3-(piperidin-1-ylmethyl)-1,3,4-oxadiazole-2(3H)-thione (IVb)

This was obtained by reacting (III, 0.01mol) and piperidine (0.015mol) as described in general procedure. IR Spectra showed bands at 3105 (Ar-H), 3068 (ArC-H), 2945 (C-H), 1632 (C=N) 1322 (C=S), 1260 (C-O-C). ^{1}H NMR chemical shift at ($CDCl_3$, δ ppm) 7.46 (s,4H, Aromatic), 5.12 (S,2H, N-CH_2-N), 4.18 (m,1H, CH), 3.84 (t, 4H, piperidine), 3.10 (m,2H, piperidine), 2.94 (t, 4H,piperidine), 2.45 (d,2H CH_2), 1.88-1.96 (m,1H of CH), 1.18 (d, 3H, CH_3), 1.02 (d, 6H, CH_3).

2.2.3.5-(1-(4-isobutylphenyl)ethyl)-3-((2-methyl piperidin-1-yl)methyl)-1,3,4-oxadiazole-2(3H)-thione (IVc)

This was obtained by reacting (III, 0.01mol) and 2-methyl piperidine (0.015mol) as described in general procedure. IR Spectra showed bands at 3115 (Ar-H), 3018 (ArC-H), 2985 (C-H), 1622 (C=N) 1336 (C=S), 1266 (C-O-C). ^{1}H NMR chemical shift at ($CDCl_3$, δ ppm) 7.16 (s,4H, Aromatic), 5.22 (S,2H, N-CH_2-N), 4.08 (m,1H, CH), 2.80-2.72(m, 1H, piperidine), 2.18-1.14(m, 6H, piperidine), 1.87-1.85(d, 3H, 2-methyl piperidine), 1.65(m, 2H, piperidine), 2.55 (d,2H CH_2), 1.78-1.86 (m,1H of CH), 1.08 (d, 3H, CH_3), 1.12 (d, 6H, CH_3).

2.2.4.5-(1-(4-isobutylphenyl)ethyl)-3-(piperazin-1-ylmethyl)-1,3,4-oxadiazole-2(3H)-thione(IVd)

This was obtained by reacting (III, 0.01mol) and piprazine (0.015mol) as described in general procedure. IR Spectra showed bands at 3145 (Ar-H), 3018 (ArC-H), 2955 (C-H), 1641 (C=N) 1338(C=S), 1252 (C-O-C). ^{1}H NMR chemical shift at ($CDCl_3$, δ ppm) 7.16 (s,4H, Aromatic), 5.22 (S,2H, N-CH_2-N), 4.08 (m,1H, CH), 3.95 (s, 1H, piperazine-NH), 3.18-3.14(t, 4H, piperazine), 3.07-3.05(t, 4H, piperazine), 2.47 (d,2H CH_2), 1.88-1.86 (m,1H of CH), 1.06 (d, 3H, CH_3), 1.02 (d, 6H, CH_3).

2.2.5.5-(1-(4-isobutylphenyl)ethyl)-3-((4-methyl piperazin-1-yl)methyl)-1,3,4-oxadiazole-2(3H)-thione (IVe)

This was obtained by reacting (III, 0.01mol) and N-methyl piprazine (0.015mol) as described in general procedure. IR Spectra showed bands at 3095 (Ar-H), 3008 (ArC-H), 2975 (C-H), 1621 (C=N) 1388(C=S), 1272 (C-O-C). ^{1}H NMR chemical shift at ($CDCl_3$, δ ppm) 7.26 (s,4H, Aromatic), 5.34 (S,2H, N-CH_2-N), 4.18 (m,1H, CH), 3.28-3.22(t, 4H, piperazine), 3.17-3.13(t, 4H, piperazine), 2.88 (s, 3H, piperazine-CH_3), 2.77 (d,2H CH_2), 1.82-1.76 (m,1H of CH), 1.12 (d, 3H, CH_3), 1.08 (d, 6H, CH_3).

2.2.6.3-((diethylamino)methyl)-5-(1-(4-isobutyl phenyl)ethyl)-1,3,4-oxadiazole-2(3H)-thione (IVf)

This was obtained by reacting 1 (III, 0.01mol) and diethyl amine (0.015mol) as described in general procedure. IR Spectra showed bands at (Ar-H), 3065 (ArC-H), 2942 (C-H), 1623 (C=N) 1378(C=S), 1252 (C-O-C). ^{1}H NMR chemical shift at ($CDCl_3$, δ ppm) 7.06 (s,4H, Aromatic), 5.04 (S,2H, N-CH_2-N), 4.08 (m,1H, CH), 3.06-2.70(m, 4H, CH_2, CH_2), 2.67 (d,2H CH_2), 1.82-1.78 (m,1H of CH), 1.59-1.48(t, 6H, CH_3, CH_3), 1.22 (d, 3H, CH_3), 1.18 (d, 6H, CH_3).

2.2.7.3-((diphenylamino)methyl)-5-(1-(4-isobutyl phenyl)ethyl)-1,3,4-oxadiazole-2(3H)-thione (IVg)

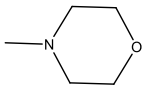
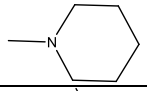
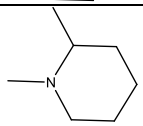
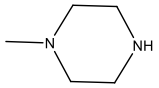
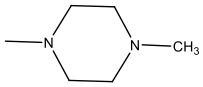
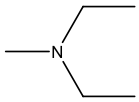
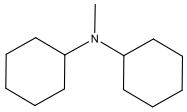
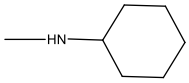
This was obtained by reacting (III, 0.01mol) and diphenyl amine(0.015mol) as described in general procedure. IR Spectra showed bands 3145 (Ar-H), 3075 (ArC-H), 2912 (C-H), 1623 (C=N) 1320(C=S), 1216 (C-O-C). ^{1}H NMR chemical shift at ($CDCl_3$, δ ppm) 7.76 (s,4H, Aromatic), 7.12-6.95 (m,10H of diphenyl amine) 5.04 (S,2H, N-CH_2-N), 4.08 (m,1H, CH), 2.67 (d,2H CH_2),

1.82-1.78 (m,1H of CH), 1.22 (d, 3H, CH_3), 1.18 (d, 6H, CH_3).

2.2.8.3-(cyclohexylamino)-5-(1-(4-isobutylphenyl) ethyl)-1,3,4-oxadiazole-2(3H)-thione (IVh)

This was obtained by reacting (III, 0.01mol**)** and cyclohexyl amine (0.015mol) as described in general procedure. IR Spectra showed bands 3165 (Ar-H), 3028 (ArC-H), 2965 (C-H), 1641 (C=N) 1388(C=S), 1254 (C-O-C). ^{1}H NMR chemical shift at ($CDCl_3$, δ ppm) 7.66 (s,4H, Aromatic), 5.02 (S,2H, N-CH_2-N), 4.18 (m,1H, CH), 2.57-2.25 (m,11H cyclohexyl amine), 2.22 (s,1H NH) 2.17 (d,2H CH_2), 1.60-1.56 (m,1H of CH), 1.04 (d, 3H, CH_3), 1.02 (d, 6H, CH_3).

Table 1: Physicochemical data of the different synthesized title compounds (IVa-h)

Compound	R	M.P. (°C)	Yield (%)	Rf*	Molecular formula	Molecular Weight
IVa		132-134	77.5	0.40	$C_{19}H_{27}N_3O_2S$	361
IVb		126-128	71.9	0.41	$C_{20}H_{29}N_3OS$	359
IVc		138-140	70.7	0.39	$C_{21}H_{31}N_3OS$	373
IVd		118-120	72.7	0.54	$C_{19}H_{28}N_4OS$	360
IVe		112-114	76.4	0.53	$C_{20}H_{30}N_4OS$	374
IVf		140-142	66.2	0.34	$C_{19}H_{29}N_3OS$	347
IVg		152-154	77.5	0.53	$C_{27}H_{29}N_3OS$	443
IVh		106-108	73.8	0.64	$C_{21}H_{31}N_3OS$	373

All compounds were recrystallized by methanol; Stationary phase - silica gel G.; Mobile phase- ethyl acetate: chloroform (4:1). Visualizing agent- iodine vapours.*

2.3. Anti-inflammatory activity

Table No- 2 reveals acute anti-inflammatory activity of title compounds IVa-h at a dose of 10 mg/kg by carrageenan induced rat paw edema method [13]. As shown in table 2, the entire investigated compound exhibited moderate to good anti-inflammatory activity. The anti-inflammatory activity of those compounds was comparable to that of the standard drug ibuprofen. Compounds that showed good anti-inflammatory activity profile were further evaluated for analgesic, antipyretic and ulcerogenic activity.

2.4. Analgesic activity

Table No- 3 reveals the analgesic activity of title compounds at the dose of 10 mg/ kg by acetic acid

induced writhing test in mice [14]. From the result, it was noticed that all compounds possess significant analgesic activity.

2.5. Antipyretic activity

Furthermore, the encouraging results from anti-inflammatory and analgesic activity prompted us to carry out the antipyretic activity in yeast-induced pyrexia [15] for 10 mg/kg in rats. Results of antipyretic activity are given in table no 4.

2.6. Ulcerogenic activity

The major drawback of NSAIDs is their gastric ulcer formation due to gastric irritation. The extent of ulcerogenic effect was evaluated for compounds IVa and IVd in rat stress model [16] at the therapeutic dose (i.e. 10 mg/kg). The gastric ulcerogenic potential was evaluated by calculating the ulcer index in treated and control animals. Result are given in Table no-5 which indicate that the compounds IVa (Ulcer index 0.83) and IVd (Ulcer index 1.08) induces less ulcer compared to standard drug Ibuprofen (Ulcer index 2.25). Hence gastric tolerance to these compounds was better than that of standard drug.

3. RESULTS AND DISCUSSION

The ibuprofen was converted to its methyl esters (I) by esterification. This methyl ester was reacted with hydrazine hydrate, gave carbohydrazide (2-(4-isobutylphenyl)propanehydrazide) (II). This carbohydrazide was treated with CS_2/ KOH in methanol gave 1,3,4-oxadiazole with ibuprofen moiety (5-(1-(4-isobutylphenyl)ethyl)-1,3,4-oxadiazole-2(3H)-thione) (III). The purity of the compounds was confirmed by melting point, TLC and structure was confirmed by IR and ^{1}HNMR spectral data.

Treatment of 5-(1-(4-isobutylphenyl)ethyl)-1,3,4-oxadiazole-2(3H)-thione (III) with various amines in presence of formaldehyde gave the title compounds IVa-h. The purity of these compounds were assessed by melting point, TLC and structure were confirmed by IR, and ^{1}HNMR. Physicochemical data of the different synthesized title compounds (IVa-h) are given in Table 1.

All these newly synthesized compounds (IVa-h) were screened for acute anti-inflammatory activity using carrageenan induced paw edema method and analgesic activity by acetic acid induced writhing method.

Table 2: **Anti-inflammatory activity of title compounds (IVa-h) by carrageenan induced paw edema.**

Compound	Paw volume		% Inhibition of edema	
	3 hr	5 hr	3 hr	5 hr
Control	2.49±0.13	2.90±0.07	-	-
IVa	0.67±0.01	0.84±0.01	73.2*	71.0*
IVb	0.87±0.07	1.03±0.06	65.2*	64.5*
IVc	0.85±0.02	1.50±0.13	65.9*	48.3*
IVd	1.19±0.04	1.65±0.02	53.2*	48.1*
IVe	0.92±0.07	1.02±0.07	62.1*	64.8*
IVf	1.22±0.01	1.65±0.03	52.0*	43.1*
IVg	1.51±0.05	1.84±0.01	39.4*	37.6*
IVh	1.45±0.08	1.71±0.09	42.8*	41.0*
Ibuprofen	0.86±0.09	1.12±0.14	65.6*	61.4*

*Data analyzed by one way ANOVA followed by Dunnett's 't' test, (n = 6), *P < 0.001 significant from control.*

Table 3: Analgesic activity compounds IVa, IVb, IVc and IVe by acetic acid induced writhing method.

Compound	No. of writhes in 15 min.	% Protection
Control	29	-
IVa	5.50	81.0*
IVb	5.18	82.2*
IVc	7.50	74.1*
IVe	6.67	77.0*
Ibuprofen	6.50	77.6*

*Data analyzed by one way ANOVA followed by Dunnett's 't' test, (n = 6), *P < 0.001 significant from control*

Results of acute anti-inflammatory studies showed compounds IVa, IVb, IVc, and IVe showed promising activity and in analgesic studies, compounds IVa, IVc and IVe showed better activity as compared to standard drug ibuprofen. The potent compounds were further screened for antipyretic and ulcerogenic activities.

Compounds IVa and IVb showed good antipyretic activity and less ulcerogenic activity as compared to standard drug ibuprofen.

Further detailed studies are needed to know the mechanism of action and site of action of these compounds.

Table 4: Antipyretic activity of IVa, IVb, IVc and IVe by yeast induced pyrexia

Compound	Mean temperature in °C at intervals[b]						
	0[a]	1	2	3	4	5	TI[c]
Control	38.30	38.26	38.20	38.11	38.00	37.89	- 1.04
IVa	38.13	37.98^{ns}	37.69***	37.03***	37.79***	36.78***	- 4.38
IVb	38.10	37.70***	37.55***	37.13***	37.06***	37.15***	- 3.91
IVc	38.27	37.72***	37.94**	37.80*	37.44***	37.20***	- 2.81
IVe	38.01	37.74*	37.77*	37.36***	37.06***	37.11**	- 3.06
Paracetamol	38.37	38.10*	37.90***	37.76***	37.51***	37.36***	- 3.22

*Data analyzed by one way ANOVA followed by Dunnett's 't' test, (n = 6), *P< 0.05, ** P< 0.01, ***P<0.001 significant from control; [a]Eighteenth hour after yeast injection was considered as 0 h.; [b]Temperature was recorded hourly from 0 to 5 h after dosing.; [c]Temperature index (TI) is the sum of mean temperature changes from the 0 h.*

Table 5: Ulcer index of IVa and IVb

Compound	Ulcer index(±SEM)
Control	0.75 (±0.17)
IVa	1.83 (±0.36)*
IVb	1.08 (±0.24)*
Diclofenac	2.25 (±0.25)^{a}

Data analyzed by one way ANOVA followed by Dunnett's 't' test, (n = 6).;
$^{a}P < 0.001$, as compared to control. $^{*}P < 0.001$, as compared to standard.

4. CONCLUSION

Various oxadiazole Mannich bases derived from ibuprofen was prepared with the objective of developing better anti-inflammatory molecules with minimum ulcerogenic activity and also to evaluate their analgesic and antipyretic activity. All these newly synthesized compounds IVa-h were screened for acute anti-inflammatory activity using carrageenan induced paw edema method and analgesic activity by acetic acid induced writhing method. Results of acute anti-inflammatory studies showed compounds IVa, IVb, IVc and IVe showed promising activity. The title compounds were also found to have significant analgesic activity in the acetic acid induced writhing model and antipyretic activity in yeast-induced pyrexia model. In addition, the tested compounds were also found to possess less degree of ulcerogenic potential as compared to ibuprofen. Thus these compounds constitute an interesting template for the evaluation of new inflammatory inhibitors and may be helpful for the design of new therapeutic tools against inflammation.

5. ACKNOWLEDGEMENT

We express our thanks to Indian Institute of science education and research, Bhopal and Indian institute of science, Bangalore for providing spectral analysis of synthesized compounds. We are grateful to Deshpande Laboratoties Pvt. Ltd., Bhopal, for permission to screen the pharmacological activities of synthesized compounds.

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