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SYNTHESIS AND BIOLOGICAL EVALUATION OF 2- PYRIDINE, 5- SUBSTITUTED 1, 3, 4- OXADIAZOLE DERIVATIVES AS NON-ACIDIC ANTI INFLAMMATORY AND ANALGESIC AGENTS

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ABSTRACT

A series of novel 1,3,4 - oxadiazole derivatives **B1-B13** have been synthesized. These series is devoid of carboxylic group as is seen in conventional non steroidal anti inflammatory drugs. The structures of these compounds were established by IR, 1H NMR, mass spectral data and elemental analysis. These compounds were evaluated for *in vitro* human red blood cell stabilization, *in vivo* anti inflammatory, analgesic and acute ulcerogenic activity.

Derivatives **B6, B7** and **B11** exhibited good anti inflammatory activity and derivatives **B2, B6** and **B8** exhibited good analgesic activity in carrageen induced rat paw edema and acetic acid induced writhing in mice, respectively. All compounds exhibited very low ulcerogenicity compared with the standard diclofenac sodium.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs and are the main drugs used in the treatment of arthritis, pain and inflammatory disorders. The main mechanism of action of NSAIDs is related to its ability to suppress the prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme prostaglandin endoperoxidases, more commonly known as cyclooxygenase (COX) [1].In early 1990's it was reported that COX enzyme exists in two isoforms, COX-I and COX-II, which are regulated and expressed differently, COX-I is constitutive and COX-II is inducible [2]. COX-1 enzyme is constitutively expressed in the kidneys, platelets and stomach. It provides cytoprotection to gastric mucosa and is important for platelet function, where as inducible COX-II mediates inflammation [3]. The commonly used NSAIDs cause the inhibition of both enzymes. In fact most of them show greater selectivity for COX-I than COX-II [4]. Hence, long term therapy with non-selective NSAIDs may elicit GI complications ranging from stomach irritation to life threatening GI ulceration and bleeding [5]. As COX-II enzyme was found to be over expressed during inflammatory conditions hence, drug research was focused on developing drugs with selective COX-II inhibition. Coxibs were marketed as new generation anti-inflammatory with safety profile, but careful prospective examination of coxibs has revealed unexpected cardiovascular adverse effects [6].

Most of the clinically used NSAID's at anti-inflammatory dose causes gastric mucosal damage by direct contact of –COOH group in them. This side effect limits the use of NSAID's as safer drug in treatment of inflammation. Several studies have described the replacement of carboxylate function of NSAID with less acidic azoles: oxazole, oxadiazole [7], thiazole [8] and thiadiazole, which resulted in an increased anti-inflammatory activity with reduced ulcerogenicity.

Various 1,3,4 oxadiazole derivatives were reported to posses anti-inflammatory activity[9, 10]. Here an attempt is to synthesize new, safer and potent agents for treatment of inflammatory diseases. We designed acid bioester 2, 5 disubstituted 1,3,4- oxadiazole moiety and evaluated for anti-inflammatory, analgesic and ulcerogenic activity.

MATERIALS AND METHODS

Melting points were recorded on a Buchi capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 157 spectrometer using KBr pellets. The ^{1}HNMR spectra was obtained on a Bucker WM-400 (400 MHZ FT NMR) spectrophotometer using $CDCl_3$ and DMSO-d_6 as solvents with TMS as an internal reference. Chemical shifts (δ) are expressed in ppm. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. Elemental analyses were performed on Perkin Elmer Auto system 240c analyzer and were within±0.4% of the theoretical values. Diclofenac sodiun was procured from Zydus Cadila, Ahmadabad. The other chemicals were purchased from Aldrich and Merck and were of analytical grade.

Ethical Approval- The proposed protocols were approved before the experimentation by the CPCSEA approved IAEC of P Wadhwani College of Pharmacy, Yavatmal.

Chemistry

The derivatives were synthesized as per the scheme I and2 [11].

1. Synthesis of Ethyl Pyridine-2-carboxylate (**2**)

Reflux Pyridine-2-carboxylic acid(10g) with etanolic HCl(100ml) for 5 hr. Reaction mixture was cooled to room temperature. Methanol was removed on rotavapour under reduced pressure. The residue was dissolved in ethyl acetate 200 ml. The ethyl acetate solution was washed with saturated sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, filtered and concentrated in vacuum. Off white solid 2 was obtained.

2. Synthesis of Pyridine-2-carbohydrazide (**3**)

The ester 2 (8g, 0.05 mol) was charged into 80 ml ethanol and refluxed with 10 ml hydrazine hydrate for 4 hours. Reaction mixture was cooled to room temperature. Methanol was removed on rotavapour under reduced pressure. Solid precipitates were filtered under suction on buchner funnel. Product was washed with 10 ml diethylether to get 6 g off white solid 3.

3. Synthesis of Ethyl oxo [2-(Pyridin-2-yl carbonyl)hydrazine]acetate (**4**)

Pyridine-2-carbohydrazide 3 (5.0g, 3.67 mmol) was dissolved in 70 ml dichloromethane and triethylamine(16ml) and add ethyl oxalylchloride(8.97g, 5.50 mmol) at 0^o C . The reaction mixture was stirred for 4 h at room temprature. The DCM solution was washed with saturated sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, filtered and concentrated in vacuum. Off white solid (4) was obtained (5.2g).

4. Synthesis of Ethyl 5-(Pyridin-2-yl)-1, 3, 4-oxadiazole-2-carboxylate (**B1**)

Ethyl oxo [2-(Pyridin-2-yl carbonyl)hydrazine]acetate (4) (5g, 2.1 mmol), triethylamine (9 ml), p-tolunesulphonic acid chloride(4g, 2.1mmol) dissolved in 50 ml chloroform and refluxed for 16 hours. Reaction mixture was cooled to room temperature. The DCM solution was washed with saturated sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, filtered and concentrated in vacuum. 4g white solid B1 was obtained.

Yield: 4g (80%); mp.: 71-74 ^oC; IR (KBr,cm^{-1}): 3011 (Ar-CH), 1725 (C=O); ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.55-7.2 (m, 4H, pyridine-H), 4.30-4.25 (q, 2H, CH_2), 1.30-1.28 (t, 3H, CH_3); ESI-MS (*m/z*): 220 (M+1); Molecular weight:-219; Molecular Formula:- $C_{10}H_9N_3O_3$.

5. Synthesis of 5-(Pyridin-2-yl)-1, 3, 4-oxadiazole-2-carboxamide (**B2**)

Ethyl 5-(Pyridin-2-yl)-1, 3, 4-oxadiazole-2-carboxylate B1 (1.0 g, 0.44 mmol) was dissolved in 10 ml of acetonitrile. To this, conc. ammonia (10 ml) was added dropwise and reaction mixture was heated on water bath at 50-60 °C for 8 h. Reaction mixture was cooled to room temperature. The solid was separated by filtration and dried to obtain white crystals.

Yield: 0.7g (70%); mp.: 98-101 °C; IR (KBr, cm^{-1}): 3423, 3210 (NH_2), 3020 (Ar-CH), 1675(C=O); ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.42-7.45 (m, 4H, Ar-H), 4.90 (bs, 2H, NH_2);; ESI-MS (*m*/*z*): 191 (M+1); Molecular weight:-190; Molecular Formula:- $C_8H_6N_4O_2$.

6. Synthesis of 5-(Pyridin-2-yl)-1, 3, 4-oxadiazole-2-carbonitrile (**B3**)

To the solution of 5-(Pyridin-2-yl)-1, 3, 4-oxadiazole-2-carboxamide B2 (0.5 g, 2.45 mmol) in 10 ml dimethylformamide, oxalyl chloride (0.46 g, 3.67 mmol) was added and the reaction mixture was stirred at 0-5 °C for 30 min. Then, pyridine (0.58 g, 7.35 mmol) was added and stirred again for 10 min. The reaction mixture was poured on ice water and extracted with dichloromethane. The organic layer concentrated to obtain white crystals

Yield: 0.35g (72 %); mp.: 74-77 °C; IR (KBr,cm^{-1}): 3040 (Ar-CH), 2239 (CN); ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.33-7.20 (m, 4H, Ar-H); ESI-MS (*m*/*z*): 173 (M+1); Molecular weight:-172; Molecular Formula:- $C_8H_4N_4O$.

7. Synthesis of 2-[5-(2H-tetrazole-5-yl)-1, 3, 4-oxadiazole-2-yl] Pyridine (**B4**)

To 5-(Pyridin-2-yl)-1, 3, 4-oxadiazole-2-carbonitrile B3 (0.25 g, 1.34 mmol), trimethylsilyl azide (0.3 g, 2.68 mmol) and dibutyltin (20 mg) in 10 ml toluene were added, the reaction mixture was refluxed for 24 h. The excess of toluene was removed by distillation and extracted with ethyl acetate. The organic layer obtained was washed with water and concentrated to get white colored compound.

Yield: 0.15 g (67%); mp.: 108-111 °C; IR (KBr,cm^{-1}): 3310 (NH), 3030 (Ar-CH), 1640 (C=N); ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.34-8.14 (m, 4H, Ar-H); ESI-MS (*m*/*z*): 216 (M+1); Molecular weight:-215; Molecular Formula:- $C_8H_5N_7O$.

8. Synthesis of 5-(Pyridin-2-yl)-1, 3, 4-oxadiazole-2-carbohydrazide (**B5**)

Ethyl 5-(Pyridin-2-yl)-1, 3, 4- oxadiazole-2-carboxylate (B1) (2.5 g, 10.0 mmol) was dissolved in 10ml ethanol in a conical flask, to this hydrazine hydrate (1.61 g, 30.0 mmol) was added and stirred for 2 h at room temperature. The precipitate appeared in the flask was filtered and recrystallised from ethanol to yield white crystalline product.

Yield: 2.0g (91%); mp.: 155-158 °C; IR (KBr,cm^{-1}): 3310, 3245 (NH_2), 3160 (NH), 1665(C=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.70 (s, 1H, NH), 7.34-8.19 (m, 4H, Ar-H), 4.49 (bs, 2H, NH_2)ESI-MS (*m*/*z*): 206 (M+1); Molecular weight:-205; Molecular Formula:- $C_8H_7N_5O_2$.

9. Synthesis of 5'-(Pyridin-2-yl)-2, 2'-bi-1, 3, 4-oxadiazole-5(4H)-one (**B6**)

A mixture of B5 (0.25 g, 1.11 mmol) and N, N'-carbonyldiimidazole (0.27 g, 1.71 mmol) in dioxane (10 ml) was heated under reflux for 8 h. After cooling, the solvent was removed under reduced pressure and the residue was triturated with water. The solid product formed was filtered off and recrystallized from isopropyl alcohol to yield colorless crystals.

Yield: 0.15 g (79 %); mp.:123-126 °C; IR (KBr,cm^{-1}): 3180 (NH), 3010 (Ar-CH), 1690 (C=O), 1622 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.42 (s, 1H, NH), 7.50-8.23 (m, 4H, Ar-H)ESI-MS (*m*/*z*): 232 (M+1); Molecular weight:-231; Molecular Formula:- $C_9H_5N_5O_3$.

10. Synthesis of 5-(Pyridin-2-yl)-2, 2'-bi-1,3,4-oxadiazole (**B7**)

A mixture of B5 (0.25 g, 1.1 mmol) and triethylorthoformate (5 ml) was refluxed for 5 h. The excess of the solvent was distilled off under reduced pressure and the solid product obtained was filtered off and recrystallized from ethanol to yield white crystalline product.

Yield: 0.17g (81%); mp.: 138-141 °C; IR (KBr,cm^{-1}): ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.38 (s, 1H, oxadiazole-H), 7.53 -8.31 (m, 4H, Ar-H)ESI-MS (*m*/*z*): 216 (M+1); Molecular weight:-215; Molecular Formula:- $C_9H_5N_5O_2$.

11. Synthesis of 5'-(Pyridin-2-yl)-2, 2'-bi-1, 3, 4-oxadiazol-5-amine (**B8**)

To sodium bicarbonate (0.96 g, 1.25 mmol) in 5 ml of water was added to a solution of B5 (0.25 g, 1.11 mmol) in 10 ml of dioxane at room temperature. After 5 min, cyanogens bromide (0.133 g, 1.25 mmol) was added. After 3 h of stirring, the solid precipitates were filtered under suction on Buchner funnel and dried in vacuum. The solid was recrystallized from isopropyl alcohol to afford brown-colored product.

Yield: 0.19 g (80%); mp.: 201-204 °C; IR (KBr, cm^{-1}): 3380, 3124 (NH_2), 3020 (Ar-CH), 1615 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.50-8.46 (m, 4H, Ar-H), 7.37 (bs, 2H, NH_2); ESI-MS (*m*/*z*): 231 (M+1); Molecular weight:- 230; Molecular Formula:- $C_9H_6N_6O_2$.

12. Synthesis of Ethyl 5-amino-1-{[5-(pyridin-2-yl)-1, 3, 4-oxadiazol-2-yl] carbonyl}-1H-pyrazole-4-carboxylate (**B9**)

A mixture of B5 (0.25 g, 1.1 mmol) and ethyl (ethoxymethylene) cyanoacetate (0.19 g, 1.1 mmol) in ethanol (10 ml) was heated under reflux for 7 h. The reaction mixture was cooled and solvent was concentrated on rotavapour under reduced pressure and the solid residue was recrystallized from ethanol to yield compound give compound B9 as white crystals.

Yield: 0.2 g (71 %); mp.: 174-177 ^{o}C; IR (KBr,cm^{-1}3340, 3190 (NH$_2$), 3030 (Ar-CH), 1705 (C=O), 1630 (C=N); ^{1}H NMR (400 MHz, DMSO-d$_6$,δ, ppm): 8.97 (s, 1H, pyrazole-H), 8.00 (bs, 2H, NH$_2$), 7.53-8.54 (m, 4H, Ar-H), 4.20-4.25 (q, 2H, CH$_2$), 1.28 (t, 3H, CH$_3$)ESI-MS (*m*/*z*): 329 (M+1); Molecular weight:-328; Molecular Formula:- $C_{14}H_{12}N_6O_4$.

13.Synthesis of(5-amino-3-phenyl-1H-pyrazol-1-yl)[5-(pyridine-2-yl)-1,3,4-oxadiazol-2-yl]methanone(**B10**)

A mixture of B5 (0.25 g, 1.1 mmol) and benzoyl acetonitrile (0.145 g, 1.1 mmol) in absolute ethanol (10 ml) was refluxed for 5 h. After cooling, the solvent was removed on rotavap under reduced pressure and the solid residue was recrystallized from ethanol to yield B10 as pale yellow crystals.

Yield: .21 g (76%); mp.: 189-192 ^{o}C; IR (KBr,cm^{-1}): -3380, 3215 (NH$_2$), 3020 (Ar-CH), 1700 (C=O), 1610 (C=N); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 8.70 (s, 1H, pyrazole-H), 7.25 (bs, 2H, NH$_2$), 7.83-8.49 (m, 4H, Ar-H), 7.40-7.56 (m, 5H, Ar-H); ESI-MS (*m*/*z*): 333 (M+1); Molecular weight:-332; Molecular Formula:- $C_{17}H_{12}N_6O_2$.

14. Synthesis of 5'(Pyridin-2-yl)-2,2'-bi-1,3,4-oxadiazole-5(4H)-thione (**B11**)

A mixture of B5 (0.5 g, 2 mmol) and carbon disulfide (3 ml) in pyridine (10 ml) was refluxed on oil bath for 6 h. After cooling, the solvent was evaporated under reduced pressure and the residue was triturated with an ice-water mixture and neutralized with diluted hydrochloric acid. The separated solid product was filtered, washed with water, dried and crystallized from ethanol to afford B11 as pale yellow crystals.

Yield: 0.48g (91%); mp.:151-154 ^{o}C; IR (KBr,cm^{-1}) 3210 (NH), 3015 (Ar-CH), 1628 (C=N),1315 (C=S); ^{1}H NMR (400 MHz, DMSO-d$_6$,δ, ppm): 10.55 (s, 1H, NH), 7.53-8.54 (m, 4H, Ar-H); ESI-MS (*m*/*z*): 248 (M+1); Molecular weight:-247; Molecular Formula:- $C_9H_5N_5O_2S$.

15. Synthesis of 5-{[2-(piperidin-1-yl)ethyl]sulfanyl}-5'-(pyridine-2-yl)-2,2'-bi-1,3,4-oxadiazole (**B12**)

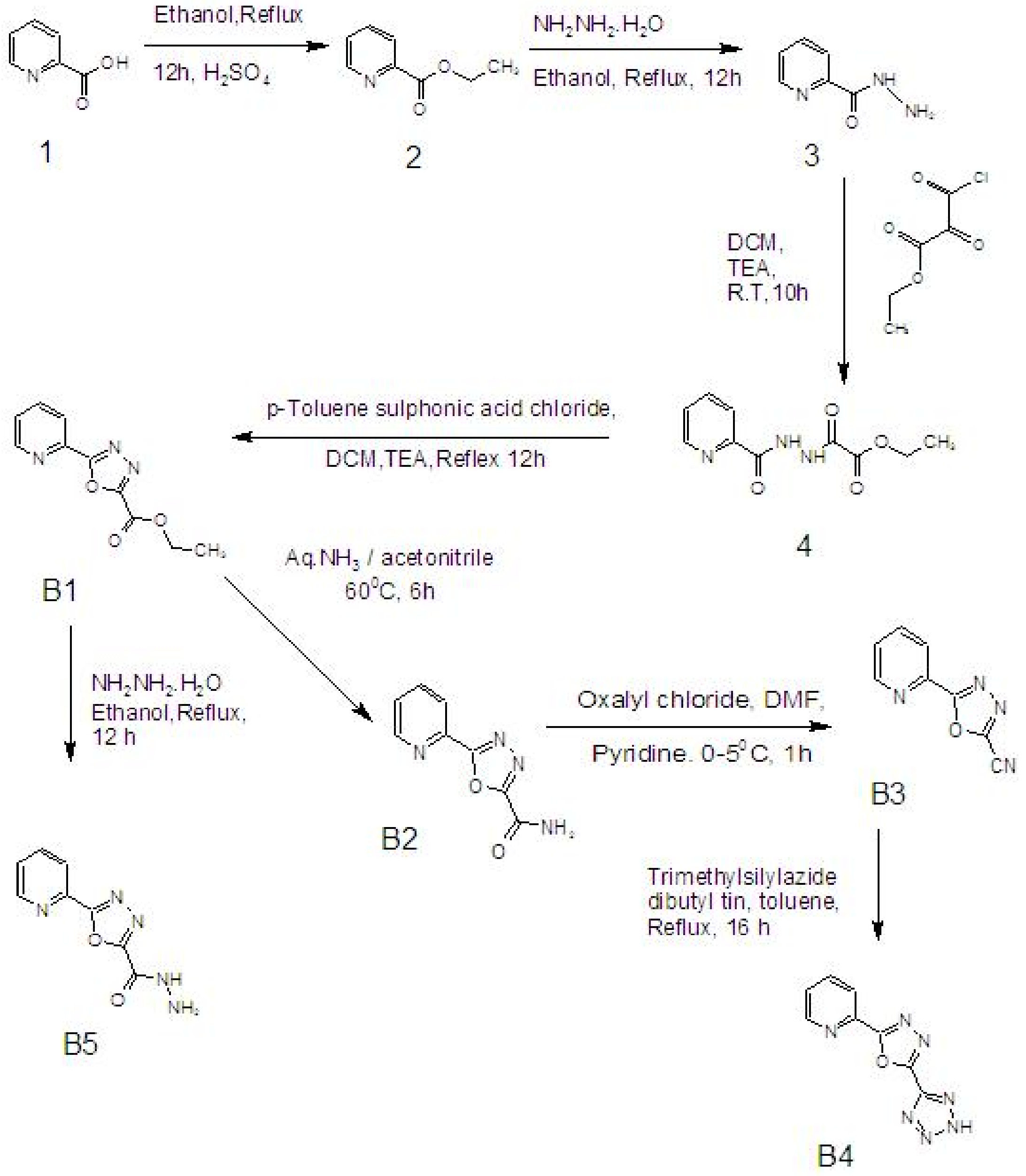
A mixture of the oxadiazolinethione B11 (0.2 g, 0.72 mmol), fused sodium acetate (0.31 g, 3.8 mmol) and 4-(2-chloroethyl) piperdinyl hydrochloride (0.14 g, 0.7mmol) in ethanol (10 ml) was refluxed for 8 h. The solvent was removed under reduced pressure and the solid product was washed with water, collected by filtration to yield B12 as white crystals.

Yield: 0.15g (74%); mp.:161-164 ^{o}C; IR (KBr,cm^{-1}): 3015 (Ar-CH), 1625 (C=N); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 7.37-8.56 (m, 5H, Ar-H), 3.72 (t, 4H, 2CH$_2$), 3.42 (t, 2H, $S\underline{CH_2}CH_2N$), 2.81 (t, 2H, $SCH_2\underline{CH_2}N$), 2.45 (t, 4H, 2CH$_2$), 1.35 (m, 2H, CH$_2$); ESI-MS (*m*/*z*): 359 (M+1); Molecular weight:-358; Molecular Formula:- $C_{16}H_{18}N_6O_2S$.

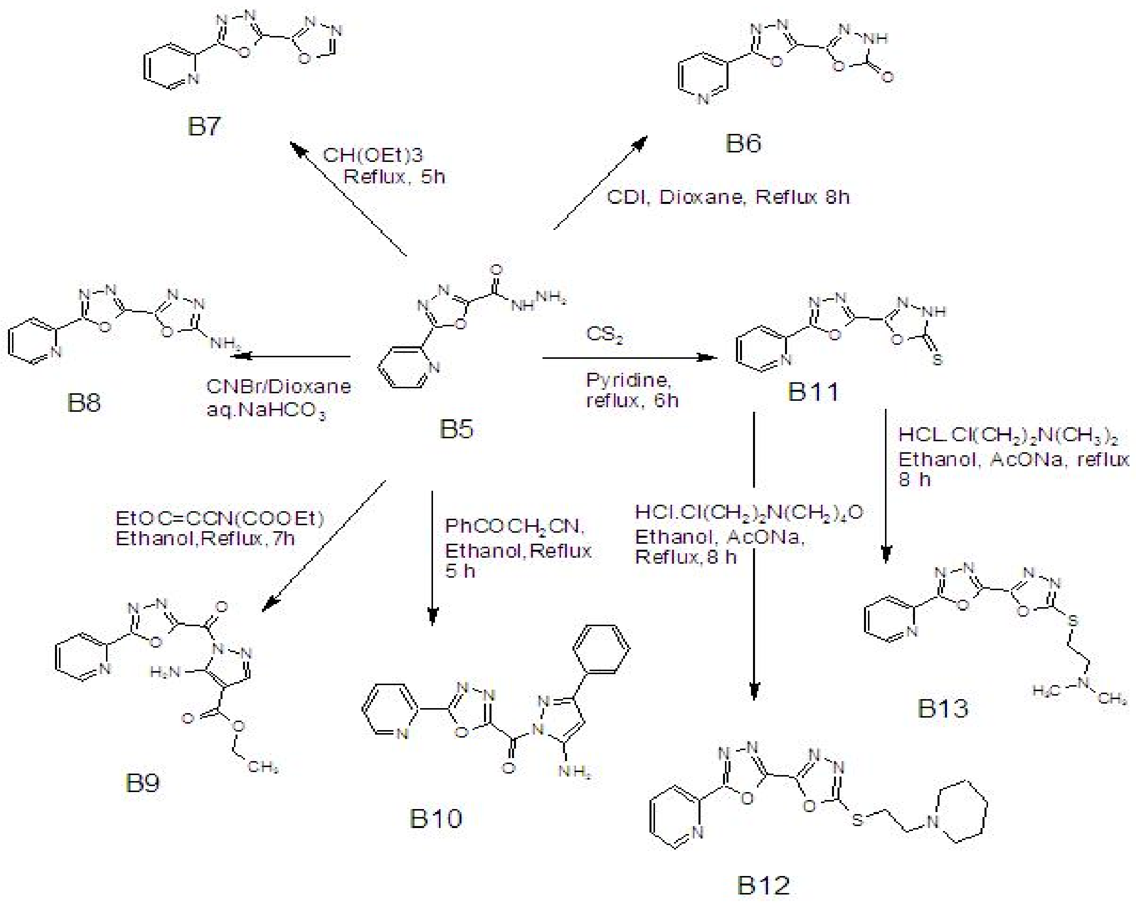
16. Synthesis of N, N-dimethyl-2-{[5'-(pyridine-2-yl)-2,2'-bi-1,3,4-oxadiazole-5-yl]sulfanyl}ethanamine (**B13**)

A mixture of the oxadiazolinethione B11 (0.2 g, 0.7 mmol), fused sodium acetate (0.31 g, 3.8 mmol) and 2-diethylaminoethyl chloride hydrochloride (0.11 g, 0.7mmol) in ethanol (10 ml) was heated under reflux for 8 h. The solvent was removed under vacuum and the solid was washed with water, filtered to yield compound B13 as white crystals.

Yield: 0.16g (80%); mp.:171-174 °C; IR (KBr,cm^{-1}): 3030 (Ar-CH),1632 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$,δ, ppm): 7.47-8.14 (m, 4H, Ar-H), 3.49 (t, 2H, $S\underline{CH_2}CH_2N$), 2.99 (t, 2H, $SCH_2\underline{CH_2}N$), 2.64-2.70 (q, 4H, $2CH_2$), 1.07 (t, 6H, $N(CH_3)_2$);; ESI-MS (*m*/*z*): 319 (M+1); Molecular weight:-318; Molecular Formula:- $C_{13}H_{14}N_6O_2S$.



Scheme 1: Synthesis of compounds B1 - B5



Scheme 2: Synthesis of Compounds B6-B13

PHARMACOLOGICAL EVALUATION

Evaluation of *In vitro* HRBC Membrane Stabilizing Activity

The lysosomal enzymes produced during inflammation produce kinds of disorders. The extracellular activity of these enzymes is related to acute or chronic inflammation. The NSAID, Diclofenac acts by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Because HRBC membrane is similar to lysosomal membrane composition, the inhibition of hypotonicity induced HRBC membrane lyses is taken as a measure of anti inflammatory activity of drugs [12].

Preparation of Human Red Blood Cells (HRBC) Suspension: Fresh whole human blood was collected from healthy volunteer who has not taken any NSAID for 2 weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water). The blood was centrifuged at 3000rpm at room temperature for 15 min. The supernatant (plasma and leukocyte) were carefully removed while the packed cells were washed three times with isosaline (0.85% w/v, pH 7.2). The process process of washing and centrifugation were repeated 5 times until the supernatant were clear. The volume of the blood was measured and reconstituted as 10% v/v erythrocyte suspension with isosaline [13].

Membrane stabilizing activity: The membrane stabilizing activity assay was carried out using 10%v/v HRBC suspension, while diclofenac sodium was used as standard drug. The assay mixture consisted of 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.25 %w/v NaCl], 0.5 ml HRBC suspension [10 % v/v] with 1 ml of test compound(**B1-B13**) or standard drug diclofenac sodium solution each at 50µg/ml concentration and the final reaction mixtures were made up to 4.5ml with isosaline.

To determine the anti inflammatory activity by HRBC membrane stabilization method the following solution were used:

1. Test solution (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v), 1ml phosphate buffer (pH 7.4), and 1ml of test compound (50µg/ml) in normal saline and 0.5ml of 10%v/v human red blood cell suspension.
2. Test control (4.5ml) consist of 2ml of hypotonic saline (0.25%w/v), 1ml of phosphate buffer (7.4 pH) , 1ml of isotonic saline and 0.5ml of 10%v/v human red blood cell suspension (produce 100% hemolysis).
3. Standard solution (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v), 1ml of phosphate buffer (7.4pH), 1ml of diclofenac sodium (50µg/ml) in normal saline and 0.5ml of 10%v/v human red blood cell suspension.
4. Drug control (4.5ml) consist of 2ml of hypotonic saline (0.25%w/v), 1ml of phosphate buffer (pH 7.4),1ml of test compound or diclofenac sodium (50µg/ml) in normal saline and 0.5ml of isotonic saline (0.85%, pH 7.2)
5. The reaction mixtures were incubated at 37^{o}C for 30 min and centrifuged at 3000 rpm for 20 min. The absorbance of the hemoglobin content of supernatant solution was measured spectrophotometrically at 560 nm. Each experiment was carried out in triplicate and the average was taken. The percentage membrane stabilization was calculated using the following formula[14]:

$$\%\ \text{membrane stabilization} = 100 - \frac{(\text{Abs of test solution} - \text{Abs of drug control})}{\text{Abs of test control}} \times 100$$

Abs = Absorbance Results are presented in **Table 1**

Evaluation of *In vivo* Pharmacological Activity

Synthesized compounds (**B1-B13**) were investigated for in vivo anti-inflammatory and analgesic activities and most active representatives (**B2, B6, B7, B8, B11**) of the series were investigated for acute ulcerogenicity. Diclofenac sodium was used as a reference standard at a dose 25mg/kg for anti-inflammatory and analgesic and 80mg/kg for ulcerogenicity studies. The experiments were performed on Albino rats of Wistar strain of either sex, weighing 180-200 g for anti-inflammatory and ulcerogenic activity and Albino mice of either sex weighing 25-30 g for analgesic activity. The animals were divided into groups (control, reference and test groups) of 6 animals each. The test compounds and the standard drugs were administered in the form of a suspension (using 1% carboxymethylcellulose) in distill water by oral route of administration for analgesic, anti-inflammatory and ulcerogenicity studies. The animals were maintained in colony cages at 25±2°C, relative humidity 45–55%, under a 12 h light–dark cycle; they were fed standard animal feed. All the animals were acclimatized for a week before use.

Evaluation Anti-inflammatory activity

Anti-inflammatory activity was evaluated using carrageenan induced rat paw edema method [15]. Carrageenan solution (0.1% in sterile 0.9% NaCl solution) in a volume of 0.1 ml was injected subcutaneously into the sub plantar region of the right hind paw of each rat. One group was kept as control and the animals of the other group were pretreated with the test drugs and standard drug 1 h before the carrageenan treatment. The paw volume of the all groups of rats were measured using the mercury displacement technique with the help of digital plethysmometer immediately before and 1, 2 and 3 h after carrageenan injection. The edema was expressed as a mean reduction in paw volume (ml) after treatment with tested compounds, percent of edema inhibition was obtained as follows:

$$\text{Percent inhibition} = (V_t - V_c)\text{control} - (V_t - V_c)\text{tested compound} / (V_t - V_c)\text{control} \times 100$$

Where Vt = volume of edema at specific time interval and Vc= volume of edema at zero time interval.

The results are presented in **Table 2**.

Evaluation of Analgesic activity

Analgesic activity was evaluated using acetic acid induced writhing method [16]. After 50 min of the oral administration of test compound and standard drug, each animal was injected with 0.25 ml of 0.6% v/v aqueous acetic acid solution intraperitonially. After 10 min of acetic acid injection, the numbers of muscular contractions (writhing consisting of arching of the back, elongation of body and extension of hind limbs) in mice were counted for a period of 15 min. A significant reduction in the number of writhing by any treatment as compared to control animals was considered as a positive analgesic response. The average number of writhes in each group of treated mice was compared with that of the control. The % analgesic activity was expressed according to the formula:

$$\% \text{ Analgesic activity} = [n - n' / n \times 100]$$

Where, n = the number of writhes in control group of mice and

n'= the number of writhes in test and standard group of mice.

The observations are tabulated as **Table 3**.

Evaluation of acute ulcerogenesis

Acute ulcerogenesis test was done according to literature method [17]. Albino rats were divided into groups consisting of 6 animals in each group. Ulcerogenic activity was evaluated after p.o. administration of test compounds or standard at the dose of 80mg/kg. Control rats received p.o. administration of vehicle (suspension of 1%CMC). Food but not water was removed 24hr before administration of the test compounds. After the drug treatment, the rats were fed with normal diet for 17 h and then sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. The mucosal damage is examined by means of a magnifying glass. For each stomach, the mucosal damage was assessed according to the following scoring system : 0.0 score is given to normal stomach (no injury, bleeding and latent injury), 0.5 score is to latent injury or widespread bleeding(>2mm), 1.0 score to slight injury (2-3 dotted lines), 2.0 score for severe injury (continuous lined injury or 5-6 dotted injuries), 3.0 score to very severe injury (several continuous lined injuries) and 4.0 for wide spread injury or widened injury. The mean score of each treated group minus the mean score of control was regarded as severity index of gastric mucosal damage. Observations are tabulated as **Table 4**.

Statistical analysis

Statistical analysis of the biological activity of the synthesized compounds was evaluated using one- way analysis of variance (ANOVA). In all cases, post hoc comparisons of the means of individual groups were performed using Dunnett's test. A significance level of $P<0.05$ denoted significance in all cases. All values are expressed as mean±SEM (standard error of mean). Statistical analysis was carried out using Graph Pad Prism (Graph Pad Prism 3.0 version).

RESULT AND DISCUSSION

Synthesis of compounds **B1-B13** is outlined in scheme **1** and **2**. Oxadiazole derivatives were characterized by IR, NMR and MS spectra.

HRBC Stabilization Activity

The HRBC stabilization activity of derivatives **B1- B13** and reference standard diclofenac sodium were evaluated at a concentration of 50µg/ml (**Table 1**). The compounds showed the percent membrane stabilization activity in the range of 25.2% and 76% while the standard diclofenac showed 74.2% stabilization.

Table 1: Effect of derivatives **B1- B13** on HRBC stabilization

Compound	Concentration (µg/ml)	% Membrane stabilization
Diclofenac	50	74.2
B1	50	39.9
B2	50	46.8
B3	50	32.7
B4	50	49.4
B5	50	26.1
B6	50	54.3
B7	50	63.7
B8	50	48.6
B9	50	28.2
B10	50	25.2
B11	50	76.0
B12	50	37.2
B13	50	42.5

Anti inflammatory Activity

The anti inflammatory activity (**Table 2**) of derivatives **B1-B13** was evaluated by carrageneen induced paw edema in rats at a concentration of 25mg/kg. Diclofenac sodium was taken as standard. The anti inflammatory activity of compounds **B6**, **B7** and **B11** were highest.

Table 2: Anti inflammatory activity of derivatives **B1-B13**, against carrageenan induced paw edema

Compound	Mean change in paw volume (ml) after treatment (± SEM)			% Inhibition		
	1h	2h	3h	1h	2h	3h
Control	0.72 ± 0.02	1.21± 0.03	1.53 ± 0.06	-----	-------	------
Diclofenac	0.42 ± 0.03*	0.51± 0.02*	0.49 ± 0.04 *	42.32	57.55	67.81
B1	0.48 ± 0.05*	0.66 ± 0.04*	0.76 ± 0.03*	33.62	45.21	50.03
B2	0. 46 ± 0.04*	0.62 ± 0.05*	0.72 ± 0.03*	36.35	48.64	53.24
B3	0.53 ± 0.03*	0.79 ± 0.05*	0.97 ± 0.07*	26.23	34.68	36.41
B4	0.45 ± 0.05*	0.61 ± 0.06*	0.70 ± 0.04*	37.33	49.56	54.38
B5	0.60 ±0.04*	0.96 ± 0.06*	1.18 ± 0.07*	16.37	20.46	22.23
B6	0.44 ±0.03*	0.53 ± 0.03*	0.54 ± 0.02*	38.66	55.94	64.53
B7	0.45 ±0.02*	0.58 ± 0.02*	0.63 ± 0.03*	37.69	52.46	59.06
B8	0.51 ±0.07*	0.80 ± 0.05*	0.80 ± 0.04*	29.18	34.19	47.54
B9	0.60 ±0.04*	0.92 ± 0.04*	1.09 ± 0.08*	16.41	23.85	28.48
B10	0.58 ±0.05*	0.87 ± 0.08*	1.03 ± 0.06*	19.67	28.44	32.39
B11	0.42 ±0.04*	0.49 ±0.06*	0.45 ± 0.06*	41.37	59.67	70.38
B12	0.60 ±0.05*	0.94 ± 0.06*	1.06 ± 0.07*	16.98	21.98	30.54
B13	0.59 ±0.07*	0.93 ±0.05*	1.00 ± 0.03*	17.92	22.89	34.36

Data analyzed by one-way ANOVA followed by Dunnett's test, (n=6), *P<0.05 significant from control. Dose levels: Test compounds and diclofenac sodium (25 mg/kg, p. o.).

Analgesic Activity

Analgesic activity was evaluated for **B1-B13** by inhibition of acetic acid induced writhing in mice at 25mg/kg (**Table 3**). Diclofenac sodium was taken as standard. The analgesic activity of **B2**, **B6** and **B8** were similar to the standard diclofenac sodium.

Table 3: Analgesic activity of compounds (**B1-B13**) against acetic acid induced writhing model

Compound	No. of Wriths	% inhibition
Control	24.5±1.20	------
Diclofenac	9.5±0.76*	61.02
B1	13.8±0.60*	43.32
B2	9.2±0.66*	62.11
B3	14.5±0.46*	40.45
B4	14.3±0.66*	41.64
B5	19.8±0.94*	19.22
B6	10.3±0.66*	57.59
B7	15.1±0.94*	38.31
B8	10.1±0.98*	59.05
B9	18.5±1.14*	24.31
B10	19.6±1.17*	19.78
B11	13.7±1.11*	43.87
B12	13.3±0.98*	45.76
B13	14.5±0.71*	40.83

Data analyzed by one-way ANOVA followed by Dunnett's test, (n=6), $^{*}P < 0.05$ significant from control. Dose levels: Test compounds and diclofenac sodium (25 mg/kg, p. o.).

Acute ulcerogenicity

Acute ulcerogenicity activity was evaluated for compounds **B2, B6, B7, B8 and B11** and was compared with diclofenac sodium and CMC(1%) (Table 4). All the compounds exhibited less ulcerogenicity when compared with standard and the ulcer index was between 0.59 and 0.93

Table 4: Evaluation of ulcerogenicity

Compound	Dose mg/kg, p.o.	Ulcer score	Ulcer index
Control	CMC 1% w/v	0.16±0.10	-----
Diclofenac	80	2.25±0.17*	2.09
B2	80	$1.00 \pm 0.22^{*a}$	0.84
B6	80	$1.08 \pm 0.20^{*a}$	0.93
B7	80	$0.83 \pm 0.10^{*a}$	0.67
B8	80	$0.75 \pm 0.59^{*a}$	0.59
B11	80	$0.75 \pm 0.59^{*a}$	0.59

Data analyzed by one-way ANOVA followed by Dunnett's test, (n=6),
$^{*}P < 0.05$ significant from control, $^{a}P < 0.05$ significant from diclofenac sodium

CONCLUSION

The oxadiazole derivatives **B6**, **B7** and **B11** exhibited good anti inflammatory activity and the compounds **B2**, **B6** and **B8** exhibited good analgesic activity when compared with diclofenac sodium. All these compounds also were less ulcerogenic than the standard drug. All these derivatives possessing pyridine ring at fifth position and 1, 3, 4- oxadiazole at second position of oxadiazole ring exhibited most prominent and consistent anti inflammatory activity. Hence, the isosteric replacement of carboxylate function with oxadiazole resulted in reduced ulcerogenicity and **B6**, **B7** and **B11** retained anti-inflammatory activity.

REFERENCES

1. Palomer A, Cabre F, Espinosa A., Identification of novel cyclooxygenase-2 selective inhibitors using pharmacophore models, Journal of Medicinal Chemistry, 2002; 45(7): 1402-1411.
2. Parsit P, Reindeau D., Selective Cycloxygenase -2 Inhibitors, Annual Reports in Medicinal Chemistry, 1997; 32: 211-220.
3. Marco LL, Cena C, Medana C, Lazzarato L, Morini G, Coruzzi G, Manarini S, Fruttero R, Gasco A., A new class of ibuprofen derivatives with reduced gastrotoxicity, Journal of Medicinal Chemistry, 2001; 44: 3463-3468.
4. Jackson LM, Hawkey CJ., Gastrointestinal effects of COX-2 inhibitors, Expert Opinion on Investigational Drugs, 1999; 8: 963-71.
5. Allison MC, Howatson AG, Torrance CJ, Lee FD, Russell IG., Gastrointestinal damage associated with the use of non steroidal anti-inflammatory drugs, New England Journal of Medicine, 1992; 327: 749-754.
6. Donge JM, Pratico D., Adverse cardiovascular effects of coxibs, Journal of Medicinal Chemistry, 2005; 48(7): 2251-2257.
7. Akhter M, Husain A, Azad B, Ajmal M., Aroyl propionic acid based 2,5, disubstituted 1,3,4-oxadiazoles: Synthesis and their anti-inflammatory and analgesic activities, European Journal of Medicinal Chemistry, 2009; 44(6): 2372-2378.
8. Giri RS, Thaker HM, Rogers D, Sudersanam V, Vasu KK., Design, synthesis and characterization of novel 2-(2,4-disubstituted- thiazole- 5-yl)-3-aryl-3*H*-quinazoline-4-one derivatives as inhibitors of NF-kB and AP-1 mediated transcription activation and as potential anti-inflammatory agents, European Journal of Medicinal Chemistry, 2009; 44(5): 2184-2189.
9. Omar FA, Mahfouz NM, Rahman MA., Design, Synthesis and anti-inflammatory activity of some 1,3,4-oxadiazole derivatives, European Journal of Medicinal Chemistry, 1996; 31(10): 819-825.

10. Somani RR., Bhanushali UV., Synthesis and evaluation of anti-inflammatory, analgesic and ulcerogenic potential of NSAIDs bearing 1,3,4-oxadiazole scaffold, Indian Journal of Pharmaceutical Sciences, 2011; 73(6):634-640.
11. Storr RC, Gilchrist TL., Houben-Weyl Method of molecular transformation: Science of synthesis: Hetarenes and related ring systems. Vol 13, Thieme New York, ISBN 0-86577-952-X, 219-249.
12. Chou CT., The anti inflammatory effect of Tripterygium wilfordihook on adjuvant induced paw edema in rats and inflammatory mediators release, Phytotherapy Research, 1997; 11: 152-154.
13. Oyedapo OO, Akinpelu BA, Akinwunmi KF, Adenyinka MO, Sipeolu FO., Red blood cell membrane stabilization potentials of extracts of Lanata camara and its fraction, International Journal of Plant Physiology and Biochemistry, 2010; 2(4): 46-51.
14. Awe EO, Mankinde JM, Adeloye OA, Banjoko SO., Membrane stabilizing activity of Russelia equisetiformis, schlecht and chan, Journal of Natural Products, 2009; 2: 03-09.
15. Winter CA, Risley EA, Nuss GN., Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs, Proceedings of Society for Experimental Biology and medicine, 1962; 111: 544–547.
16. Koster R, Anderson M, De Beer EJ., Acetic acid for analgesic screening. Federation Proceedings, 1959; 18: 412-418.
17. Cioli V, Putzolu S, Rossi V, Sorza Barcellona P, Corradino C., The role of direct tissue contact in the production of gastrointestinal ulcers by anti-inflammatory drugs in rats, Toxicolology and Applied Pharmacology,1979; 50(2): 283-289.