Research Article **ISSN: 2349 – 7114**

Asian Journal of Research in Pharmaceutical Sciences and Biotechnology

Journal home page: www.ajrpsb.com

SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF PYRAZINE DERIVATIVES

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ABSTRACT

The five different types of pyrazine derivatives were synthesized called as 5-methylpyrazine-2-carbohydrazide. The structures of the synthesized compounds were characterized on the basis of IR, ^{1}HNMR and Mass spectral data. Among all synthesized compounds all compounds are screened for their anti-inflammatory activity by paw edema method, Diclofenac sodium is employed as a reference standard, the percentage inhibition of pyrazine derivatives and diclofenac were 96.71 % and 98.68 % respectively. From the results it is concluded that, compound II and V exhibited potent, when compare to rest of compounds exhibited mild to moderate anti inflammatory activity.

KEY WORDS

Anti - inflammatory, Carohydrazide and Methylpyrazine.

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INTRODUCTION

The widespread occurrence of simple pyrazine molecules in nature, especially in the flavours of many food systems, their effectiveness at very low concentrations as well as the still increasing applications of synthetic pyrazines in the flavour and fragrance industry are responsible for the high interest in these compounds[1]. Certain pyrazines, especially dihydropyrazines, are essential for all forms of life due their DNA strand breakage activity and/or by their influencing of apoptosis[2]. Synthetic

pyrazine derivatives are also useful as drugs (antiviral, anticancer, antimycobacterial, etc.) fungicides, and herbicides[3]. Furthermore, a simple pyrazine compound, 3- amino-6-chloro-pyrazine-6-carboxylic acid, has shown anti-auxin behaviour. The importance of the pyrazine (1, 4-diazine) ring for the biological Herbicides, activity can be evaluated primarily according to the size of the studied molecules. In relatively small compounds, the pyrazine ring is necessary for biological action due to its resemblance (bioisosterism) to the naturally occurring compounds (e.g. nicotinamide, or pyrimidine nucleic bases). In bulky compounds the introduction of the pyrazine ring brings specific chemical and physicochemical properties for the molecule as a whole, such as basic and slightly aromatic character. A fully comprehensive study of the pyrazines including reactivity and synthesis is beyond the scope of this work but can be found in the literature[4, 5]. Herbicides are generally considered as growth inhibitors, thus their different inhibitory responses have been studied in various culture systems. Plant tissue and cell cultures provide model systems for the study of various molecular, physiological, organism and genetic problems. These systems have been used in the study of herbicides and other xenobiotics[6].

The pyrazine ring is a part of many polycyclic compounds of biological and/or industrial significance; examples are quinoxalines, phenazines, and bio-luminescent natural products pteridines, flavins and their derivatives. All these compounds are characterized by a low lying unoccupied π-molecular orbital and by the ability to act as bridging ligand. Due to these two properties 1,4-diazines, and especially their parent compound pyrazine, possess a characteristic reactivity. Pyrazine is a weak diacid base (pK1 = 0.57; pK2 = -5.51), weaker than pyridine, due to the induction effect of the second nitrogen[7].

Pyrazine nucleus possesses remarkable pharmaceutical importance and biological activities, some of their derivatives occur as natural products. In view of our ongoing interest in the synthesis of some new potentially bioactive pyrazine derivatives. The synthesis of compound 2-(piperidin-4-ylmethoxy) pyrazine derivatives has been attracted widespread attention due to their diverse pharmacological properties like anti-inflammatory, antibiotic, antifungal, herbicidal, antitubercular, etc. To approach this goal synthesis of some new 2-(piperidin-4 yl methoxy) pyrazine derivatives have been undertaken[8]. Inflammation is a body defense reaction to eliminate or limit the spread of an injurious agent and is characterized by five cardinal signs, redness (*rubor*), swelling (*tumor*), heat (*calor*), pain (*dolor*) and loss of function (*function laesa*). The inflammatory process involves a cascade of events elicited by numerous stimuli that include infectious agents, ischemia, antigen-antibody interaction and thermal or physical injury. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of acute and chronic inflammation, pain and fever. But the greatest disadvantage in presently available synthetic drugs is that they cause gastrointestinal irritation and reappearance of symptoms after discontinuation[9].

MATERIALS AND METHOD

General Procedure for synthesis of 5-methylpyrazine-2-carbohydrazide

5-Methylpyrazinoic acid was dissolved in ethanol and few drops of conc. H_2SO_4 was added and refluxed for 24 hrs; hydrazine hydrate 100% (3.0 mol) was added to it and further refluxed for a period of 8 hrs. The excess of solvent was distilled off to get the resulting product. The product was crystallized from aqueous ethanol [10]. A solution of aromatic/substituted aldehyde (0.05 mol) in ethanol was added to a solution of so obtained 5-methylpyrazinoic acid hydrazide (0.05 mol) in 10 ml ethanol. The mixture was refluxed for 4 hrs. After cooling the mixture, the precipitate was filtered, dried and recrystallised from aq. Ethanol [11, 12] schematic synthesis was shown in Figure No.1.

Anti-Inflammatory Screening

Healthy adult albino rates of Wistar strain weighing 150-200 g were obtained from. The animal house was well ventilated and animals had 12 hour day and night schedule with temperature between 11-

20^0C. The animals were housed in large spacious hygienic cages during the course of the experimental period.

Test Compounds

Compound (I, II, III, IV and V) 75 mg/kg, 150 mg/kg, b. wt., Diclofenac sodium used as a reference standard (100mg/kg) orally were suspended in 0.5 % w/v dispersion of CMC in distilled water.

Methods

Carrageenan Induced Hind Paw Edema in Rats

This is acute model for screening anti-inflammatory drugs. Albino Wistar rats were divided into five groups of six each. They were starved overnight with water *ad libitum* prior to the day of experiment. Acute inflammation was induced by injecting 0.05 mL of 1 % suspension of carrageenan in0.5%w/v dispersion of CMC in distilled water into sub-planter region of the left hind paw as per the techniques. A mark was applied on the leg at the malleolus to facilitate subsequent readings. The paw edema volume was measured by mercury displacement with the help of a plethysmograph before as well as 0, 30, 60, 120, 180 minutes after carrageenan injection. The animals were treated with COMPOUND (I, II, III, IV, V) 75 mg/kg, 150mg/kg, 0.5% CMC (3 mL/kg, p.o.) treated animals were served as control and diclofeac sodium (100 mg/kg, p.o.) was treated as standard. Mean increase in paw volume was measured. The percentage inhibition of edema in various groups was calculated using the formula:

$$\% \text{ Paw edema inhibition} = 1 - \frac{\text{Edema volume in drug treated group}}{\text{Edema volume in control group}} \times 100$$

Statistical analysis

The results were expressed as the mean ± SEM. The results obtained from the present study were analyzed using one way ANOVA followed by Dunnett's multiple comparison tests. Data was computed for statistical analysis by using Graph pad prism software.

RESULTS AND DISCUSSION

Anti-inflammatory activity

Anti-inflammatory activity of the selected new compounds was screened by caragennan induced acute rat hind paw edema method. Diclofenac sodium used as standard drug, which showed 98.68 % inhibition. The results of the anti-inflammatory study reveal that all the five compounds have shown significant anti-inflammatory effect which was evident by significant reduction in the paw volume when compared to control group. In that compound II and compound V showed significant activity when compare to rest of three was shown in Table No.1 and Figure No.2. The Carrageenan-induced paw oedema as an *in vivo* model of inflammation was selected to assess the anti-inflammatory activity of synthetic product particularly in the acute phase of inflammation. Oedema formation due to carrageenan in the rat is a biphasic event. The early phase is related to the production of histamine, serotonin and possibly cyclooxygenase products and kinin like substances reaching peak at 180 min. The second phase of oedema is due to the release of prostaglandins, free radicals, proteases and lysosomes. The second phase is sensitive to most clinically effective anti-inflammatory drugs. Oral administration of the pyrazine derivates suppressed the edematous response after 3 hours and this effect continued upto 5 h. The observed effect was similar to that of diclofenac. NSAIDS block the synthesis of prostaglandins by inhibiting cyclooxygenase. The results of the present study indicated that pyrazine derivatives significantly inhibited the formation of rat hind paw oedema in the late phase. The effect of pyrazine derivatives may be due to influence on inflammatory mediators and also on pathway of prostaglandins synthesis.

SPECTERAL DATAS FOR PYRAZINE DERIVATIVES

Compound - I

5-methyl-N'-[3-phenylprop-2-en-1-ylidene] pyrazine-2-carbohydrazide

UV ëmax (CH3OH):271; IR (KBr): 3143 (NH), 3012 (Ar-CH), 1626 (C=O), 1487 (C=N), 785, 680 (CH out of planebending); 1H NMR (300 MHz, CDCl3): 8.38-9.35(m, 2H,pyrazine), 7.10-7.38 (m, 5H, aromatic), 7.51 (s, 1H, =CH)6.90-7.09(s, 2H, CH=CH), 10.56 (s, 1H, N-H), 2.68 (s, 3H,CH3); MS (ESI): *m/z* 289.1128 [M+Na]+. Anal. Calcd.

forC15H14N4O:C, 67.65; H, 5.30; N, 21.04. Found: C, 67.61;H, 5.27; N, 21.00.

Compound - II

5-methyl-N'-[furan-3-ylmethylidene] pyrazine-2-carbohydrazide

UV ëmax (CH3OH):330; IR (KBr): 3302 (NH), 3007 (Ar-CH), 1674 (C=O), 1485 (C=N), 785, 740 (CH out of planebending); 1H NMR (300 MHz, CDCl3): 8.35-8.44 (m, 2H,pyrazine), 6.47-6.85 (m, 3H, furyl), 7.50 (s, 1H, =CH)10.54 (s, 1H, N-H), 2.66 (s, 3H, CH3); MS (ESI): *m/z*253.1978 [M+Na]+. Anal. Calcd. for C11H10N4O2: C,57.39; H, 4.38; N, 24.34. Found: C, 57.56; H, 4.32; N,24.33.

Compound - III

5-methyl-N'-[(4- methoxyphenyl)methylidene] pyrazine-2-carbohydrazide

UV ëmax (CH3OH):326; IR (KBr): 3288 (NH), 2926 (Ar-CH), 1678 (C=O), 1508 (C=N), 823 (CH out of planebending); 1H NMR (300 MHz, CDCl3): 8.17-8.58 (m,2H,pyrazine), 6.87-7.20 (m, 4H, aromatic), 7.69 (s, 1H, =CH),3.84 (s, 3H, -OCH3), 10.49 (s, 1H, N-H), 2.56 (s, 3H,CH3); MS (ESI): *m/z* 271.1104 [M+1]+. Anal. Calcd. forC14H14N4O2: C, 62.21; H, 5.22; N, 20.73. Found: C, 62.19;H, 5.27; N, 20.70.

Compound - IV

5-methyl-N'-[(2-chlorophenyl) methylidene] pyrazine-2-carbohydrazide

UV ëmax (CH3OH):267; IR (KBr): 3287 (NH), 3013 (Ar-CH), 1676 (C=O), 1587 (C=N), 823 (CH out of planebending), 779 (C-Cl); 1H NMR (300 MHz, CDCl3): 8.64-9.07 (m, 2H, pyrazine), 7.21-7.38 (m, 4H, aromatic), 7.80(s, 1H, =CH), 10.75 (s, 1H, N-H), 2.66 (s, 3H, CH3); MS(ESI): *m/z* 275.1811 [M+1]+. Anal. Calcd. forC13H11ClN4O: C, 56.84; H, 4.04; N, 20.40. Found: C,56.80; H, 4.01; N, 20.43.

Compound - V

5-methyl-N'-[(3-chlorophenyl) methylidene] pyrazine-2-carbohydrazide

UV ëmax (CH3OH):272; IR (KBr): 3289 (NH), 2926 (Ar-CH), 1686 (C=O), 1587 (C=N), 835 (CH out of planebending), 613 (C-Cl); 1H NMR (300 MHz, CDCl3): 8.72-9.02 (m, 2H, pyrazine), 7.82-8.04 (m, 4H, aromatic), 7.42(s, 1H, =CH),10.44 (s, 1H, N-H), 2.86 (s, 3H, CH3); MS(ESI): *m/z* 275.1498 [M+1]+. Anal. Calcd. forC13H11ClN4O: C, 56.84; H, 4.04; N, 20.40. Found: C,56.88; H, 4.06; N, 20.39.

Table No.1: Anti-Inflammatory effect of pyrazine derivatives carrageenin induced paw edema in rats

S.No	Groups	Mean paw volume in ml							Percentage inhibition
		0 min		15 min	30 min	60 min	120 min	180 min	
1	Control	0.64±0.2		0.58±0.1	0.78±0.4	0.80±0.1	0.84±0.2	0.96±0.6	Vc = 0.76
2	Compound-I	75 mg	0.50±0.1	0.52±0.6	0.50±0.8	0.58±0.4	0.60±0.4	0.64±0.8	59.21
		150mg	0.41±0.2	0.42±0.2	0.44±0.4	0.46±0.8	0.51±0.6	0.52±0.4	71.05
3	Compound-II	75 mg	0.52±0.1	0.48±0.4	0.40±0.6	0.36±0.2	0.30±0.1	0.21±0.2	81.84
		150mg	0.40±0.2	0.36±0.6	0.32±0.4	0.24±0.3	0.22±0.2	0.12±0.1	95.26
4	Compound-III	75 mg	0.60±0.1	0.62±0.6	0.60±0.8	0.68±0.4	0.70±0.4	0.74±0.8	45.26
		150mg	0.51±0.2	0.50±0.2	0.54±0.4	0.54±0.8	0.51±0.6	0.62±0.4	61.05
5	Compound-IV	75 mg	0.64±0.2	0.68±0.1	0.68±0.4	0.80±0.1	0.84±0.2	0.96±0.6	30.78
		150mg	0.52±0.1	0.48±0.4	0.44±0.6	0.36±0.2	0.34±0.1	0.30±0.2	78.15
6	Compound-V	75 mg	0.51±0.1	0.46±0.4	0.41±0.6	0.35±0.2	0.28±0.1	0.21±0.2	82.89
		150mg	0.42±0.2	0.35±0.6	0.31±0.4	0.23±0.3	0.18±0.2	0.10±0.1	96.71
7	Diclofenac	100mg	0.42±0.1	0.34±0.1	0.28±0.2	0.20±0.6	0.16±0.1	0.10±0.2	98.68

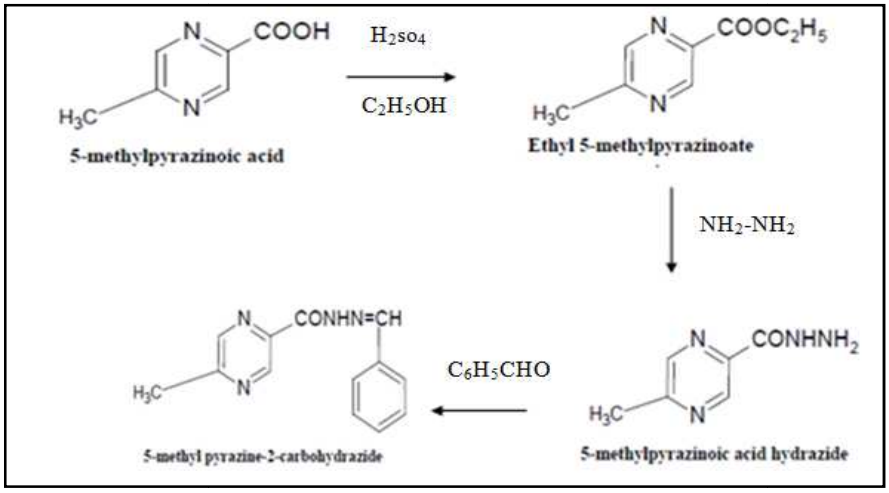

Figure No.1: schematic synthesis of pyrazine derivatives

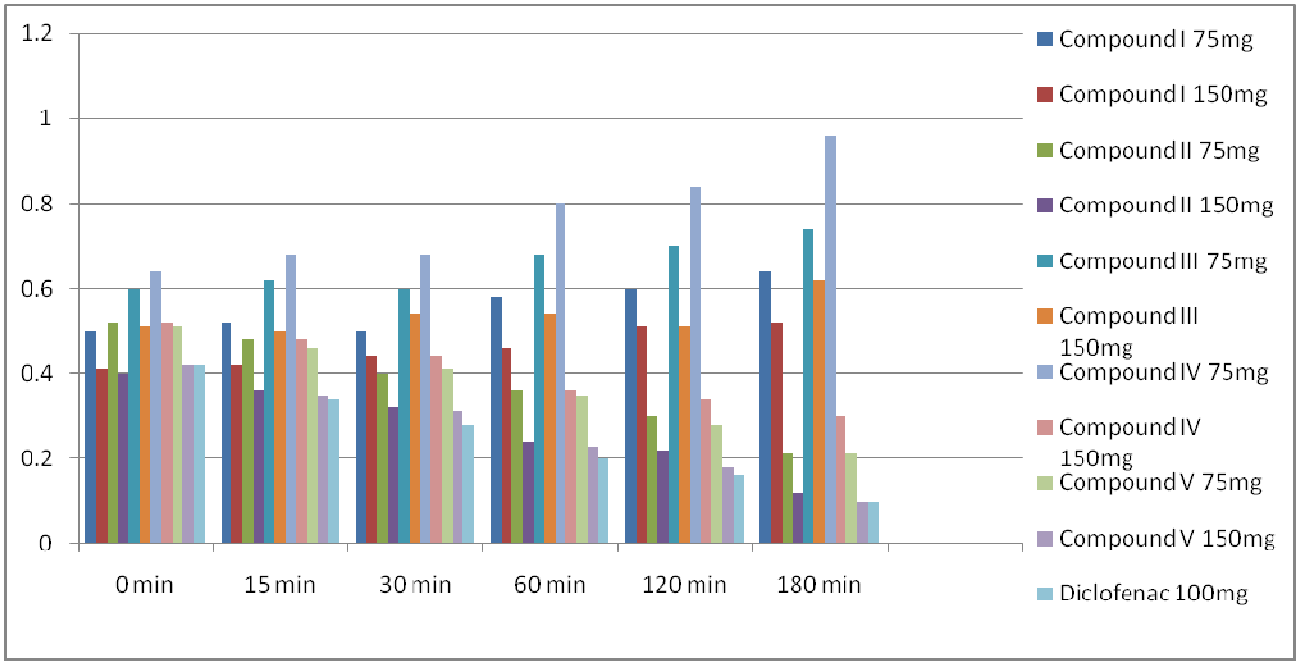

Figure No.2: Effect of Anti-Inflammatory activity of pyrazine derivatives

CONCLUSION

Five pyrazine derivatives have been synthesized, characterized by IR, ^{1}HNMR and Mass spectral data for all five compounds and all compounds are screened for their anti-inflammatory using paw-odema method, diclofenac employed as a reference standard. From the results obtained it is concluded that compound II and V shown potent and compound I, III, IV shown mild to moderate anti-inflammatory activity when compared to control and almost equipotent activity when compared to standard diclofenac sodium. Compounds II and V containing electron withdrawing groups like furan, cyclo hexachloride which may favored potent anti-inflammatory activity when compared to compounds I, III and IV. If work is futher continued with

different substituted heterocyclic compounds, you may get potent pharmacophore which may promote significant anti-inflammatory activity.

ACKNOWLEDGEMET

The authors are thankful to Sir C R Reddy Educational institutions for providing necessary facilities.

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