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Synthesis and pharmacological activities of some new 1-substituted-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles

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ABSTRACT

Several-1-carbothioamide-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles **(2a-d)**, 1-(pyridine-4-ylcarbonyl)-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles **(3a-d)**, were synthesized. The structures of the newly synthesized compounds were supported by IR, ^{1}H NMR and Mass spectral data. These compounds were investigated for their, anti-inflammatory, analgesic, ulcerogenic, lipid peroxidation, antibacterial and antifungal activities. Some of the synthesized compounds showed potent anti-inflammatory activity along with minimal ulcerogenic effect and lipid peroxidation, compared to ibuprofen and flurbiprofen. Some of the tested compounds also showed moderate antimicrobial activity against tested bacterial and fungal strains.

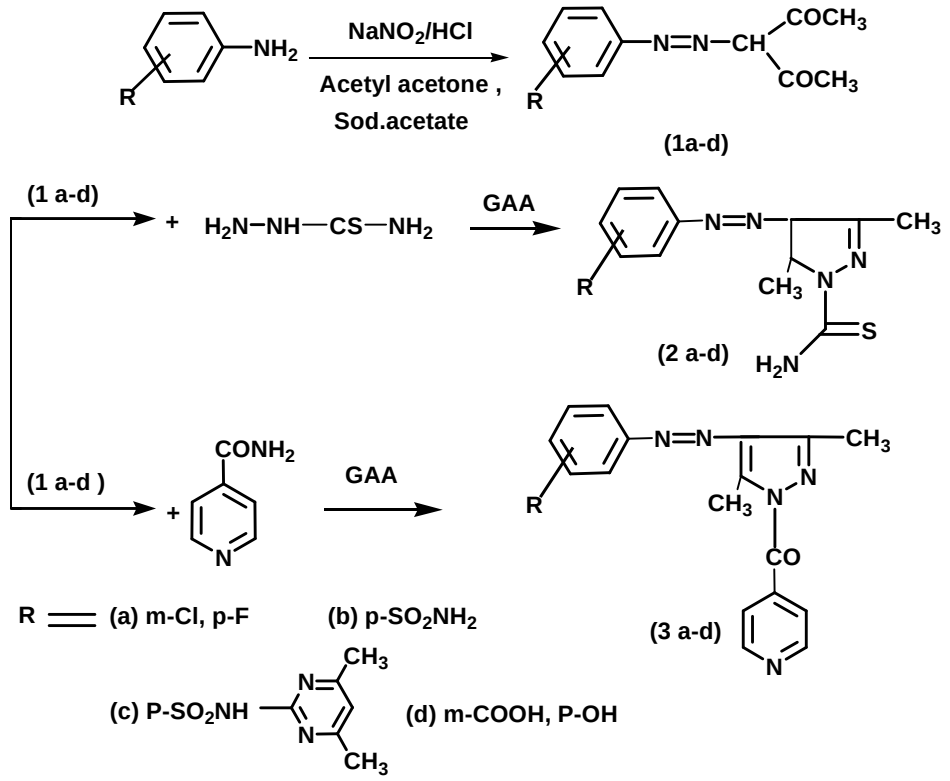
KEYWORDS

Pyrazoles;
Anti-inflammatory;
Ulcerogenicity;
Lipid peroxidation;
Hepatotoxic effect.

INTRODUCTION

Currently, available non-steroidal anti-inflammatory drugs (NSAIDs) like, ibuprofen, flurbiprofen, fenbufen and naproxen exhibit gastric toxicity. Long-term use of these drugs has been associated with gastro-intestinal (GI) ulceration, bleeding and nephrotoxicity[1]. The GI damage from NSAIDs is generally attributed to two factors, i.e. local irritation by the carboxylic acid moiety, common to most NSAIDs (topical effect) and decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining GI health and homeostasis[2,3]. The pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting cyclooxygenases (COXs)[2,4]. The chronic use of NSAIDs including ibuprofen may elicit appreciable GI toxicity[5]. Therefore synthetic approaches based upon NSAIDs chemical modification has been taken with the aim of improving NSAID safety profile. In view of the potential biological activities[6-9] of arylazo pyrazoles we report herein the synthesis of some new-1-(substituted)-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles. The synthesis involves treatment of acetyl acetone with different diazonium salt in the presence of sodium acetate when 3-[(substituted phenyl) diazenyl] pentane-2,4-dione **(1a-d)** are obtained. The later on treatment with carbothioamide, pyridine-4-ylcarbonyl, furnished 1-(substituted)-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles (Scheme 1). In view of the reported antimicrobial activity of pyrazole, these compounds were also tested for their antibacterial and antifungal activity against

some selected microbes. Two selected compounds were also studied for their hepatotoxic effects on rat liver. The structures of the various compounds were assigned on the basis of IR, ^{1}H NMR and Mass spectral data.



Scheme 1

EXPERIMENTAL

Carbothioamide-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles **(2a-d)**, 1-(pyridine-4-ylcarbonyl)-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles **(3a-d)**, were obtained by cyclisation of intermediates **(1a-d)** with 5-chloro-6-fluoro-1,3-benzothiazole-2-yl-thiosemicarbazide and N-4H-1,2,4-triazol-4-ylhydrazine. The intermediates **(1a-d)** were obtained by diazotization reaction of substituted aniline. The reaction sequences are outlined in Scheme 1.

EXPERIMENTAL PROTOCOLS

Melting points were determined in open capillary tubes. IR spectra were recorded on a perkin-Elmer 157 spectrometer and ^{1}HNMR spectra on a Bucker WM-400 (400 MHZ FT NMR) spectrophotometer using TMS (Tetramethyl Silane) as internal reference (chemical shift in δ ppm). The physical data of the compounds are presented in TABLE 1.

TABLE 1 : Physical data of the compounds (2a-d & 3a-d)

Compound	Mol. Formula	Mol. Weight	Yield %	Melting point	N % Found	N % Calculated
2a	$C_{12}H_{11}ClFN_5S$	311.76	65	239?C	22.51	22.46
2b	$C_{12}H_{14}N_6O_2S_2$	338.40	45	245?C	24.90	24.83
2c	$C_{18}H_{20}N_8O_2S_2$	444.53	43	265?C	25.40	25.21
2d	$C_{13}H_{13}N_5O_3S$	319.33	41	227?C	21.80	21.93
3a	$C_{17}H_{13}ClFN_5O$	357.76	62	250?C	19.67	19.58
3b	$C_{17}H_{16}N_6O_3S$	384.41	47	247?C	21.97	21.86
3c	$C_{23}H_{22}N_8O_3S$	490.53	46	256?C	22.90	22.84
3d	$C_{18}H_{15}N_5O_4$	365.24	43	260?C	19.25	19.17

3-[(substituted phenyl) diazenyl] pentane-2,4-dione (1a-d)

Substituted aniline (0.05 mol) was dissolved in dil HCl (40 ml, 1:1). The contents were stirred and cooled (0-2°C) and a cold solution of sodium nitrite (6.0g in 15 ml of water) was added to it slowly maintaining the temperature between 0-2°C. The cold diazotized solution was added drop wise to a well cooled and stirred

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mixture of acetyl acetone (0.05 mol) and sodium acetate (4g, dissolved in 5 ml of 50 % ethanol). The stirring was continued for 1.5 hr and crystals separated were filtered, washed with water, dried and crystallized from ethanol to yield (1a-d). IR (KBr) 1a-d: 2993-3012 cm^{-1}, CH stretching, 1661-1693 cm^{-1}, C=O stretching and 1536-1573 cm^{-1}, N=N stretching vibration; ^{1}HNMR ($CDCl_3$) 1a: δ 1.78 (s, 1H,N–CH). The signals of two $COCH_3$ were obtained as singlets at δ 2.35 and δ 2.43, δ 7.37-7.51 (m, 3H, Ar-H); 1d: δ 1.65 (s, 1H,N–CH). The signals of two $COCH_3$ were obtained as singlets at δ 2.47 and δ 2.59, δ 6.93-7.35 (m, 3H, Ar-H) and δ 10.2 (s, 1H, COOH).

1-carbothioamide-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles (2a-d)

3-[(substituted phenyl) diazenyl] pentane-2, 4-dione (1a-d 0.001 mol) and thiosemicarbazide (0.001 mol) were dissolved in glacial acetic acid (10 ml) and the solution was refluxed for 20 hr. The resulting solid was purified by repeated washing with acetic acid and crystallization from acetic acid to get 2a-d. IR (KBr) 2a-d: 1038-1080 cm^{-1} (C=S), 1574-1613 cm^{-1} (N=N) and at 2958-3018 cm^{-1} (CH stretching); ^{1}HNMR ($CDCl_3$) 2a: δ 2.17 (s, 3H of CH_3 attached to the 3rd position of the pyrazole ring), δ 4.14 (s, 2H, S=C–NH_2), δ 7.38-8.12 (m, 3H,Ar-H).

1-(pyridine-4-ylcarbonyl)-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles (3a-d)

3-[(substituted phenyl) diazenyl] pentane-2,4-dione (1a-d 0.001 mol) and 2 isonicotinic acid hydrazide (0.001 mol) were dissolved in glacial acetic acid (10 ml) and the solution was refluxed for 20 hr. The resulting solid was filtered, washed with acetic acid and recrystallized from acetic acid to obtain 3a-d. IR (KBr) 3a-d: 1577-1614 cm^{-1} (N=N) and 1630-1666 (C=O), 2980-3013 (CH stretching); ^{1}HNMR ($CDCl_3$) 3b: merged singlet observed at δ 2.46, indicating the presence of 3 and 5 methyl group attached to pyrazole ring. δ 7.85-7.88 (m, 4H, pyridyl) and δ 7.10-7.70 (m, 4H, Ar-H); 3d: δ 6.96-7.43 (m, 3H, Ar-H), δ 7.44-7.82 (m, 4H, pyridyl) and 10.4 (s, 1H,COOH).

RESULT AND DISCUSSION

Spectral characterization of the compounds

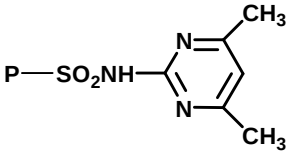
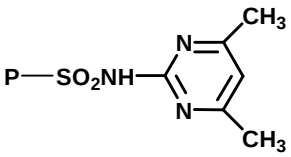
The IR spectrum of the compounds **(1a-d)** showed peaks at 2993-3012 cm^{-1}, CH stretching; 1661-1693 cm^{-1}, C=O stretching and 1536-1573 cm^{-1}, -N=N- stretching vibrations. The NMR spectrum of the compound **(1a)** showed a singlet at 1.78 indicating the presence of N-CH proton. The signals of two $COCH_3$ were obtained as singlets at δ 2.35 and δ 2.43. In the aromatic region multiplete were obtained at δ 7.37-7.51 indicating the presence of 3 aromatic protons. The NMR spectrum of the compound **(1d)** showed a singlet at δ 1.65 indicating the presence of N-CH proton. The protons of two $COCH_3$ were obtained as singlets at δ 2.47 and δ 2.59. Furthermore multiplet were obtained at 6.93-7.35 indicating the presence of 3 aromatic protons. The COOH proton was obtained as a singlet at δ 10.2. The IR spectrum of the compounds **(2a-d)** showed peaks at 2958-3018 cm^{-1}, CH stretching, 1574-1613 cm^{-1}, -N=N- stretching and 1038-1080 cm^{-1}, C=S stretching vibrations. The NMR spectrum of the compound **(2a)** showed a singlet observed at δ 2.17, indicating the presence of methyl groups attached to the 3rd position of the pyrazole ring. A singlet was observed at δ 4.14 due to 2 protons of S=C–NH_2. In the aromatic region a multiplet of 3 protons was observed at δ 7.12-7.38. The IR spectrum of the compounds **(3a-d)** showed peaks at 2980-3013 cm^{-1}, CH stretching, 1577-1614 cm^{-1}, -N=N- stretching and 1630-1666 cm^{-1}, C=O stretching vibrations. The NMR spectrum of the compound **(3b)** showed a merged singlet of methyl protons attached to the 3rd and 5th position of the pyrazoles ring at δ 2.46. In the aromatic region a multiplet was obtained at 7.85-7.88 indicating the presence of 4 pyridyl protons. In the aromatic region a multiplet was obtained at δ 7.10-7.70 indicating the presence of 4 aromatic protons. The NMR spectrum of the compound **(3d)** showed a singlet of COOH protons at δ 10.4. In the aromatic region two multiplet was obtained at δ 6.96-7.43 and 7.45-7.82 indicating the presence of 3 aromatic protons and 4 pyridyl protons respectively.

Pharmacological results and discussion

The anti-inflammatory activity of the synthesized compounds **(2a-d)** and **(3a-d)** was evaluated by carrageenan induce paw edema method of Winter et al.[10]. The compounds **(2a-d)**, **(3a-d)** were tested at an equimolar oral dose relative to 70 mg/kg ibuprofen. The tested compounds showed anti-inflammatory activity

ranging from 18.17-80.29 %, whereas standard drug ibuprofen and flurbiprofen showed 80.38 and 80.29% inhibition respectively after 4 h (TABLE 2). The anti-inflammatory activity of pyrazole derivatives is in the range of 18.17-80.29%. It was observed that the pyrazole derivatives **(2a)** (80.29%) has shown the activity almost equal to standard drug, ibuprofen (80.38%). Compound **(2d)** and **(3c)** showed moderate activity. Test compounds that exhibited potent anti-inflammatory activity **(2a)**, **(2d)** and **(3c)** were further evaluated for their analgesic and ulcerogenic activities. All the compounds showed moderate analgesic activity in comparison to their standard drugs. The tested compounds showed significant reduction in ulcerogenic activity ranging from 0.333 ± 0.10 to 0.833 ± 0.24[e], whereas the standard drug ibuprofen and flurbiprofen showed high severity index of 2.000 ± 0.13 and 1.666 ± 0.24 respectively. In general, the tested compounds showed a better GI safety profile compared to standard drugs (TABLE 2).

TABLE 2 : Anti-inflammatory and antimicrobial activity of the compounds (2a-d & 3a-d)

Compound	R	Anti-inflammatory activity[#]		Antimicrobial activity MIC[##]		
		Difference in paw volume after 4 hr	% inhibition ± SEM	*S. aureus*	*E. coli*	*C . albicans*
2a	m-Cl, p-F	0.08 ± 0.008	80.29 ± 1.51	200	200	200
2b	p-SO_2NH_2	0.27 ± 0.013	38.63 ± 3.05[d]	200	25	100
2c		0.18 ± 0.008	57.57 ± 1.91[d]	200	100	12.5
2d	m-COOH, p-OH	0.09 ± 0.008	78.78 ± 1.91	200	12.5	200
3a	m-Cl, p-F	0.36 ± 0.018	18.17 ± 4.23[d]	50	200	200
3b	p-SO_2NH_2	0.15 ± 0.013	65.14 ± 3.03[c]	25	25	- -
3c		0.09 ± 0.013	78.78 ± 3.03	100	25	50
3d		0.21 ± 0.012	50.75 ± 2.73[d]	25	50	50
Ibuprofen		0.08 ± 0.012	80.38 ± 2.62	X X	X X	X X
Flurbipro- phen		0.08 ± 0.009	80.29 ± 2.25	X X	X X	X X
Ketocona- zole		X X	X X	X X	X X	6.25
Ofloxacin		X X	X X	6.25	6.25	- -
Control		0.44 ± 0.016		- -	- -	- -

- - Did not show any activity; X X Not tested; [#]Relative to their respective standard and data were analyzed by ANOVA followed by dunnett's multiple comparision test for n=6; [c]p<0.05, [d]p<0.01; [##]µg ml^{-1}

It has been reported in literature that compounds showing less ulcerogenic activity also showed reduced malondialdehyde (MDA) content, a byproduct of lipid peroxidation[11,12]. Therefore, an attempt was made to correlate the decrease in ulcerogenic activity of the compounds with that of lipid peroxidation. All the compounds screened for ulcerogenic activity were also analyzed for lipid peroxidation. The lipid peroxidation was measured as n mole of MDA/100 mg of tissue. The ibuprofen and flurbiprofen exhibited maximum lipid peroxidation 7.79 ± 0.13 and 7.51 ± 0.68 respectively, whereas control group showed 3.25 ± 0.05. It was found that all the cyclised derivatives showing less ulcerogenic activity also showed reduction in lipid peroxidation (TABLE 3). Thus these studies showed that synthesized compounds have inhibited the induction of gastric mucosal lesions and the results further suggested that their protective effect might be related to the inhibition of lipid peroxidation in gastric mucosa.

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TABLE 3 : Analgesic, ulcerogenic and lipid peroxidation activity of selected compounds.

Compound	Analgesic Activity[#]			Ulcerogenic activity (Severity index ± SEM)[##]	nmol MDA content ± SEM / 100 mg tissue[##]
	Pre-treatment/ normal 0 h (s)	Post-treatment/ after 4 h (s)	% Inhibition		
2a	1.810 ± 0.186	2.467 ± 0.290	36.2	0.333 ± 0.10 [d]	5.04 ± 0.53 [d]
2d	0.992 ± 0.076	1.362 ± 0.139	37.3[c]	0.75 ± 0.25 [d]	5.65 ± 0.36[e]
3b	1.156 ± 0.067	1.706 ± 0.069	48.1[a]	0.833 ± 0.24[e]	5.82 ± 0.50[e]
Control	- -	- -	- -	0.00	3.25 ± 0.05
Ibuprofen	1.361 ± 0.086	2.37 ± 0.131	74.1[a]	2.000 ± 0.13	7.79 ± 0.13
Flurbiprofen	1.15 ± 0.060	1.95 ± 0.097	69.5[a]	1.666 ± 0.24	7.51 ± 0.68

[#]Relative to normal and data were analyzed by paired student's *t* test for n=6; [a]p<0.0001, [b]p<0.001, [c]p<0.01; [##]Relative to their respective standard and data were analyzed by ANOVA followed by dunnett's multiple comparision test for n=6; [d]p<0.01, [e]p<0.05.

The compounds **(2a)** derivatives of pyrazole showing potent anti-inflammatory activity with reduced ulcerogenicity and lipid peroxidation was further studied for their hepatotoxic effect. The compound was studied for their effect on biochemical parameters (serum enzymes, total protein and total albumin) and histopathology of liver. As shown in TABLE 4, activities of liver enzymes SGOT, SGPT, alkaline phosphatase and total protein, total albumin were almost remains same with respect to control values. The histopathological studies of the liver samples do not show any significant pathological changes in comparison to control group (Figure 1). No hepatocyte necrosis or degeneration was seen in the samples.

TABLE 4 : Effect of compound on serum enzymes, total proteins and total albumin.

Compound	SGOT Units/ml[#]	SGPT Units/ml[#]	Alkaline Phophatase[#]	Total protein g/dl[#]	Total albumin g/dl[#]
Control	148.67 ± 1.50	27.67 ± 0.84	13.06 ± 0.25	1.80 ± 0.01	1.67 ± 0.01
2a	147.50 ± 0.34	28.17 ± 0.83	15.18 ± 0.13[a]	1.89 ± 0.07	1.80 ± 0.05[b]

[#]Relative to control and data were analyzed by students's *t* test for n=6; [a]p<0.0001, [b]p<0.01

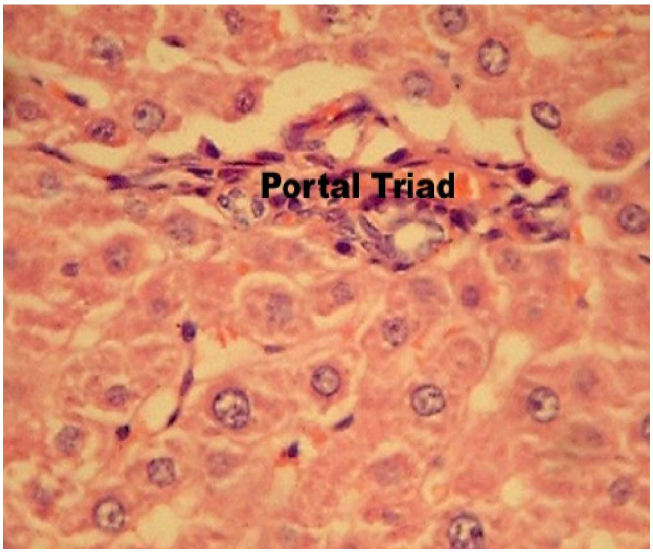

Figure 1 : High power (400x) photomicrograph of section of liver from control group animal showing portal triad structures.

Compounds **(2a-d)** and **(3a-d)** have been evaluated for their *in-vitro* anti-microbial activity against Staphylococcus aureus (S. aureus, ATCC-29737), as an example of gram-positive bacteria, Escherchia coli (E. coli, ATCC-8739) as an example of gram-negative bacteria and Aspergilus niger (A. niger) as a representative of fungi using cup plate technique. DMF (N,N-Dimethyl formamide) was run as a control and test was performed at 200, 100, 50, 25 µg/ml concentration. Ofloxacin and Ketoconazole was used as a standard drug. The micro dilution susceptibility test in nutrient agar media (Hi-Media), Sabroaud's dextrose agar media were used for determination of antibacterial and antifungal activities respectively. The minimal inhibitory concentration (MICs, μgmL^{-1}) of the tested compounds were recorded in TABLE 2. The results revealed that most of the newly synthesized pyrazoles derivatives bearing isonicotinic acid hydrazide moiety **(3a-d)** exhibited promising anti-bacterial activity. Out of the tested compound, compound **(3a)** having 3-chloro, 4-fluoro groups in the phenyl ring exhibited remarkable antibacterial activity against *E. coli* (gram negative bacteria), S. aureus (gram positive bacteria) and A. niger fungi whereas com-

pound **(3d)** having COOH, OH group at meta and para position of the phenyl ring showed MIC 50 μgmL^{-1} against S. aureus, E. coli, as compared with the broad spectrum antibiotics Ofloxacin (MIC 10.0 μgmL^{-1} against S. aureus and 12.5 μgmL^{-1} against E. coli). The antifungal screening results have shown that the compound **(2a)**, **(3a)**, **(3c)** and **(3d)** exhibited good activity (MIC 25 μgmL^{-1}) against *A. niger*, as compared with the standard drug Ketoconazole (MIC 12.5 μgmL^{-1}).

Pharmacology

Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity using carrageenan induced hind paw edema method of Winter et al.[10]. The experiment was performed on Albino rats of Wistar strain of either sex, weighing 180-200 gm. The animals were randomly allocated into groups of six animals each. One group was kept as control, received only 0.5% carboxymethyl cellulose solution. Group II and Group III were kept as standard and receives ibuprofen (70 mg/kg p.o.) and flurbiprofen (10 mg/kg p.o.) respectively. 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, 1 h after the administration of the test compounds and standard drugs. The right hind paw volume was measured before and after 4 h of carrageenan treatment by means of a plethysmometer. The percent anti-inflammatory activity was calculated according to the following formula.

$$\text{Percent anti} - \text{inf lammatory activity} = \left[V_c - \frac{V_t}{V_c} \right] \times 100$$

where, V_t represents the mean increase in paw volume in rats treated with test compounds and V_c represents the mean increase in paw volume in control group of rats.

Analgesic activity

Analgesic activity was evaluated by tail immersion method[13]. Swiss albino mice allocated into different groups consisting of six animals in each, of either sex, weighing 25-30 gm were used for the experiment. Analgesic activity was evaluated after oral administration of the test compounds **(2a-d)** and **(3a-d)** at an equimolar dose relative to 70 mg/kg ibuprofen. Test compounds and standard drugs were administered orally as suspension in carboxymethyl cellulose solution in water (0.5 % w/v). The analgesic activity was assessed before and after 4 h interval of the administration of test compounds and standard drugs. The lower 5 cm portion of the tail was gently immersed into thermostatically controlled water at 55 ± 0.5oC. The time in second for tail withdrawal from the water was taken as the reaction time with a cut of time of immersion, set at 10 seconds for both control as well as treated groups of animals.

Acute ulcerogenicity

Acute ulcerogenicity was determined according to Cioli et al.[14]. The animals were allocated into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after oral administration of the test compounds **(2a)** and **(2d)** at an equimolar dose relative to 210 mg/kg ibuprofen and test compounds **(3c)** at an equimolar dose relative to 30 mg/kg flurbiprofen. Control group received only 0.5% carboxymethylcellulose solution. Food but not water was removed 24 h 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 opens along the greater curvature, washed with distilled water and cleaned gently by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass. For each stomach the mucosal damage was assessed according to the following scoring system:

0.5: redness, 1.0: spot ulcers, 1.5: hemorrhagic streaks, 2.0: ulcers > 3 but = 5, 3.0: ulcers >5. The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage.

Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa et al.[15]. After screening for ulcerogenic activity, the gastric mucosa was scraped with two glass slides, weighed (100 mg) and homogenized in 1.8 mL of 1.15% ice cold KCl solution. The homogenate was supplemented with 0.2 mL of 8.1% sodium dodecyl sulphate (SDS), 1.5 mL of acetate buffer (pH 3.5) and 1.5 mL of 0.8% thiobarbituric acid (TBA). The mixture was heated at 95°C for 60 minutes. After cooling the reactants were supplemented with 0.2 mL of 8.1% sodium dodecyl

sulphate (SDS), 1.5 mL of acetate buffer (pH 3.5) and 1.5 mL of 0.8% thiobarbituric acid (TBA). The mixture was heated at 95°C for 60 minutes. After cooling the reactants were supplemented with 5 ml of the mixture of *n*-butanol and pyridine (15:1v/v), shaken vigorously for 1 min and centrifuged for 10 min at 4000 rpm. The supernatant organic layer was taken out and absorbance was measured at 532 nm on UV spectrophotometer. The results were expressed as n mol MDA/100 mg tissue, using extinction coefficient 1.56×10^5 cm^{-1} M^{-1}.

Hepatotoxic studies

The study was carried out on Wistar albino rats of either sex weighing 150-200gm. Animal were devided in to three groups, six rats in each. Group 1 was kept as control and receives only vehicle (0.5% w/v solution of carboxymethylcellulose in water), group 2 and 3 received compound **(2a)**, in 0.5 % w/v solution of carboxymethylcellulose in water for 15 days. After the treatment (15 days) blood was obtained from all the groups of rats by puncturing the retro-orbital plexus. Blood samples were allowed to clot for 45 min at room temperature and serum was separated by centrifugation at 2500 rpm for 15 min and analyzed for various biochemical parameters.

Assessment of liver function

Assessment of liver function such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by a reported method[16]. The alkaline phosphatase, total protein and total albumin were measured according to the reported procedures[17-19]. Data is recorded in TABLE 4.

Histopathological studies of liver

The histopathological studies were carried out by reported method[20]. The rats were sacrificed under light ether anesthesia after 24 h of the last dosage, the liver were removed and washed with normal saline and stored in formalin solution. Section of 5-6 microns in thickness were cut, stained with haematoxylin and eosin and then studied under an electron microscope (Figure 1-3).

Antibacterial and antifungal activities

Antibacterial activity of the synthesized compounds were determined in vitro by using dish diffusion method[21] against *Staphylococcus aureus* (gram-positive), *Escherichia coli* (gram negative) at 25, 50, 100 and 200 μg ml^{-1} concentration respectively, in the nutrient agar media by measuring the zone of inhibition in mm. Standard antibiotic Ofloxacin was used as reference drug at 25 and 50 μg ml^{-1} concentration.

Similarly, the antifungal activity of the synthesized compounds were determined in vitro by dish diffusion method against fungal strain *A. niger* at 25, 50, 100 and 200 μg ml^{-1} concentration in sudroad dextrose medium by using ketoconazole as standarad drug at 25 and 50 μg ml^{-1} concentration. The zone of inhibition was measured in mm. The compounds which showed

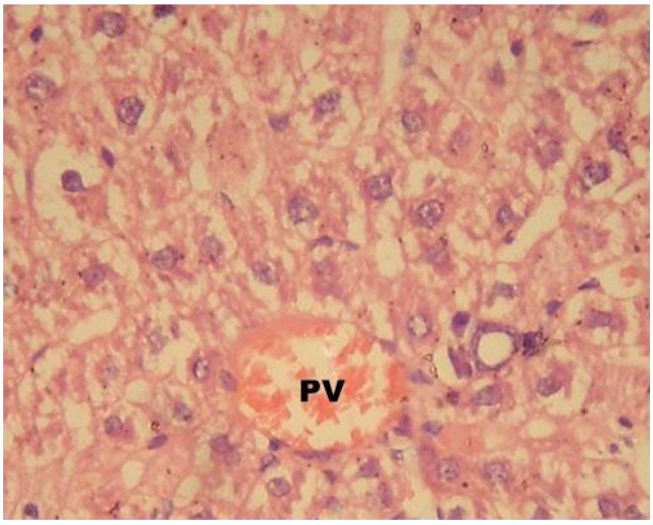

Figure 2 : High power (400x) photomicrograph of section of liver from experimental group animal showing normal portal triad structures.

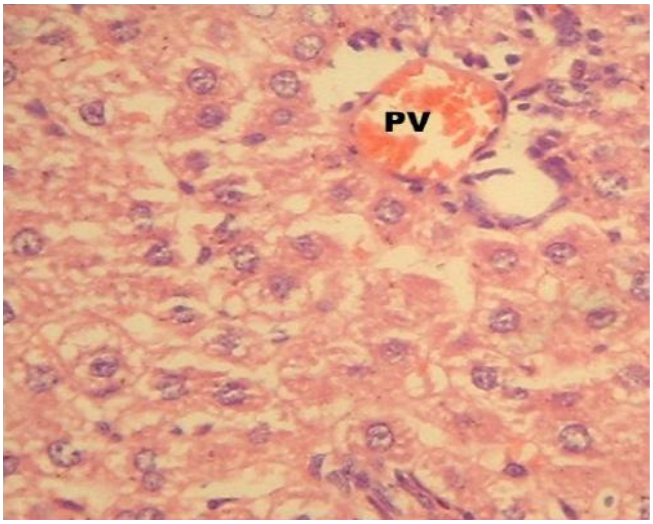

Figure 3 : High power (400x) photomicrograph of section of liver from experimental group animal showing normal portal triad structures.

inhibition at 25 μg ml^{-1} concentration were further tested at 12.5 and 6.25 μg ml^{-1} concentrations. DMF was used as solvent to prepare the desired concentration of the synthesized compounds.

CONCLUSION

In summary, we have described synthesis and pharmacological activity 1-(substituted)-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles derivatives. It was observed that the pyrazole derivatives **(2a)** has shown the activity almost equal to standard drug, ibuprofen and some of the compounds showed moderate analgesic activity in comparison to their standard drugs. The compounds **(2a)** was further studied for their hepatotoxic effect. Both the compounds were studied for their effect on biochemical parameters and histopathology of liver. The histopathological studies of the liver samples do not show any significant pathological changes in comparison to control group. All compounds were also tested for their antimicrobial activity.

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