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ORIGINAL ARTICLE

Noval 1-substituted-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazole derivatives: Synthesis and pharmacological activity

Sabir Hussain *, Deepika Kaushik

Department of Chemistry, Rawal Institute of Engineering & Technology (M.D. University Rohtak), Faridabad 121002, India

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KEYWORDS

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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**, 1-(5-chloro-6-fluoro-1,3-benzothiazole-2-yl)thiocarbamoyl-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles **4a–d** and 1-[(1,2,4-triazole-4-yl) carbothioamide]-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles **5a–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.

1. 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 (Kimmey, 1992). 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 (Smith et al., 1998; Hawkey et al., 2000). The pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting cyclooxygenases (COXs) (Smith et al., 1998; Warner et al., 1999). The chronic use of NSAIDs including ibuprofen may elicit appreciable GI toxicity (Lanza, 1998). 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 (Ahuwalia and Mittal, 1989; Werbal and

* Corresponding author. Tel./fax: +91 0129 3064100.
E-mail address: 03.sabir@gmail.com (S. Hussain).

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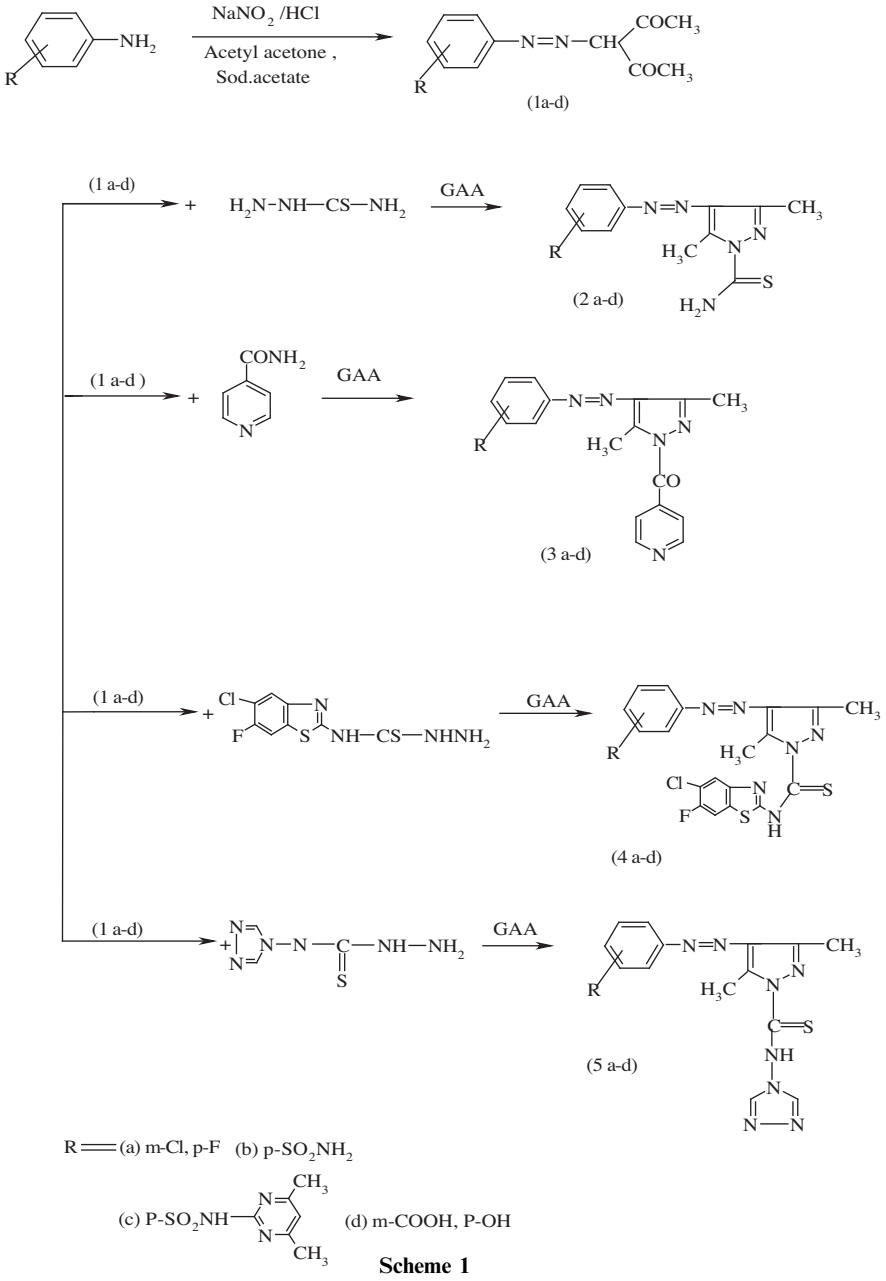
Elslager, 1968; Garg and Prakash, 1971; Kabra et al., 1977) of arylazo pyrazoles we report herein the synthesis of some new-1-(substituted)-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles. The synthesis involves treatment of acetyl acetone with different diazonium salt in the presence of sodium acetate when 3-[(substituted phenyl) diazenyl] pentane-2, 4-dione **1a–d** are obtained. The later on treatment with carbothioamide, pyridine-4-ylcarbonyl, 5-chloro-6-fluoro-1,3-benzothiazole-2-yl-thiocarbamoyl and 1,2,4-triazole-4-yl carbothioamide 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 activities 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.

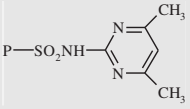
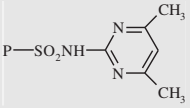
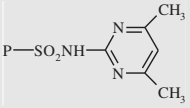
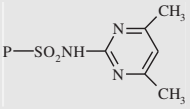
2. Chemistry

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**), 1-(5-chloro-6-fluoro-1,3-benzothiazole-2-yl) thiocarbamoyl-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles (**4a–d**) and 1-[(1,2,4-triazole-4-yl) carbothioamide]-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles (**5a–d**) were obtained by cyclization of intermediates **1a–d** with thiosemicarbazide, isonicotinic acid hydrazide, 5-chloro-6-fluoro-1,3-benzothiazole-2-yl-thiosemicarbazide and N-4H-1,2,4-triazol-4-ylhydrazine. The interme-



Scheme 1

Table 1 Anti-inflammatory and antimicrobial activity of the compounds (**2a–d** & **3a–d**, **4a–d** & **5a–d**).

Compound	R	Anti-inflammatory activity[#]		Antimicrobial activity		
		Difference in paw volume	% Inhibition ± SEM	MIC[##]		
		After 4 h		*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	*m*-COOH, *p*-OH	0.21 ± 0.012	50.75 ± 2.73[d]	25	50	50
4a	*m*-Cl, *p*-F	0.23 ± 0.011	47.72 ± 2.56[d]	25	50	25
4b	p-SO_2NH_2	0.10 ± 0.014	76.51 ± 3.65	50	200	200
4c		0.08 ± 0.009	80.29 ± 2.25	200	100	50
4d	*m*-COOH, *p*-OH	0.14 ± 0.012	68.17 ± 3.10[c]	100	25	- -
5a	*m*-Cl, *p*-F	0.31 ± 0.009	28.02 ± 2.17[d]	200	200	200
5b	*p*-SO_2NH_2	0.20 ± 0.011	53.02 ± 2.54[d]	200	50	200
5c		0.15 ± 0.013	65.90 ± 3.05[c]	- -	100	25
5d	*m*-COOH, *p*-OH	x x	x x	- -	25	200
Ibuprofen		0.08 ± 0.012	80.38 ± 2.62	x x	x x	x x
Flurbiprophen		0.08 ± 0.009	80.29 ± 2.25	x x	x x	x x
Ketoconazole		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 comparison test for $n = 6$.

[c] $p < 0.05$.

[d] $p < 0.01$.

[##] μg ml^{-1}.

diates **1a–d** were obtained via coupling acetylacetone with diazotized aromatic amines. The reaction sequences are outlined in Scheme 1.

3. Result and discussion

3.1. 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 δ 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 multiplets were obtained at δ 7.37–7.51 indicating the presence of three 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 multiplets were obtained at 6.93–7.35 indicating the presence of three 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 two protons of S═C–NH_2. In the aromatic region a multiplet of three protons was observed at δ 7.12–7.38. The IR spectrum of the

Table 2 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]
4c	1.482 ± 0.153	2.200 ± 0.162	48.4[a]	0.417 ± 0.08 [d]	4.82 ± 0.25[d]
5a	1.217 ± 0.120	1.843 ± 0.147	51.4[b]	1.000 ± 0.31[d]	5.78 ± 0.97[e]
5b	1.171 ± 0.084	1.620 ± 0.088	38.3[c]	0.667 ± 0.10[d]	5.12 ± 0.29
5d	1.860 ± 0.223	2.513 ± 0.202	53.1[b]	0.666 ± 0.16[d]	5.62 ± 0.29[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 comparison test for $n = 6$.
[d] $p < 0.01$.
[e] $p < 0.05$.

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 positions of the pyrazole ring at δ 2.46. In the aromatic region a multiplet was obtained at δ 7.85–7.88 indicating the presence of four pyridyl protons. In the aromatic region a multiplet was obtained at δ 7.10–7.70 indicating the presence of four aromatic protons. The NMR spectrum of the compound **3d** showed a singlet of COOH protons at δ 10.4. In the aromatic region two multiplets were obtained at δ 6.96–7.43 and 7.45–7.82 indicating the presence of three aromatic protons and four pyridyl protons respectively. The IR spectrum of the compounds (**4a**–**d**) showed peaks at 2979–3018 cm^{-1}, CH stretching, 1530–1560 cm^{-1}, –N═N– stretching and 1564–1616 cm^{-1}, C═C stretching of aromatic ring. The NMR spectrum of the compound **4a** showed two singlets at δ 2.61 and δ 3.18 indicating the presence of methyl protons attached at 3rd and 5th positions of the pyrazole ring. In the aromatic region a complex multiplet was obtained at δ 7.12–7.48 indicating the presence of 5 aromatic protons. The IR spectrum of the compounds (**5a**–**d**) showed peaks at 2981–3013 cm^{-1}, CH stretching; 1508–1567 cm^{-1}, –N═N– stretching and 1050–1086 cm^{-1}, C═S stretching. The NMR spectrum of the compound **5d** showed two singlets at δ 2.72 and δ 3.28 indicating the presence of methyl protons attached to the 3rd and 5th positions of the pyrazole ring. A multiplet of 3 protons was observed in the aromatic region at δ 7.22–7.54. Furthermore, a singlet for S═C–NH proton was also observed at δ 4.16.

3.2. Pharmacological results and discussion

The anti-inflammatory activity of the synthesized compounds **2a-d, 3a-d** and **4a**–**d** was evaluated by carrageenan induce paw edema method of Winter et al. (1962). The compounds (**2a-d**, **3a-d)** were tested at an equimolar oral dose relative to 70 mg/kg ibuprofen and the compounds (**4a-d**, **5a-c)** were tested at an equimolar oral dose relative to 10 mg/kg flurbiprofen. The tested compounds showed anti-inflammatory activity ranging from 18.17% to 80.29%, whereas standard drug ibuprofen and flurbiprofen showed 80.38 and 80.29% inhibition respectively after 4 h (Table 1). 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%) and **4c** (80.29%) have shown the activity almost equal to standard drug, ibuprofen (80.38%). Compounds **2d**, **3c** and **4b** showed moderate activity. Test compounds that exhibited potent anti-inflammatory activity **2a, 2d, 3c, 4b**, and **4c** 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 1.000 ± 0.31, whereas the standard drug ibu-

Table 3 Effect of compounds 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]
4c	137.00 ± 0.72[a]	26.50 ± 0.72	17.07 ± 0.11[a]	0.90 ± 0.09[a]	0.71 ± 0.07[a]

[#] Relative to control and data were analyzed by Students's t test for $n = 6$.
[a] $p < 0.0001$.
[b] $p < 0.01$.

profen 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).

It has been reported in the literature that compounds showing less ulcerogenic activity also showed reduced malondialdehyde (MDA) content, a byproduct of lipid peroxidation (Naito et al., 1998; Pohle et al., 2001). 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 cyclized derivatives showing less ulcerogenic activity also showed reduction in lipid peroxidation (Table 2). 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.

The compounds **2a** and **4c**, derivatives of pyrazole showing potent anti-inflammatory activity with reduced ulcerogenicity and lipid peroxidation were further studied for their hepatotoxic effect. Both the compounds were studied for their effect on biochemical parameters (serum enzymes, total protein and total albumin) and histopathology of liver. As shown in Table 3, activities of liver enzymes SGOT, SGPT, alkaline phosphatase and total protein, total albumin almost remain same with respect to control values, except for compound **4c** in which total protein and total albumin were markedly reduced. The histopathological studies of the liver samples do not show any significant pathological changes in comparison to control group. No hepatocyte necrosis or degeneration was seen in any of the samples.

Compounds (**2a–d**,**3a–d, 4a–d** and **5a–d**) have been evaluated for their in vitro anti-microbial activity against *Staphylococcus aureus* (ATCC-29737), as an example of gram-positive bacteria, *Escherichia* coli (ATCC-8739) as an example of gram-negative bacteria and *Aspergilus 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 were used as standard drugs. The micro dilution susceptibility test in nutrient agar media (Hi-Media), Sabroaud's dextrose agar media were used for the determination of antibacterial and antifungal activities respectively. The minimal inhibitory concentration (MICs, μg mL^{-1}) of the tested compounds were recorded in Table 1. The results revealed that most of the newly synthesized pyrazole derivatives bearing isonicotinic acid hydrazide moiety (**3a–d**) exhibited promising anti-bacterial activity. Out of the tested compound, compound **3a** and **5a** 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 compound **3d** having COOH, OH group at meta and para positions of the phenyl ring showed MIC 50 μg mL^{-1} against *S. aureus, E. coli,* as compared with the broad spectrum antibiotics Ofloxacin (MIC 10.0 μg mL^{-1} against *S. aureus* and 12.5 μg mL^{-1} against *E. coli)*. The antifungal screening results have shown that the compounds **2a**, **3a**, **3c**, **3d**, and **4a** exhibited good activity (MIC 25 μg mL^{-1}) against *A. niger*, as compared with the standard drug ketoconazole (MIC 12.5 μg mL^{-1}).

4. Pharmacology

4.1. Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity using carrageenan induced hind paw edema method of Winter et al. (1962). 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 standards and receive 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.

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

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 the control group of rats.

4.2. Analgesic activity

Analgesic activity was evaluated by tail immersion method (Janssen et al., 1963). 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 and test compounds (**4a–d** and **5a–c**) at an equimolar dose relative to 10 mg/kg flurbiprofen. 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 ± 0.5 °C. 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 s for both control as well as treated groups of animals.

4.3. Acute ulcerogenicity

Acute ulcerogenicity was determined according to Cioli et al. (1979). 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**, **4b** and **4c**) 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 $\leqslant 5$, 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.

4.4. Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa et al. (1979). 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 sulfate (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 min. After cooling the reactants were supplemented with 0.2 mL of 8.1% sodium dodecyl sulfate (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 min. After cooling the reactants were supplemented with 5 ml of the mixture of *n*-butanol and pyridine (15:1 v/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 nmol MDA/100 mg tissue, using extinction coefficient $1.56 \times 10^5\ cm^{-1}\ M^{-1}$.

4.5. Hepatotoxic studies

The study was carried out on Wistar albino rats of either sex weighing 150–200 gm. Animals were divided into 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), groups 2 and 3 received compound **2a** and **4c**, respectively, 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.

4.6. Assessment of liver function

Assessment of liver function such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) was estimated by a reported method (Reitman and Frankel, 1957). The alkaline phosphatase, total protein and total albumin were measured according to the reported procedures (King and Armstrong, 1934; Reinhold, 1953; Varley, 1988). All the data are recorded in Table 3.

4.7. Histopathological studies of liver

The histopathological studies were carried out by the reported method (Luna, 1968). The rats were sacrificed under light ether anesthesia after 24 h of the last dosage, the liver was removed and washed with normal saline and stored in formalin solution. Section of 5–6 μm in thickness was cut, stained with hematoxylin and eosin and then studied under an electron microscope.

4.8. Antibacterial and antifungal activities

Antibacterial activity of the synthesized compounds was determined in vitro by using dish diffusion method (Barry, 1976) against *S. aureus* (gram-positive), *E. 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} concentrations.

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

5. Conclusion

In summary, we have described the synthesis and pharmacological activity of 1-(substituted)-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles derivatives. It was observed that the pyrazole derivatives **2a** and **4c** have shown the activity almost equal to the standard drug, ibuprofen and some of the compounds showed moderate analgesic activity in comparison to their standard drugs. The compounds **2a** and **4c** were 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 did not show any significant pathological changes in comparison to the control group. All compounds were also tested for their antimicrobial activity.

6. Experimental protocols

Melting points were determined in open capillary tubes. IR spectra were recorded on a Perkin-Elmer 157 spectrometer and ^{1}H NMR spectra on a Bucker WM-400 (400 MHz FT NMR) spectrophotometer using TMS (Tetramethyl Silane) as internal reference (chemical shift in δ ppm). Purity of the compounds was checked by TLC (Thin Layer Chromatography) on silica gel plates and spots were visualized by the exposure to iodine vapors. The physical data of the compounds prepared are presented in Table 1.

6.1. 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 (6.0 g in 15 ml of water) was added to it slowly maintaining the temperature between 0 °C and

2 °C. The cold diazotized solution was added drop wise to a well cooled and stirred mixture of acetyl acetone (0.05 mol) and sodium acetate (4 g, dissolved in 5 ml of 50% ethanol). The stirring was continued for 1.5 h 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}H NMR** ($CDCl_3$) 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).

*6.2. 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 h. 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}H NMR** ($CDCl_3$) 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).

*6.3. 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 h. 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}H NMR** ($CDCl_3$) 3b: merged singlet observed at δ 2.46, indicating the presence of 3 and 5 methyl groups 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).

*6.4. 1-(5-Chloro-6-fluoro-1,3-benzothiazole-2-yl) thiocarbamoyl-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles (**4a–d**)*

3-[(Substituted phenyl) diazenyl] pentane-2,4-dione (**1a–d**, 0.001 mol) and 5-chloro-6-fluoro-1,3-benzothiazole-2-yl-thiosemicarbazide (0.001 mol) were dissolved in glacial acetic acid (10 ml) and the solution was refluxed for 20 h. The resulting solid was filtered, washed with acetic acid and recrystallized from acetic acid to obtain **4a–d**. IR (KBr) 4a–**d**: 2979–3018 cm^{-1} (CH stretching), 1530–1560 cm^{-1} (N═N stretching), 1564–1616 cm^{-1} (C═C stretching of aromatic ring); ^{1}H NMR ($CDCl_3$) **4a**: two singlets at δ 2.61 and δ 3.18 indicating the presence of methyl protons attached at 3rd and 5th positions of the pyrazole ring. δ 7.12–7.48 (complex m, 5H, Ar-H).

*6.5. 1-[(1,2,4-Triazole-4-yl) carbothioamide]-3,5-dimethyl-4-[(substituted phenyl) diazenyl] pyrazoles (**5a–d**)*

3-[(Substituted phenyl) diazenyl] pentane-2,4-dione (**1a–d**, 0.001 mol) and N-4H-1,2,4-triazol-4-ylhydrazine (0.001 mol) were dissolved in glacial acetic acid (10 ml) and the solution was refluxed for 20 h. The reaction mixture was cooled and the compound separated was filtered, washed with acetic acid. It was further purified by recrystallization from acetic acid to get (**5a–d**). IR (KBr) **5a–d**: 1050–1086 cm^{-1} (C═S), 1508–1567 cm^{-1} (N═N) and 2981–3013 cm^{-1} (CH stretching); ^{1}H NMR ($CDCl_3$) **5d**: δ 4.16 (s, 1H, S═C–NH), two singlet at δ 2.72 and δ 3.28 indicating the presence of methyl protons attached at 3rd and 5th positions of the pyrazole ring and δ 7.22–7.54 (m, 3H, Ar-H).

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