

Synthesis, anti-inflammatory and antioxidant activities of some new pyrazole derivatives

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Received on:21-06-2014; Revised on: 18-07-2014; Accepted on:17-08-2014

ABSTRACT

Background: Pyrazole is an important class of heterocyclic compound, has been shown to exhibit diverse biological and pharmacological activities such as anti-cancer, antioxidant, anti-inflammatory, antimicrobial, etc. **Methods:** In this study, a series of novel pyrazole derivatives bearing hydrazone moiety have been synthesized via the reaction of the pyrazole carbohydrazone with different aldehydes. The structures of all compounds were confirmed via a wide range of spectroscopic techniques including IR, ¹H NMR, and mass spectra. All synthesized compounds have been tested for their *in vitro* antioxidant activities using 1,1-biphenyl-2-picrylhydrazyl (DPPH) as a free radical scavenging reagent, and *in vivo* anti-inflammatory activities utilizing a standard acute carrageenan-induced paw edema. **Results:** The data reported herein indicates that compound 5a, 5c and 9 has emerged as potentially active compounds as anti-inflammatory and antioxidant compounds. **Conclusions:** All synthesized compounds were found to possess antioxidant & anti-inflammatory activities and could be useful as a template for future development through modification or derivatization to design more potent biologically active compounds.

KEYWORDS: Pyrazole, anti-inflammatory activity, Antioxidant activity, DPPH, Carrageenan.

1. INTRODUCTION

In the past decade, interest in pyrazole chemistry has significantly increased mainly due to the discovery of the interesting properties exhibited by a great number of pyrazole derivatives. Pyrazole, a five-membered heterocycle containing two adjacent nitrogen atoms, is a motif found in a number of small molecules that possess a wide range of agricultural and pharmaceutical activities.^{1,2} Derivatives of pyrazoles are found to show good antibacterial,³ analgesic,⁴ radioprotective,⁵ anti-convulsant⁶, anti-depressant,⁷ anti-inflammatory,^{8,9} antifungal,¹⁰ herbicidal,¹¹ insecticidal,¹² anti-HCV and antiviral activity.¹³ Furthermore, some pyrazoles have been implemented as antileukemic,^{14,15} antitumor^{16,17} and antiproliferative activity.^{18,19} In recent years, several drugs including patented ones are developed from the pyrazole derivatives. For instance, **celecoxib** demonstrates anti-inflammation effect and inhibits COX-2; **rimonabant** functions as cannabinoid receptor and is utilized in obesity treatment; **fomepizole** inhibits alcohol dehydrogenase; and **sildenafil** inhibits phosphodiesterase (Figure 1).

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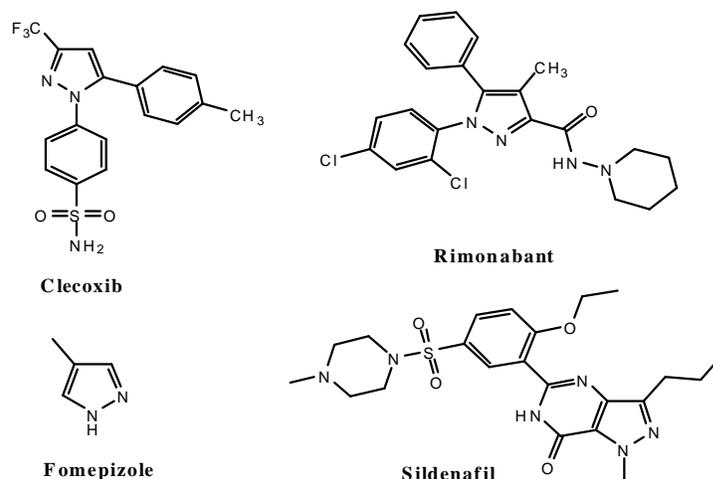


Figure 1. Several drug molecules which include pyrazole scaffold.

A number of hydrazide–hydrazone derivatives have been claimed to possess interesting bioactivity such as antibacterial, antifungal, anti-convulsant, anti-inflammatory, anti-malarial, analgesic, anti-platelets, anti-tuberculosis and anticancer activities.^{20,21} A few of pyrazole-carbohydrazone derivatives have also been reported.²²⁻²³

The extensive studies have been focused on pyrazole derivatives because of their diverse chemical reactivity, accessibility and wide range of biological activities. In view of the above facts, and in con-

tinuation of our search on pharmacologically active heterocyclic compounds, we report herein synthesis, anti-inflammatory and antioxidant activities of some new pyrazole-carbohydrazidehydrazones derivatives.

2. Chemistry

Unless otherwise stated, all the reagents and reactants were purchased from commercial suppliers; melting points were determined using a Büchi B-545 digital capillary melting point apparatus and used without correction. The FT-IR spectra were recorded on VERTEX 70 spectrometer using KBr discs. Spectroscopic data were recorded as follows: ¹H-NMR spectra were acquired on a BrukerAvance 300 (300 MHz) in DMSO-d₆ using TMS as an internal standard. Chemical shifts are given in δ ppm. Mass spectra were collected using a API 3200 LC/MS/MS system and thin layer chromatography on silica gel plates, using a mixture of hexan and ethyl acetate as the eluent. Iodine or UV lamp was used as a visualizing agent. In the following section, these abbreviations are used: 's' for singlet, 'd' for doublet, 'dd' for double doublet and 'm' for multiplet are used for the NMR assignments.

2.1. General procedure for the preparation of substituted-1H-pyrazol-3-carbohydrazide hydrazone 5a-c:

To a solution of derivatives **4** (1 mmol) in 10 ml of ethanol, it was added an equimolar amount of the appropriate benzaldehyde derivative in the presence of acetic acid. The mixture was maintained under reflux for 2 h, until TLC indicated the end of reaction. Then, the reaction mixture was poured in cold water, and the precipitate formed was filtered out washed with ethanol and recrystallized from methanol/DMF.

N'-(2,4-dichlorobenzylidene)-5-methyl-1H-pyrazol-3-carbohydrazide 5a :

Yellow solid, yield 95% ; M.p. 258-260°C (methanol/DMF); IR (KBr, ν(cm⁻¹)) : 3326 (NH), 1684 (C=O) ; ¹H-NMR (300 MHz, DMSO-d₆, δ(ppm)) : 2.27 (s, 3H), 6.49 (s, 1H), 7.41 (dd, 1H, J=8.41, J=1.81 Hz), 7.68 (d, 1H, J=1.81 Hz), 7.97 (d, 1H, J=8.41 Hz), 8.86 (s, 1H), 11.98 (s, 1H), 13.10 (s, 1H); MS: m/z = 297.1 (M-H⁺).

N'-(4-dimethylaminobenzylidene)-5-phenyl-1H-pyrazol-3-carbohydrazide 5b:

Yellow solid, yield 78% ; M.p. 262-264°C (methanol/DMF); IR (KBr, ν(cm⁻¹)) : 3219 (NH), 1648 (C=O); ¹H-NMR (300 MHz, DMSO-d₆, δ(ppm)) : 2.95 (s, 6H), 6.72 (s, 1H), 7.16-6.72 (m, 9H), 8.35 (s, 1H), 11.35 (s, 1H), 13.72 (s, 1H) ; MS: m/z = 334.5 (M-H⁺).

N'-(2,4-dichlorobenzylidene)-5-phenyl-1H-pyrazol-3-carbohydrazide 5c :

Yellow solid, yield 62% ; M.p. 234-236°C (methanol/DMF); IR (KBr,

ν(cm⁻¹)) : 3182 (NH), 1657 (C=O); ¹H-NMR (300 MHz, DMSO-d₆, δ(ppm)) : d 7.21 (s, 1H), 7.21-8.04 (m, 8H), 8.91 (s, 1H), 12.11 (s, 1H), 13.81 (s, 1H); MS: m/z = 360.9 (M-H⁺).

2.2. Procedure for the preparation of *N'*-(2,4-dichlorobenzylidene)-2-(3,5-dimethyl-1H-pyrazol-1-yl)acetohydrazide 9 :

To a solution of derivative **8** (1 mmol) in 10 ml of ethanol, it was added an equimolar amount of the 2,4-dichlorobenzaldehyde in the presence of acetic acid. The mixture was maintained under reflux for 2 h, until TLC indicated the end of reaction. Then, the mixture was poured in cold water, and the precipitate formed was filtered out washed with ethanol and recrystallized from methanol/DMF. Yellow solid, yield 70% ; M.p. 204-206°C (Methanol/DMF); IR (KBr, ν(cm⁻¹)) : 3414 (NH), 1684 (C=O); ¹H-NMR (300 MHz, DMSO-d₆, δ(ppm)) : 2.42 (s, 3H), 2.51 (s, 3H), 5.83 (s, 2H), 6.39 (s, 1H), 7.21 (dd, 1H, J₁=8.71, J₂=2.11 Hz), 7.82 (d, 1H, J=2.11 Hz), 7.89 (d, 1H, J=8.71 Hz), 8.88 (s, 1H), 11.78 (s, 1H); MS: m/z = 325.2 (M-H⁺).

3. Biological experiments

3.1. *In vitro* antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging: Free radical scavenging activity of the synthesized compounds were measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) according to the method of Sanchez-Moreno.²⁵ 0.2 mM solution of DPPH in methanol was prepared and 0.5 ml of this solution was added to 2.5 ml of synthesized compounds (increasing from 15.625 to 500 µg ml⁻¹) and were allowed to stand at room temperature for 30 min. The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm. Radical scavenging activity (RSA) was calculated using the formula:

$$\% \text{ RSA} = [(A_c - A_t) / A_c] \times 100$$

Where A_c is the absorbance of the control sample (DPPH solution without test sample) and A_t is the absorbance of the test sample (DPPH solution + test compound). Test was performed in triplicate, and the results were averaged.

3.2. *In vivo* anti-inflammatory activity

Compounds **5a**, **5b**, **5c** and **9** were screened for their *in vivo* anti-inflammatory activity by the carrageenan-induced paw edema standard method. Adult albino rats of either sex (pregnant female animals were excluded) weighing 180-220 g were divided into 10 group of 6 animals each. To reduce the variability of edema response, rats were fasted overnight, then on the next day (day of experiment), animals were uniformly hydrated by giving 3 ml of water per rat orally. Indomethacin (reference standards) and the tested compounds (50 and 100 mg/kg body weight) were suspended in saline solution and given

orally 1 h before induction of inflammation. The control group was given saline solution.

Carrageenan paw edema was induced according to a modified method²⁶ by subcutaneous injection of 1% solution of carrageenan in saline (0.1 ml/rat) into the subplanter region of the right hind paw of rats. The thickness of rat paw was measured by digital plethysmograph (Ugobasile, Italy) at different time intervals, after 0, 0.5, 1, 2, 3 and 4 h of carrageenan injection. The edema was determined from the difference between the thickness of injected and non-injected paws. Data were collected, checked, revised, and analyzed. Quantitative variables from normal distribution were expressed as mean \pm standard deviation (SD). The significant difference between groups was tested by using one way ANOVA followed by variance test at $P < 0.05$ and < 0.01 .

The anti-inflammatory activity was expressed as percentage inhibition of edema thickness in treated animals in comparison with the control group (Table 1).

$$\% \text{ inhibition of edema} = [(V_c - V_t) / V_c] \times 100$$

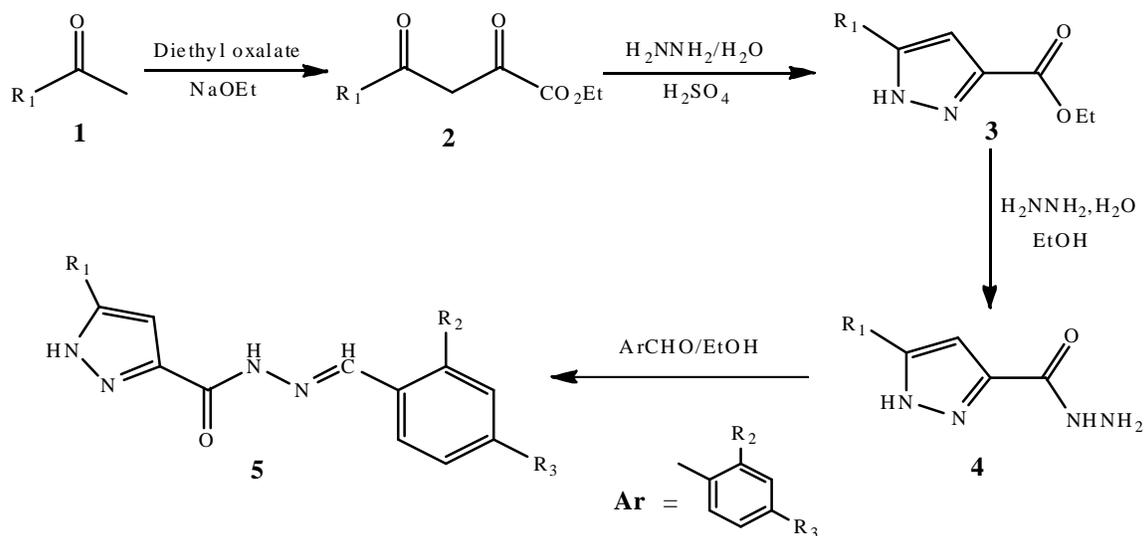
Where V_c is the Mean increase in paw volume of control group; V_t is the Mean increase in paw volume of treated group.

4. RESULTS AND DISCUSSION

4.1. Synthesis

Synthesis of N' -[(aryl) methylene]-5-substituted-1H-pyrazole-3-carbohydrazide (**5a-c**) is outlined in **Scheme 1**. Starting compounds, ethyl 3-substituted-1H-pyrazole-5-carboxylate (**3**) were readily prepared by the reaction of ethyl-2,4-dioxo-4-substituted-butanoate (**2**), which can be obtained from commercially available acetone or acetophenone (**1**) and diethyl oxalate, with hydrazine in the presence of sulfuric acid at room temperature.²⁴ The reaction of ethyl 3-substituted-1H-pyrazole-5-carboxylate (**3**) with hydrazine hydrate in ethanol afforded 3-substituted-1H-pyrazole-5-carbohydrazide (**4**). Compounds (**5a-c**) were obtained in good yields by condensing of (**4**) with functionalized aromatic aldehydes at reflux in ethanol. The structure of these stable and crystalline compounds was fully characterized by usual methods (IR, ¹H-NMR and mass spectrometry).

Thus, for example **5a** ($R = \text{CH}_3$, $\text{Ar} = 2,4\text{-diCl-C}_6\text{H}_4$), obtained as yellow crystal, gave a $[\text{M}+\text{H}]$ -ion peak at m/z 297.1 in the ESI-MS, in the accord with the molecular formula $\text{C}_{12}\text{H}_{10}\text{Cl}_2\text{N}_4\text{O}$. In The IR spectra, the carbonyl group absorptions in hydrazide moiety and NH bands in CONH were observed in the 1684 cm^{-1} and $3330\text{-}2950 \text{ cm}^{-1}$ region, respectively. The ¹H NMR spectra indicated the chemical shift of the NH in the pyrazole at $\delta = 13.10$ ppm in the form of singlet peak.



Comp.	R ₁	R ₂	R ₃	Yield (%)
5a	CH ₃	Cl	Cl	95
5b	C ₆ H ₅	H	N(CH ₃) ₂	78
5c	C ₆ H ₅	Cl	Cl	62

Scheme 1. Preparation of compounds (5a-c).

Another NH proton in the CONH appeared at $\delta = 11.98$ ppm in the form of singlet peak. The chemical shift of the N=CH appeared at $\delta = 8.86$ ppm in the form of singlet peak. The doublet peaks at $\delta = 7.97$ ppm is C₃ aromatic proton signals in 2,4-dichlorophenyl moiety (J = 8.7 Hz). Two aromatic protons signal in 2,4-dichlorophenyl moiety appeared at the range of $\delta = 7.67$ and 7.68 ppm as doublet peaks (J = 2.1 Hz) and $\delta = 7.48$ ppm as double doublet peaks (J = 1.8 and J = 2.1 Hz). The singlet appeared at $\delta = 6.49$ ppm is consistent with the proton in pyrazole moiety. A singlet signal appeared at $\delta = 2.27$ ppm are consistent with the protons in CH₃.

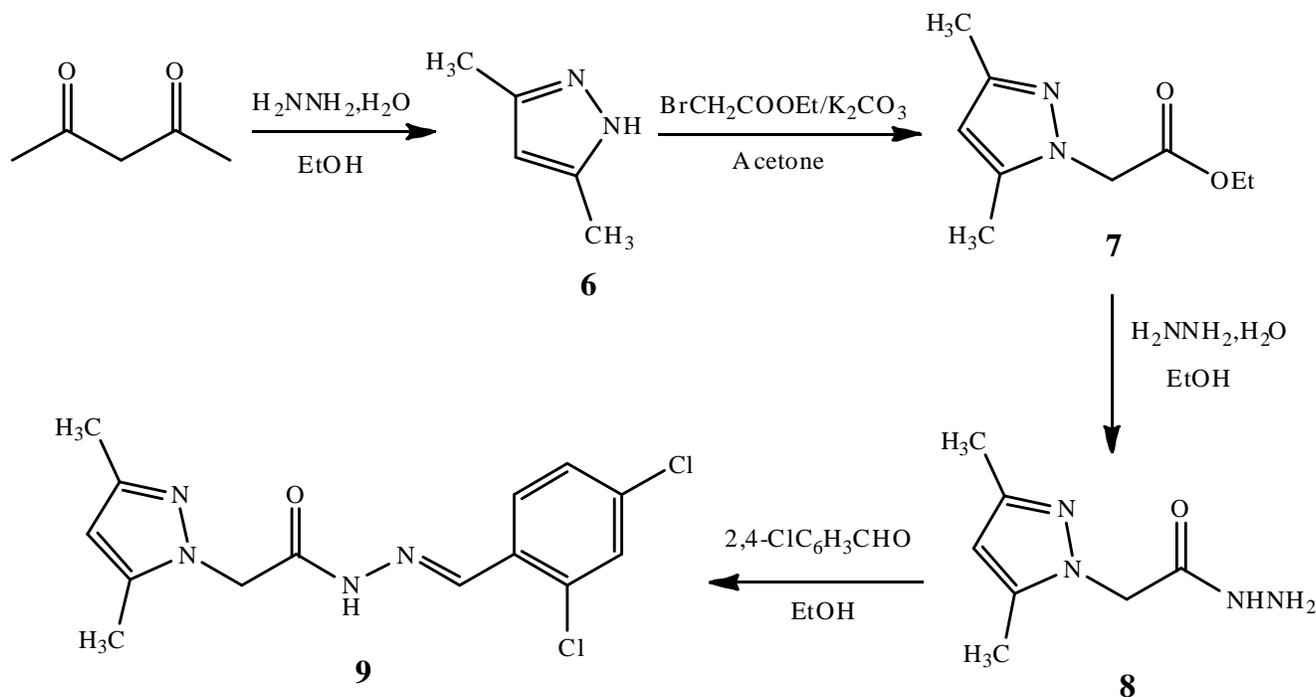
Synthesis of N'-(2,4-dichlorobenzylidene)-2-(3,5-dimethyl-1H-pyrazol-1-yl)acetohydrazide (**9**) is outlined in **Scheme 2**. Starting compounds, 2-(3,5-dimethyl-1H-pyrazol-1-yl)acetohydrazide (**8**), were readily prepared by the reaction of ethyl 2-(3,5-dimethyl-1H-pyrazol-1-yl) acetate (**7**), which can be obtained from 3, 5-dimethylpyrazole (**6**) and ethyl 2-bromoacetate, with hydrazine in ethanol. The reaction of 2-(3,5-dimethyl-1H-pyrazol-1-yl)acetohydrazide(**8**) with 2,4-dichlorobenzaldehyde in the presence of acetic acid at refluxing in ethanol afforded N'-(2,4-dichlorobenzylidene)-2-(3,5-dimethyl-1H-pyrazol-1-yl)acetohydrazide (**9**) in good yield. The structure was determined by IR, ¹H NMR and mass spectrometry.²⁴

4.2. Antioxidant activity

All the synthesized compounds were screened for free radical scavenging activity by DPPH method.²⁵ All compounds have exhibited free radical scavenging capacity by comparison with the standard Trolox. The DPPH radical scavenging capacity of the **5a** product was almost similar to the trolox (P<0.001) with a percentage inhibition of $88.09 \pm 0.63\%$. The **5c** product (at the highest concentration 500 $\mu\text{g/ml}$) shows a higher activity with a percentage inhibition of 84.21%, but this action is significantly lower than that of Trolox. The **5b** product shows a relatively low activity ($41.02 \pm 1.13\%$) followed by the **9** product with a percentage inhibition of $26.12 \pm 1.07\%$, which is practically ineffective, (p> 0.05). Among the tested compounds, **5a** and **5c** have exhibited promising radical scavenging activity, as compared with standard. The variation exhibited in DPPH scavenging capacity could be attributed to the effect of different substitutions.

4.3. Anti-inflammatory activity

All the synthesized compounds (**5a-c**) and **9** were tested for their anti-inflammatory activity using carrageenan-induced rat hind paw edema method.²⁶ The protocol of animal experiments was approved by the Institutional Animal Ethics Committee (IAEC). The edema hind paw was induced by injection of 0.1 mL of 1% carrageenan solution into



Scheme 2. Preparation of compound 9.

subplanter region of right hand paw. Anti-inflammatory activity was calculated at hourly intervals up to 4h after injection and presented in Table 1 as the mean paw volume (mL) as well as the percentage anti-inflammatory activity (% Inhibition of edema). Most of the synthesized compounds showed appreciable inhibition of the edema size in comparison with Indomethacin. The pyrazole derivatives **5b** and **5c** (50 mg/kg) showed good anti-inflammatory activity with a percent inhibition of 74.07% and 80.24% respectively, the anti-inflammatory activity of **5b** and **5c** at the dose of 100mg/kg showed excellent protection against inflammation (81.48% and 92.59% inhibition, respectively) in comparison with Indomethacin. It shows that the compound has significant ($P < 0.01$; $P < 0.001$) anti-inflammatory effect and the results were compared with indomethacin (5mg/kg) and show percentage paw volume reduction of 75.93 % (Table 1). The compounds **5a** and **9** at the dose of 50mg/kg exhibited an anti-inflammatory activity that became significant ($P < 0.01$) 4 hours after the injection of carrageenan with a maximum effect of 71.85% and 62.96% respectively.

Carrageenan is commonly used as an experimental model for inflammation; it is attributed to the release of histamine, serotonin and kinin. Furthermore it is related to the release of prostaglandin and bradykinins so the effect of the synthesized compounds against inflammation produced by these individual mediators was studied.²⁷⁻²⁸ The inflammation model of a carrageenan induced edema is usually used to assess the activity of products in resisting the pathological changes associated with acute inflammation.²⁹⁻³⁰ The inflammatory response is usually quantified by increase in paw size (edema) which is maximal around 4-5 h postcarrageenan injection.³¹

The results obtained from the carrageenan-induced paw edema shows that paw edema was markedly inhibited by the administration of the synthesized compounds in dose - response relationship. The effect observed, which was time-dependent, lasted for at least 4 h. The inhibitory values of edema at indicating that the synthesized compounds are active at doses ranging from 50-100 mg/kg and can inhibit a acute inflammatory process.

Table 1 Anti-inflammatory activity of the tested compounds using carrageenan-induced paw edema in rats

Compound	Dose (mg/kg)	Percentage inhibition of edema ± SE				
		30 min	60 min	120 min	180 min	240 min
Control	0.5	0.00	0.00	0.00	0.00	0.00
Indomethacin	5	31.5±0.45	54±0.37	60.5±0.56	67±0.12	75.93±0.28
5a	50	2.72±0.41	45.09±0.32	67.45±0.41	67.17±0.11	80.24±0.29
	100	40.00±0.46	31.42±0.12	61.15±0.57	71.28±0.80	92.59±0.87
5b	50	16.81±0.58	13.27±0.30	41.20±0.50	44.61±0.54	74.07±0.12
	100	18.18±0.11	28.40±0.23	45.93±0.32	53.84±0.56	81.48±0.15
5c	50	19.69±0.17	22.12±0.45	45.93±0.49	41.53±0.23	71.85±0.25
	100	3.63±0.11	7.56±0.12	33.33±0.22	35.28±0.12	53.08±0.56
9	50	0.00±0.20	7.56±0.10	46.45±0.16	44.61±0.37	62.96±0.63
	100	0.00±0.21	18.20±0.27	27.55±0.23	7.69±0.12	23.70±0.61

All values are represented as means of 6 experiments ± SD. Statistical analysis was carried out by one-way ANOVA analysis of variance test at $P < 0.05$ and < 0.01 . Compared normal control vs edema. Control rats and treated rats.

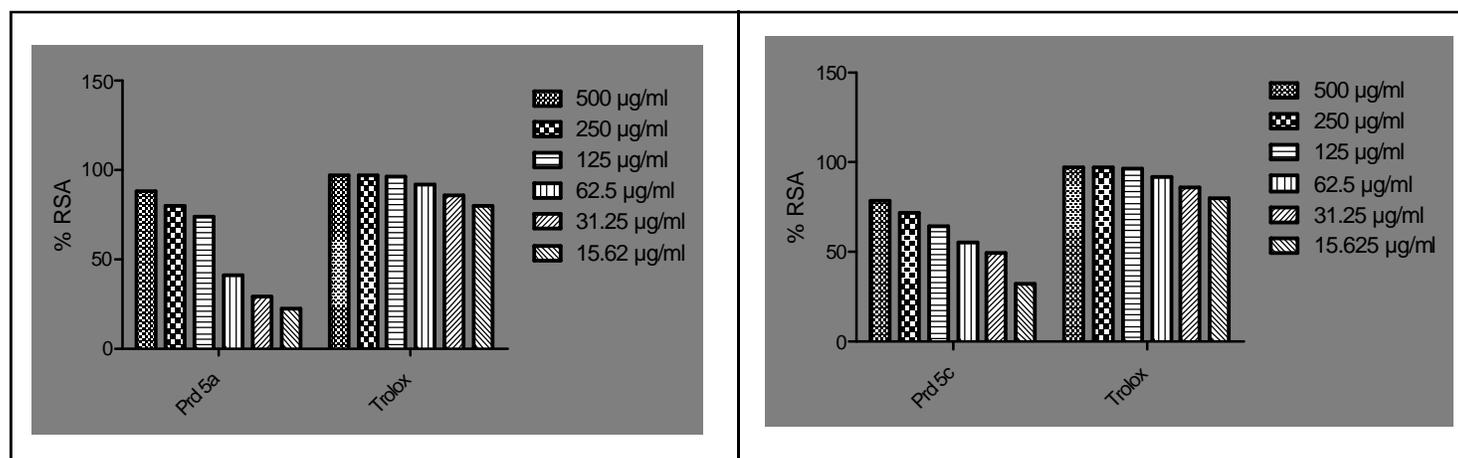


Figure 2. Free-radical scavenging activity of synthesized compounds measured using the DPPH assay (5a and 5c): Values are means ± SD of three determinations with respect to positive control (Trolox).

5. CONCLUSION

In summary, a novel series of pyrazole derivatives bearing hydrazone moieties were synthesized. The novel drugs were characterized by spectral data (IR, ¹H NMR, and mass spectra). All synthesized compounds were found to possess antioxidant & anti-inflammatory activities and could be useful as a template for future development through modification or derivatization to design more potent biologically active compounds. The new skeleton may also possess other biological activities of the parent ring systems.

Conflicts of interest

All authors have none to declare.

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Source of support: Nil, Conflict of interest: None Declared