Synthesis and antimicrobial activity of some derivative of hydrazine from salicylic acid

Under the supervisor

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Done By

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ABSTRACT

In our project, we prepare tow new hydrazone compounds of 3-methoxysalicylic acid, which obtained by reaction aldehyde ‹4-nitrobenzaldehye ‹3-nitrobenzaldehye with appropriate hydrazide to obtain hydrazone that is yellow color). The prepared compounds were characterized by using FT-IR spectroscopy and \determining the melting point. Anti-inflammatory activity of the compounds were determined. in-vitro by human red blood cell (HRBC) membrane stability method, the compounds showed a significant activity to protection of the cell membrane .

Introduction

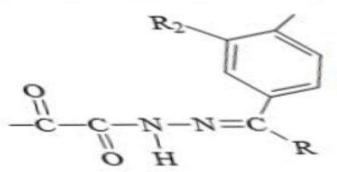
Hydrazones are a class of organic compounds which possess the structure R1R2C=NNH2 .They are related to ketone and aldehyde in which oxygen has been replaced with NNH2 group. These azometine NHN=CH- proton constitute an important class of compounds for new drug development. Hydrazones are formed by the reaction of hydrazine or hydrazid with aldehyde and ketonrs

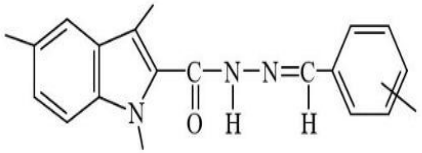
The C=N bond of hydrazone and terminal nitrogen atom containing a lone pair of electron is responsible for the physical and chemical properties. The C-atom in hydrazone has both electrophilic and nucleophilic character and both the N-atoms are nucleophilic although the amino type nitrogen is more reactive. Due to these properties hydrazones are widely used in organic synthesis.

Biological Activity :

1.Anticonvulsant Activity Ex Acetyl Hydrazone

2.Antideprassant Activity Ex Isocarbaxzide



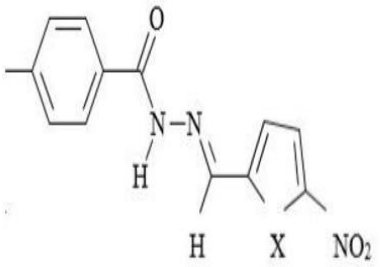


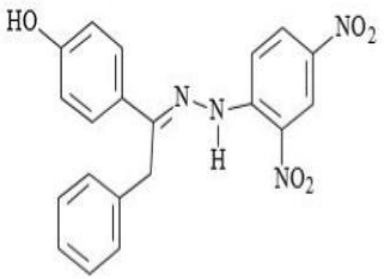
3.Antmicrobial ex nifuroxazide

4.Antimalarial

5.Antimycobacterial ex rifampin,HIN

6.Antitumor ex diphenolic hydrazone





Chemical reactvity:

1.Reaction with electrophilic reagents

Reaction with carbon, nitrogen, halogen electrophiles

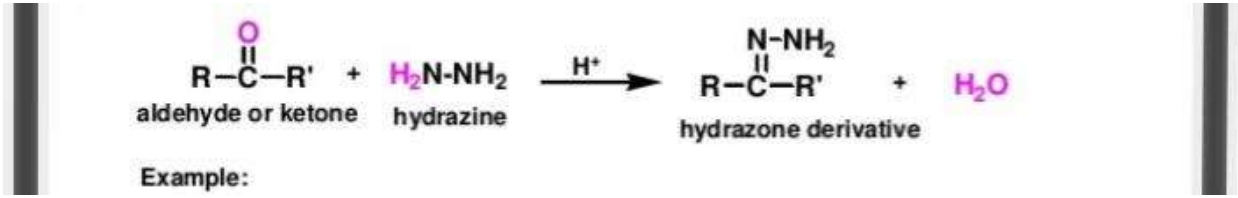
2.Reaction with nucleophilic reagents

Reaction with carbon, nitrogen, and oxygen

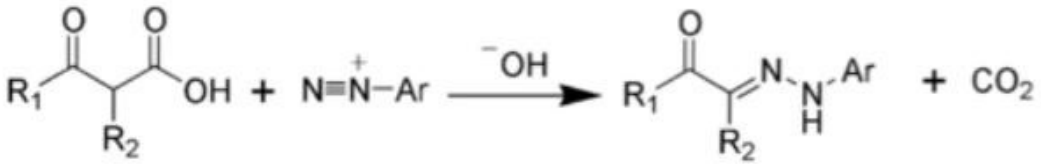
3.Reduction

Methods to preparing hydrazone:

1.Condensation for functional aldehyde and ketone with hydrazine . as the following reactio



2.The Japp-klingemann reaction



Experimental

1. Experimental part:

-Materials

3-methoxysalicylic acid, absolute ethanol, sodium bicarbonate, chloroform، $H2SO4$ ،4-Nitrobenzaldehyde ،3-nitrobenzaldehyde .

Apparatus: refleux, melting point apparatus .

2.2 methods of synthesis

2.2.1 Synthesis of ester of 3-methoxysalicylic acid:

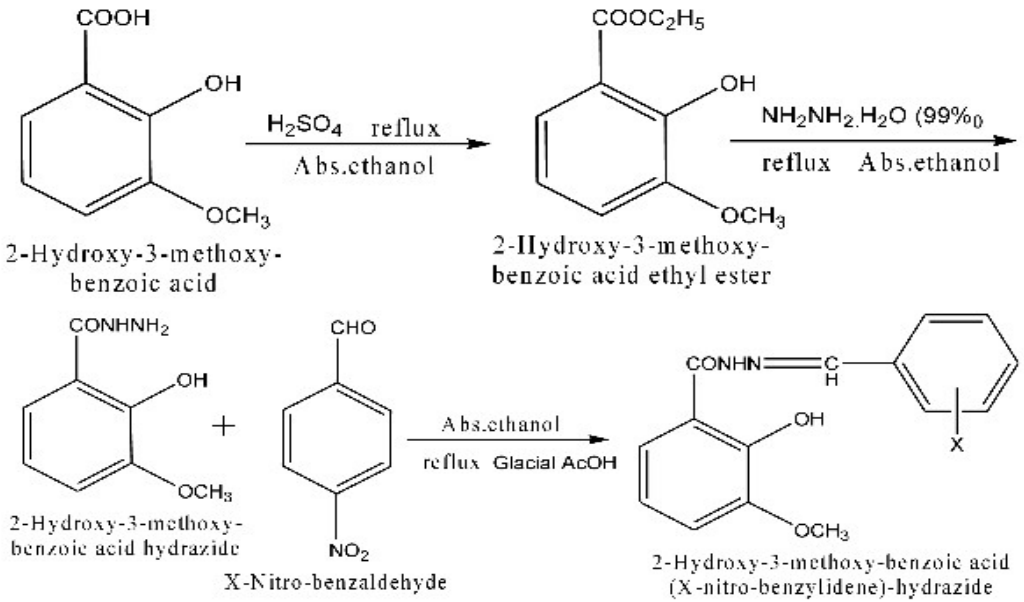
(1g) of salicylic acid ،1ml of H_2SO_4 as catalyst and 25ml of absolute ethanol, then refluxed for 6 hrs. The solvent was concentrated under reduced pressure. The desired ester extracted (twice) with chloroform and saturated sodium bicarbonate solution by using separating funnel to remove unreacted salicylic acid .

2.2.2 Synthesis of 3-methoxysalicylic acid hydrazide

An appropriate the ester of 3-methoxysalicylic acid(0.01mol) was dissolved in absolute ethanol (20ml) and added hydrazine hydrate (99%,0.015 mol) while shaking. The reaction mixture was stirred well, refluxed for 3hrs and left in the refrigerator for 3h. The resultant yellow crystalline solid was filtered, washed repeatedly with small portions of cold water and finally with a small quantity of cold alcohol. The product was dried and purified by recrystallization from ethanol .

2.2.3 Synthesis of 3-methoxysalicylic acid hydrazones

An appropriate the of 3-methoxysalicylic acid hydrazide (0.01mol) was dissolved in absolute ethanol (20ml) and added 3 -or 4 - nitrobenzaldehyde (0,01mol) with 2ml of glacial acetic acid. The reaction mixture was refluxed for 5hrs and left in the overnight. The resultant crystalline solid was filtered, washed cold alcohol. The product was dried and purified by recrystallization from ethanol.



Scheme-1: Synthetic routs of the hydrazones

In vitro anti-inflammatory activity

HRBC method was used to estimation in vitro anti-inflammatory activity. Blood was collected from healthy volunteers; the blood was mixed with equal volume of sterilized Alsever's solution. The blood solution centrifuged at3000 rpm and the packed cells were separate. The packed cells were washed with isosaline solution and 10 %v/v suspension was prepared by complete the volume with isosaline.

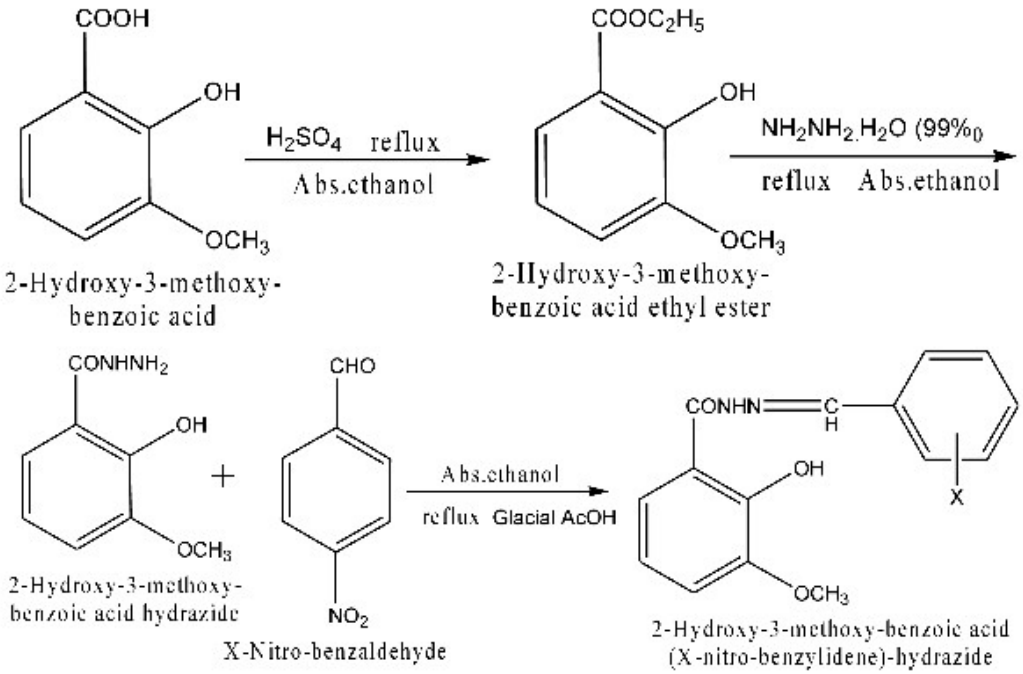
Tested compounds(75 mg) was dissolve in 1ml of ethanol. Samples of each compound, control and sodium diclofenac were mixed with (1ml) phosphate buffer(2ml) of hyposaline and(0.5ml) of HRBC suspension. All the assay mixtures were incubate d36.5 for ºC 30 minutes and centrifuged at 3000 rpm for 10minutes. The supernatant liquid was decanted and haemoglobin content was estimate by spectrophotometer at 560 nm. The percentage of haemolysis protection was estimate by assuming the haemolysis produced in the control as 100% ،according to following equation]25 .

Percentage protection=100)-Ac-As/Ac.

Were Ac= Absorption of control and As= Absorption of sample

Results and Discussion

The compounds under study were prepared in three steps. The first step involved the preparation of aster from the reaction of salicylic acid derivative with ethyl alcohol using sulfuric acid as a catalyst. The second step involved the preparation of the acid hydrazide from the reaction of ester with hydrazine hydrate) 99 (%in the alcoholic medium. The third step involved the preparation of the final compounds, hydrazones, from the reaction of benzaldehyde derivatives with the acid hydrazide.



Scheme-1: Synthetic routs of the hydrazones

The melting point of the compounds prepared in the research, which expresses the purity of the compounds as well as their difference from the melting points of the reactive substances, as shown in Table 2

Table-2: properties of synthesized hydrazones

Compound	M.P °C	Appearance	Yield %
A	80-81	yellow	82
B	68-70	pale yellow	91

T-IR Spectra

The IR spectra of hydrazones are represented in the Figures 1and 2and the analysis results are summarized in Table1 .The IR spectra of prepared compounds as shown in figures1 and2 ،indicate the formation of the Schiff base product by the absence of the carbonyl group) 1700cm−1 (band and the appearance of a strong band in the vibrations 1593cm−1 and 1604cm−1 attributed to the C=N group] 26 .[In addition, the absence of the stretching vibration of NH) (3400-3500 cm-1)confirmed that the title compounds were obtained via condensation reaction.

In the IR spectra, observed that both synthesized hydrazones display two absorption bands in the regions 1348 and (1350cm-) and (1462)and(1460cm-1)which may attributed to the symmetrical and asymmetrical stretching vibrations of NO2 group

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Compd.	C=N (Str.)	N-H (Str.)	Ar. C=C	NO_2 (Str.)		Ar-H		Aliphatic C-H (Str.)
				Sym.	Asym.	(Str.)	(O. O. P.) Bend.	
A	1593	3321	1517	1348	1460	3100	831	2839
B	1604	3221	1525	1350	1462	3082	835	2841

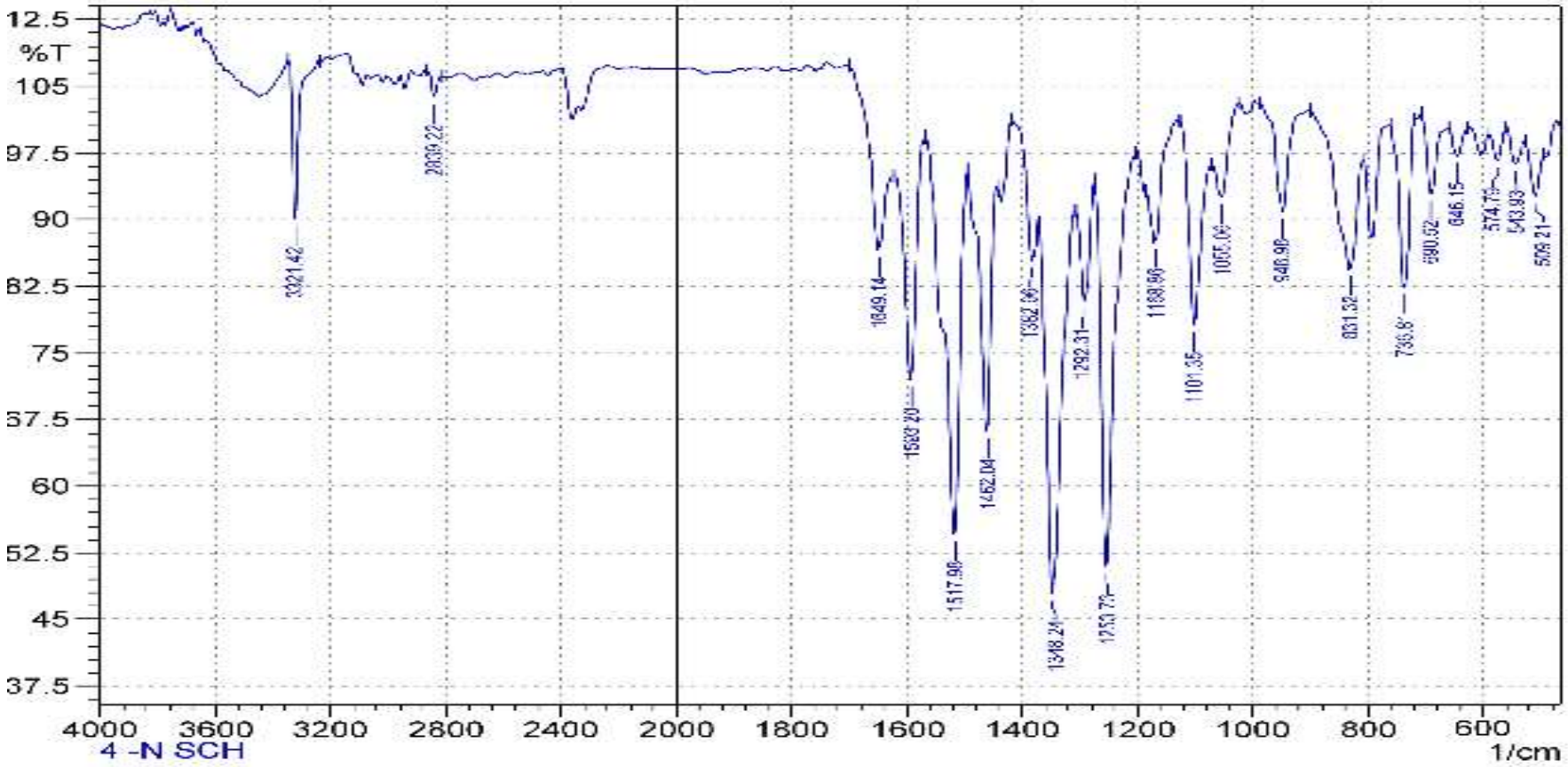
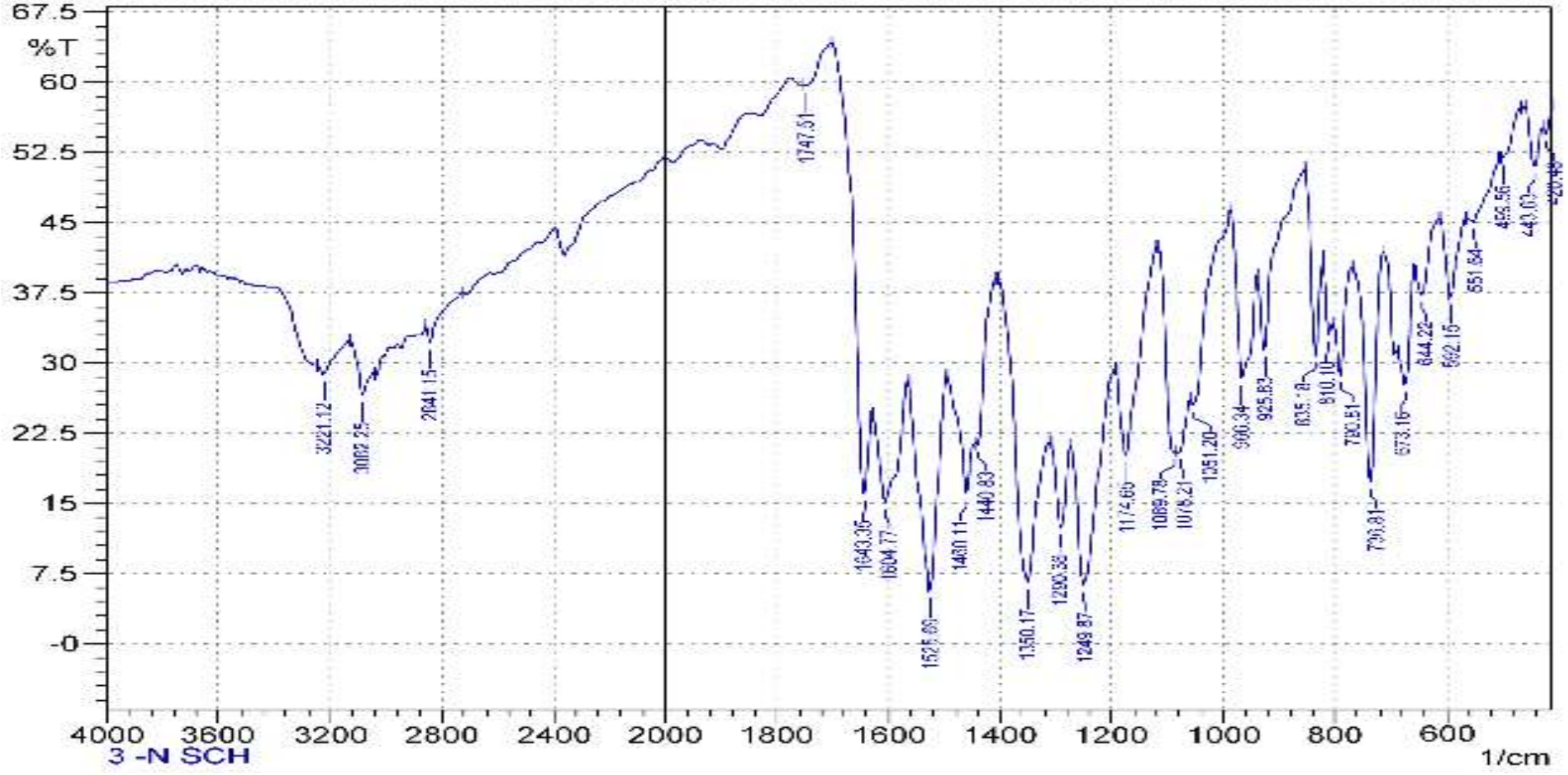


Figure-1: FT-IR spectrum of hydrazone A


67.5
%T
60
52.5
45
37.5
30
22.5
15
7.5
-0
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3600
3200
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2000
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1600
1400
1200
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800
600
1/cm
3 -N SCH
3221.12
3082.25
2941.15
1747.51
1643.05
1604.77
1525.69
1430.11
1440.83
1350.17
1290.38
1249.87
1174.60
1089.78
1018.21
1051.20
960.34
925.83
810.10
780.51
796.81
673.16
644.22
592.15
551.64
495.56
440.00

In vitro anti-inflammatory activity

The synthesized hydrazone compounds (A and B) showed a significant anti-inflammatory activity by HRBC membrane stabilization method. The results of the activity are listed in Table 3

Table 3: protection of HRbC membrane of compounds at dose 75mg

The compound	Protection %
A	57.53
B	71.78
Sodium diclofenac	74.48

From the results, compound B showed high activity compared with other compound. The active compounds B contain a nitro group at meta position. The action of the hydrazone compounds could be related to the binding of compounds with erythrocyte membrane, especially phospholipids. Since the compounds has polar and nonpolar groups can bonded with same groups of phospholipids. This prevents membrane damage by physical interaction of osmotic pressure differences, which is causative to hemolysis of red blood cells .


Thank
You!