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"SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 1, 3, 4-THIADIAZOLE DERIVATIVES AS AN ANTI-INFLAMMATORY AGENTS"

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ABSTRACT
Inflammation is a defensive mechanism of the body to any toxic, chemical irritant, injury as well as infection; process may changing from a localized to a generalized. The symptoms of inflammation generally redness, heat, pain, edema and loss of function. Non-steroidal anti-inflammatory drugs commonly prescribed worldwide for management or reduce of pain and inflammation due capability to block COX enzyme which is production of prostaglandin mediators that's responsible for inflammation. Continuous use of non-steroidal anti-inflammatory drugs has been produce certain adverse effects in patients but two are commonly such as gastric intestinal ulceration and renal toxicity because inhibition of cytoprotective COX-1 enzyme. In order to developing NSAIDs drugs with minimum these side effects, this may be accompanied by drugs without contain acidic group (carboxylic as functional group). Thiadiazole derivatives and its analogous which show anti-inflammatory and analgesic activity and also reduce gastrointestinal ulceration as well as various other adverse reactions. That's why to synthesized compounds having the 1, 3, 4-thidiazole nucleus with substitution on 2 and 5 position in order to find out their potential as analgesic and anti-inflammatory agents and maybe increase selectivity and reduce adverse effect. Azo compound is prepared by diazotization reaction between 2 amino 5-[(4-methoxy phenyl)-1, 3, 4-thiadiazole and various coupling component to get target product. The purification of compound was done by recrystallization with ethanol and further characterized by spectroscopic technique such as FTIR spectroscopy. Significant biological activity showed of all synthesized compound against carrageenan induced rat paw edema method

KEYWORDS: Non-steroidal anti-inflammatory, 1, 3, 4-Thiadiazole, Anti-inflammatory activity.

INTRODUCTION
Inflammation is a defensive mechanism of the body to any toxic, chemical irritant, injury. The symptoms of inflammation generally redness, heat, pain, edema and loss of function due to fluid, protein and leukocytes accumulation that result from dilation of the blood vessel which lead increasing blood supply inflamed site. This inflammatory response is mediated process due to release various physiological and pathological mediators. The mediators are chemical substance, main function in inflammation that is release of plasma protein which is arise from cell such as platelets, neutrophils, monocytes or macrophages and mast cell. These are release by any noxious stimulation where it bind to cell on target receptors in specific manner which is result in increased vascular permeability, increase smooth muscle contraction and produce pain. For example of mediators like as prostaglandins, nitrous oxide, interferon, tumour necrosis factor a (TNF–a), thromboxane's and cytokines.[1,13,15]

No steroidal anti-inflammatory (NSAIDs) including Aspirin, Diclofenac, phenylbutazone and various other NSAID is for management of pain, inflammation and fever associated disease in human as well as for veterinary medicine because its capability to block COX enzyme which is production of prostaglandin mediators that's responsible for inflammation. Cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) is two isoenzymes of cyclooxygenase (COX). Continuous use of non-steroidal anti-inflammatory drugs has been produce certain adverse effects in patients but two are commonly such as gastric intestinal ulceration and renal toxicity because inhibition of cytoprotective COX-1 enzyme.[13,18] In order to developing NSAIDs drugs with minimum these side effects, this may be accompanied by drugs without contain acidic group (carboxylic as functional group). From the in-depth literature review, it was found that thiadiazole derivatives and its analogous which show anti-inflammatory and analgesic activity and also reduce gastrointestinal ulceration as well as various

other adverse reactions. That's why to synthesized compounds having the 1, 3, 4-thidiazole nucleus with substitution on 2 and 5 position in order to find out their potential as analgesic and anti-inflammatory agents. And maybe increase selectivity and reduce adverse effect.[4,13,18]

MATERIALS AND METHODS

All chemicals obtained from Loba Chemie Pvt, Fine lab Islampur and melting point of compound was determined in open capillaries and are uncorrected. Infrared (IR) spectra was recorded on Shimadzu 8400S and Mass spectra were recorded on Brucker 100 MHz.

Methods give comprehensive information about synthetic scheme and following way step by step explanation of the work carried out.

- Synthetic scheme is very simple, inexpensive, during course of chemical reaction all factor considered such as temperature, reaction time and time to time observation of reaction vessel due some changes
- Synthetic rout may be optimized in order to get desirable product yield with minimum effort and effluent.
- Method used for chemical reactions based on various parameter such as time, temperature, reactivity of reactant etc
- Scheme represent the general synthetic procedure and the possible end product, where "R, R1"denote the substituents to be added and (1), (2) denotes the intermediates formed.
- Probable 'R, R1' group to be added. And (P) denotes end products

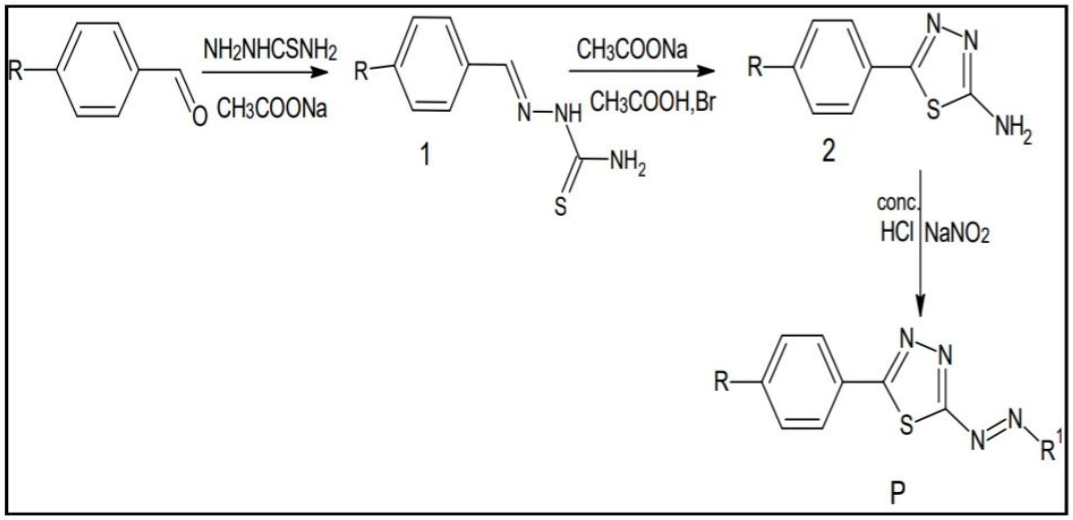
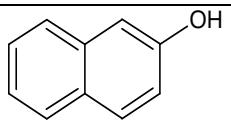
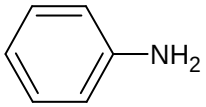
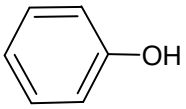


Figure 1. General synthetic scheme of Reaction where Intermediate 1, 2 and P is represent as final product as per include below table.

Table 1: Represent probable R1 group to be added.

Compound code	R	R1	Name of substituent
P1	OCH3		2- Naphthol
P2	OCH3		Aniline
P3	OCH3		Phenol

Procedure for synthesis

1. **Synthesis of thiosemicarbazone from thiosemicarbazide**

Add 0.01 thiosemicarbazide and 0.01M crystalline sodium acetate in 250 ml RBF and add 10 ml water. Then 0.5 gm. 4 methoxy benzaldehyde slowly added with vigorous stirring. Resulting mixture of reaction turbid then added methanol until to obtained clear solution then shake for 30 min and keep the reaction for 1hrs. Keep a reaction mixture for 1 day in refrigerator.

Precipitate thiosemicarbazone from cold solution. Filtrate it and recrystallization by means ethanol as a solvent.

2. 2 amino 5-[(4-methoxy phenyl)-1, 3, 4-thiadiazole

Take Intermediate (1) 0.01M and 0.01M crystalline sodium acetate where added in 40 ml of glacial acetic acid in a 250 ml RBF**.** RBF equipped with a separating funnel for the bromine addition. Prepare bromine solution in separating funnel (in that 0.8 ml added to 6 ml glacial acetic acid GAA). Then add drop by drop bromine solution to reaction mixture of first step in RBF while stirring magnetically. After 1 hour a magnetically stirring, the solution was slowly poured in 100 ml beaker containing crushed ice. Then resulting solid was precipitated, dried and recrystallization by using hot ethanol.

3. Synthesis of Azo compound

Preparation of an Azo compounds to require two organic compound diazonium salt and substituted compound like as beta Napthol, phenol, aniline etc. as coupling compounds.

Mechanism of this reaction in which diazonium salt as a react electrophile rich coupling component like 2-Napthol, phenol, Aniline through nucleophile aromatic substituent to get desired derivatives.

1, 3, 4 thiadiazole azo compound were prepared by diazotization reaction between intermediate (2) with different coupling component in presence of 1M HCL, 5 M sodium nitrate and 0.1M NaOH resulting target product is formed.

Where diazonium salt as (solution A) was prepared by take intermediate (2) 0.01M and dissolve in 15 ml of 5M HCL with continuous stirring. Above mixture cooled at 0°C in water bath containing ice or refrigerator. Then it add 15 ml 1M sodium nitrate solution with continuous stirring and temperature must be 0°C. The solution (B) of coupling component was prepared by taking 0.01M substituted compound dissolve in 15 ml of 1 M NaOH at 0°C in refrigerator. Solution (A) was added slowly to solution (B) with continuous stirring while temperature kept at 0°C. After one half hours temperature at 0°C target product was precipitated then neutralized, filtered and washed with several time of water.

Table 2. IR Spectral data of synthesised compound P1.

Functional group	Standard frequency cm^{-1}	Observe Frequency cm^{-1}
C-H stretch	3040-3010	3012cm
C-C stretch	1300-800	1168 cm^{-1}
C=N stretch	1665-1350	1628 cm^{-1}
C=S stretch	1249-1050	1243 cm^{-1}
N-H stretch	3500-3400	3192 cm^{-1}
O-H stretch	3385-32021	3274 cm^{-1}
N=N stretch	1518-1500	1507cm $^{-1}$

Table 3. IR Spectral data of synthesised compound P2.

Functional Group	Standard frequency cm^{-1}	Observe Frequency cm^{-1}
C-H stretch	3040-3010	3085
C-C stretch	1172-1157	1171
C=N stretch	1691-1649	1690
C=S stretch	1275-1050	1275
N-H stretch	3400-3000	3302
N=N stretch	1538-1500	1531

Table 4. IR Spectral data of synthesised compound P3.

Functional group	Standard frequency cm^{-1}	Observe Frequency cm^{-1}
C-H stretch	3040-3010	3283
C-C stretch	800-1300	1166
C=N stretch	1691-1350	1690
C=S stretch	1250-1050	1235
N-H stretch	3500-3400	3478
O-H stretch	3385-3201	3305
N=N stretch	1518-1500	1503

- **Mass spectral study**

The mass spectra of azo dyes show the presence of Molecular ion peak at m/z 363.15, 312.26 and 313.25 for P1, P2and P3 respectively which are in agreement with the proposed structures.

BIOLOGICAL ACTIVITY

Screening of anti-inflammatory activity of synthesized derivatives is done by utilizing of "Paw edema method", in which produce edema into all group of rat of the paw after injecting 0.1ml of 1% of carrageenan solution.

Synthesized compound given to selected group by oral dose before carrageenan inflamed solution and standard given one group by orally. Then anti-inflammatory activity is determined by comparing of treatment groups and control group.

$$= \left[\frac{v_c - v_t}{v_c}\right] 100$$

Where V indicate the percentage difference in increased volume of paw edema t, v_c Paw edema volume those have receive carrageenan 1% solution (control group), v_t Indicate increased volume of paw edema those groups receive test compound in 0, 1, 2, 3, 4, 5 hours.

- **% inhibition of activity for P1**

Table 5. (% inhibition of P1)

Groups	Paw edema volume in ml						% of inhibition
	Hours						
	Dose/kg	1	2	3	4	5	
Group-I	Normal	1.75±0.001	1.78±0.009	1.81±0.085	1.85±0.004	1.88±0.256	
Group-II	50mg/kg	1.57±0.058	1.48±0.005	1.42±0.003	1.39±0.015	1.35±0.09	20.23
Group-III	100mg/kg	1.60±0.004	1.53±0.001	1.46±0.007	1.42±0.004	1.39±0.002	18.44
Group-IV	200mg/kg	1.62±0.045	1.57±0.08	1.53±0.005	1.47±0.004	1.41.±0.007	16.03
Group-V	10mg/kg	1.52±0.04	1.44±0.005	1.50±0.002	1.47±0.001	1.38±0.004	19.33

- **% inhibition of activity for P2**

Table 6. (% inhibition of P2)

Groups	Paw edema volume in ml						% of inhibition
	Hours						
	Dose/kg	1	2	3	4	5	
Group-I	Normal	1.75±0.005	1.77±0.051	1.81±0.041	1.84±0.003	1.87±0.021	
Group-II	50mg/kg	1.71±0.012	1.69±0.002	1.65±0.025	1.59±0.003	1.56±0.015	8.88
Group-III	100mg/kg	1.67±0.015	1.65±0.003	1.68±0.021	1.61±0.011	1.57±0.063	9.44
Group-IV	200mg/kg	1.66±0.021	1.61±0.033	1.64±0.036	1.58±0.005	1.51±0.032	11.11
Group-V	10mg/kg	1.54±0.04	1.41±0.02	1.52±0.007	1.39±0.005	1.34±0.087	20

- **% inhibition of activity for P3**

Table 7. (% inhibition of P3)

Groups	Paw edema volume in ml						% of inhibition
	Hours						
	Dose/kg	1	2	3	4	5	
Group-I	Normal	1.76±00.5	1.78±0.011	1.82±0.111	1.85±0.122	1.88±0.14	
Group-II	50mg/kg	1.91±052	1.70±0.05	1.32±0.125	1.50±0.144	1.90±0.784	8.28
Group-III	100mg/kg	1.81±001	1.53±415	1.44±015	1.38±0.145	1.57. ±0.08	14.91
Group-IV	200mg/kg	1.94±0.01	1.77±0.021	1.30±0.123	1.53±0.235	1.73.±0.132	8.83
Group-V	10mg/kg	1.51±0.012	1.44±0.01	1.51±0.321	1.46±005	1.38±0.009	19.33

RESULT AND DISCUSSION

All synthesised Azo compound is prepared by diazotization reaction between 2 amino5-[(4-methoxy phenyl-1, 3, 4-thiadiazole and various coupling component such as beta Napthol, Aniline and Phenol to obtain the desired compound. The purification of compound was done by recrystallization with ethanol and further characterized by spectroscopic technique such as FTIR, MASS. Significant biological activity showed of all synthesized compound against carrageenan induced rat paw edema method.

Screening anti-inflammatory activity for synthesized compounds is done by with help carrageenan induce paw edema method and result was synthesized P1 - P3 compounds shows that significant biological activity where,

- P1 compound showed nearest anti-inflammatory activity that of the standard drugs.
- P2 as well as P3 compound showed near about same activity but less active than that of standard drug i.e. it may less effective as that of indomethacin

That means it's confirmed that the all synthesized compounds are having significant Anti-inflammatory activity.

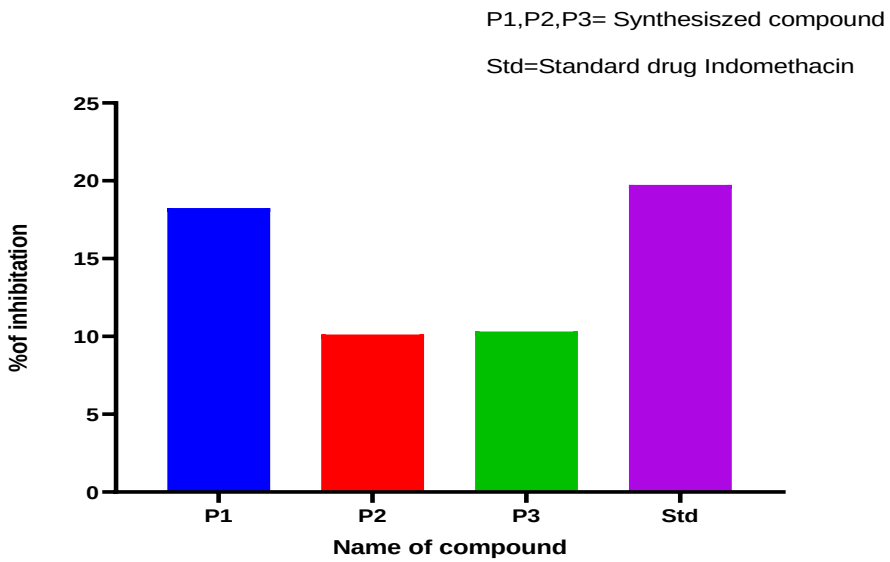


Figure 2. Graph for anti-inflammatory activity.

Table 8. Characterization data of synthesised compound.

Compound code	Molecular Formula	M.P	% yield	% COX Inhibition
P1	$C_9H_{14}N_3O_1S_1$	180-190°C	45.55 %	18.247%
P2	$C_{14}H_{10}N_5O_1S_1$	198-204°C	56.69 %	10.128%
P3	$C_{14}H_{10}N_4O_1S_1$	172-180°C	57.15%	10.211%

CONCLUSION

This was conclude that synthesized compound with good yield. All synthesized compound possess, Significant anti-inflammatory activity, these are all compound synthesized via diazotization reaction of thiadiazole nucleus.

The anti-inflammatory activity done against Wister albino rat of the different doses of synthesized compound and Indomethacin entreated rats which was showed that inhibition of paw edema in rats in all observed time intervals by Carrageenan induced paw edema. Significant activity was observed in rats treated with thiadiazole derivatives at different dose. Biological activity possessing due to Azo coupling reaction that have may decreases certain side effect associated with non-steroidal anti-inflammatory drug All point of the view all synthesized compounds possess the desired biological activity i. e. Anti-inflammatory activity.

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