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SYNTHESIS AND EVALUATION OF ANTI INFLAMMATORY ACTIVITY OF SOME NOVEL 1, 2, 4-TRIAZOLE-3- THIOL DERIVATIVES

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ABSTRACT

Developing a new drug from original idea to the launch of a finished product is a complex process. Numbers of triazole derivatives as clinical drugs or candidates have been frequently employed for the treatment of various types of diseases, which have proved the importance of this heterocyclic nucleus in drug design and discovery. Various derivatives are prepared from potassium dithio carbazinate by a four step process. The structures of final synthesized compounds were assigned on the basis of IR spectral data. All the newly synthesized compounds were screened for their *in-vitro* anti-inflammatory properties. The para amino benzaldehyde derivatives and chlorobenzaldehyde derivatives of 1,2,4- Triazol- 3 thiols shows significant results.

Key Words: 1, 2, 4- Triazol- 3 thiols, potassium dithio carbazinate, antiinflammatory.

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INTRODUCTION

Medicinal chemistry is the application of chemical research techniques to the synthesis of pharmaceuticals. Developing a new drug from original idea to the launch of a finished product is a complex process Numbers of triazole (Fig-1) derivatives as clinical drugs or candidates have been frequently employed for the treatment of various types of diseases, which have proved the importance of this heterocyclic nucleus in drug design and discovery. Recently many endeavours were made to involve the triazole into the anthelmintic, antihypertensive, anti HIV, antibacterial, antifungal, antimigrane, analgesic, and anticonvulsant design, which have brought lots of active compounds. This work is an attempt to synthesize novel triazole derivatives in the design and development of anti-inflammatory agents. Triazoles are five membered heterocyclic ring systems having three nitrogen atoms. All triazoles are of synthetic origin and there is no report of detection of this ring System in nature. It has two isomeric forms viz 1, 2, 3-Triazole and 1, 2, 4- Triazoles (1, 2).

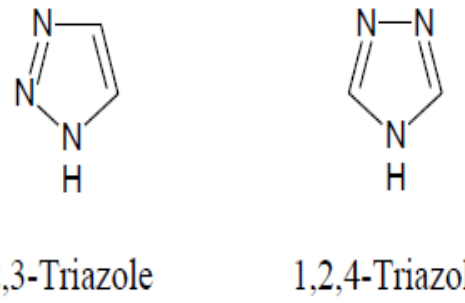

Fig-1 Structures of 1,2,3-Triazole and 1,2,4-Triazoles

MATERIAL AND METHODS

Scheme of synthesis (Fig-2)

Step 1- Preparation of potassium dithio carbazinate (2) To a continuously stirred solution of 0.15 mol of potassium hydroxide (8.4 gm) and 0.1 mol of isoniazid (1) (18.5 gm) in absolute ethanol (75 ml), 0.15 mol carbon disulphide (11.2 gm) was added drop wise. After complete addition, the mixture was agitated for 8 hrs. The precipitate was collected by filtration, washed with ether to obtain crude suspension of potassium dithio carbazinate. The potassium salts were employed for the preparation of corresponding triazoles without further purification as the dithiocarbazinates were moisture sensitive.

Step 2- Preparation of 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (3) A 0.02M suspension of potassium dithiocarbazinate (2) (4.44g), 0.04M hydrazine hydrate (2ml,) and water (80ml) was refluxed for 3 hrs. The colour of the reaction mixture changed to green, hydrogen sulphide was evolved and a homogenous solution resulted. A white solid was precipitated by dilution with cold water (100ml) and acidification with concentrated hydrochloric acid. The product was filtered, washed with cold water (2×30 ml) recrystallised from ethanol and dried under oven.

Step 3- Preparation of 4-hydrazinyl-5-(pyridin-4-yl)-4H-1,2,4-triazole-3- thiol (4) 0.03 mol of compound (3) is added to a solution prepared by adding 6 ml of conc. HCl drop wise, with stirring, to 6 ml of hydrazine hydrate at 5-10 oC. Then, 24 ml ethylene glycol was added and refluxed for 3 hr. On cooling, it gives 4-hydrazinyl-5- (pyridin-4-yl)-4H-1,2,4-triazole-3-thiol which was filtered, washed with water recrystallised from ethanol and was then dried under oven.

Step 4-Preparation of 4-{(2E)-2-[1-(substituted phenyl) ethylidene] hydrazinyl}-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (5) To a solution of 1.5 mM of 4-hydrazinyl-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (4) in 20 ml absolute ethanol,2.2 mM of appropriate benzaldehyde derivatives and 2-3 drops glacial acetic acid were added and refluxed on water bath for 10 hours

On cooling, it produced the corresponding hydrazone, which was filtered, washed with water and recrystallised from ethanol. It was then dried under oven.

Step 5- Preparation of 4-[(2E)-2-(substituted benzylidene)hydrazinyl]-5- (pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (6) To a solution of 1.5 mM of 4-hydrazinyl-5-(pyridin-4-yl)-4H-1,2,4-triazole-3- thiol (4) in 20 ml absolute ethanol, 2.2 mM of appropriate acetophenone derivative and 2-3 drops glacial acetic acid were added and refluxed on water bath for 10 hours. On cooling, it gives the corresponding hydrazone, which was recrystallized from ethanol and dried.

Step 6-Preparation of 3-(phenyl)-1-[3-(pyridin-4-yl)-5-sulfanyl-4H-1,2,4- triazol-4-yl]-1H-pyrazole-4-carbaldehyde (7) 5 mmol of compounds (6) were dissolved in Vilsmeier Haack reagent [1.5 ml POCl3 in 6 ml DMF] and stirred at room temperature for 4 hr. Then the contents were poured over crushed ice (previously neutralized with sodium bicarbonate). A solid separated out, which was filtered, washed with water, dried recrystallized from ethanol and dried under oven.

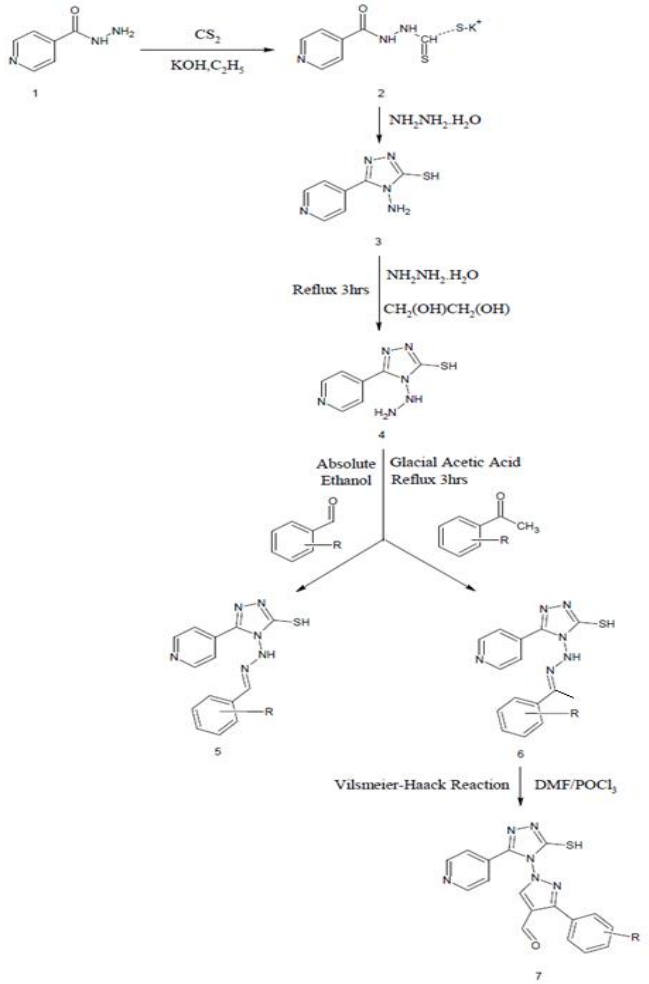

Fig-2 Scheme of synthesis

In-Vitro **Anti- Inflammatory studies (7-11)**

Protein Denaturation- 5ml 0.2% w/v bovine serum albumin in Tris HCl buffer saline and different concentrations of synthesized compounds were taken in test tubes and heated at 72°C for 5 minutes, cooled for 10 minutes. The absorbance of these solutions was determined at 660nm. The experiment repeated with standard (*Ibuprofen*) also. The IC_{50} was calculated and compared with standard.

Proteinase Inhibitory Action- The reaction mixtures (2ml) contained 0.06 mg trypsin, 1ml 25mM Tris HCl buffer (pH7.4) and 1ml aqueous solution of synthesized compounds of different concentration (100,200,300,400,500µg/ml). The mixtures were incubated at 37ºC for 5min.Then 1ml of 0.8%(w/v) casein was added. The mixtures were incubated for an additional 20 minutes. Then 2ml of 70 %(w/v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged. Absorbance of the supernatant was read at 280nm against buffer as blank. $IC_{50}$was calculated and compared with standard (*Ibuprofen*)

RESULTS AND DISCUSSION

Spectral studies- The characterization requires the identification of molecular framework, the nature of functional groups that are present and their location within the skeletal structure and finally the establishment of any stereo chemical relationships, which might exist. In the resent work, IR spectroscopic analysis was carried out to confirm the structures of newly synthesized compounds, and all the compounds were in agreement with their molecular structures.

SPECTRAL FEATURES OF SAMPLE 'PABZ' (Fig-3)

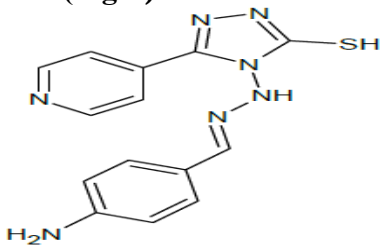

Fig-3 Proposed structure of 4-[(2*E*)-2-(4- aminobenzylidene)hydrazin yl] -5-(pyridin-4-yl)-4*H*- 1, 2,4-triazole-3-thiol

The suggested groups of the derivative 6d were confirmed by IR Spectra shown in the fig-4 Absorption between 3498cm^{-1} and 3486cm^{-1} indicate the presence of $–NH_2$ group. Absorption between 1306cm^{-1} and 1258cm^{-1} indicate the presence of aromatic C-N group. Absorption between 1074 cm^{-1} and 1026cm^{-1} indicate the presence of aliphatic C-N group. Absorption between 2674 cm^{-1} and 2643 cm^{-1} indicate the presence SH group (5, 6).

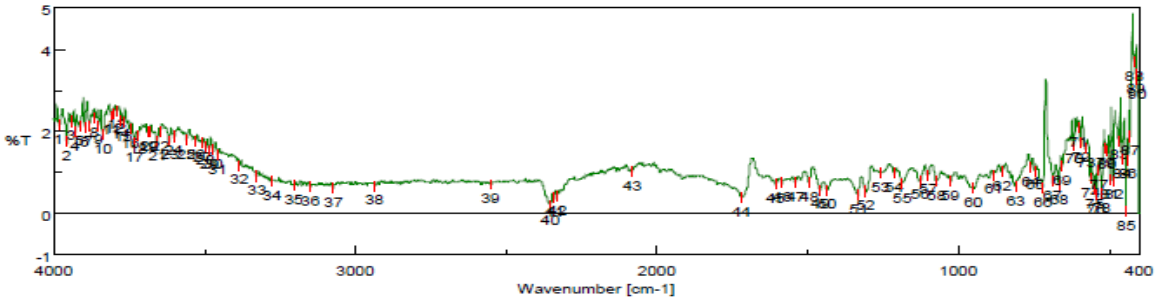

Fig-4 IR spectra of compound PABZ

STRUCTURAL FEATURES OF SAMPLE 'PCBZ' (Fig-5)

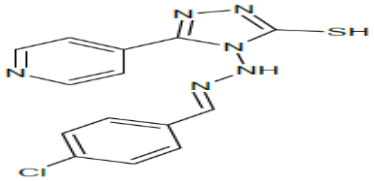

Fig-5 Proposed structure of 4-[(2*E*)-2-(4- chlorobenzylidene)hydrazi nyl]- 5-(pyridin-4-yl)-4*H*- 1,2,4-triazole-3-thiol

IR in cm^{-1}- Absorption between 3498cm^{-1} and 3486cm^{-1} indicate the presence of –NH_2 group. Absorption between 1306cm^{-1} and 1258cm^{-1} indicate the presence of aromatic C-N group· Absorption between 1074 cm^{-1} and 1026cm^{-1} indicate the presence of aliphatic C-N group. Absorption between 808cm^{-1} and 753 cm^{-1} indicate the presence of C-Cl group, 2643 cm^{-1} indicate the presence SH group (Fig-6).

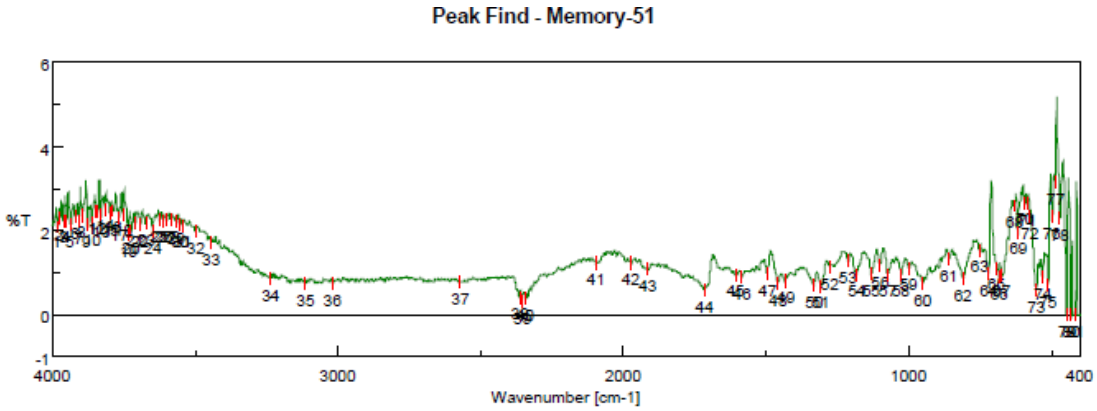

Fig- 6 IR spectra of compound PCBC

Anti- Inflammatory studies

The Anti- Inflammatory studies of synthesized compounds were carried out and the IC_{50} Values of synthesized compounds was calculated and tabulated (table-1) below and compared with standard.

Table-1 Anti-inflammatory studies of synthesized derivatives

S. No	Extract/ Standard	IC_{50} Values (mcg/ml)	
		Protein Denaturation Method	Proteinase Inhibitory Action
1	Standard *(Ibuprofen)*	52	61
2	PABZ	87	98
3	PCBC	76	80

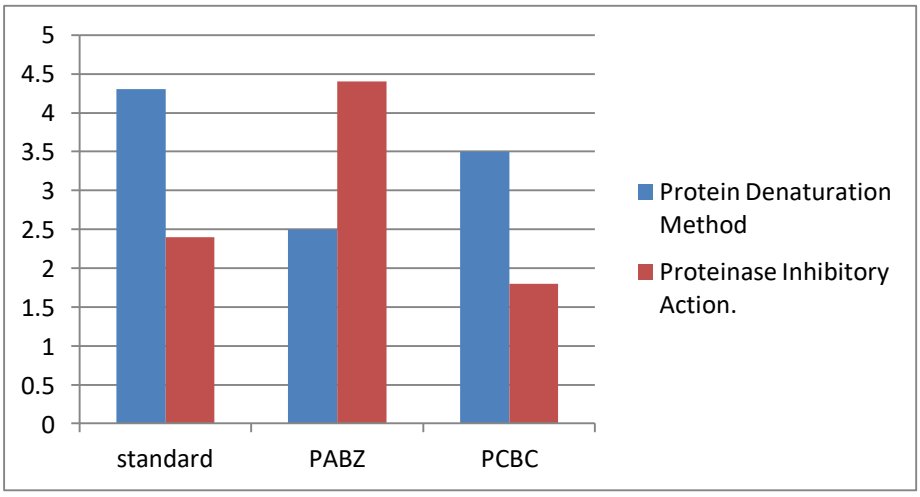

Fig-7 Graphical representation of anti-inflammatory studies

The anti inflammatory activities by inhibition of protein denaturation and proteinase inhibitory assay showed that the para amino benzaldehyde derivatives and chloro-benzaldehyde derivatives of 1, 2, 4- Triazol- 3 thiols possess potent anti inflammatory activity (Fig-7).

CONCLUSION

Medicinal chemistry is the application of chemical research techniques to the synthesis of pharmaceuticals. Developing a new drug from original idea to the launch of a finished product is a complex process. Numbers of triazole derivatives as clinical drugs or candidates have been frequently employed for the treatment of various types of diseases, which have proved the importance of this heterocyclic nucleus in drug design and discovery. Various derivatives are prepared from potassium dithio carbazinate by a four step process. The structures of final synthesized compounds were assigned on the basis of IR spectral data. All the newly synthesized compounds were screened for their *in-vitro* anti-inflammatory properties. The para amino benzaldehyde derivatives and chloro-benzaldehyde derivatives of 1,2,4- Triazol- 3 thiols shows significant results.

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