# Design, Synthesis and Biological Activity of Certain 3,4-Disubstituted-5-mercapto-1,2,4-triazoles and Their Hydrazino Derivatives

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3-Aryloxy methyl-4-(N-pyrazin-2'yl carboxamido)-5-mercapto-1,2,4-triazoles ($3a_1$-$a_{14}$) were prepared starting from potassium dithio carbazinates ($2a_1$-$a_{14}$). These triazoles were then employed in the synthesis of 3-aryloxy methyl-4-(N-pyrazin-2'yl carboxamido)-5-hydrazino-1,2,4-triazoles ($4a_1$-$a_{14}$). All the newly synthesized compounds were characterized by analytical, IR, NMR spectral studies. The compounds were screened for their antibacterial, antifungal, anti-inflammatory and analgesic properties. Most of the compounds have shown significant antifungal activity while few have shown excellent anti-inflammatory and analgesic activity. An attempt is made to study the structure activity relationship (SAR).

**Key Words :** Triazoles, Anti-fungal, Anti-bacterial, Anti-inflammatory, Analgesic activity

## Introduction

A number of heterocyclic systems incorporating 1,2,4-triazole nucleus fused with other heterocycles possess a broad spectrum of biological activities.[1-4] A survey of literature also revealed that substituted 1,2,4-triazoles and their N-bridged heterocycles have received considerable attention during last two decades as they are endowed with variety of biological activities and have a wide range of therapeutic properties.[5,6] The synthesis of these heterocycles has received considerable attention in recent years.[7] Prompted by the above facts and as a part of our program aimed at developing new biologically active compounds, a convenient synthesis of hitherto unreported title compounds incorporating pyrazin-2-yl carboxamido, 1,2,4-triazole and -$NHNH_2$ moieties together was devised. Apart from their chemical interest, these compounds could also be a subject of studies as pharmacological agents.

## Results and Discussion

The aryloxy acid hydrazides (**$1a_1$-$a_{14}$**) were prepared from the corresponding esters by a reaction with hydrazine hydrate following known method.[8] The hydrazides were then converted into their corresponding potassium dithiocarbazinates (**$2a_1$-$a_{14}$**) by reaction with carbon disulphide in presence of alcoholic potassium hydroxide.[9] The required 3-aryloxy-4-(N-pyrazin-2'-yl carboxamido)-5-mercapto-1,2,4-triazoles (**$3a_1$-$a_{14}$**) were prepared in excellent yields in one pot reaction by heating a mixture of potassium dithiocarbazinates (**$2a_1$-$a_{14}$**) and pyrazinic acid hydrazide for 6-8 hr when profuse evolution of hydrogen sulphide was observed. The reaction (**$3a_1$-$a_{14}$**) with hydrazine hydrate (99%) in absolute ethanol furnished 3-aryloxy methyl-(N-pyrazin-2'-yl carboxamido)-5-hydrazino-1,2,4-triazoles (**$4a_1$-$a_{14}$**). The compounds have been characterized on the basis of elemental analysis and spectral data.

**Table 1**. Characterization data of compounds (**$3a_1$-$a_{14}$**) and (**$4a_1$-$a_{14}$**)

| Sl. No. | Compound | Ar | Melting Point (°C) | Yield (%) |
|---|---|---|---|---|
| 1 | **$3a_1$** | Phenyl | 226 | 68 |
| 2 | **$3a_2$** | 2-methyl phenyl | 232 | 74 |
| 3 | **$3a_3$** | 3-methyl phenyl | 220 | 65 |
| 4 | **$3a_4$** | 4-methyl phenyl | 208 | 80 |
| 5 | **$3a_5$** | 2-chloro phenyl | 218 | 70 |
| 6 | **$3a_6$** | 4-chloro phenyl | 224 | 72 |
| 7 | **$3a_7$** | 2,4-dichloro phenyl | 252 | 68 |
| 8 | **$3a_8$** | 2-bromo phenyl | 226 | 64 |
| 9 | **$3a_9$** | 4-bromo phenyl | 244 | 68 |
| 10 | **$3a_{10}$** | 4-amino phenyl | 229 | 64 |
| 11 | **$3a_{11}$** | 2-nitro phenyl | 245 | 74 |
| 12 | **$3a_{12}$** | 4-nitro phenyl | 178 | 78 |
| 13 | **$3a_{13}$** | 1-naphthyl | 175 | 72 |
| 14 | **$3a_{14}$** | 2-naphthyl | 217 | 76 |
| 15 | **$4a_1$** | Phenyl | 235 | 70 |
| 16 | **$4a_2$** | 2-methyl phenyl | 230 | 78 |
| 17 | **$4a_3$** | 3-methyl phenyl | 235 | 70 |
| 18 | **$4a_4$** | 4-methyl phenyl | 250 | 83 |
| 19 | **$4a_5$** | 2-chloro phenyl | 224 | 68 |
| 20 | **$4a_6$** | 4-chloro phenyl | 240 | 73 |
| 21 | **$4a_7$** | 2,4-dichloro phenyl | 242 | 70 |
| 22 | **$4a_8$** | 2-bromo phenyl | 280 | 69 |
| 23 | **$4a_9$** | 4-bromo phenyl | 266 | 71 |
| 24 | **$4a_{10}$** | 4-amino phenyl | 202 | 62 |
| 25 | **$4a_{11}$** | 2-nitro phenyl | 270 | 74 |
| 26 | **$4a_{12}$** | 4-nitro phenyl | Decomposes at 265 | 76 |
| 27 | **$4a_{13}$** | 1-naphthyl | 184 | 69 |
| 28 | **$4a_{14}$** | 2-naphthyl | 241 | 73 |

The compounds gave satisfactory C, H and N analysis.

**Table 2.** Antibacterial and antifungal activity of triazole heterocycles (**3a1-a14** and **4a1-a14**)

| Sl. No | Compound | Antibacterial activity (% relative inhibition) con. in μg/mL | | | | | | | | | | | | | | | | Antifungal activity (% relative inhibition) con. in μg/mL | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Bacillus substilis* | | | | *Staphylococcus aures* | | | | *Escherichia coli* | | | | *Pseudomonas auriginosa* | | | | *Colletotricum gleosporioides penz* (Poisoned food technique) | |
| | | 50 | | 100 | | 50 | | 100 | | 50 | | 100 | | 50 | | 100 | | 50 | 100 |
| | | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | | |
| 1 | **3a1** | 15 | 13 | 17 | 16 | 10 | 09 | 12 | 10 | 15 | 13 | 16 | 14 | 05 | 05 | 08 | 07 | 40 | 42 |
| 2 | **3a2** | 14 | 11 | 16 | 13 | 09 | 08 | 12 | 11 | 15 | 13 | 16 | 13 | 06 | 06 | 08 | 08 | 30 | 34 |
| 3 | **3a3** | 10 | 09 | 12 | 11 | 08 | 07 | 12 | 11 | 12 | 11 | 13 | 11 | 06 | 05 | 09 | 07 | 32 | 36 |
| 4 | **3a4** | 18 | 15 | 20 | 17 | 13 | 12 | 15 | 14 | 17 | 14 | 18 | 15 | 07 | 05 | 10 | 07 | 40 | 42 |
| 5 | **3a5** | 16 | 15 | 20 | 18 | 12 | 11 | 14 | 14 | 15 | 13 | 18 | 17 | 05 | 05 | 07 | 07 | 54 | 56 |
| 6 | **3a6** | 18 | 17 | 20 | 18 | 10 | 10 | 12 | 11 | 18 | 36 | 12 | 19 | 06 | 04 | 08 | 07 | 73 | 75 |
| 7 | **3a7** | 15 | 14 | 16 | 17 | 11 | 10 | 13 | 12 | 14 | 13 | 16 | 14 | 07 | 07 | 11 | 10 | 40 | 41 |
| 8 | **3a8** | 12 | 10 | 14 | 13 | 09 | 08 | 12 | 11 | 13 | 11 | 14 | 13 | 05 | 05 | 06 | 06 | 30 | 33 |
| 9 | **3a9** | 13 | 11 | 14 | 12 | 08 | 07 | 11 | 10 | 12 | 11 | 15 | 13 | 08 | 07 | 09 | 09 | 40 | 43 |
| 10 | **3a10** | 17 | 16 | 18 | 15 | 11 | 10 | 14 | 13 | 15 | 14 | 18 | 17 | 07 | 07 | 10 | 09 | 38 | 40 |
| 11 | **3a11** | 18 | 16 | 18 | 18 | 10 | 09 | 12 | 11 | 17 | 15 | 18 | 17 | 05 | 05 | 06 | 06 | 25 | 28 |
| 12 | **3a12** | 18 | 17 | 18 | 17 | 09 | 08 | 11 | 10 | 18 | 15 | 20 | 19 | 06 | 06 | 08 | 07 | 38 | 40 |
| 13 | **3a13** | 13 | 12 | 14 | 12 | 10 | 09 | 13 | 12 | 14 | 14 | 16 | 15 | 07 | 07 | 09 | 07 | 30 | 32 |
| 14 | **3a14** | 14 | 13 | 15 | 14 | 11 | 09 | 13 | 11 | 14 | 12 | 14 | 13 | 06 | 05 | 08 | 06 | 33 | 35 |
| 15 | **4a1** | 32 | 30 | 35 | 31 | 30 | 30 | 32 | 30 | 34 | 31 | 34 | 32 | 30 | 30 | 31 | 31 | 42 | 44 |
| 16 | **4a2** | 31 | 30 | 34 | 30 | 30 | 30 | 32 | 31 | 35 | 32 | 36 | 31 | 30 | 30 | 30 | 30 | 44 | 46 |
| 17 | **4a3** | 30 | 29 | 34 | 31 | 32 | 30 | 32 | 30 | 31 | 30 | 33 | 30 | 29 | 28 | 30 | 29 | 40 | 41 |
| 18 | **4a4** | 38 | 36 | 40 | 37 | 33 | 31 | 34 | 32 | 32 | 31 | 35 | 34 | 31 | 30 | 32 | 31 | 44 | 46 |
| 19 | **4a5** | 36 | 34 | 38 | 36 | 32 | 31 | 34 | 33 | 34 | 32 | 35 | 34 | 30 | 30 | 30 | 30 | 36 | 39 |
| 20 | **4a6** | 38 | 36 | 40 | 38 | 33 | 30 | 34 | 31 | 32 | 31 | 35 | 33 | 32 | 30 | 33 | 30 | 44 | 46 |
| 21 | **4a7** | 34 | 32 | 36 | 31 | 32 | 31 | 32 | 32 | 34 | 31 | 38 | 32 | 30 | 30 | 32 | 30 | 64 | 68 |
| 22 | **4a8** | 30 | 30 | 33 | 30 | 32 | 30 | 31 | 31 | 31 | 31 | 33 | 30 | 29 | 29 | 30 | 30 | 42 | 44 |
| 23 | **4a9** | 31 | 30 | 33 | 31 | 34 | 32 | 30 | 30 | 30 | 30 | 34 | 31 | 30 | 29 | 30 | 29 | 72 | 75 |
| 24 | **4a10** | 35 | 31 | 37 | 34 | 31 | 30 | 33 | 31 | 33 | 30 | 34 | 32 | 30 | 30 | 31 | 29 | 43 | 44 |
| 25 | **4a11** | 36 | 32 | 37 | 34 | 31 | 29 | 32 | 31 | 32 | 30 | 36 | 32 | 31 | 30 | 32 | 29 | 72 | 76 |
| 26 | **4a12** | 36 | 33 | 38 | 36 | 32 | 29 | 35 | 31 | 35 | 32 | 36 | 32 | 30 | 30 | 30 | 30 | 44 | 46 |
| 27 | **4a13** | 35 | 31 | 35 | 32 | 33 | 28 | 33 | 32 | 33 | 30 | 33 | 31 | 30 | 29 | 31 | 30 | 38 | 40 |
| 28 | **4a14** | 36 | 32 | 37 | 35 | 35 | 30 | 36 | 32 | 35 | 32 | 36 | 30 | 30 | 29 | 30 | 30 | 42 | 44 |

[a]values in comparison with streptomycin. [b]values in comparison with gentamycin. % relative inhibition = $\frac{\text{inhibition of the test compound}}{\text{inhibition of the standard drug}} \times 100$

## Biological Activities

**Antibacterial and Antifungal Activity.**[10] All the compounds synthesized (**3a1-a14**) and (**4a1-a14**) have been screened for in vitro antibacterial activity against the organisms *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas auriginosa* by cup plate method at 50 and 100 μg/mL concentrations. DMSO was used as solvent control. Streptomycin and Gentamycin were used as standard drugs.

The compounds (**3a1-a14**) and (**4a1-a14**) were also tested for antifungal activity against the fungal organism *Colletotrichum gleosporioides penz* by poisoned food technique in potato dextrose agar (PDA) medium.

3-aryloxy-4-(N-pyrazin-2'-yl carboxamido)-5-mercapto-1,2,4-triazoles (**3a1-a14**) have exhibited weak antimicrobial activity (Table 2). However their hydrazino derivatives (**4a1-a14**) have shown moderate antimicrobial activity (Relative inhibition 30-40%) (Table 2).

Antifungal screening result has indicated that some members of the triazoles series like **3a5**, **3a6**, **4a7**, **4a9**, **4a11**, and **4a12** possess good antifungal activity (Relative inhibition 54-75%). Hydrazino triazoles (**4a1-a14**) obtained by the replacement of mercapto group by hydrazino group in triazoles (**3a1-a14**) showed quite significant antifungal activity (Relative inhibition 64-76%) against the fungal strain used for the study (Table 2).

Hydrazino triazoles which failed to show significant antibacterial activity however showed excellent antifungal activity. Perhaps, the introduction of -$NHNH_2$ group by replacing mercapto group of triazoles and the presence of electron withdrawing groups in the various positions of the phenyl ring in the 3rd position of triazole system may be responsible for the enhanced antifungal activity. It was

further observed that aryloxy methyl substituent at 3rd position of triazole ring with the substitution in the para position exhibited much enhanced activity compared to the ortho position substituents. Hydrazino triazole molecule designed consists of different moieties and active groups substituted in the triazole ring system that is believed to act as carrier for toxic agents into cells of pathogenic organisms, leading to high intra cellular concentrations of the toxicant which ultimately causes cell death.

**Anti-inflammatory and Analgesic Activity.**[11,12] The triazoles (**3a$_1$-a$_{14}$**) and their hydrazino derivatives (**4a$_1$-a$_{14}$**) were screened for their anti-inflammatory activity using rat hind paw method of Winter[11] *et al.* modified by Dhawan and Srimal.[12] The compounds were also screened for analgesic activity using Eddy's hot plate technique.[13]

Among the triazole series compounds **3a$_2$**, **3a$_4$**, **3a$_{12}$**, **3a$_{13}$** and **3a$_{14}$** showed significant activity (Table 3). Hydrazino triazoles **4a$_2$**, **4a$_4$**, **4a$_6$**, **4a$_8$**, **4a$_9$**, **4a$_{10}$**, **4a$_{13}$**, and **4a$_{14}$** showed higher activity than their parent triazoles. Thus in general, majority of triazoles and their hydrazino derivatives exhibited significant activity, in some cases equipotent and in few cases, even superior than the standard. Rest of the compounds in both the series exhibited moderate activity. In the literature it is shown that triazole scaffold containing compounds exhibits anti-inflammatory activity mediated through inhibition of cyclooxygenase-I and II (COX-I and II) enzyme depending on the position and kind of substituents on the 1,2,4-triazole system.[14] The compounds under study are speculated to show anti-inflammatory activity through COX-I and/or COX-II. Investigations are pending to demonstrate their inhibitory potencies towards COX-I and COX-II inhibition.

The analgesic studies of all the synthesized compounds revealed that the compound **3a$_{14}$** and **4a$_4$** showed moderate activity and rest of the compounds of both the series **3** and **4** showed weak activity (Table 3).

## Experimental

**Biological Screening**

**Antibacterial Screening:**

Standard nutrient agar medium:

**Table 3.** Anti-inflammatory and Analgesic activity of triazoles (**3a$_1$-a$_{14}$**) and their hydrazino derivatives (**4a$_1$-a$_{14}$**)

| Sl. No. | Compound | Anti-inflammatory Activity | | | Analgesic Activity | | |
|---|---|---|---|---|---|---|---|
| | | Dose Mg/kg | Paw edema volume | % Reduction | Dose Mg/kg | Maximum Average reaction time (sec) | Analgesic activity |
| 1 | Control | – | 0.59 ± 0.03 | – | – | 3.83 ± 0.31 | – |
| 2 | Ibuprofen | 200 | 0.15 ± 0.02 | 74.58 | 20 | 9.83 ± 0.33 | 256.66 |
| 3 | **3a$_1$** | 200 | 0.21 ± 0.00 | 64.41 | 20 | 5.33 ± 0.33 | 139.00 |
| 4 | **3a$_2$** | 200 | 0.16 ± 0.01 | 72.88 | 20 | 5.33 ± 0.33 | 169.71 |
| 5 | **3a$_3$** | 200 | 0.21 ± 0.01 | 64.41 | 20 | 5.33 ± 0.33 | 95.82 |
| 6 | **3a$_4$** | 200 | 0.08 ± 0.01 | 86.44 | 20 | 5.33 ± 0.33 | 187.21 |
| 7 | **3a$_5$** | 200 | 0.20 ± 0.01 | 66.10 | 20 | 5.33 ± 0.33 | 82.77 |
| 8 | **3a$_6$** | 200 | 0.19 ± 0.01 | 67.80 | 20 | 5.33 ± 0.33 | 121.93 |
| 9 | **3a$_7$** | 200 | 0.22 ± 0.01 | 62.71 | 20 | 5.33 ± 0.33 | 152.22 |
| 10 | **3a$_8$** | 200 | 0.21 ± 0.01 | 64.41 | 20 | 5.33 ± 0.33 | 86.94 |
| 11 | **3a$_9$** | 200 | 0.22 ± 0.01 | 62.71 | 20 | 5.33 ± 0.33 | 117.49 |
| 12 | **3a$_{10}$** | 200 | 0.22 ± 0.01 | 62.71 | 20 | 5.33 ± 0.33 | 95.82 |
| 13 | **3a$_{11}$** | 200 | 0.19 ± 0.01 | 67.80 | 20 | 5.33 ± 0.33 | 152.22 |
| 14 | **3a$_{12}$** | 200 | 0.16 ± 0.01 | 72.88 | 20 | 5.33 ± 0.33 | 161.10 |
| 15 | **3a$_{13}$** | 200 | 0.17 ± 0.01 | 71.19 | 20 | 5.33 ± 0.33 | 152.22 |
| 16 | **3a$_{14}$** | 200 | 0.15 ± 0.01 | 74.58 | 20 | 5.33 ± 0.33 | 204.44 |
| 17 | **4a$_1$** | 200 | 0.19 ± 0.01 | 67.80 | 20 | 5.17 ± 0.31 | 134.99 |
| 18 | **4a$_2$** | 200 | 0.15 ± 0.01 | 74.58 | 20 | 6.50 ± 0.34 | 169.71 |
| 19 | **4a$_3$** | 200 | 0.20 ± 0.01 | 66.10 | 20 | 4.50 ± 0.22 | 117.49 |
| 20 | **4a$_4$** | 200 | 0.07 ± 0.01 | 88.13 | 20 | 8.00 ± 0.36 | 208.88 |
| 21 | **4a$_5$** | 200 | 0.19 ± 0.01 | 67.80 | 20 | 3.50 ± 0.34 | 91.38 |
| 22 | **4a$_6$** | 200 | 0.15 ± 0.01 | 74.58 | 20 | 6.00 ± 0.36 | 156.66 |
| 23 | **4a$_7$** | 200 | 0.20 ± 0.01 | 66.10 | 20 | 4.17 ± 0.31 | 108.88 |
| 24 | **4a$_8$** | 200 | 0.17 ± 0.01 | 71.19 | 20 | 4.50 ± 0.22 | 117.49 |
| 25 | **4a$_9$** | 200 | 0.15 ± 0.01 | 74.54 | 20 | 4.83 ± 0.31 | 126.11 |
| 26 | **4a$_{10}$** | 200 | 0.16 ± 0.01 | 72.88 | 20 | 4.17 ± 0.31 | 108.88 |
| 27 | **4a$_{11}$** | 200 | 0.20 ± 0.01 | 66.10 | 20 | 3.83 ± 0.31 | 99.99 |
| 28 | **4a$_{12}$** | 200 | 0.19 ± 0.01 | 67.80 | 20 | 7.33 ± 0.61 | 191.38 |
| 29 | **4a$_{13}$** | 200 | 0.15 ± 0.01 | 74.58 | 20 | 5.17 ± 0.54 | 134.99 |
| 30 | **4a$_{14}$** | 200 | 0.15 ± 0.01 | 74.58 | 20 | 6.83 ± 0.31 | 178.33 |

| | |
|---|---|
| Meat extract (bacteriological) | ---- 1.0% |
| Peptone | ---- 1.0% |
| Sodium chloride | ---- 0.5% |
| Agar | ---- 2.0% |
| Water | ---- 100 mL |

Meat extract was taken and made up the volume to 100 mL with water and to this were added weighed quantities of peptone, salt and agar. The contents were dissolved by heating and the mixture was filtered and pH was adjusted to 7.5. The medium was sterilized by autoclaving at 121° for 15 minutes, cooled to 45° and then poured in 20 mL quantities to petridishes. A loopful of an overnight broth culture was spread evenly over the whole part with a sterile cotton-wool swab.

The culture plates were dried in the incubator with the lid until its surface was free from visible moisture without further delay, known concentration of the drug was applied as discs (prepared by uniformly punching out 6 mm discs from Whatmann filter paper (No. 41) and impregnating with drug (100 discs in 1 mL) with adequate spacing to the surface of the culture plates with sterile fine pointed forceps and pressed gently to ensure full contact with the medium.

It was then transferred to the incubator for 24 hours at 37° C. At the end of 24 hours the diameter of zone of inhibition produced were measured (Table 2).

**Antifungal Screening:** *In vitro* evaluation of some of the triazole derivatives synthesized was done by "Poisoned food technique" against the fungal organism *Collectotrichum gleosporioides penz.* at the concentration levels of 50 µg/mL and 100 µg/mL with greseofulvin as standard drug for comparison.

The efficacy of compounds was tested against the fungal organism *Collectotrichum gleosporioides penz.* by assessing the percent inhibition. The test compounds were uniformly incorporated aseptically to standard Potato-Dextrose-Agar (PDA) medium, cooled to 45 °C so as to give the required concentrations. Twenty ml of this poisoned medium was poured into flat bottom petriplates. One cm diameter of culture discs of *Collectotrichum gleosporioides* were kept at the centre of each petriplate containing the test compounds and the plates were then incubated at 28 ± 1 °C for seven days. Three replications were maintained for each treatment. The growth of the fungus without any compound served as control The radial growth of the colony was recorded when maximum growth was observed in control and further percent inhibition was calculated by using the following formula of Vincent (1927) for each chemical

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Percent inhibition
C = Radial growth of fungus in control
T = Radial growth of fungus in treatment.

Angular transformations were made for the data and analyzed statistically. The readings are represented in Table 2.

**Antiinflammatory Activity:** The albino rats were divided into 11 groups containing 6 animals each. The animals were fasted for 12 hours prior to the experiment and they were supplied with water.

On the day of the experiment, the animals were weighed and marked. A mark was made on the right hind paw just beyond the tibia-tarsal junction, so that every time the paw is dipped in the mercury column up to marked level to ensure constant paw volume. Then the paw volume of each rat was measured by mercury displacement method.

The animals of group –1 were treated with Acacia suspension as Control. The group –2 animals were treated with Ibuprofen 200 mg/kg as a standard drug, which was injected half an hour prior to the injection of formalin. The animals of the groups 3 to 11 were injected with 1,2,4-triazole derivatives, in a dose of 200 mg/kg body weight, half an hour prior to injection of formalin. Then 0.1 mL of formalin was injected subcutaneously into the right hind paw of all the animals in all groups. The paw volume of all animals in all groups was measured at 60, 120, 240 and 360 minutes intervals, after formalin administration.

The differences in the paw volumes (*i.e.* oedema volumes) of each animals of all the groups were calculated and compared with the changes in the oedema volumes of control and the drug treated animals. The results were expressed as percentage reduction in oedema volume, which can be calculated by using the formula:

$$\text{Percent Reduction} = \frac{C_{vt} - t_{vt}}{C_{vt}} \times 100$$

Where,

$C_{vt}$ = oedema volume of control animals at time 't'
$t_{vt}$ = oedema volume of drug treated animals at time 't'

The results are compiled in the Table 3.

***Analgesic Activity:*** 1) Albino mice of either sex were selected and divided into eleven groups, containing six animals in each group. These animals were fasted for twenty four hours, prior to the experiment.

2) Animals of Group – I considered as Control, were administered with 3% Acacia suspension.

3) Animals of Group – II were treated with standard drug, *i.e.*, Ibuprofen (20 mg/kg), which is considered as standard group.

4) Animals of Group – III, IV, V, VI, VII, VIII, IX, X and XI were treated with drugs (20 mg/kg) respectively.

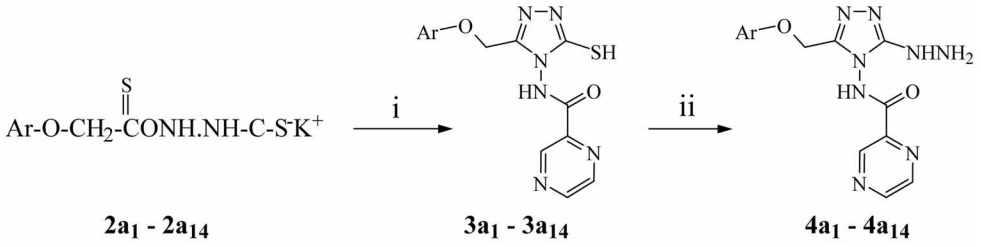
5) The reaction time for each mouse was recorded at time interval of 0, 30, 60, 120, 240 and 360 minutes after the administration of test substances by using Eddy's hot plate.

The % analgesic activity (PAA) was calculated by the following formula

$$PAA = (T_2/T_1) \times 100$$

$T_1$ is the reaction time before treatment and $T_2$ is the reaction time after the treatment. The results are shown in Table 3.

**Chemistry.** All the compounds in the study were synthesized by following Scheme 1. Melting points were determin-




**Scheme 1**. Reagents: i) Pyrizinic acid hydrazide, water, reflux, 5-6 hrs; ii) Hydrazine hydrate, ethanol, reflux.

ed on a Toshniwal apparatus in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel-G plates using chloroform-ethyl acetate (1:1) solvent system as irrigant and iodine vapour as visualizing agent. IR spectra in KBr ($cm^{-1}$) were recorded on a Schimadzu FTIR-8000 series spectrophotometer and $^1H$ NMR spectra (DMSO-$d_6$) on EM 390 MHz spectrometer using TMS as internal standard (Chemical shifts are expressed in $\delta$ ppm). Mass spectra were recorded on a Jeol JMSD-300 Mass Spectrometer operating at 70 eV. All the compounds showed satisfactory micro analytical results for C, H and N.

Potassium dithiocarbazinates (**$2a_1$-$a_{14}$**) were prepared by reacting aryloxy acid hydrazides (**$1a_1$-$a_{14}$**) with carbon disulphide in the presence of alcoholic KOH, following the reported procedure and were used directly for the next step without further purification (Yields were between 75-82%).

**3-Aryloxy-4-(N-pyrazin-2'-yl carboxamido)-5-mercapto-1, 2, 4-triazoles (3$a_1$-$a_{14}$):** A suspension of potassium dithiocarbazinates (2, 0.1 mol) pyrazinic acid hydrazide (0.1 mol) and water (5 mL) was heated under reflux for 5-6 hr when hydrogen sulphide was evolved and a clear solution resulted. Dilution of the reaction mixture with cold water (50 mL) and subsequent acidification with HCl gave the requir-ed product which was filtered, washed with water and crystallized from aqueous ethanol. The compounds of the series (**$3a_1$-$a_{14}$**) were prepared following the same procedure and characterized (Table 1).

**3-Phenoxymethyl-4-(N-pyrazin-2'-yl-carboxamido)-5-mercapto-1,2,4-triazoles (3$a_1$)**

**IR**: 3250 (NH of CONH), 3050 (aromatic C-H stretch-ing), 2910 ($OCH_2$), 2610 (SH), 1665 (CONH), 1635 (NH in plane bending), 1620 (C=N), 1605 (C=C), 1490 (C-N), 1098 (C-O-C), 740 (monosubstituted benzene), 695 (C-S); **$^1H$ NMR** (DMSO-$d_6$): 5.50 (2H, s, $OCH_2$), 6.60-7.50 (4H, m, Ar-H). 8.80-9.20 (3H, m, Ar-H), 10.60 (1H, s, NH of CONH), 13.80 (1H, s, SH); **MS:** m/z 304 ($M^+$). [Found C, 55.17; H, 3.79; N, 25.57; $C_{14}H_{12}N_6O_2S$ requires C, 55.26; H, 3.94; N, 27.63%].

**3-[(4-Methylphenoxy)methyl]-4-(N-pyrazin-2'-yl-carbox-amido)-5-mercapto-1,2,4-triazoles (3$a_4$)**

**IR**: 3248 (NH of CONH), 3066 (aromatic C-H stretch-ing), 2950 (C-H stretching), 2905 ($OCH_2$), 2608 (SH), 1662 (CONH), 1640 (NH in plane bending), 1615 (C=N), 1608 (C=C), 1486 (C-N), 1130 (C-O-C), 840 (1,4 disubstituted benzene), 700 (C-S); **$^1H$ NMR** (DMSO-$d_6$): 2.20 (3H, s, $-CH_3$), 5.40 (2H, s, $OCH_2$), 6.50-7.30 (4H, m, Ar-H), 8.80-9.20 (3H, m, heterocyclic protons), 10.60 (1H, s, NH of CONH), 13.80 (1H, s, SH); **MS:** m/z 318 ($M^+$). [Found C, 55.49; H, 4.35; N, 26.36; $C_{15}H_{14}N_6O_2S$ requires C, 56.60; H, 4.40; N, 26.42%].

**3-[(4-Chlorophenoxy)methyl]-4-(N-pyrazin-2'-yl-carbox-amido)-5-mercapto-1,2,4-triazoles (3$a_6$)**

**IR**: 3225 (NH of CONH), 3060 (aromatic C-H stretch-ing), 2915 ($OCH_2$), 2548 (SH), 1670 (CO of CONH), 1635 (NH in plane bending), 1615 (C=N), 1610 (C=C), 1480 (C-N), 1150 (C-O-C), 838 (1,4 disubstituted benzene), 686 (C-S); **$^1H$ NMR** (DMSO-$d_6$): 5.50 (2H, s, $OCH_2$), 6.80-7.60 (4H, m, Ar-H), 8.80-9.20 (3H, m, heterocyclic protons), 10.70 (1H, s, NH of CONH), 13.80 (1H, s, SH); **MS:** m/z 362 ($M^+$). [Found C, 46.37; H, 2.96; N, 22.99; $C_{14}H_{11}N_6ClO_2S$ requires C, 46.41; H, 3.04; N, 23.20%].

**3-[(4-Nitrophenoxy)methyl]-4-(N-pyrazin-2'-yl-carbox-amido)-5-mercapto-1,2,4-triazoles (3$a_{12}$)**

**IR**: 3260 (NH of CONH), 3060 (aromatic C-H stretch-ing), 2906 ($OCH_2$), 2600 (SH), 1674 (CO of CONH), 1635 (NH in plane bending), 1620 (C=N), 1608 (C=C), 1525 and 1354 ($NO_2$), 1494 (C-N), 1150 (C-O-C), 842 (1,4 disubstitu-ted benzene), 700 (C-S); **$^1H$ NMR** (DMSO-$d_6$): 5.60 (2H, s, $OCH_2$), 6.80-7.50 (4H, m, Ar-H), 8.80-9.20 (3H, m, hetero-cyclic protons), 10.70 (1H, s, NH of CONH), 13.80 (1H, s, SH); **MS:** m/z 349 ($M^+$). [Found C, 48.06; H, 3.08; N, 23.96; $C_{14}H_{11}N_7O_4S$ requires C, 48.14; H, 3.15; N, 24.10%].

**3-Aryloxy methyl-4-(N-pyrazin-2'-yl-carboxamido)-5-hydrazino-1,2,4-triazoles (4$a_1$-$a_{14}$):**

Triazole (3. 0.01 mol), hydrazine hydrate 99% (0.01 mol) in absolute ethanol (50 mL) were refluxed on steam till the evolution of hydrogen sulphide stopped. The solid obtained was filtered washed with water, dried and crystallized from ethanol get the crystals of required product. All the com-pounds of the series were prepared following the same procedure and characterized (Table 1).

**3-Phenoxymethyl-4-(N-pyrazin-2'-yl-carboxamido)-5-hydrazino-1,2,4-triazoles (4$a_1$)**

**IR**: 3268 (NH and $NH_2$), 3058 (aromatic C-H stretching), 2908 ($OCH_2$), characteristic absorption band for SH at 2610 disappeared, 1665 (CO of CONH), 1632 (NH in plane bend-ing), 1618 (C=N), 1600 (C=C), 1490 (C-N), 740 (monosub-stituted benzene), 695 (C-S); **$^1H$ NMR** (DMSO-$d_6$): 5.50 (2H, s, $OCH_2$), 5.80 (2H, s, $NH_2$), 6.50-7.50 (4H, m, Ar-H), 8.80-9.20 (3H, m, heterocyclic protons), 9.90 (1H, s, NH) 10.60 (1H, bs, NH of CONH), the signal for -SH at 13.80

disappeared. [Found C, 47.56; H, 4.44; N, 36.68; $C_{14}H_{14}N_8O_2$ requires C, 47.68; H, 4.64; N, 37.08%].

**3-[(4-Methylphenoxy)methyl]-4-(N-pyrazin-2'-yl-carboxamido)-5-hydrazino-1,2,4-triazoles ($4a_4$)**

**IR**: 3256, 3362 (NH and $NH_2$), 3054 (aromatic C-H stretching), 2942 (C-H stretching of $CH_3$), 2905 ($-OCH_2$), The characteristic absorption band for SH at 2608 is disappeared, 1656 (CO of CONH), 1632 (NH in plane bending), 1610 (C=N), 1602 (C=C), 1478 (C-N), 1140 (C-O-C), 842 (1,4 disubstituted benzene); **$^1$H NMR** (DMSO-$d_6$): 2.20 (3H, s, $-CH_3$), 5.40 (2H, s, $OCH_2$), 5.80 (2H, s, $NH_2$), 6.50-7.30 (4H, m, Ar-H), 8.80-9.20 (3H, m, heterocyclic protons), 9.80 (1H, s, NH) 10.60 (1H, bs, NH of CONH), The signal for -SH at 13.80 disappeared. [Found C, 49.27; H, 4.96; N, 35.36; $C_{15}H_{16}N_8O_2$ requires C, 49.39; H, 4.43; N, 33.13%].

**3-[(4-Chlorophenoxy)methyl]-4-(N-pyrazin-2'-yl-carboxamido)-5-hydrazino-1,2,4-triazoles ($4a_6$)**

**IR**: 3260, 3380 (NH and $NH_2$), 3048 (aromatic C-H stretching), 2910 ($-OCH_2$), The characteristic absorption band for SH at 2598 is disappeared, 1662 (CO of CONH), 1630 (NH in plane bending), 1618 (C=N), 1605 (C=C), 1482 (C-N), 1180 (C-O-C), 842 (1,4 disubstituted benzene); **$^1$H NMR** (DMSO-$d_6$): 5.40 (2H, s, $OCH_2$), 5.80 (2H, s, $NH_2$), 6.50-7.30 (4H, m, Ar-H), 8.80-9.20 (3H, m, heterocyclic protons), 9.90 (1H, s, NH) 10.60 (1H, bs, NH of CONH), The signal for -SH at 13.80 disappeared. [Found C, 42.49; H, 4.36; N, 33.08; $C_{14}H_{15}N_8O_2Cl$ requires C, 42.60; H, 4.43; N, 33.13%].

**3-[(4-Nitrophenoxy)methyl]-4-(N-pyrazin-2'-yl-carboxamido)-5-hydrazino-1,2,4-triazoles ($4a_{12}$)**

**IR**: 3280, 3380 (NH and $NH_2$), 3058 (aromatic C-H stretching), 2902 ($-OCH_2$), The characteristic absorption band for SH at 2600 is disappeared, 1662 (CO of CONH), 1630 (NH in plane bending), 1615 (C=N), 1599 (C=C), 1530, 1360 ($NO_2$), 1478 (C-N), 1170 (C-O-C), 848 (1,4 disubstituted benzene); **$^1$H NMR** (DMSO-$d_6$): 5.60 (2H, s, $OCH_2$), 5.90 (2H, s, $NH_2$), 6.80-7.50 (4H, m, Ar-H), 8.80-9.20 (3H, m, heterocyclic protons), 9.70 (1H, s, NH), 10.70 (1H, bs, NH of CONH). [Found C, 41.28; H, 3.66; N, 36.19; $C_{14}H_{13}N_9O_4$ requires C, 41.49; H, 3.75; N, 36.31%].

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