

# Synthesis and Biological Evaluation of certain N-bridged 1,2,4 – Triazole analogues.

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## Abstract:

3-(2-amino-3,5-dibromophenyl) – 4- amino 5- mercapto – 1,2,4 – triazole (**6**) when condensed with substituted aryl/aryloxy/hetero aryl acids in presence of  $\text{POCl}_3$  gave 3-(2-amino-3,5-dibromophenyl)-6-substituted (3,4-b) (1,3,4) triazolothiadiazoles (**7a<sub>1</sub> – a<sub>25</sub>**). The compound **6** when condensed with dicarboxylic acids like oxalic acid, succinic acid and tartaric acid in presence of  $\text{POCl}_3$  yielded 6,6 bis- 3-(2-amino-3,5 – dibromo phenyl) – 1,2,4 triazolo (3,4-b) (1,3,4) thia diazoles (**8,9 & 10**). The structures of the newly synthesized compounds have been established on the basis of their spectral data. The broad spectrum of activity exhibited by triazoles and their N-bridged analogues prompted us to evaluate their antibacterial, antifungal, antitubercular and anticancer activity. Few compounds showed significant antitubercular and anticancer activity. Majority of the compounds exhibited excellent antibacterial and antifungal activity.

**Key Words:** Triazolothiadiazole, antimicrobial, antitubercular, anticancer.

## INTRODUCTION

Heterocyclic compounds containing 1,2,4-triazole and thiazole nucleus possess a diversity of useful biological effects. Further it was also reported that substituted 1,2,4 triazoles and their N-bridged heterocycles have received considerable attention during past two decades as they are endowed with variety of biological activities and have a wide range of therapeutic properties<sup>1-4</sup>. Various 3- substituted 4-amino 5-mercapto -1,2,4-triazoles have been studied extensively for the past several years because of their broad spectrum of biological activity and variety of medicinal applications<sup>5-8</sup>. The amino and mercapto groups of these compounds serve as readily accessible nucleophilic centres for the preparation of N-bridged heterocycles. The 1,3,4-thiadiazoles which display diverse biological activities, possible due to the presence of toxophoric>N-C-S moiety. The 1,2,4 triazolo-(3,4 -b) – 1,3,4 thiazole derivatives obtained by fusing biolabile 1,2,4-triazole and 1,3,4-thiazole rings together are reported to possess antibacterial, antifungal, antitubercular, anti-inflammatory, analgesic, antiviral plant growth regulatory properties. Further it has been reported that many biological active natural and synthetic products have interesting molecular symmetry. Recently some bistriazole derivatives endowed with excellent biological activity have also been reported<sup>9-10</sup>. Prompted by the above facts and as part of our programme aimed at developing new biologically active compounds, we designed the synthesis and biological evaluation of series of novel 3-(2-amino-3,5- dibromo phenyl)-6-substituted (3,4b) (1, 3, 4) – triazole thiazoles (**7a<sub>1</sub> – a<sub>25</sub>**) and 6,6-bis-3(2- amino-3,5-dibromo phenyl)-1,2,4-triazolo (3,4b) (1, 3, 4) thiazoles<sup>8-11</sup>. (**8,9 & 10**) Apart from their chemical interest these compounds could also be a subject of studies as pharmacological agents.

## EXPERIMENTAL:

Melting points were determined using open capillary tubes in paraffin oil bath Polmon Digital Melting point. Apparatus (model MP 96). Purity of the compounds was checked by TLC on pre-coated silica gel sheets obtained from Merck, Germany. Visualization of the spots on TLC plates was achieved either by exposure to Iodine vapour or UV light. Reaction completion and purity was monitored by TLC. IR spectra in KBr ( $\text{cm}^{-1}$ ) were recorded on Perkin Elmer Infrared spectrophotometer (Model Spectrum 100) with NaCl optics. Samples were scanned in KBr pellets. <sup>1</sup>H NMR spectra were recorded on BrukerAvance 300 MHz

and 400 MHz instrument using TMS as internal standard (chemical shifts are expressed in  $\delta$  ppm). Mass spectra were recorded on Perkin Elmer PE SCIEX – API – 2000 Mass spectrophotometer.

The parent triazole 3-(2-amino-3,5 – dibromo phenyl)-4-amino-5-mercapto 1,2,4 – triazole was prepared by 2 methods and comparative study of the 2 methods is carried out.

## METHOD A

### Preparation of 3, 5 – dibromoanthranilic acid hydrazide (3)

3,5-Dibromo methyl anthranilate (0.01 mol) in ethanol (30ml) and hydrazine hydrate (99%, 0.03 mol) was refluxed for 8 hrs. The mixture was cooled, the product obtained was filtered and was crystallised from ethanol (Yield 88%).

### Preparation of potassium dithiocarbazinate (4)

A mixture of 3,5-dibromoanthranilic acid hydrazide (0.01mol), KOH (0.03mol) were added to anhydrous alcohol (70ml) and  $\text{CS}_2$  and was stirred for 12 hrs. The solid product was filtered, dried, washed with ether and directly used for the preparation of triazole.

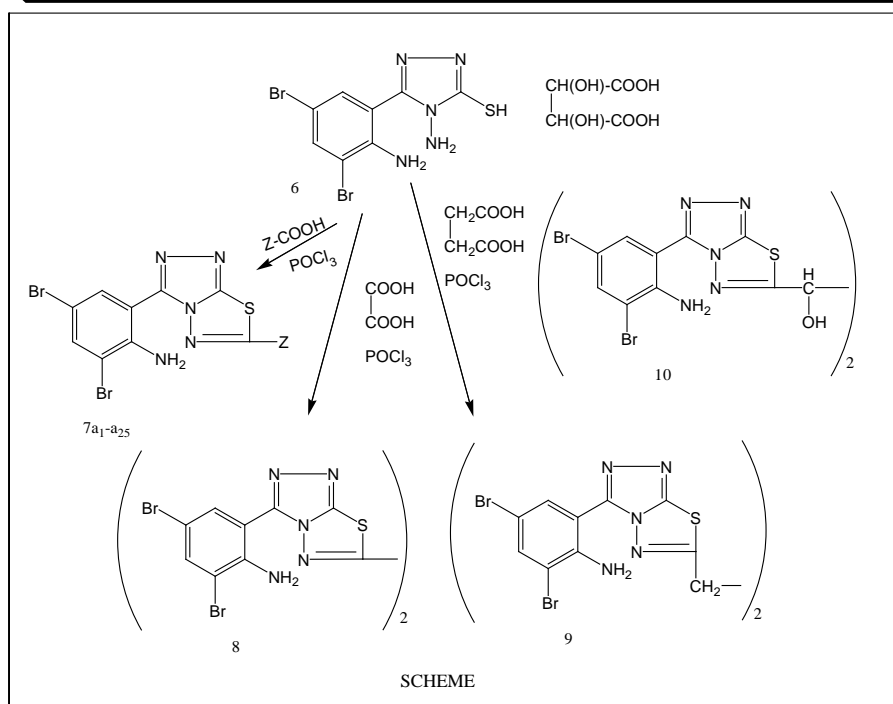
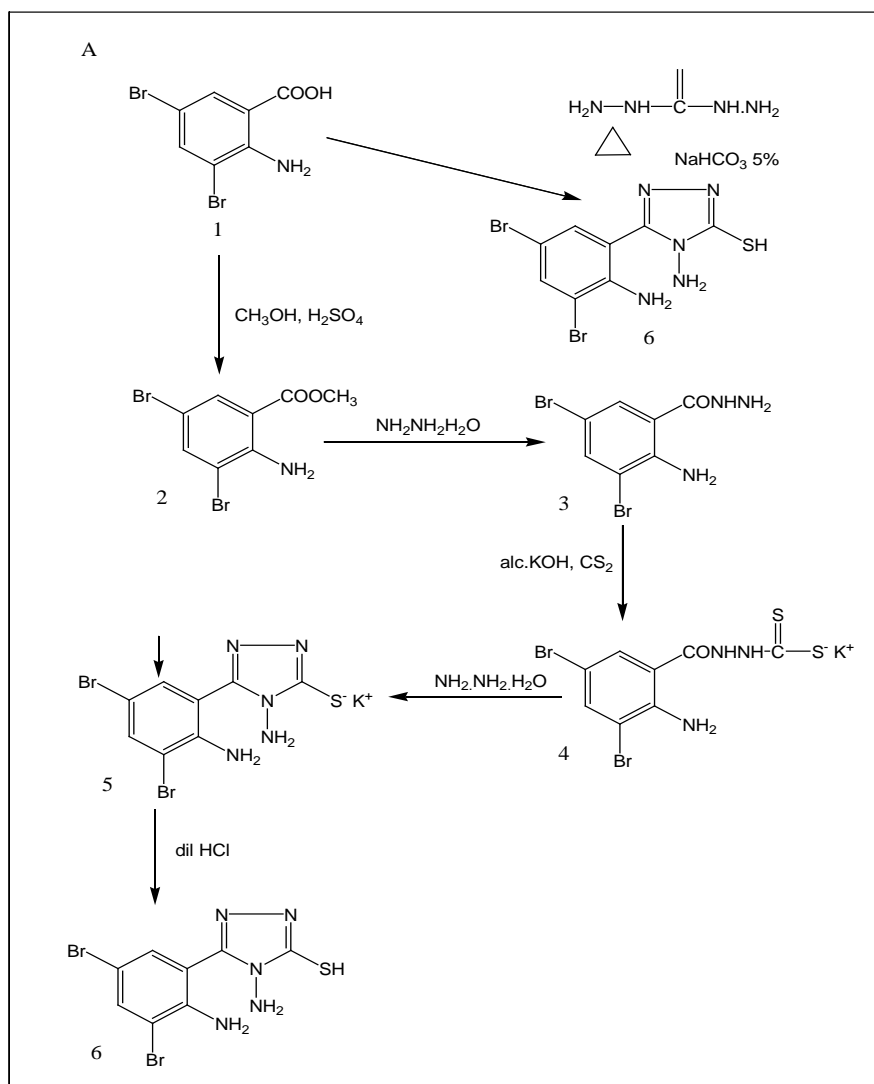
### Preparation of 3- (2 – amino-3,5– dibromo phenyl)-4-amino-5- mercapto, 1, 2,4-triazole (6)

The mixture of dithiocarbazinate (4) and hydrazine hydrate (99%) in the ratio 1:3 was heated at 155°C till  $\text{H}_2\text{S}$  was evolved. The product was added to water and acidified with hydrochloric acid (25%). The required triazole (6) that is separated was purified by crystallization using ethanol (yield 70%, mp 78-80°C).

## METHOD B:

The well triturated mixture of 3,5-dibromoanthranilic acid and thiocarbonylhydrazide in equimolar proportion was fused for 2 hrs. It was cooled, washed with sodium bicarbonate 5% solution, again washed with water and the dried compound was recrystallized from ethanol. [Yield 85%, mp 78°-80°].

**6 IR (KBr)  $\text{Vcm}^{-1}$  :** 3465, 3349 ( $\text{NH}_2$ ), 3074 (aromatic C-H str), 1612 (C=N), 1571, 1531 & 1451 (C=C ring str), 1303 (C=S), 879 (substituted phenyl ring), 690 (C-Br), <sup>1</sup>H NMR  $\delta$  3.04 (1H, s, SH), 3.65 – 3.89 (2H, s,  $\text{NH}_2$ ), 6.17 (2H, s, 2H of N –  $\text{NH}_2$ ), 7.94 – 9.02 (2H, m, Ar-H), mass  $m/z$  366 ( $m^+$ ), 368 ( $m^+ + 2$ ) other important fragment ions are at  $m/z$  = 320, 292, 278 (base peak), 252, 198, 149, etc.



**Preparation of 3-(2-amino-3,5-dibromo phenyl)-6-substituted (3,4b) (1,3,4) - triazolethiadiazoles (7a<sub>1</sub> - a<sub>25</sub>) and 6,6'-bis-3-(2-amino-3,5-dibromo phenyl)-1,2,4-triazolo (3,4b) (1,3,4) thiazoles (8, 9 and 10)**

Equimolar mixture of triazole (6) and carboxylic acid in POCl<sub>3</sub> (10ml) was heated for 7 hrs. Excess of POCl<sub>3</sub> was removed by distillation under vacuum. The product obtained was dissolved in water and treated with sodium bicarbonate solution (5%) to remove unreacted acid. The product was again washed with water and purified by recrystallization using ethanol. The compounds 8, 9 & 10 were also prepared using the same procedure by heating the mixture of triazole (2mol) and dicarboxylic acids like oxalic acid, succinic acid and tartaric acid respectively (1mol) for about 7 hrs. The purity of the compounds was established by TLC silcagel G plates using n-hexane and ethyl acetate (1:1) as elute and observed in UV light the compounds thus prepared are listed in table - 1.

**7a<sub>2</sub> IR, (KBr), cm<sup>-1</sup>:** 3467 and 3354 (NH<sub>2</sub>), 3070 (aromatic C-H str), 1640 (NH bending of NH<sub>2</sub>), 1616 (C=N), 1593, 1567, 1525 & 1488, (C=C ring str), 1402 (C-N), 1280 (N-N=C). The peak at 1303 for C=S of parent triazole 6 is disappeared, 875 and 843 (substituted phenyl rings), 680 (C-S), 656 (C-Br), 556 (C-C1), HNMR: δ 3.87 (2H, s, NH<sub>2</sub>), 7B - 9.01 (6H, m, Ar - H), mass : m/z 488 (mt)

**7a<sub>4</sub> IR (KBr) cm<sup>-1</sup>:** 3460 (NH<sub>2</sub>), 3092 aromatic C - H str, 1640 (N - H bending of NH<sub>2</sub>), 1624 (C = N), 1590, 1580, 1541 (C = C ring str), 1541 and 1345 (NO<sub>2</sub>), 1437 (C - N), the peak at 1303 for C = S of parent triazole 6 is disappeared, 1280 (N - N = C), 895 & 881 (substituted phenyl rings), 680 (C - S), 656 (C - Br), 556 (C - C1). HNMR: δ 3.87 (2H, s, NH<sub>2</sub>), 7.13 - 8.92 (6H, m, Ar - H), mass : m/z 543 (m+).

**7a<sub>5</sub> IR (KBr), cm<sup>-1</sup>:** 3420 (NH<sub>2</sub>), 3068 (aromatic C - H str), 2953 and 2867 C - H str of -CH<sub>3</sub>, 2929 & 2830 C - H str of >C H<sub>2</sub>,

1596, 1556, 1508 & 1463 (C = C ring str), 1314 (C - N), 1278 (>N - N = C). The peak at 1303 for C = S of parent triazole 6 is disappeared, 870, 848 (substituted phenyl rings) 595 (C - Br), HNMR : δ 0.89 - 0.92 (6H, d, 2XCH<sub>3</sub> of -CH (CH<sub>3</sub>)<sub>2</sub>), 1.56 - 1.66 (3H, d, CH<sub>3</sub> of (CH-CH<sub>3</sub>)), 1.81-1.94 (1H, m CH of -CH (CH<sub>3</sub>)<sub>2</sub>), 2.31 - 2.5 (2H, s, CH<sub>2</sub> of CH<sub>2</sub> - CH (CH<sub>3</sub>)<sub>2</sub>), 3.19 - 3.9 (1H, m, CH of CH - CH<sub>3</sub>), 4.1 (2H, s, NH<sub>2</sub>), 7.14 - 8.5 (6H, m, Ar-H), mass : m/z 535 (m+) & 537 (m<sup>+</sup>+2)

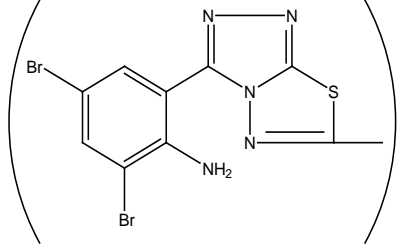
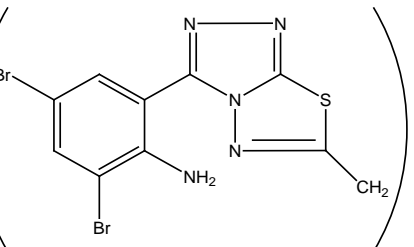
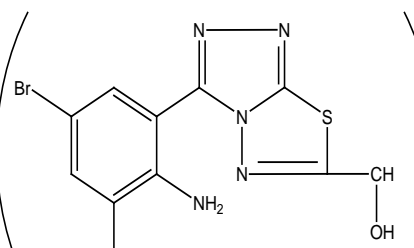
**7a<sub>6</sub> IR (KBr) cm<sup>-1</sup>:** 3471 & 3468 (NH<sub>2</sub>), 3071 (aromatic C-H str), 1602 (C = N and C = C), 1362 (C - N), 1277, (>N - N = C), the peak at 1303 for C = S of parent triazole 6 is disappeared, 871 substituted phenyl ring, 682 (C-S), 666 (C-Br), HNMR : δ 4.20 (4H, s, 2X NH<sub>2</sub>), 7.12 - 8.70 (6H, m, Ar - H), mass m/z 626 (m<sup>+</sup>) & 628 (m<sup>+</sup>+2).

**7a<sub>9</sub> IR (KBr) cm<sup>-1</sup>:** 3366 (NH<sub>2</sub>), 3063 (aromatic C-H str), 1596 (C=N), 1584, 1563, 1495 (C=C ring str), 1371 (C-N), 1276 (>N - N=C), the peak at 1303 for C=S of parent triazole 6 is disappeared. 874 & 724 (substituted phenyl rings), 699 (C-S), 630 (C-Br), HNMR: δ 4.25 (2H, s, NH<sub>2</sub>), 3.80 (1H, s, >CH) 7.00 - 8.52 (12H, m, Ar-H). Mass: m/z 543 (m+).

**7a<sub>10</sub> IR (KBr) cm<sup>-1</sup>:** 3473 & 3371 (NH<sub>2</sub>), 3243 (NH), 3073 (aromatic C-H str), 2950 & 2843 (C - H str of CH<sub>3</sub> asymmetric and symmetric, 1640 (NH-bending) 1613 (C = N), 1575, 1507, 1457 (C=C ring str), 1433 & 1366 (C-H bending of CH<sub>3</sub> asymmetric and symmetric). The peak at 1303 for C=S of parent triazole 6 is disappeared, 1277 (>N-N=C), 875 & 725 (substituted phenyl rings), 680 (C-S), 608 (C-Br), HNMR: δ 2.33 - 2.35 (6H, s, 2X CH<sub>3</sub>), 4.98 - 5.30 (2H, bb, NH<sub>2</sub>) 7.06 - 8.41 (9H, m, Ar - H), 9.14 (1H, s, NH), Mass, m/z 572 (m+), other important fragment ion peaks are observed at m/z 294, 219, 131, 296.8 & 78.7.

**Table 1: Characterization of 3-(2-amino-3,5-dibromo phenyl)-6-substituted (3,4-b) (1,3,4) triazolo thiazoles. (7a<sub>1</sub>-a<sub>25</sub>) 6,6'-bis-3(2'-amino-3'-5'-dibromo phenyl)-1,2,4-triazolo (3,4-b) (1,3,4)-thiazoles (8,9,10)**

S.No.	Code	Z	Mol formula	M.P (°c)
1	7a <sub>1</sub>	2 - Chloro phenyl	C <sub>15</sub> H <sub>8</sub> N <sub>5</sub> Br <sub>2</sub> SCl	140
2	7a <sub>2</sub>	4 - Chloro phenyl	C <sub>15</sub> H <sub>8</sub> N <sub>5</sub> Br <sub>2</sub> SCl	158
3	7a <sub>3</sub>	Pentafluoro phenyl	C <sub>15</sub> H <sub>4</sub> N <sub>5</sub> Br <sub>2</sub> SF <sub>5</sub>	110
4	7a <sub>4</sub>	3, 5 - Dixitro phenyl	C <sub>15</sub> H <sub>7</sub> O <sub>4</sub> N <sub>7</sub> Br <sub>2</sub> S	120
5	7a <sub>5</sub>	4 - Isobutyl phenyl ethyl	C <sub>21</sub> H <sub>21</sub> N <sub>5</sub> Br <sub>2</sub> S	80
6	7a <sub>6</sub>	2 - Amino - 3, 5 - dibromo phenyl	C <sub>15</sub> H <sub>8</sub> N <sub>6</sub> Br <sub>4</sub> S	170
7	7a <sub>7</sub>	2 - (2', 6' - dichloroanilino benzyl)	C <sub>22</sub> H <sub>14</sub> N <sub>6</sub> Br <sub>2</sub> SCl <sub>2</sub>	110
8	7a <sub>8</sub>	2 - Chlorobenzyl	C <sub>16</sub> H <sub>10</sub> N <sub>5</sub> Br <sub>2</sub> SCl	60
9	7a <sub>9</sub>	Diphenyl methyl	C <sub>22</sub> H <sub>15</sub> N <sub>5</sub> Br <sub>2</sub> S	170
10	7a <sub>10</sub>	2 - (2', 3' - dimethyl aniline phenyl)	C <sub>24</sub> H <sub>18</sub> N <sub>6</sub> Br <sub>2</sub> S	140
11	7a <sub>16</sub>	2 - Amino - 4 - nitro phenyl	C <sub>15</sub> H <sub>9</sub> O <sub>2</sub> N <sub>7</sub> Br <sub>2</sub> S	170
12	7a <sub>11</sub>	Phenoxy methyl	C <sub>16</sub> H <sub>11</sub> ON <sub>5</sub> Br <sub>2</sub> S	98
13	7a <sub>12</sub>	2 - ChloroPhenoxy methyl	C <sub>16</sub> H <sub>10</sub> ON <sub>5</sub> Br <sub>2</sub> SCl	140
14	7a <sub>13</sub>	3 - ChloroPhenoxy methyl	C <sub>16</sub> H <sub>10</sub> ON <sub>5</sub> Br <sub>2</sub> SCl	98
15	7a <sub>14</sub>	4 - ChloroPhenoxy methyl	C <sub>16</sub> H <sub>10</sub> ON <sub>5</sub> Br <sub>2</sub> SCl	102
16	7a <sub>15</sub>	2, 4 - Dichlorophenoxy methyl	C <sub>16</sub> H <sub>10</sub> ON <sub>5</sub> Br <sub>2</sub> SCl	120
17	7a <sub>17</sub>	4 - Amino Phenoxy methyl	C <sub>16</sub> H <sub>12</sub> ON <sub>7</sub> Br <sub>2</sub> S	160
18	7a <sub>18</sub>	2 - Methyl Phenoxy methyl	C <sub>17</sub> H <sub>13</sub> ON <sub>5</sub> Br <sub>2</sub> S	118
19	7a <sub>19</sub>	4 - Methyl Phenoxy methyl	C <sub>17</sub> H <sub>13</sub> ON <sub>5</sub> Br <sub>2</sub> S	120
20	7a <sub>20</sub>	4 - BromoPhenoxy methyl	C <sub>16</sub> H <sub>10</sub> ON <sub>5</sub> Br <sub>2</sub> S	128
21	7a <sub>21</sub>	4 - Nitro Phenoxy methyl	C <sub>16</sub> H <sub>10</sub> O <sub>3</sub> N <sub>6</sub> Br <sub>2</sub> S	170
22	7a <sub>22</sub>	1 - Naphthoxy methyl	C <sub>20</sub> H <sub>13</sub> N <sub>5</sub> Br <sub>2</sub> S	142
23	7a <sub>23</sub>	2 - Naphthoxy methyl	C <sub>20</sub> H <sub>13</sub> N <sub>5</sub> Br <sub>2</sub> S	134
24	7a <sub>24</sub>	Pyridinyl	C <sub>14</sub> H <sub>8</sub> N <sub>6</sub> Br <sub>2</sub> S	246
25	7a <sub>25</sub>	2 - ChloroPyridinyl	C <sub>14</sub> H <sub>7</sub> N <sub>6</sub> Br <sub>2</sub> SCl	140

S.No.	Code	Structure	Mol. formula	M.P (°C)
26	8		$C_{18}H_8N_{10}Br_4S_2$	170
27	9		$C_{20}H_{12}N_{10}Br_4S_2$	110
28	10		$C_{20}H_{12}O_2N_{10}Br_4S_2$	102

**7a<sub>11</sub> IR (KBr) cm<sup>-1</sup>:** 3472, 3370 (NH<sub>2</sub>), 3073 (aromatic C – H str), 1599 (C=N), 1599, 1572, 1532, 1508 (C = C ring str), 1532 & 1348 (NO<sub>2</sub>), the peak at 1303 for C=S of parent triazole 6 is disappeared, 1277 (N–N=C), 870, 787 (substituted phenyl rings), 681 (C–S), 616 (C–Br), HNMR : δ 3.86 (2H, s, NH<sub>2</sub>), mass : m/z 513 (m+). Other important fragment ion peaks are observed at m/z 304, 149 & 105.

**7a<sub>19</sub> IR (KBr) cm<sup>-1</sup>:** 3445 (NH<sub>2</sub>), 3070 (aromatic C–H str), 2950 & 2858 (C–H str of CH<sub>3</sub> and OCH<sub>2</sub>), 1607 (C=N), 1586, 1555, 1508 (C = C ring str), 1435 and 1340 (C–H bending of CH<sub>3</sub> & OCH<sub>2</sub>) 1308 (C–N), the peak at 1303 for C=S of parent triazole 6 is disappeared. 1121 (C–O–C), 875 & 816 (substituted phenyl rings) 659 (C–Br), HNMR: δ 1.60( 3H, s, CH<sub>3</sub>), 3.85 (2H, s, OCH<sub>2</sub>), 4.25 (2H, s, NH<sub>2</sub>), 7.20 – 8.55 (6H, m, Ar-H) Mass: m/z 497 (m+). The other important fragment ion peaks are observed at m/z 376, 138, 97, 89 & 59.

**7a<sub>21</sub> IR (KBr) cm<sup>-1</sup>:** 3472 (NH<sub>2</sub>), 3073 (aromatic C–H str), 2947 & 2851 (C–H str of OCH<sub>2</sub> asymmetric and symmetric), 1638 (NH bending), 1613 (C=N), 1592, 1558 1507 (C=C ring str), 1558 & 1341 (NO<sub>2</sub>), 1457 & 1341 (C–H bending of OCH<sub>2</sub> asymmetric and symmetric), 1277 (N – N = C), the peak at 1303 for C = S of parent triazole 6 is disappeared, 1110 (C–O–C), 869 & 824 (substituted phenyl rings), 705 (C–S), 680 (C-Br), HNMR : δ 3.80 (2H, s, OCH<sub>2</sub>) 4.30 (2H, s, NH<sub>2</sub>), 7.20 – 8.50 (6H, m, Ar-H), Mass: m/z 524 (m+), the other important fragment ion peaks are observed at m/z 339, 218, 138, 97, 89 & 59.

**7a<sub>25</sub> IR (KBr) cm<sup>-1</sup>:** 3445 (NH<sub>2</sub>), 3069 (aromatic C – H str), 1640 (NH bending), 1622 (C = N), 1583, 1556, 1508 (C = C ring str), 1402 (C – N), the peak at 1303 for C = S of parent triazole 6 is disappeared, 896 & 881 (substituted phenyl & heteroaryl rings) 698 (C – S), 678 (C – Br), 547 (C – C1) HNMR: δ 4.20 (2H, s, NH<sub>2</sub>), 7.45 – 8.60 (4H, m, 2-aryl & 2-heteroaryl protons) Mass: m/z = 489 (m+).

#### BIOLOGICAL ACTIVITIES.

##### Antibacterial & antifungal activities,

The invitro antibacterial & antifungal activities of the synthesized compounds **7a<sub>1</sub> – a<sub>25</sub>, 8, 9 & 10** was carried out by well diffusion method by punching template on Mullar Hinton agar well size of 6mm was made on agar with a holding capacity of 50 µl. Three standard bacterial strains viz E.coli(ATC NO 8739), P.aeruginosa (ATCC NO 9027) and S.aureus (ATCC NO 6538) were used for this purpose. Inaculum size of these standard strains were matched with 0.5 Mac Farlands comparator to get 1.5 X 10<sup>5</sup> organisms/ml. Lawn culture is made on mullar Hinton agar plate with standard strain. Known quantity of each sample was dissolved in 1000ml. DMSO solvent in a sterile screw capped Bijou bottle. 50 ml of solvent dissolved sample was charged into the wells of inoculated mullar Hinton Agar Incubation of plates was done for 12 hrs at 37°C & later looked for Zone of inhibition around the well. The diameter of inhibitory zone was measured and recorded in millimeter and concentration of test and standard were taken at 10 mg/ml.

The compounds were also screened for their antifungal activity by preparing the solution of compounds in DMSO solvent of concentrations 75, 50, 25 & 10 µg/ml. the selected few compounds of having significant activity were further screened for their antifungal activity following minimum inhibition concentration MIC procedure against the organism. Afumigatus ATCC NO 13073 using fluconazole as a reference standard. 9 dilutions of each drug were prepared (with brain heart infusion) BHI for MIC. In the initial tube 20 µl of drug was added in to the 380 µl of BHI broth for dilutions 200 µl of BHI broth was added in to the next 9 tubes separately. Then from the initial tube 200 µl was transferred to the first tube containing 20 µl of BHI broth. This was considered as 10<sup>-1</sup> dilution. From 10<sup>-1</sup> diluted tube 200 µl was transferred to second tube to make 10<sup>-2</sup> dilution. The serial dilution was repeated up to 10<sup>-9</sup> dilution for each drug. From the

maintained stock cultures of required organisms, 5µl was taken and added into 2ml of BHI broth. In each serially diluted tube 200µl of above culture suspension was added. The tubes were incubated for 24 hrs and observed for turbidity.

Among the triazole derivatives screened for antibacterial activity, it was observed that majority of triazole derivatives exhibited moderate to excellent activity against the organism E.Coli in comparison with the standard drugs Ciprofloxacin, Gentamycin & Tobramycin. It was found that the compounds **7a<sub>1</sub>**, **7a<sub>5</sub>**, **7a<sub>12</sub>**, **7a<sub>13</sub>**, **7a<sub>15</sub>**, **7a<sub>16</sub>**, **7a<sub>20</sub>** & **7a<sub>24</sub>** have exhibited significant activity in comparison with the standard Ciprofloxacin.

It was observed in general that triazolothiadiazole derivatives with the substituents at 6<sup>th</sup> position like 2 – amino – 3, 4 – dibromo phenyl, 4 – isobutyl phenyl ethyl, 2 – chlorophenyl, 2, 4 – dichlorophenyl methyl & a bistriazolothiadiazole derivative bearing CHOH showed much significant activity. Perhaps the electron withdrawing group's at 6<sup>th</sup> position contributes in enhancing the antibacterial activity.

Antifungal screening result indicates that majority of the triazolothiadiazoles except **7a<sub>8</sub>**, **7a<sub>18</sub>**, **7a<sub>19</sub>**, & **09** possess significant activity. The presence of N – C – S linkage in the fused system may be responsible for better antifungal activity. The antifungal activity of triazolothiadiazole derivatives is much better than bis derivatives (**8**, **9** & **10**). MIC result also indicates that the representative compounds of the series tested exhibit significant antifungal activity even at lower concentration. The compounds **7a<sub>1</sub>**, **7a<sub>3</sub>**, **7a<sub>5</sub>**, **7a<sub>6</sub>**, **7a<sub>7</sub>**, **7a<sub>9</sub>**, **7a<sub>14</sub>**, **7a<sub>15</sub>** & **7a<sub>16</sub>** showed

activity even at 8µl/ml. the compounds **7a<sub>3</sub>**, **7a<sub>5</sub>** & **7a<sub>7</sub>** showed activity at 4µg/ml.

Majority of the compounds of this series appeared to be novel antibacterial & antifungal agents and active ones can be used as antibacterial & antifungal agents in various forms like capsules, tablets, power, ointment, lotions, creams etc after carrying out the toxicity studies.

#### Antitubercular activity<sup>14</sup>

The evaluation of antitubercular activity was performed using the standard strain mycobacterium tuberculosis H<sub>37</sub> RV & middlebrook 7H – 9 broths following the standard procedure. The growth of mycobacterium tuberculosis strain (100000 organisms/ml) was measured after a period of 3 weeks Streptomycin & Pyrazinamide were used as standards.

The parent triazole (**6**) and its few representative triazolothiadiazole derivatives **7a<sub>1</sub>**, **7a<sub>3</sub>**, **7a<sub>11</sub>**, **7a<sub>15</sub>**, **7a<sub>16</sub>**, **7a<sub>19</sub>**, **7a<sub>21</sub>**, **7a<sub>22</sub>** & **7a<sub>24</sub>** were screened against mycobacterium tuberculosis H<sub>37</sub> RV at (Concentrations 5, 10 & 25 µg/ml. The compound **7a<sub>24</sub>** should equipotent activity in comparison with standard drugs at all the concentrations. The compounds **6**, **7a<sub>1</sub>**, **7a<sub>11</sub>**, **7a<sub>19</sub>**, & **7a<sub>22</sub>** showed activity at 10 & 25µg/ml concentrations. Perhaps the heterocyclic moiety as substituent at 6<sup>th</sup> position of triazolothiadiazole ring system may be responsible for antitubercular property.

**Table 2: Antibacterial & Antifungal activity of triazolo (7a<sub>1</sub> – a<sub>25</sub>, 8, 9, 10)**

Sl No	Compound	Antibacterial activity			Antifungal activity			
		Zone of Inhibition (mm)			Zone of Inhibition (mm) & concentration (µg/ml)			
		Saureus	E Coli	Paeruginosa	75 µg/ml	50 µg/ml	25 µg/ml	10 µg/ml
1	7a <sub>1</sub>	00	26	00	34	32	32	28
2	7a <sub>2</sub>	00	00	00	36	33	33	30
3	7a <sub>3</sub>	22	22	00	36	34	34	32
4	7a <sub>4</sub>	24	24	00	34	28	28	23
5	7a <sub>5</sub>	22	26	00	40	40	40	35
6	7a <sub>6</sub>	38	22	00	740	740	740	38
7	7a <sub>7</sub>	26	24	00	38	36	32	30
8	7a <sub>8</sub>	00	22	00	32	26	26	18
9	7a <sub>9</sub>	22	24	00	36	34	34	32
10	7a <sub>10</sub>	00	00	00	35	29	24	20
11	7a <sub>11</sub>	00	24	22	24	23	23	20
12	7a <sub>12</sub>	00	26	24	28	25	21	18
13	7a <sub>13</sub>	00	30	00	740	740	38	34
14	7a <sub>14</sub>	00	24	12	38	36	36	34
15	7a <sub>15</sub>	00	28	00	34	30	30	26
16	7a <sub>16</sub>	00	26	00	34	32	32	30
17	7a <sub>17</sub>	00	22	24	34	32	32	30
18	7a <sub>18</sub>	00	24	24	24	18	15	11
19	7a <sub>19</sub>	00	20	20	22	19	14	10
20	7a <sub>20</sub>	00	28	28	26	22	20	17
21	7a <sub>21</sub>	00	00	00	28	23	21	19
22	7a <sub>22</sub>	00	24	00	30	30	30	28
23	7a <sub>23</sub>	00	00	00	00	00	00	00
24	7a <sub>24</sub>	22	26	00	28	26	26	23
25	7a <sub>25</sub>	24	24	00	34	34	32	28
26	8	00	24	24	32	28	26	20
27	9	00	20	22	24	20	15	11
28	10	00	28	24	26	24	21	20
29	Ciprofloxacin	30	28	27				
30	Gentamycin	34	30	35				
31	Tobramycin	30	32	34				

**Table 2 (b): Antifungal Activity by MIC method concentration (µg/ml)**

S No	Compound	Concentration (µg/ml)									
		500	250	125	62.5	31.25	16	8	4	2	
1	7a <sub>1</sub>	S	S	S	S	S	S	S	R	R	
2	7a <sub>2</sub>	S	S	S	S	S	S	R	R	R	
3	7a <sub>3</sub>	S	S	S	S	S	S	S	S	S	
4	7a <sub>5</sub>	S	S	S	S	S	S	S	S	S	
5	7a <sub>6</sub>	S	S	S	S	S	S	S	R	R	
6	7a <sub>7</sub>	S	S	S	S	S	S	S	S	S	
7	7a <sub>9</sub>	S	S	S	S	S	S	S	R	R	
8	7a <sub>13</sub>	S	S	S	S	S	S	R	R	R	
9	7a <sub>14</sub>	S	S	S	S	S	S	S	R	R	
10	7a <sub>15</sub>	S	S	S	S	S	S	S	S	R	
11	7a <sub>16</sub>	S	S	S	S	S	S	S	S	R	
12	7a <sub>22</sub>	S	S	S	S	S	R	R	R	R	
13	7a <sub>25</sub>	S	S	S	S	S	R	R	R	R	

**Table 3: Antitubercular activity of triazolothiadiazoles**

Sl No	Compound	Concentration		
		5µg/ml	10µg/ml	25µg/ml
1	6	R	S	S
2	7a <sub>1</sub>	R	S	S
3	7a <sub>3</sub>	R	R	R
4	7a <sub>11</sub>	R	S	S
5	7a <sub>15</sub>	R	R	R
6	7a <sub>16</sub>	R	R	R
7	7a <sub>19</sub>	R	S	S
8	7a <sub>21</sub>	R	R	R
9	7a <sub>22</sub>	R	S	S
10	7a <sub>24</sub>	S	S	S

S – Sensitive, R – Resistance

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**REFERENCES**

- Mathew V, Keshavayya J & Vaidya V. P Heterocyclic system containing Bridge Head nitrogen atom: Synthesis and Pharmacological activities of some substituted 1,2,4 triazolo (3,4-b) (1,3,4) thiadiazoles, Eur. J. Medchem 2006, 41, 1048.
- Udupi R.H, Bhat A. R synthesis of 4 (N-pyridylcarboxamido)-5-mercapto -3-substituted-1, 2,4-triazoles for possible antitubercular activity, Indian J. Heterocyclic chem 1996, 6, 41.
- Udupi R.H, Purushottamachar P and Bhat A. R, studies on antitubercular agents, synthesis of 4-pyridyl-3-substituted-1,2,4-triazolo (3,4-b) (1,3,4) thiadiazolidines, Indian J. Heterocyclic chem 2000, 9, 287.
- Udupi R. H, Suresh G. V, Shetty S. R & Bhat A. R, synthesis & biological evaluation 3-substituted -1-[2-(4'-isobutyl phenyl) propionomido]-1,2,4-triazoles & their derivatives, J. Indian chemSoc 2000, 77, 302.
- Udupi R.H, Sudheendra G, Bheemachari, Srinivasulu N, Rajesh Varnekar & Purushottamachar P, Design, synthesis & biological activity of certain 3, 4 - disubstituted-5-mercapto-1,2,4-triazoles & their hydrazine derivatives, Bull, Korean ChemSoc 2007, 28, 12.
- NehaSinghal, P. K. Sharma, Rupesh Dudhe & Nitin Kumar – Recent advancement of triazole derivatives & their biological significance, J. chem pharm. Res., - 2011, 3 (2) : 126 – 133.
- B. Shivarama Holla, B. SooryanarayanaRao, B. K. Sarojini, P.M. Akberali, N. Suchetha Kumari, synthesis and studies on some new fluorine containing triazolo – thiadiazines as possible antibacterial, antifungal & anticancer agents Eur. J. Med. Chem, - 2006, 41, 657 – 663.
- Udupi R.H, Ashok Kushnoor & Bhat A.R, synthesis & biological evaluation of some substituted 1,2,4-triazoles, J.Ind chemical Society, 1999, 76, 461 462.
- B. Shivaram Holla, K. Narayan Poojary, B. Sooryanarayana Rao & M. K. Shivananda New bis – amino mercapto triazoles & bistriazolothiadiazoles as possible anticancer agents Eur. J. Med. Chem, 2002, 17, 511 – 517.
- Udupi R.H, B. Rajeeva, N. Srinivasulu, T. Y. Pasha, S. Ramachandra Shetty & Bhat A.R, synthesis & biological evaluation of some 4-amino-3-[4 isobutyl phenyl]-ethyl-5-mercapto-1,2,4-triazoles, Indian J. heterocyclic chem 2004, 13, 233 – 236.
- Udupi R.H, B. Rajeeva, N. Srinivasulu, T. Y. Pasha, S. Ramachandra Shetty & Bhat A.R, synthesis & biological evaluation of some new 4-amino-3-[2 - (2',6'-dichlorophenyl)-amino]-benzyl-5-mercapto-1,2,4 triazole derivatives, Indian J. Heterocyclic chem 2004, 13, 237 – 240.
- Cruickshank R, Duguid J. P, Marmoin B. P, swan H. A, "the practice of medical microbiology" 12<sup>th</sup> Ed, Churchill living stone, London, 1975, 2, 190.
- Fair Brother R. W, Martyn G. the disc technique for determining sensitivity to the antibiotics. J. Clin, Patho, 1951, 4, 374 – 377.
- Pandeya S.N, Yogeshwari, P.Sriram, Ananthan. S, Sci.Pharm, 2002, 70, 39.