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

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Abstract:
3-(2́-amino-3́,5́–dibromophnyl) – 4- amino 5- mercapto – 1,2,4 – triazole (6) when condensed with substituted aryl/aryloxy/ hetero aryl acids in presence of $pocl_3$ gave 3 –(2́–amino-3́,5́ dibromophenyl)–6–substituted (3,4–b) (1,3,4) triazolothiadiazoles ($7a_1 – a_{25}$).The compound 6 when condensed with dicarboxylic acids like oxalic acid, succinic acid and tartaric acid in presence of $pocl_3$ yielded 6,6́ bis– 3-(2–amino-3́,5́ – 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 thiadiazole 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[1-4]. 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[5-8]. The amino and mercapto groups of these compounds serve as readily accessible ncleophilic 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 thiadiozole derivatives obtained by fusing biolabile 1,2,4-trizole and 1,3,4-thiodiazole 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[9-10]. 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–(2́-amino–3́,5́– dibromo phenyl)–6–substituted (3,4b) (1, 3, 4) – triazole thiadiozoles (**$7_{a1} – a_{25}$**) and 6,6́ –bis–3(2́- amino–3́,5́-dibromo phenyl)–1,2,4–triazolo (3,4b) (1, 3, 4) thiadiazoles[8-11]. (**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 complation and purity was monitored by TLC. IR spectra in KBr (cm^{-1}) were recorded on Perkin Elmer Infrared spectrophotometer (Model Spectrum 100) with Nacl optics. Samples were scanned in KBr pellets.[!]HNMR spectra were recorded on BrukerAvance 300 MHz and 400 MHz instrument using TMS as internal standard (chemical shifts are expressed in δ ppm). Mass spectra were recorded on Perkin Elmer PE SCIEX – API – 2000 Mass spectrophotometer.

The parent triazole 3–(2́-amino-3́,5́ –dibromo phenyl)–4–amino–5–mercapto 1,2,4 – triazole was prepared by 2 methods and comparative study of the 2 metods 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 CS_2 and was stirred for 12 hrs. The solid product was filtered, dried, washed with either and directly used for the preparation of triazole.

Preparation of 3- (2́ –amino–3́,5́– 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 H_2S 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 thiocarbohydrazide 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) Vcm^{-1} : 3465, 3349 (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), HNMR δ3.04 (1H, $_S$, SH), 3.65 – 3.89 (2H, $_S$, NH_2), 6.17 (2H, $_S$, 2H of N – NH_2), 7.94 – 9.02 (2H, m, $Ar._H$), mass m/z366 (m+), 368 ($m^+ + 2$) other important fragment ions are at m/z= 320, 292, 278 (base peak), 252, 198, 149, etc.

Preparation of 3 (2́–amino-3́,5́–dibromo phenyl)–6–substituted (3,4b) (1,3,4) – triazolethiadiazoles ($7a_1 – a_{25}$) and 6, 6́ bis–3 (2́–amino-3́,5́– dibromo phenyl) -1,2,4–triazolo (3,4b) (1,3,4) thiadiazoles (8, 9 and 10)

Equimolar mixture of triazole (6) and carboxylic acid in $POCl_3$ (10ml) was heated for 7 hrs. Excess of $POCl_3$ 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 oxylic 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.

$7a_2$ IR, (KBr), cm^{-1}: 3467 and 3354 (NH_2), 3070 (aromatic C–H str). 1640 (NH bending of NH_2), 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 m- Br), 556 (C–C1), ´HNMR δ3.87 (2H, s, NH_2), 7B – 9.01 (6H, m, Ar - H), mass : m/2 488 (mt)

$7a_4$ IR (KBr) cm^{-1}: 3460 (NH_2), 3092 aromatic C – H str, 1640 (N – H bending of NH_2), 1624 (C = N), 1590, 1580, 1541 (C = C ring str), 1541 and 1345 (NO_2), 1437 (C – N), the peak at 1303 for C = S of parent triazole 6 is disappeared, 1280 (N – N = C), 895 & 881 (substituted phynl rings), 680 (C – S), 656 (C – Br),556 (C – C1). ´HNMR: δ 3.87 (2H, s, NH_2) 7.13 – 8.92 (6H, m, Ar – H), mass : m/z 543 (m+).

$7a_5$ IR (KBr), cm^{-1}: 3420 (NH_2), 3068 (aromatic C – H str), 2953 and 2867 C – H str of $–CH_3$, 2929 & 2830 C - Hstr of >C H_2, 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_3 of –CH $(CH_3)_2$), 1.56 – 1.66 (3H, d, CH_3 of (CH-CH_3), 1.81-1.94 (1H, m CH of – CH $(CH_3)_2$, 2.31 – 2.5 (2H, s, CH_2 of CH_2 – CH $(CH_3)_2$, 3.19 -3.9 (1H, m, CH of CH – CH_3), 4.1 (2H, s, NH_2), 7.14 – 8.5 (6H, m, Ar-H), mass : m/z 535 (m+) & 537 (m^++2)

$7a_6$ IR (KBr) cm^{-1}: 3471& 3468 (NH_2), 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- B_r), ´HNMR : δ 4.20 (4H, s, 2X NH_2),7.12 – 8.70 C(H, m, Ar – H), mass m/z 626 (m^+) & 628 (m^++2).

$7a_9$ IR (KBr) cm^{-1}: 3366 (NH_2), 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_2), 3.80 (1H, s, >CH) 7.00 – 8.52 (12H, m, Ar–H). Mass: m/z 543 (m+).

$7a_{10}$ IR (KBr) cm^{-1}: 3473 &3371 (NH_2), 3243 (NH), 3073 (aromatic C–H str), 2950 & 2843 (C – H str of CH_3 asymmetric and symmetric, 1640 (NH–bending) 1613 (C = N), 1575, 1507, 1457 (C= Cring str), 1433 & 1366 (C–H bending of CH_3 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:δ2.33 – 2.35 (6H, s, 2X CH3), 4.98 – 5.30 (2H, bb, NH_2) 7.06 – 8.41 (9H, m, Ar – H), 9.14 (1H, s, NH), Mass, m/z572 (m+), other important fragment ion peaks are observed at m/z294, 219, 131, 296.8 & 78.7.

$7a_{11}$ IR (KBr) cm^{-1}: 3472, 3370 (NH_2), 3073 (aromatic C – H str), 1599 (C=N), 1599, 1572, 1532, 1508 (C = C ring str), 1532 & 1348 (NO_2), 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_2), mass : m/z513 (m+). Other important fragment ion peaks are observed at m/z 304, 149 & 105.

$7a_{19}$ IR (KBr) cm^{-1}: 3445 (NH_2), 3070 (aromatic C–H str), 2950 & 2858 (C–H str of CH_3 and OCH_2), 1607 (C=N), 1586, 1555, 1508 (C =C ring str), 1435 and 1340 (C–H bending of CH_3 & OCH_2) 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_3), 3.85 (2H, s, OCH_2), 4.25 (2H, s, NH_2), 7.20 – 8.55 (6H, m, Ar-H) Mass: m/z497 (m+). The other important fragment ion peaks are observed at m/z 376, 138, 97, 89 & 59.

$7a_{21}$ IR (KBr) cm^{-1}: 3472 (NH_2), 3073 (aromatic C–H str), 2947 & 2851 (C–H str of OCH_2 asymmetric and symmetric), 1638 (NH bending), 1613 (C=N), 1592, 1558 1507 (C=C ring str), 1558 & 1341 (NO_2), 1457 & 1341 (C–H bending of OCH_2 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_2) 4.30 (2H, s, NH_2), 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.

$7a_{25}$ IR (KBr) cm^{-1}:3445 (NH_2), 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_2), 7.45 – 8.60 (4H, m, 2-aryl & 2-heteroaryl protons)´ Mass: m/z = 489 (m+).

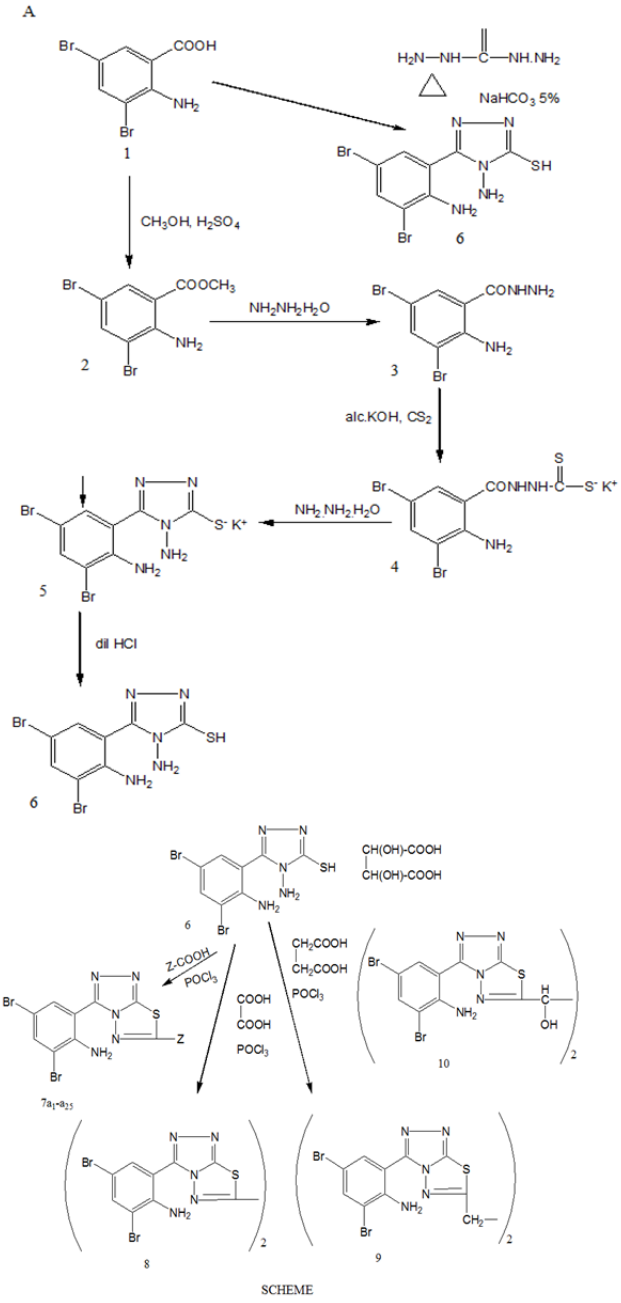


Table 1: Characterization of 3–(2́–amino-3́, 5́–dibromo phenyl)–6–substituted (3,4–b) (1,3,4) triazolo thiadiazoles. ($7a_1$-a_{25}) 6,6'-bis-3(2'-amino-3'-5'-dibromo phenyl)-1,2,4-triazolo (3,4-b) (1,3,4)-thiasiazoles (8,9,10)

S.No.	Code	Z	MoI formula	M.P (˚c)
1	$7a_1$	2 – Chloro phenyl	$C_{15}H_8N_5Br_2Scl$	140
2	$7a_2$	4 – Chloro phenyl	$C_{15}H_8N_5Br_2Scl$	158
3	$7a_3$	Pentafluoro phenyl	$C_{15}H_4N_5Br_2SF_5$	110
4	$7a_4$	3, 5 – Dixitro phenyl	$C_{15}H_7O_4N_7Br_2S$	120
5	$7a_5$	4 – Isobutyl phenyl ethyl	$C_{21}H_{21}N_5Br_2S$	80
6	$7a_6$	2 – Amino – 3, 5 – dibromo phenyl	$C_{15}H_8N_6Br_4S$	170
7	$7a_7$	2 - (2́, 6́ – dichloroanilino benzyl)	$C_{22}H_{14}N_6Br_2Scl_2$	110
8	$7a_8$	2 – Chlorobenzyl	$C_{16}H_{10}N_5Br_2Scl$	60
9	$7a_9$	Diphenyl methyl	$C_{22}H_{15}N_5Br_2S$	170
10	$7a_{10}$	2 - (2́, 3́–dimethyl aniline phenyl)	$C_{24}H_{18}N_6Br_2S$	140
11	$7a_{16}$	2 – Amino – 4 – nitro phenyl	$C_{15}H_9O_2N_7Br_2S$	170
12	$7a_{11}$	Phenoxy methyl	$C_{16}H_{11}ON_5Br_2S$	98
13	$7a_{12}$	2 – ChloroPhenoxy methyl	$C_{16}H_{10}ON_5Br_2Scl$	140
14	$7a_{13}$	3 – ChloroPhenoxy methyl	$C_{16}H_{10}ON_5Br_2Scl$	98
15	$7a_{14}$	4 – ChloroPhenoxy methyl	$C_{16}H_{10}ON_5Br_2Scl$	102
16	$7a_{15}$	2, 4 – Dichlorophenoxy methyl	$C_{16}H_{10}ON_5Br_2Scl$	120
17	$7a_{17}$	4 – Amino Phenoxy methyl	$C_{16}H_{12}ON_7Br_2S$	160
18	$7a_{18}$	2 – Methyl Phenoxy methyl	$C_{17}H_{13}ON_5Br_2S$	118
19	$7a_{19}$	4 – Methyl Phenoxy methyl	$C_{17}H_{13}ON_5Br_2S$	120
20	$7a_{20}$	4 – BromoPhenoxy methyl	$C_{16}H_{10}ON_5Br_2S$	128
21	$7a_{21}$	4 – Nitro Phenoxy methyl	$C_{16}H_{10}O_3N_6Br_2S$	170
22	$7a_{22}$	1 – Naphthoxy methyl	$C_{20}H_{13}N_5Br_2S$	142
23	$7a_{23}$	2 – Naphthoxy methyl	$C_{20}H_{13}N_5Br_2S$	134
24	$7a_{24}$	Pyridinyl	$C_{14}H_8N_6Br_2S$	246
25	$7a_{25}$	2 – ChloroPyridinyl	$C_{14}H_7N_6Br_2Scl$	140

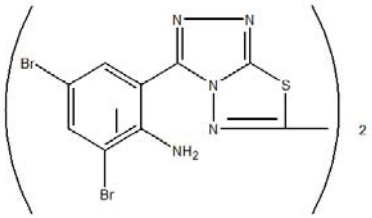
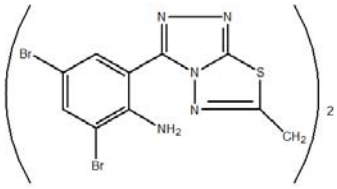
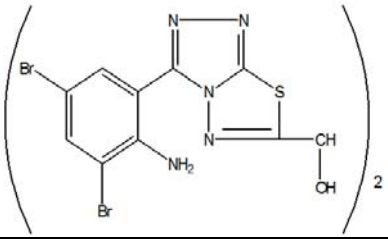
S.No.	Code	Structure	MoI. formula	M.P (˚C)
26	8		$C_{18}H_8N_{10}Br_4S_2$	170
27	9		$C_{20}H_{12}N_{10}Br_4S_2$	110
28	10		$C_{20}H_{12}O_2N_{10}Br_4S_2$	102

Table 2: Antibacterial & Antifungal activity of triazolo ($7a_1 – a_{25}$, 8, 9, 10)

Sl No	Com-pound	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	$7a_1$	00	26	00	34	32	32	28
2	$7a_2$	00	00	00	36	33	33	30
3	$7a_3$	22	22	00	36	34	34	32
4	$7a_4$	24	24	00	34	28	28	23
5	$7a_5$	22	26	00	40	40	40	35
6	$7a_6$	38	22	00	740	740	740	38
7	$7a_7$	26	24	00	38	36	32	30
8	$7a_8$	00	22	00	32	26	26	18
9	$7a_9$	22	24	00	36	34	34	32
10	$7a_{10}$	00	00	00	35	29	24	20
11	$7a_{11}$	00	24	22	24	23	23	20
12	$7a_{12}$	00	26	24	28	25	21	18
13	$7a_{13}$	00	30	00	740	740	38	34
14	$7a_{14}$	00	24	12	38	36	36	34
15	$7a_{15}$	00	28	00	34	30	30	26
16	$7a_{16}$	00	26	00	34	32	32	30
17	$7a_{17}$	00	22	24	34	32	32	30
18	$7a_{18}$	00	24	24	24	18	15	11
19	$7a_{19}$	00	20	20	22	19	14	10
20	$7a_{20}$	00	28	28	26	22	20	17
21	$7a_{21}$	00	00	00	28	23	21	19
22	$7a_{22}$	00	24	00	30	30	30	28
23	$7a_{23}$	00	00	00	00	00	00	00
24	$7a_{24}$	22	26	00	28	26	26	23
25	$7a_{25}$	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	Ciprofloxin	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	$7a_1$	S	S	S	S	S	S	S	R	R
2	$7a_2$	S	S	S	S	S	S	R	R	R
3	$7a_3$	S	S	S	S	S	S	S	S	S
4	$7a_5$	S	S	S	S	S	S	S	S	S
5	$7a_6$	S	S	S	S	S	S	S	R	R
6	$7a_7$	S	S	S	S	S	S	S	S	S
7	$7a_9$	S	S	S	S	S	S	S	R	R
8	$7a_{13}$	S	S	S	S	S	S	R	R	R
9	$7a_{14}$	S	S	S	S	S	S	S	R	R
10	$7a_{15}$	S	S	S	S	S	S	S	S	R
11	$7a_{16}$	S	S	S	S	S	S	S	S	R
12	$7a_{22}$	S	S	S	S	S	R	R	R	R
13	$7a_{25}$	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	$7a_1$	R	S	S
3	$7a_3$	R	R	R
4	$7a_{11}$	R	S	S
5	$7a_{15}$	R	R	R
6	$7a_{16}$	R	R	R
7	$7a_{19}$	R	S	S
8	$7a_{21}$	R	R	R
9	$7a_{22}$	R	S	S
10	$7a_{24}$	S	S	S

S – Sensitive, R – Resistance

BIOLOGICAL ACTIVITIES.

Antibacterial & antifungal activities,

The invitro antibacterial & antifungal activities of the synthesized compounds **$7a_1 – a_{25}$, 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 strainsviz E.coli(ATC NO 8739), P.aeruginosa (ATCC NO 9027) and S.aureus (ATCC NO 6538) were used for this purpose. Inacolum size of these standard strains were matched with 0.5 Mac Farlands comparator to get 1.5 X 10^5organisms/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 & 10µg/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 380µl of BHI broth for dilutions 200 µl of BHI broth was added in to the next 9 tubes separately. Then from the initial tube 200µl was transferred to the first tube containing 20µl of BHI broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube 200µl was transferred to second tube to make 10^{-2}dilution.The serial dilution was repeated up to 10^{-9} 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 **$7a_1$, $7a_5$, $7a_{12}$, $7a_{13}$, $7a_{15}$, $7a_{16}$, $7a_{20}$& $7a_{24}$** have exhibited significant activity in comparision with the standard Ciprofloxacin.

It was observed in general that triazolethiadiazole derivatives with the substituents at 6^{th} 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 with drawing group's at 6^{th} position contributes in enhancing the antibacterial activity.

Antifungal screening result indicates that majority of the triazolothiadiazoles except **$7a_8$, $7a_{18}$, $7a_{19}$, & 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 beeter 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 **$7a_1$, $7a_3$, $7a_5$, $7a_6$, $7a_7$, $7a_9$, $7a_{14}$, $7a_{15}$& $7a_{16}$** showed activity even at 8µl/ml. the compounds **$7a_3$, $7a_5$& $7a_7$** 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[14]

The evaluation of antitubercular activity was performed using the standard strain mycobacterium tuberculosis H_{37} 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 **$7a_1$**, **$7a_3$**, **$7a_{11}$**, **$7a_{15}$**, **$7a_{16}$**, **$7a_{19}$**, **$7a_{21}$**, **$7a_{22}$ & $7a_{24}$** were screened against mycobacterium tuberculosis H_{37} RV at (Concentrations 5, 10 & 25 µg/ml. The compound **$7a_{24}$** should equipotent activity in comparison with standard drugs at all the concentrations. The compounds **6, $7a_1$**, **$7a_{11}$**, **$7a_{19}$**, **& $7a_{22}$** showed activity at 10 & 25µg/ml concentrations. Perhaps the heterocyclic moiety as substituent at 6^{th} position of triazolo thiadiazole ring system may be responsible for antitubercular property.

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