PART-A

CHAPTER-I

SECTION-III

Synthesis and antimicrobial evaluation of 6-(substitutedphenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles.
INTRODUCTION

Thiadiazole derivatives have played an important role in pharmaceutical industries and exhibited various biological activities due to the presence of N=C-S group. In thiadiazole ring system, one sulphur and two nitrogen atoms are present in a five membered ring. According to their position, thiadiazole systems are classified as 1,2,3-thiadiazole (I), 1,2,4-thiadiazole (II) and 1,3,4-thiadiazoles (III).

![Chemical Structures of Thiadiazole Systems]

Among these three types of thiadiazoles 1,3,4-thiadiazole(III) is very well known.

SYNTHETIC ASPECT

Literature survey reveals that several publications and patents described the synthesis of 1,3,4-thiadiazole as under.

1. S. Shelke et al. have reported green synthesis for some novel Triazolothiadiazoles having lesser reaction time and higher yield.

![Chemical Structure of Triazolothiadiazole Synthesis]

2. A. A. Hassan et al. have prepared 1,3,4-thiadiazoles by the cyclization of tetracyanoethene and 4-phenyl thiosemicarbazides.

![Chemical Structure of Thiadiazole Synthesis]

3. Li-xue Zhang et al. have synthesized 1,3,4-thiadiazoles by the cyclization of aromatic acid with triazoles in the presence of POCl3.
4. Q. Bano\textsuperscript{6} and co-workers have been prepared 6-phenyl amino-1,3,4-thiadiazole by reacting triazole with amino acid.

5. El. Ashry\textsuperscript{7} and co-workers have developed an efficient conventional heating and microwave assisted protocol for fused heterocycles.

6. Taha\textsuperscript{8} et al. accomplished the synthesis of triazolothiadiazoles by using 4-amino-4H-3-methylthio{7H-1,2,4triazolo[1,5-d]tetrazolo-6-y1}-1,2,4 triazole-5-thiol as key intermediate.

**THERAPEUTIC ASPECT**

1. Antiviral\textsuperscript{9}
2. Herbicidal\textsuperscript{10}
3. Antihelmintic\textsuperscript{11}
4. Antitumor\textsuperscript{12}
5. Antibacterial\textsuperscript{13}
6. Antiinflammatory\textsuperscript{14}
7. Antitubercular\textsuperscript{15}
8. Analgesic\textsuperscript{16}
9. Anticancer\textsuperscript{17}
10. CNS depressant\textsuperscript{18}
11. Diuretics\textsuperscript{19}
12. Hypoglycemic agents\textsuperscript{20}
1. R. Bhat\textsuperscript{21} et al. have synthesized new series of thiadiazoles evaluated on \textit{in vitro} growth of microorganisms causing microbial infection.

![Thiadiazole Molecule](image)

2. V. Jatav\textsuperscript{22} et al. have synthesized a series of 3-[5-substitutedphenyl-1,3,4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H)-ones and evaluated for anticonvulsant, sedative-hypnotic and CNS depression activities.

![Quinazoline Molecule](image)

3. Ibrahim\textsuperscript{23} et al. have optimized the biological response of new lead Triazolothiadiazoles derivatives and compound demonstrated inhibitory effects on the growth of wide cancer cell lines.

![Triazolothiadiazole Molecule](image)

4. Hu\textsuperscript{24} and co-workers developed series of C-3/C-3 bis-fluoroquinolone containing heterocycles cross linked with Triazolothiadiazoles nucleus against murine leukemia cell line (L1210), human leukocytoma cell line (HL60).

![Fluoroquinolone Molecule](image)

5. Mathew\textsuperscript{25} et al. synthesized a series of 3,6-di substituted 1,2,4triazolo[3,4-b][1,3,4]thiadiazoles and tested for analgesic activity.
6. C.G. Bonde\textsuperscript{26} et al. have synthesized and reported a series of Triazolothiadiazoles containing pyrazine nucleus and synthesized compounds were demonstrated having good antimycobacterial activities.

7. K. Lam\textsuperscript{27} and co-workers have designed, synthesized and evaluated Triazolothiadiazoles derivatives as tyrosinase inhibitors.

K.M. Thaker\textsuperscript{28} have synthesized 2-\((3'5'\text{-dichlorobenzo}[b]\text{thiophen}-2'\text{-yl})\text{-5 ary lamino-1,3,4thiadiazoles from triazole. S.L. Vasoya\textsuperscript{29} have synthesized some new thiosemicarbazide and 1,3,4-thiadiazole heterocycles bearing the benzo[b]thiophene nucleus as potent antitubercular and antimicrobial agents.

The chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention due to the synthetic and effective biological importance. 1,3,4-Thiadiazole nucleus is associated with a broad spectrum of biological activities possibly by their virtue of pharmacophoric N=C=S moiety. Prompted by the pharmacological importance of these molecules we have synthesized some new triazolothiadiazole derivatives which have been described in following section.

\textbf{SECTION-III:} \textbf{SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 6-(SUBSTITUTEDPHENYL)-3-(PYRAZIN-2-YL)-[1,2,4]TRIAZOLO[3,4-B][1,3,4]THIADIAZOLES.}
SECTION-III: SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 6-(SUBSTITUTEDPHENYL)-3-(PYRAZIN-2-YL)-[1,2,4]TRIAZOLO[3,4-B][1,3,4]THIADIAZOLES.

N-Bridged heterocyclic compounds derived from 1,2,4-triazoles have found several applications in the field of medicine, agriculture and industry. In view of these finding it appeared of interest to synthesize some newer Triazolothiadiazoles. Triazolothiadiazoles have been prepared by cyclocondensation of 4-amino-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol with different aryl acids in the presence of phosphorous oxychloride as shown in reaction scheme.

**Reaction scheme**

Where R= 4-OCH₃, 4-Cl, 2-Br, 2,4-diCl, 3-OCH₃, 2-F etc.
Plausible mechanism
EXPERIMENTAL SECTION

[A] Synthesis of methyl pyrazine-2-carboxylate (2)
As per Part-A, Chapter-I(Section-I) page no.26

[B] Synthesis of pyrazine-2-carbohydrazide (3)
As per Part-A, Chapter-I(Section-I) page no.26

[C] Synthesis of potassium-2-(pyrazine-2-carbonyl)hydrazinecarbodithioate (4)
As per Part-A, Chapter-I(Section-I) page no.26

[D] Synthesis of 4-amino-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol (5)
As per Part-A, Chapter-I(Section-I) page no.26

[E] Synthesis of 6-(Substitutedphenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles 7(a-j)

An equimolar mixture of 4-amino-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol (5) (10 mmol) and substituted aryl acids (6a-6j) (10 mmol) in phosphorous oxychloride (8 v/v) was heated up to 90-95°C with continuous stirring. The completion of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled and poured on to ice, precipitates were filtered, dried and recrystallized from ethanol to give triazolo [3,4-b][1,3,4]thiadiazole in analytical pure form. Physical constants of newly synthesized triazolo [3,4-b][1,3,4]thiadiazole derivatives 7a-7j are recorded in Table I.
TABLE I: PHYSICAL CONSTANTS OF 6-SUBSTITUTEDPHENYL-3-(PYRAZIN-2-YL)-[1,2,4]TRIAZOLE[3,4-B][1,3,4]THIADIAZOLES. 7(a-j)

![Chemical structure of the compound](image)

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<th>Sr. No.</th>
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<tr>
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ANALYTICAL DATA

6-(4-methoxyphenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole(7a)  IR (ν max cm⁻¹, KBr): 3043 (C-H str.), 2929(C-H str.), 1662(C=N str.), 1537(C=C str.), 1433 (C-H bend), 1122(C-O-C str.), 989(sp²C-H str.), 670(1,3-di substituted). El⁺ m/z: 310.06 Anal. Calcd for C₁₄H₁₀N₆OS: C, 54.18%; H, 3.25%; N, 27.08%; O, 5.16%; S, 10.33%.

6-(4-chlorophenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole(7b)  IR (ν max cm⁻¹, KBr): 3083(C-H str.), 2918(C-H str.), 1658(C=N str.), 1525(C=C str.), 1402 (C-H bend), 968(sp²C-H str.), 814(1,4-di substituted)). El⁺ m/z: 314.75 Anal. Calcd for C₁₃H₁₁ClN₆S: C, 49.61%; H, 2.24%; Cl, 26.70%; N, 26.70%; S, 10.19%.

6-(2-bromophenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole(7c) IR (ν max cm⁻¹, KBr): 3084 (C-H str.), 2932(C-H str.), 1648(C=N str.), 1526(C=C str.), 1402 (C-H bend), 889(sp²C-H str.), 745(1,2-di substituted)). El⁺ m/z: 359.20 Anal. Calcd for C₁₃H₁₂BrN₆S: C, 44.71%; H, 1.73%; Br, 22.24%; N, 23.40%; S, 8.93%.

6-(2,4-dichlorophenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole(7d) IR (ν max cm⁻¹, KBr): 3095 (C-H str.), 2922(C-H str.), 1701(C=N str.), 1558(C=C str.), 1489 (C-H bend), 989(sp²C-H str.), 742(C-Cl str.)). El⁺ m/z: 349.19 Anal. Calcd for C₁₃H₁₂Cl₂N₆S: C, 44.71%; H, 1.73%; Cl, 20.21%; N, 24.07%; S, 9.18%.

6-(3-methoxyphenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole(7e) IR (ν max cm⁻¹, KBr): 3043 (C-H str.), 2929(C-H str.), 1662(C=N str.), 1537(C=C str.), 1433 (C-H bend), 1122(C-O-C str.), 989(sp²C-H str.), 670(1,3-di substituted). ¹H NMR 400 MHz (CDCl₃): 9.66(1H, s (pyrazine)) 8.81 (1H, s(pyrazine)), 8.70(1H, s(pyrazine)), 8-3.9-8.37(1H, dd), 7.58-7.54(1H, t), 7.17-7.08(2H, m), 4.08(3H, s). ¹³C NMR (100 MHz, CDCl₃): 163.7 (-N-C(S)=N ), 157.3(C, triazole ring), 157.8(C-O), 144.7(CH, pyrazine ring), 144.4(CH,pyrazine ring), 143.3(S-C=N) 142.6(C, pyrazine ring), 142.3(CH, pyrazine ring), 134.0(CH, phenyl ring), 128.5(CH, phenyl ring), 128.7(CH, phenyl ring), 111.8(CH,phenyl ring), 56.0(OCH₃). El⁺ m/z: 310.06 Anal. Calcd for C₁₄H₁₄O₆N₆S: C, 54.18%; H, 3.25%; N, 27.08%; O, 5.16%; S, 10.33%.

6-(2-fluorophenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole(7f) IR (ν max cm⁻¹, KBr): 3064 (C-H str.), 2924(C-H str.), 1614(C=N str.), 1539(C=C str.), 1438 (C-H
bend), 852(sp²C-H str.), 760(1,2-di substituted)). EIM⁺ m/z: 298.29 Anal. Calcd for C₁₃H₇FN₆S C, 52.34%; H, 2.37%; F, 6.37%; N, 28.17%; S, 10.75%.

6-(4-bromophenyl)-3-(pyrazin-2-yl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole(7g) IR (v max cm⁻¹, KBr): 3114 (C-H str.), 2971(C-H str.), 1602(C=N str.), 1531(C=C str.), 1490 (C-H bend), 828(sp²C-H str.), 814(1-4-di substituted)). EIM⁺ m/z: 359.20 Anal. Calcd for C₁₃H₇BrN₆S: C, %; H, 1.96%; Br, 22.24%; N, 23.40%; S, 8.93%.

6-(2-chlorophenyl)-3-(pyrazin-2-yl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole(7h) IR (v max cm⁻¹, KBr): 3080 (C-H str.), 2922(C-H str.), 1670(C=N str.), 1591(C=C str.), 1409 (C-H bend), 979(sp²C-H str.), 852(C-Cl str.), 777(1,2-di substituted). ¹H NMR 400 MHz (CDCl₃): 9.66(1H, s (pyrazine)) 8.81 (1H.s(pyrzine)), 8.71(1H, s(pyrazine)), 8.12-8.09(1H,dd), 7.58-7.47(3H, m). ¹³C NMR (100 MHz, CDCl₃): 177.2(S-C=N), 164.8 (-N-C(S)=N ), 156.9(C, triazole ring), 145.1(CH, pyrazine ring), 144.4(CH,pyrazine ring), 143.5(C, pyrazine ring), 141.7(CH, pyrazine ring), 133.3(C, phenyl ring), 133.0(CH, phenyl ring), 131.1(CH, phenyl ring), 127.7(CH,phenyl ring), 127.6(CH, phenyl ring). EIM⁺ m/z: 314.75 Anal. Calcd for C₁₃H₇ClN₆S: C, 49.61%; H, 2.24%; Cl, 26.70%; N, 26.70%; S, 10.19%.

6-(2-methylphenyl)-3-(pyrazin-2-yl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole(7i) IR (v max cm⁻¹, KBr): 3032 (C-H str.), 2941(C-H str.), 1638(C=N str.), 1541(C=C str.), 1408 (C-H bend), 884(sp²C-H str.), 767(1,2-di substituted). EIM⁺ m/z: 294.33 Anal. Calcd for C₁₄H₁₀N₆S: C, 57.13%; H, 3.42%; N, 28.55%; S, 10.89%.

6-(4-methylphenyl)-3-(pyrazin-2-yl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole(7j) IR (v max cm⁻¹, KBr): 3063 (C-H str.), 2947(C-H str.), 1620(C=N str.), 1573(C=C str.), 1424 (C-H bend), 902(sp²C-H str.), 834(1,4-di substituted)). EIM⁺ m/z: 294.33 Anal. Calcd for C₁₄H₁₀N₆S: C, 57.13%; H, 3.42%; N, 28.55%; S, 10.89%.
IR spectrum of 6-(3-methoxyphenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (7e)

IR spectrum of 6-(2-chlorophenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (7h)
Mass spectrum of 6-(3-methoxyphenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (7e)

Mass spectrum of 6-(2-chlorophenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (7h)
$^1$H NMR spectrum of 6-(3-methoxyphenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (7e)

$^1$H NMR spectrum of 6-(2-chlorophenyl)-3-(pyrazin-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (7h)
$^{13}$C NMR spectrum of 6-(3-methoxyphenyl)-3-(pyrazin-2-yl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (7e)

$^{13}$C NMR spectrum of 6-(2-chlorophenyl)-3-(pyrazin-2-yl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (7h)
ANTIMICROBIAL ACTIVITY OF 6-SUBSTITUTEDPHENYL-3-(PYRAZIN-2-YL)-[1,2,4] TRIAZOLO[3,4-B][1,3,4]THIADIAZOLES.

All the synthesized compounds 7(a-j) were tested in vitro for their antibacterial and antifungal activity. All the glass apparatus used were sterilized before use. The broth dilution technique was used to determine the minimum inhibitory concentration (MIC) of the synthesized compounds. Bacterial strain of *Staphylococcus aureus* MTCC87, Bacillus subtilissuis MTCC 441 as a gram positive, *Escherichia coli* MTCC1302, *shigella* MTCC 11947 as a gram negative used in a present study. Fungal strains of *Aspergillus niger* MTCC 1344 and *Candida albicans* MTCC 227 were taken. DMSO was used as the solvent for the compounds and control. A blank test was carried out to check the antimicrobial activity of DMSO. Ampicillin, Ciprofloxacin and Norfloxacin were used as the standard drugs for antibacterial activity and Clotrimazole and Terbinafeine were used as the standard drug for antifungal activity.

The standard strains were procured from the Microbial Type culture minimal inhibitory collection (MTCC), Institute of Microbial Technology, India. The compounds, defined as the lowest concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth were determined by using micro dilution broth method according to NCCLS standard. Antimicrobial activity was carried out by Autus laboratory Rajkot and by Dr. Dhiman Sarkar, Scientist, National Chemical Laboratory, Pune.

**Minimal Inhibitory Concentration [MIC]**

The main advantage of the broth dilution method for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

1. Serial dilutions were prepared in primary and secondary screening.
2. The control tube containing no antibiotic is immediately subculture (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37°C overnight.
3. The MIC of the control organism is read to check the accuracy of the drug concentrations.
4. The lowest concentration inhibiting growth of the organism is recorded as the MIC.
5. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.
6. DMSO was used as a control solvent.

**Methods used for primary and secondary screening**

Each synthesized compounds were diluted in DMSO to obtain 2000 μg/ml concentration, as a stock solution. Inoculum size for test strain was adjusted to 10^8 cfu (colony forming unit) per milliliter by comparing the turbidity.

**Primary screen:** In primary screening 1000 μg/ml, 500μg/ml and 250 μg/ml concentrations of the synthesized compounds were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

**Secondary screen:** The compounds found active in primary screening were similarly diluted to obtain 200 μg/ml, 100 μg/ml, 50 μg/ml, 25 μg/ml, 12.5 μg/ml, and 6.25 μg/ml concentrations.

**Reading Result:** The highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculums. The test mixture should contain 108 cfu/ml.

The results obtained from antimicrobial susceptibility testing are depicted in Table II.
TABLE II: ANTI MICROBIAL ACTIVITY OF 6-SUBSTITUTED PHENYL-3-(PYRAZIN-2-YL)-[1,2,4]TRIAZOLO[3,4-B][1,3,4]THIADIAZOLES.

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<td>MIC(μg/ml)</td>
<td>MIC(μg/ml)</td>
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<td>T.</td>
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A: Ampiciliin, C: Ciprofloxacin, N: Norfloxacin, Cl: Clotrimazole, T: Terbinafeine
RESULT AND DISCUSSION

In the present study a series of triazolothiadiazole of 1,2,3-triazole 3-thiol possessing pyrazine nucleus 7a-7j have been synthesized by 4-amino-5-(pyrazin-2-yl)-4H-1,2,4-triazole-3-thiol, and various aryl acids in the presence of POCl₃ as a solvent. The structures of all new synthesized compounds were established by ¹H, ¹³CNMR, FT-IR and mass spectroscopy. The mass Spectrum of 7e displayed the molecular ion peak (M⁺) peak at m/z 310, which was consistent with the product structure. The ¹H NMR spectrum of 7e exhibited singlet of proton at δ 4.08 ppm which indicated the presence of –OCH₃ group. Three singlets at δ 9.65, 9.81, and 9.70 ppm represent the characteristic values of pyrazine ring. Double doublet at δ 8.39-8.36, triplet at δ7.58-7.56 and multiplet at 7.0-7.17 represent the 1-3 substituted phenyl ring. The ¹³C NMR spectrum of 7e showed (-N-C(S)=N ) peak at δ 163.7 ppm and (S-C=N) peak at δ 143.3 ppm indicate the formation of triazolothiadiazole ring. C-O peak at δ 157.8 ppm, peak at δ 56.0 ppm indicates the presence of methoxy group. The IR spectrum exhibited general absorption bands which are in good agreement with the structure.

All the synthesized compounds 7(a-j) were tested in vitro for their antibacterial and antifungal activity. Bacterial strain of *Staphylococcus aureus* and *Bacillus subtilius* as gram positive, *Escherichia coli* and *shigella* as gram negative used in a present study. Fungal strains of *Aspergillus niger* and *Candida albicans* were taken. Ampicillin, Ciprofloxacin and Norfloxacin were used as the standard drugs for antibacterial activity and Clotrimazole and Terbinafine were used as the standard drug for antifungal activity.

From the results of antimicrobial activity depicted in Table II we can say that 7i is most active with MIC of 50µg/ml against *Staphylococcus aureus* with respect to standard Ampicillin, Ciprofloxacin and Norfloxacin. While compounds 7f is most active with MIC of 50µg/ml against *Bacillus subtilius* as gram positive and *Escherichia coli* as gram negative with respect to standard Ampicillin, Norfloxacin and Ciprofloxacin respectively.

Compounds 7b and 7g are moderately active with MIC of 100µg/ml against *Staphylococcus aureus* with respect to standard.
Compounds 7d, 7e, 7h and 7j are moderately active against *Bacillus subtilius* with respect to standard.

7a is most active with MIC of 50µg/ml against *shigella* with respect to standard Ampicillin, while 7j is act as a moderately active. Remaining compounds did not show significant activity against *shigella*.

Compounds 7b, 7c, 7d and 7g are moderately active against *Escherichia coli* with respect to standard.

Looking at the antifungal results compound 7j is more active with respect to standard having MIC of 50µg/ml against *Candida albicans* while 7a, 7d, 7e and 7i are equally active with the MIC of 100µg/ml with respect to standard antifungal drugs like Clotrimazole and Terbinafine.

Compounds 7b and 7g are more active with respect to standard having MIC of 50µg/ml against *Aspergillus niger* while 7c, 7f and 7i are equally active with the MIC of 100µg/ml with respect to standard antifungal drugs like Clotrimazole and Terbinafine.

Overall we can say that 7f and 7i are most active as antibacterial agents and 7b and 7j are most active as antifungal agent.
REFERENCES