CHAPTER-4

Formation of Novel quarternary ammonium salts of Acid of Thiadiazine
4.1 Objective

Now with keeping all the above facts we wish to report synthesis of novel quarternary ammonium salts and some metal salts of Thiadiazine acid along with their characterisation and biological evaluation.

4.2 REACTION SCHEME

A) Reaction scheme for the preparation of Thiadiazine acid amine salts

![Reaction Scheme]

*Figure-4.3*
### 4.3 Physical Data Table

<table>
<thead>
<tr>
<th>Code</th>
<th>R</th>
<th>Mol.Formula</th>
<th>Mol.Wt</th>
<th>M.P °C</th>
<th>Rxn.Time (Hrs)</th>
<th>Yield (%)</th>
</tr>
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<tr>
<td>NB-21</td>
<td>Diisopropylethyl</td>
<td>$\text{C}<em>{26}\text{H}</em>{31}\text{N}_3\text{O}_4\text{S}$</td>
<td>409.54</td>
<td>224-26</td>
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<td>&gt;260</td>
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4.4 EXPERIMENTAL

4.4.1 MATERIALS AND METHODS

All the chemicals used for preparation of novel compounds were purified by means of chemical purifications. Melting points were measured by capillaries. IR spectra were analysed on Perkin elmer SP-100 spectrophotometer. III 1H-NMR of compounds were analysed by Bruker Ac-80 spectrometer instrument (300MHz in DMSO-d6 ) using Tetramethyl silane as internal standard and chemical shifts are presented in δ (ppm). The completion of the reaction was checked by TLC. Elemental analyses performed on Perkin-Elmer C, H, and N analyzer. IV

4.4.2 General method for the preparation of Thiadiazine acid amine salts

1.0g (0.0035mole) of 2-(3,5-dimethyl-1,1-dioxido-2H-1,2,6-thiadiazin-4-yl)benzoic acid was dissolved in 100ml of Ethyl acetate at 60°C. Charged (0.0071mole) of amine to the clear solution at room temperature. Immediately some hazyness appeared. Stirred the reaction mixture for 2.0 hrs at room temperature. Filtered white solid using Ethylacetate wash to get pure Salt.23

4.4.3 General method for the preparation of Thiadiazine acid Sodium/Potassium salts

1.0g (0.0035mole) of 2-(3,5-dimethyl-1,1-dioxido-2H-1,2,6-thiadiazin-4-yl)benzoic acid was dissolved in 100ml of Ethylacetate at 60°C. Charged (0.0071mole) of Sodium Methoxide/ Potassium tert-butoxide to the clear solution at RT. Immediately some hazyness appeared. Stirred the reaction mixture for 6.0 hrs at RT. Solvent distilled and the residual sticky mass stirred with Ethylacetate to get white solid. Filtered white solid using Ethylacetate wash to get pure Salt. 23
4.5 ANALYTICAL DATA

4.5.1 N,N-diisopropyl ethylamine salt: (NB-21)

Mol. Wt: 409.54, M.P.: 224-226 °C; Yield 85%; IR (cm⁻¹): 1600 (C=O acid), 3059 (N-H), 1168, 1320 (>S=O); 1520 (C=N); 2990 (Quaternary salt); 3435 (-NH₂-);

1H NMR (δppm): 1.23 (m, 15H, -CH₃-), 1.53 (s, 6H, Di-Methyl), 3.1 (m, 2H, -(CH)₂-), 3.6 (q, 2H, -CH₂-N-), 7.05-7.66 (m, 4H, Ar-H);

Elemental analysis for C₂₀H₃₁N₃O₄S; Calculated: C, 58.60; H, 7.56; N, 10.25; O, 15.62; S, 7.81 Found: C, 58.31; H, 7.30; N, 10.59; O, 15.26; S, 7.61

4.5.2 N,N-diisopropylamine salt: (NB-22)

Mol. Wt: 381.48, M.P.: 237-238 °C; Yield 83%; IR (cm⁻¹): 1621 (C=O acid), 3032 (N-H), 1116, 1316 (>S=O); 1556 (C=N); 2858 (Quaternary salt); 3421 (-NH₂-);

1H NMR (δppm): 1.15 (d, 12H, -CH₃-), 1.56 (s, 6H, Di-Methyl), 3.21 (m, 2H, -(CH)₂-), 6.90-7.41 (m, 4H, Ar-H);

Elemental analysis for C₁₈H₂₇N₃O₄S; Calculated: C, 56.62; H, 7.07; N, 11.0; O, 16.17; S, 8.38 Found: C, 56.39; H, 6.96; N, 11.14; O, 15.76; S, 8.89

4.5.3 Potassium Salt: (NB-23)

Mol. Wt: 318.39, M.P.: >260 °C; Yield 75%; IR (cm⁻¹): 1690 (C=O acid), 3147 (N-H), 1145, 1330 (>S=O); 1517 (C=N); 1H NMR (δppm): 1.51 (s, 6H, Di-Methyl), 7.04-7.65 (m, 4H, Ar-H), 12.40 (s, -SO₂-NH-);

Elemental analysis for C₁₂H₁₁KN₂O₄S; Calculated: C, 45.22; H, 3.45; K, 12.24; N, 8.79; O, 20.10; S, 10.05 Found: C, 45.35; H, 3.29; K, 12.53; N, 9.10; O, 20.09; S, 10.15
4.5.4 Sodium Salt: (NB-24)

Mol. Wt: 302.28, M.P.: >260°C; Yield 86 % ; IR ( cm⁻¹): 1665(C=O amide), 3141(NH), 1170, 1330(>S=O); 1550 (C=N), 1H NMR (δppm): 1.56 (s, 6H, Di-Methyl), 6.98-7.56(m,4H,Ar-H), 12.31(s,-SO₂-NH-); Elemental analysis for C₁₂H₁₁N₂NaO₄S; Calculated: C, 47.63; H, 3.63; Na, 7.60 N, 9.26; O, 21.17; S, 10.58 Found: C, 47.29; H, 3.31; Na, 7.86; N, 9.59; O, 21.39; S, 10.85

4.5.5 N,N-diphenylamine salt: (NB-25)

Mol. Wt: 449.52, M.P.: 211-212 °C; Yield 60 % ; IR (cm⁻¹): 1659(C=O acid), 3118 (N-H), 1173, 1322(>S=O); 1513 (C=N); 2988(Quarternary salt); 3423(-NH₂-), 1H NMR (δppm): 1.61 (s, 6H, Di-Methyl), 6.86-7.72 (m,14H,Ar-H); Elemental analysis for C₂₄H₂₃N₃O₄S; Calculated: C, 64.06; H, 5.11; N, 9.34; O, 14.23; S, 7.11 Found: C, 64.13; H, 5.62; N, 9.48; O, 14.71; S, 7.26

4.5.6 t-butylamine salt: (NB-26)

Mol. Wt: 352.42, M.P.: 238-239 °C; Yield 87 % ; IR (cm⁻¹): 1687(C=O acid), 3129 (N-H), 1141, 1310(>S=O); 1557 (C=N); 2899(Quarternary salt); 3487(-NH₂-), 1H NMR (δppm): 1.22 (s, 9H, -(CH₃)₃), 1.59 (s, 6H, Di-Methyl), 6.89-7.29(m,4H,Ar-H); Elemental analysis for C₁₆H₂₂N₅O₄S; Calculated: C, 54.48; H, 6.24; N, 11.91; O, 18.16; S, 9.08 Found: C, 54.71; H, 6.58; N, 11.70; O, 18.33; S, 9.55
4.5.7 N,N-dicyclohexylamine salt: (NB-27)

Mol. Wt: 461.61, M.P.: 216-217 °C; Yield 80 % ;
IR ( cm⁻¹): 1670(C=O acid ), 3179 (N-H), 1154,
1329(>S=O); 1527 (C=N); 2997(Quarternary salt);
3469(-NH₂), 1H NMR ( δppm ): 1.02 (m, 4H,
-(CH₂)₂), 1.10 (m,16H,-(CH₂)₈-), 2.86 (m, 2H,
-(CH₂), 1.87(s,6H-Di-Methyl); 6.86-7.40(m,4H,Ar-
H); Elemental analysis for C₂₄H₃₅N₃O₄S;Calculated:
C, 62.39; H, 7.58; N, 9.09; O,13.86;S,6.93 Found:
C, 62.62; H, 7.73; N, 9.39; O,13.42; S,6.49

4.5.8 Methylamine salt: (NB-28)

Mol. Wt: 310.34, M.P.: 241-242 °C; Yield 73 % ; IR ( cm⁻¹):
1698(C=O acid ), 3131 (N-H), 1186,
1319(>S=O); 1508 (C=N); 2890(Quarternary salt);
3386(-NH₂), 1H NMR ( δppm ): 1.60 (s, 6H, Di-
Methyl), 2.12 (s,3H,-N-CH₃), 6.90-7.32(m,4H,Ar-H);
Elemental analysis for C₁₃H₁₆N₃O₄S;Calculated: C,
50.26; H, 5.15; N, 13.53; O,20.62;S,10.31 Found: C,
50.73; H, 5.39; N, 13.06; O,20.26; S,10.35

4.5.9 Ethylamine salt:(NB-29)

Mol. Wt: 324.37, M.P.: 231-232 °C; Yield 78 % ; IR ( cm⁻¹):
1698(C=O acid ), 3148 (N-H), 1152, 1336(>S=O);
1527 (C=N); 2899(Quarternary salt); 3335(-NH₂-), 1H
NMR ( δppm ): 1.33 (s, 6H, Di-Methyl), 2.19 (s,3H,-
CH₃),2.71 (t,2H,-CH₂-), 6.90-7.32(m,4H,Ar-H);
Elemental analysis for C₁₄H₁₈N₃O₄S;Calculated: C,
51.79; H, 5.54; N, 12.94; O,19.73;S,9.86 Found: C,
51.83; H, 5.83; N, 12.70; O,19.21; S,9.49
4.5.10 Cyclohexylamine salt: (NB-30)

Mol. Wt: 378.46, M.P.: 207-208 °C; Yield 90 %; IR (cm⁻¹): 1705 (C=O acid), 3130 (N-H), 1178, 1312 (>S=O); 1520 (C=N); 2996 (Quarternary salt); 3401 (-NH₂-), 1H NMR (δ ppm): 1.07-1.58 (m, 10H, -(CH₂)₅), 1.43 (s, 6H, Di-Methyl), 2.74 (m, 1H, -NH-CH-), 7.08-7.47 (m, 4H, Ar-H);
Elemental analysis for C₁₈H₂₄N₃O₄S; Calculated: C, 57.07; H, 6.34; N, 11.09; O, 16.91; S, 8.45
Found: C, 57.48; H, 6.49; N, 10.89; O, 16.73; S, 8.10
4.6 SPECTRAL DISCUSSION

4.6.1 IR SPECTRAL STUDY

IR spectra were analysed by Perkin Elmer SP-100 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. The characteristic bands of C-H aromatic stretching frequencies were observed between 3100-3000 cm\(^{-1}\). The stretching frequency of carbonyl (\textgreater{}C=O) of acid were at 1760-1670 cm\(^{-1}\). Vibrational frequency of N-H of Thiazine ring shows band around 3100-3500 cm\(^{-1}\). At 1153-1157, 1317-1325 cm\(^{-1}\) SO\(_2\) symmetric and asymmetric stretching frequency and at 1500-1550 cm\(^{-1}\) C=N vibrational frequency of thiazine ring has been observed. Also the characteristic peak of quarternary salt around 2900 cm\(^{-1}\) has been observed. The above mentioned respective frequencies suggest the correct formation of the desired products.

4.6.2 MASS SPECTRAL STUDY

Mass spectra were recorded on Waters Q-TOF Premier model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analysis. Molecular ion peak, [M+Na], [2M+Na] and Fragment peaks were observed in agreement with molecular weight of respective compound. The probable Mass pattern for the representative compound of each series is discussed below.

N,N-diisopropyl ethylamine salt: (NB-21)

![Chemical Structure](image)

Mol.Wt : 409.54
Mol.Ion peak: 409.33
M+1: 410.09
Base Fragment: 129.11
Acid Fragment: 281.08
N,N-diisopropylamine salt: (NB-22)

4.6.3 1H-NMR SPECTRAL STUDY

$^1$H-NMR spectra of the synthesized compounds were recorded on Bruker Ac-80 spectrometer (300MHz) spectrometer. Sample solutions were made in DMSO solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from H-NMR spectrum and their chemical shift ($\delta$ppm) were in the agreement of the structure of the molecule. In some cases, aromatic protons were obtained as multiplet and the peak due to –NH of thiazadiazine ring do not appear. The spectral interpretation is discussed as under.
N,N-diisopropyl ethylamine salt: (NB-21)

1. The fifteen protons of the five (1, 2, 3, 4, 5) methyl functional groups attached to Nitrogen gives a strong multiplet at 1.20-1.25 δ ppm.
2. The proton no. 6 is surrounded by three protons of Methyl group and Nitrogen. So the downfield peak appeared at 3.57-3.60 δ ppm having quartet.
3. The proton no 7 and 8 both have the same environment of two Methyl groups and Nitrogen which gives signal at 3.09-3.11 δ ppm as multiplet.
4. The proton no. 9 and 10 are symmetrically placed. So collectively they give a singlet at 1.53 δ ppm for six protons of two Methyl groups attached to Thiadiazine ring.
5. In the region of 7.05-7.66 δ ppm the four distinct peaks due to four aromatic protons of phenyl ring attached to Thiadiazine ring can be seen in the NMR.

Thus, by assigning all the peaks as per the above given justifications and by calculating the shift value for the respective protons the proposed structure of NB-21 is confirmed.
N,N-diisopropylamine salt: (NB-22)

1. The twelve protons of four methyl groups (1, 2, 3, and 4) have same chemical environment and attached to –CH-group. So collectively they gives a signal of doublet at 1.15-1.16 δppm.

2. The proton no.5 and 6 is surrounded by six protons of two Methyl group and Nitrogen which gives multiplate at 3.21 δ ppm.

3. The proton no. 7 and 8 are symmetrically placed. So collectively they give a singlet at 1.56 δ ppm for six protons of two Methyl groups attached to Thiadiazine ring.

4. In the region of 6.90-7.41 δ ppm the four distinct peaks due to four aromatic protons of phenyl ring attached to Thiadiazine ring can be seen in the NMR.

Thus, by assigning all the peaks as per the above given justifications and by calculating the shift value for the respective protons the proposed structure of NB-22 is confirmed.
4.7 SPECTRAL PRESENTATION OF SYNTHESISED COMPOUNDS

4.7.1 IR Spectrum of NB-21

4.7.2 Mass Spectrum of NB-21
4.7.3 $^1\text{H}$ Spectrum of NB-21

4.7.4 IR Spectrum of NB-22
4.7.5 Mass Spectrum of NB-22

4.7.7 $^1$H Spectrum of NB-22
4.8 BIOLOGICAL SCREENING OF QUARTERNARY SALTS OF 2-(3,5-
DIMETHYL-1,1-DIOXIDO-2H-1,2,6-THIADIAZIN-4-YL)BENZOIC ACID

4.8.1 Biological Screening:

The biological screening of all newly synthesised compounds was carried out. Biological screening method part is described in Chapter-2 Page no 45.

All the compounds have been evaluated for their biological screening represented in Graphical Table no-B.

The biological screening was compared with standard drug viz Amoxycillin, Benzyl Penicillin Ampicillin, Norfloxacin, and antifungal activity was compared with viz Greseofulvin. The inhibition zones were measured in mm. The zones of inhibition that displayed by standard drugs are recorded in Table no-A.
4.8.2 ANTIMICROBIAL ACTIVITY ZONE OF INHIBITION OF STANDARD DRUGS AT 50MG/ML CONCENTRATION.

Table-A

Zones of inhibition in mm

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<th>S.aureus</th>
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### 4.8.3 Biological Screening Data of Quarternary Salts of Thiadiazine acid

**Table B**

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The table above presents the biological screening data of quarternary salts of thiadiazine acid. Each column represents a different substance, and each row represents a different bacterial strain. The values indicate the level of activity or presence of the substance against each strain.
4.9 REFERENCES:


