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Potential Antibacterial Agents *Part-V.* Synthesis and Structural Characterization of Some New 3-Aryl 7H, 6-(Aryl) 5H-1, 2, 4-Triazolo [3, 4-b]-1, 3, 5-Thiadiazines

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N-(Substituted benzoyl) thiosemicarbazides **11a-e** were treated with sodium hydroxide solution to give 3-aryl-5-mercapto-4H-1,2,4- triazole **111a-e** which were subjected to Mannich Reaction in presence of 2-OC_2H_5, 4-NO_2 and 2-OCH_3 substituted anilines to give 3-aryl 7H, 6-(aryl) 5H-1,2,4-triazolo [3,4-b]-1,3,5-thiadiazines **IVa-h**. The compounds, **IVa-h** were compared with oxytetracycline and ampiclox (standard antibiotics) for their antibacterial activity.

Key words: Fused ring heterocyclic compounds, Triazolothiadiazines, Intramolecular Mannich condensation.

Introduction

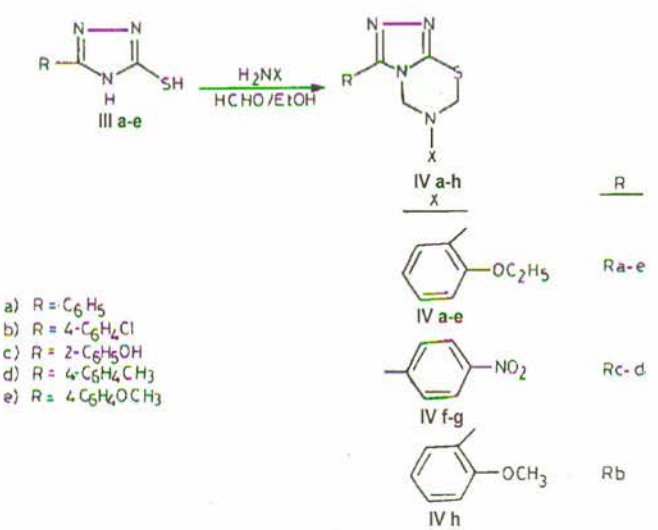
Nitrogen heterocycles as a part of fused ring systems have evoked considerable attention recently due to appreciable pharmacological activities associated with them. Further, when 1,2,4-triazole was fused with different heterocyclic rings it displayed and enhanced biological activities. Presence of different substituents e.g.chloro or methyl in these compounds also tend to augment their activities (Yadav *et al* 1994). 1,2, 4-Triazoles constitute an important class of nitrogen heterocycles: representatives possess significant medicinal activities such as hypoglycemic (Mhasalkar *et al* 1970), diuretic (Shah *et al* 1969), antibacterial (Pathak *et al* 1980) and antitubercular (Mir and Comrie 1970) together with this, they include herbicides, defoliants, growth regulators, fungicides and insecticides (Katritzky *et al* 1994). 1,2,4-Triazole nucleus has recently been incorporated into drugs including H_1/H_2 histamine receptor blockers, chlolinesterase active agents, CNS stimulants, anti-anxiety agents and sedatives (Heindel and Reid *et al* 1980).

In view of the above noted significance of 1,2,4-triazoles and their fused ring systems with thiazinone (1,2,4-triazole [3,4-b]-1,3-thiazinone) showing prominant antifungal activity (Yadav *et al* 1989) we started to synthesize triazolothiadiazines having new fused ring system. Some preliminary work of this synthesis was presented in 5th National Chemistry Conference (Sarfraz *et al* 1993). Later in 1997 similar other derivatives of triazolothiadizines were reported by Chinese scientists as antibacterial agents (Zhong and Tian-pa 1997).

Mannich reaction particularly intermolecular double Mannich reaction has been helpful to develop new heterocyclic ring systems not reported earlier when (2-α-hydroxy ethyl) benzimidazole was reacted with aromatic amine and formalin (Husain *et al* 1990). Present study deals with the synthesis, characterization and evaluation of antibacterial activity of eight new triazolothiadizines **1Va-h** Scheme 1.

Experimental

Melting points were determined on Buchi 510 melting point apparatus and are uncorrected. IR in KBr were recorded on JASCO 1RA-1 infrared spectrophotometer (λmax cm^{-1}). 1H-NMR spectra were recorded on Bruker AM-300 and 400 AM (300, 400, 500 MHz) spectrometers in C_5D_5N and DMSO-δ_6;



Scheme 1.

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chemical shifts are expressed in d_6 values. Mass spectra were recorded on Finnigan MAT 112-S and MAT 312 spectrometers connected to PDP 11/34 and MAT 188 computer.

Preparation of ***I a-e***. Following the standard procedure (Ebi *et al* 1996) hydrazine hydrate was reacted with aromatic esters to give substituted benzoylhydrazides **Ia-e**.

General procedure (Goswan et al 1984) for the preparation of ***II a-e***. To a suspension of **Ia-e** (0.01 mol) in 2% hydrochloric acid (100 ml) was added potassium thiocyanate (2g, 0.02 mol) and the mixture was refluxed with stirring for 3 h. On cooling to room temperature crystals appeared which were filtered to give **IIa-e**.

General procedure (Kalluraya et al 1994) for the preparation of ***III a-e***. Compounds **II a-e** (0.002 mol) were heated for 4 h on water bath in 4% aq. sodium hydroxide solution (50 ml). The resulting solution was neutralized with dilute hydrochloric acid, when a solid separated out which was filtered. The precipitated solid was pure enough to be used for the preparation of **IV a-e**.

General procedure for the preparation of ***IV a-h***. To a stirred and cooled solution of anilines (0.002 mol) in water (20 ml) containing acetic acid (0.4 ml) and formalin (37%, 0.2 ml) was added **III a-e** (0.002 mol). Stirring was continued for 15 min in ice bath then for 4 h at room temperature. The resulting solid was filtered. It was recrystallized with appropriate solvents. The physical analytical data is illustrated in Table 1.

Alternate procedure for the preparation of ***IV a-h***. Triazole (0.005 mol) was dissolved in 50 ml ethanol and aniline (0.005 mol) was added to this solution when a clear solution resulted, to this 1.0 ml HCl (conc) was added followed by 1 ml (0.01 mol, 30%) of formalin in methanol (50 ml). Resulting reaction mixture was refluxed for 3 h. On cooling crystals appeared which on recrystallization from appropriate solvent gave **IV a-h**.

Evaluation of antibacterial activity. Compounds **IV a-h** were tested for their antibacterial activity against both Gram-ve and Gram+ve bacteria namely, *Escherichia coli, Aeromonas sp., Enterobacter sp., Salmonella para typhi B,*

Table 1
Physical data of compound **IVa-h**

Compound	M.P.°C	Yield % (Solvent)	Mol.formula Mol.weight	IR Absorption cm^{-1} KBr	MS, m/e (%intensity)	Elemental analysis calc./found		
						C	H	N
IV a R = C_6H_5 X = 2-OC_2H_5	195	85 (C_2H_5OH)	$C_{18}H_{18}N_4OS$ 338.1204	3050, 2980 1585, 1460 1270, 735	338 (12.2) 177 (100) 149 (42.5, 120 (49.9)	63.89 63.75	5.36 5.29	16.56 16.16
IVb R = 4-C_6H_4Cl X = 2-OC_2H_5	206	83 (C_2H_5OH)	$C_{18}H_{17}N_4OSCl$ 372.0818	3000, 2900, 1670, 1580, 1440, 1270, 915	372 (100) 212 (19) 149 (83) 120 (56)	58.11 58.23	4.60 4.58	15.05 15.12
IVc R = 2-C_6H_4OH X = 2-OC_2H_5	265	90 (C_6H_6)	$C_{18}H_{18}N_4O_2S$ 354.1112	3040, 1650, 1620, 1500, 1420, 1245, 720	354 (13), 193 (46), 149 (33), 119 (100)	61.00 60.88	5.12 5.34	15.81 15.67
IVd R = 4-$C_6H_4CH_3$ X = 2-OC_2H_5	130	60 (C_2H_5OH/H_2O)	$C_{19}H_{20}N_4OS$ 352.1351	3005, 1670, 1580, 1505, 1440, 1220, 730	352 (6.6), 191 (100), 150 (38), 120 (18)	64.76 64.68	5.72 5.38	15.90 15.91
IVe R = 4-$C_6H_4OCH_3$ X = 2-OC_2H_5	140	90 (C_6H_6)	$C_{19}H_{20}N_4O_2S$ 368.1353	2900, 1670, 1590, 1220, 730	368 (2.6),207 (16), 149 (45), 120 (100)	61.95 61.73	5.47 5.37	15.21 15.45
IVf R = 2-C_6H_4OH X = 4-NO_2	260	40 (C_2H_5OH)	$C_{16}H_{13}N_5O_3S$ 355.0001	3020, 1620, 1598, 1505, 1480, 1240, 740 740	355 (1), 192 (100), 120 (16) 105 (22) 91 (29)	54.09 54.21	3.69 3.77	19.71 19.61
IVg R = 4-$C_6H_4CH_3$ X = 4-NO_2	270	68 (C_2H_5OH)	$C_{17}H_{15}N_5O_3S$ 353.0002	3000, 1600, 1505, 1460, 1270, 710	353 (2.1), 191 (100), 150 (61), 132 (45), 118 (45)	57.79 57.63	4.28 4.18	18.11 17.99
IVh R= 4-C_6H_4Cl X = 2-OCH_3	180	50 (C_6H_6)	$C_{17}H_{15}NOSCl$ 358.0634	3005, 1670, 1585, 1240, 715	358 (21) 210 (65) 152 (23), 135 (100) 120 . (42)	56.98 56.79	4.22 4.13	15.64 15.97

Table 2
Antibacterial activity results zone of inhibition (mm)

S. No.	Name of the test organisms	IV-a	IV-b	IV-c	IV-d	IV-e	IV-f	IV-g	IV-h	Oxytetra-cycline	Ampiclox
Grame-Negative											
1.	*Escherichia coli*	21	20	18.5	30	27	20	19	18	12.7	18.5
2.	*Aeromonas sp.*	23.4	20.4	22.4	-	-	-	-	-	13.3	18
3.	*Enterobacter sp.*	22	-ve	20	29	27	-	-	-	21	18.5
4.	*Pseudomonas sp.*	16.3	-ve	24.8	-	-				13.6	20.8
5.	*Salmonella para typhi B*	19	19	20.4	-	-	26	-	25	21.7	18.6
6.	*Klebsiella sp.*	19.7	19.6	19.5	-	-	20	18	20	24.8	17.4
7.	*Salmonella typhimurium*	20.5	20.8	23	-	-	-	-	-	23.3	18.3
Gram-Positive											
1.	*Bacillus subtilis*	16.2	15.1	20	31	30	24	28	26	27.7	21.1
2.	*Bacillus pumilus*	19.2	18	17	27	-	22	13	14	22.6	18.1
3.	*Streptococcus pyogen*	15	16.6	15.7	21	30	24	24	24	26.4	21.8
4.	*Streptococcus lactis*	15.2.	21	17.3	20	23	-	-	-	26.3	-ve
5.	*Staphylococcus aureus*	20.7	20.8	-	-	22.1	22	17	-	22.1	22.2

Conc, 10 mg ml^{-1} DMF; No inhibition, -ve; Diameter of the wall, 8.2 mm.

Klebsiella sp., *Salmonella typhimurium* (Gram-ve); *Bacillus subtilis*, *Bacillus pumilus*, *Streptococcus pyogenes*, *Streptococcus lactis* and *Staphylococcus aureus* (Gram +ve). The cultures of bacteria grown overnight at 37°C were used for testing antibacterial activity. The assay was carried out by overlay agar method (Mandour *et al* 1995).

The plates were formed with two different media. The thick hard layer or underlay was made with antibiotic assay medium (base agar). The overlay was made with antibiotic assay medium (seed agar). First the underlay was formed by pouring base agar (melted) plates. When this layer became hard, the seed agar tubes (melted but not too hot to kill the bacteria) were inoculated with organism and poured on hard layer or underlay. The plates were kept at room temperature to get hard. Holes were bored in the medium with the help of sterilized borer. Different dilutions of the testing compounds were poured in each hole. The compounds were dissolved in DMF and blank experiment with pure DMF was also performed. Two antibiotics Ampiclox and Oxytetracycline were used for comparison. Results are reported as zone of inhibition after 24 to 48 h of growth at 37°C (Table 2).

Results and Discussion

Substituted aryl 5-mercapto 1,2,4-triazoles **(III a-e)** were condensed with formaldehyde and ortho-phenetidine to give **IVa-e**, with papra nitroaniline to give **IV f-g** and with ortho anisidine to give **IV h**, thus resulting in the formation of desired close ring compounds. **IIIa-e** componds were synthesized by standard methods from **IIa-e** componds which were obtained from **Ia-e** componds.

IR spectra of compounds **IVa-h** showed absorption bands at ν 3050-2900 cm^{-1} for CH stretching, 1670-1580 for C=N and for benzene ring. Absence of absorptions at ν 3400-3300 and 2600-2500 cm^{-1} for NH and SH groups respectively indicated that reaction has occurred at both the functional groups (Kallaraya *et al* 1994).

The ^{1}H-NMR spectra of **IVg** (a typical example) showed signals (δ ppm) at 2.4 (s, 3H, CH_3), 5.6 (s, 2H, SCH_2) 6.6 (S,2H, NCH_2N) while the aromatic protons appeared as multiplets between δ 7.2-8.3 integrating for eight protons. Similarly ^{1}H NMR spectra **IVa-h** were consistent with the associated structures.

Further evidence for the proposed structure **IVg** was obtained from mass spectral data which had molecular ion peak at m/z 353 along with the fragment peak at m/z 191 corresponding to the parent triazole which is usually observed in such types of molecules (Yadav *et al* 1989). Other derivatives also showed similar mass fragmentation pattern. The structures assigned to **IV a-h** were confirmed from their analytical and spectral data (Table 1).

Antibacterial screening. The antibacterial activity test was performed according to the cup plate method in dimethylformamide and compared with known antibiotics (Oxytetracycline and Ampiclox). The compounds were tested against seven Gram negative and five Gram positive organisms (Table 2). The compound **IVc** was more active than the standard antibiotics against Gram negative bacteria *Aeromonas species*, *Pseudomonas species* and *Salmonella typhimurium* while compound **IVf** was more active against *Escherichia* coli and *Salmonella typhimurium*. Regarding Gram positive

bacterial the compound **IVe-f** showed activity against *Bacillus subtilus* and the compound **IV d-h** also inhibited the growth of *Streptoccus pyogen.*

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