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In Vitro Antimicrobial Activity Of 3-Thio-4-Aryl-5-Tolyl-[1, 2, 4]–Dithiazolidines [Hydrochloride]

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ABSTRACT

A series of novel 3-thio-4-aryl-5-tolyl-[1, 2, 4]-dithiazolidine [hydrochloride] have been synthesized by the interaction of several Ammonium aryl dithiocarbamate with *N*-*p*-tolyl-*S*-chloro isothiocarbamoyl chloride in refluxing chloroform medium. These compounds were screened for their antibacterial and antifungal activities against–*E. coli, P. vulgaries, S. aureus, S. typhimurium, K. pneumonie, Ps. aeruginosa, A. niger and C. albicance.*The newly synthesized compounds have been characterized by analytical and IR, ^{1}H NMR and Mass spectral studies.

I. INTRODUCTION

Any chemical substance inhibiting the growth or causing the death of micro-organisms is known as antibacterial agent. Chemical substances are used for treatment of diseases and have been known since the 1500's. The chemical substances used for the treatment of infectious diseases and diseases caused by the proliferation of malignant cell are called as chemotherapeutic agents. Antibacterial drugs that destroy bacteria or stop its growth. An antibiotic is a chemotherapeutic agent that stops the growth of micro-organisms, such as bacteria, fungi or protozoan's. "Antibiotic" is considered to be a substance which is anti-bacterial, anti-fungal, or anti-parasitical. It is very important to know the specific mechanism by which chemotherapeutic agents inhibit or kill micro-organisms. This information has wide application. It may suggest some chemical entity as superior drug e. g. similar compounds but with some modification in its configuration. It provides better understanding of the cell. More often than not, chemotherapeutic agents have been discovering by screening experiments i. e. by trial or error. New chemotherapeutic agents are intensively investigated in order to establish their mode of action.

Dithiazolidine constitutes a major role in the synthesis of various heterocyclic moieties. They act as active precursors in synthetic heterocyclic chemistry. Synthesis of a series of novel five member ring containing nitrogen and sulphur are well known[1]. A small heterocyclic ring containing nitrogen and sulphur have been under investigation for a long time because of their important properties. Synthesis, structural properties and antimicrobial activities of various [1, 2, 4]-dithiazolidine have been reported earlier[2]. The literature survey revealed that the [1,2,4]- dithiazolidine have been found to possess potent anti-tumors, anti-tuberculosis[3], anti-diabetic and anti-cancer[4] and anti inflammatory[5] properties.

Thiocarbamides and their heterocyclic derivatives have gained recently much interest as inhibitors of Human Immunodeficiency Virus (HIV)[6] and Therapeutic agents[7]. Some of the heterocyclic derivatives of thiocarbamides are found to possess diverse pharmacological activities like antifungal and anti-tubercular agents. In view of utility of thiocarbamides, N-aryl-S-chloro isothiocarbamoyl chloride have been used in synthesis of substituted [1, 2, 4] dithiazolidine by interacting with Ammonium aryl dithiocabamates. The drug containing 1, 2, 4-dithiazolidines show a diverse range of physiological activities, antimicrobial[8-9], anti-inflammatory[10-12], anti-ulcer[13-14], and anti-cancer[15]. Here is reported the synthesis of several 3-thio-4-aryl-5-tolyl-[1, 2, 4]-dithiazolidine [hydrochloride] (3a-g) have been synthesized by the interaction of several Ammonium aryl dithiocarbamate (1a-g) with *N*-*p*-tolyl-*S*-chloro isothiocarbamoyl chloride (2). The required Ammonium aryl dithiocarbamate (1a-g) were obtained by the interaction of different amines with carbon disulphide and Ammonia.

II. RESULTS AND DISCUSSION

These compounds show appreciable activity towards *E. coli, P. vulgaries, S. aureus, S. typhimurium, K. pneumonie, Ps. aeruginosa, A. niger and C. albicance.* The product was found to be non-desulphurrizable when boiled with alkaline lead acetate solution. The IR spectra of products shows bands due to Ar-H, C-H, C=N, C-C, C-N, C=S, C-S, S-S stretching and ^{1}HNMR spectra of products distinctly displayed signals due to aromatic protons and Acetyl protons. The Mass spectrum of product was also observed. The identities of these new 3-thio-4-aryl-5-tolyl-[1, 2, 4]-dithiazolidine [hydrochloride] have been established on the basis of usual chemical transformations and also IR, ^{1}H NMR and Mass spectral studies[16-18].

III. EXPERIMENTAL

General Methods

All the newly synthesized dithiazolidine were screened for their antibacterial activities against pathogenic bacteria like *Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Salmonella typhimurium, Klebsiella pneumonie, Pseudomonas aeruginosa,* and antifungal activities against *Aspergillus niger* and *Candida albicans.* 1,2,4-dithiazolidines, compounds have been synthesized as follows:

Several 3-thio-4-aryl-5-tolyl-[1, 2, 4]-dithiazolidine [hydrochloride] (3a-g) have been synthesized by the interaction of several Ammonium aryl dithiocarbamate (1a-g) with *N*-*p*-tolyl-*S*-chloro isothiocarbamoyl chloride (2). in $CHCl_3$. After condensation, the solvent was distilled off to obtain a sticky residue. This residue was triturated several times with petroleum ether (60-80^{0}C) to afford a pale yellow solid (3a-g).

All chemicals were research grade. Melting points determined are uncorrected. IR spectra were recorded in KBr on a FT-IR Perkin-Elmer RXI(4000-450cm^{-1}) spectrophotometer. ^{1}H NMR measurements were performed on a Bruker DRX-300 (300 MHz FT NMR) NMR spectrometer in $CDCl_3$ solution with TMS as internal reference. The Mass spectra were recorded on a THERMO Finnigan LCQ Advantage max ion trap Mass spectrometer. Thin layer chromatography (TLC) was performed on silica Gel G and spots were visualized by iodine vapour. The compounds describe in this paper were first time synthesized by the multistep reaction protocol.

Synthesis of Ammonium aryl dithiocarbamate[19] (1a-g)

The compound Ammonium aryl dithiocarbamate was prepared by drop wise addition of Amine [9ml] in ice cold mixture of ammonium [15ml, density 0.88] and carbon disulphide [7.5ml] followed by the vigorous shaking. The reaction mixture was allowed to stand for 30min heavy precipitate of Ammonium aryl dithiocarbamate separates out. Filter it and dry it.

Synthesis of *N*-*p*-tolyl-*S*-chloro isothiocarbamoyl chloride (2)

N-*p*-tolyl-S-chloro-isothiocarbamoyl chloride (**2**) was prepared by passing a calculated amount of chlorine from *p*-tolyl isothiocyanate.

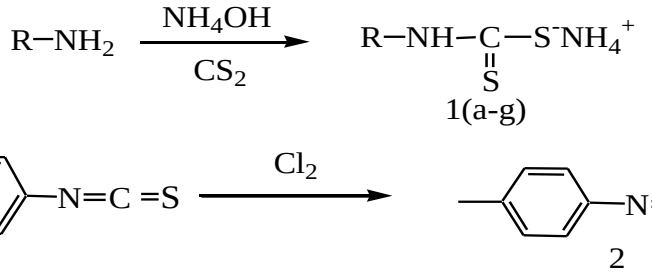
3a:- Synthesis of 3-thio-4-aryl-5-tolyl-[1, 2, 4]-dithiazolidine [hydrochloride]

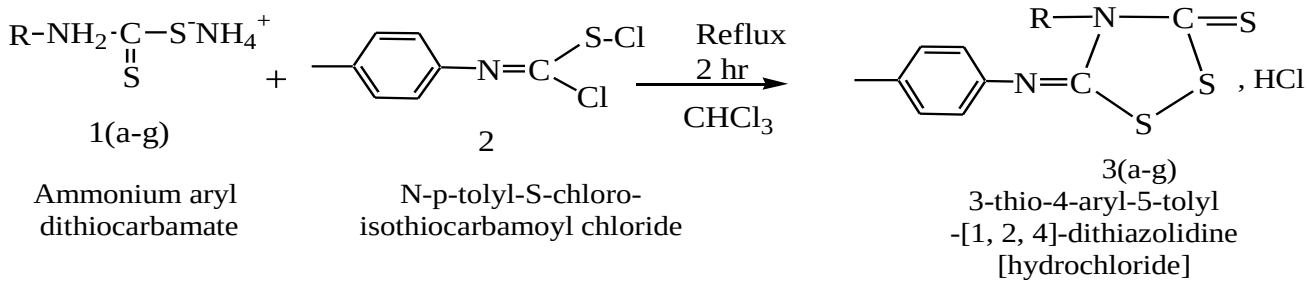
A mixture of Ammonium phenyl dithiocarbamate (1a-g) and N-tolyl-*S*-chloro isothiocyanocarbamoyl chloride was gently refluxed for 2 hr during which evolution of HCl was noticed. The progress of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was brought to room temperature and the solvent removed under reduced pressure to obtain residue. This residue was triturated several times with petroleum ether (60-80°C) to afford a pale yellow solid **(3a)**.

3a: IR (KBr) :υ 3155.5 (Ar-H), 2951.0 (C-H aliphatic), 1593.2 (C=N), 1508.3 (C-C), 1131.0 (C-N), 1143.7 (C=S), 752.2 (C-S), 503.4 (S-S), cm^{-1}; ^1H NMR (δ in ppm, $CDCl_3$): δ 7.94-7.22 (9H, m),; δ 2.358-2.353 (3H, s, CH_3) Mass (m/z): 316 (M^+), 300, 225, 211 , Anal. Calcd for $C_{15}H_{12}N_2S_3$, HCl: C, 56.96; H, 3.79; N, 8.86; S, 30.37; Found: C, 56.92; H, 3.75; N, 8.90; S, 30.35.

On the basis of all above facts the product with m. p. 122°C was assigned the structure 3-thio-4-phenyl-5-tolyl-[1, 2, 4]-dithiazolidine [hydrochloride]

When the reaction of *N*-*p*-tolyl-*S*-chloro-isothiocarbamoyl chloride was extended to several other Ammonium phenyl dithiocarbamate corresponding 3-thio-4-aryl-5-tolyl-[1, 2, 4]-dithiazolidine [hydrochloride] (3b-g) have been isolated.





Ammonium aryl dithiocarbamate | N-p-tolyl-S-chloro-isothiocarbamoyl chloride | 3-thio-4-aryl-5-tolyl -[1, 2, 4]-dithiazolidine [hydrochloride]

Where, R= (a) Phenyl, (b) *o*-Cl-phenyl, (c) *m*-Cl-phenyl, (d) *p*-Cl-phenyl, (e) *o*-tolyl, (f) *m*-tolyl (g) *p*-tolyl.

3c: IR (KBr) :υ 3032.1 (Ar-H), 2769.7 (C-H aliphatic), 1593.2 (C=N), 1541.1 (C-C), 1131.0 (C-N), 1207.4 (C=S), 715.5 (C-S), 532.3 (S-S), cm^{-1}; ^1H NMR (δ in ppm, $CDCl_3$): δ 7.94-7.22 (8H, m),; δ 2.358-2.353 (3H, s, CH_3) Mass (m/z): 350 (M^+), 335, 315, 259, Anal. Calcd for $C_{15}H_{11}N_2S_3Cl$, HCl: C, 51.42; H, 3.14; N, 8.00; S, 27.42; Found: C, 51.46; H, 3.10; N, 8.02; S, 27.46.

3g: IR (KBr) :υ 3147.8 (Ar-H), 2949.1 (C-H aliphatic), 1554.6 (C=N), 1512.1 (C-C), 1311.5 (C-N), 1143.7 (C=S), 711.7 (C-S), 532.3 (S-S), cm^{-1}; ^1H NMR (δ in ppm, $CDCl_3$): δ 7.35-7.09 (8H, m),; δ 3.53-2.30 (6H, s) Mass (m/z): 330 (M^+), 314, Anal. Calcd for $C_{16}H_{14}N_2S_3$, HCl: C, 58.18; H, 4.24; N, 8.48; S, 29.09; Found: C, 58.16; H, 4.28; N, 8.52; S, 29.02.

Table -1: Physical data for characterization of compounds (3a-g)

Compd	Yield %	R_f	M.P. ^0C	Analysis (%): Found (calcd)	
				N	S
3a	80.00	0.67	122	8.90(8.86)	30.35(30.37)
3b	71.46	0.72	110	8.03(8.00)	27.40(27.42)
3c	69.39	0.48	101	8.02(8.00)	27.46(27.42)
3d	55.34	0.51	123	7.96(8.00)	27.38(27.42)
3e	83.00	0.55	138	8.42(8.48)	29.06(29.09)
3f	82.88	0.60	127	8.46(8.48)	29.10(29.09)
3g	75.00	0.63	170	8.52(8.48)	29.02(29.09)

C and H analysis was found satisfactory in all cases.

IV. MATERIALS AND METHODS

The antimicrobial activities of these compounds were determined in vitro by using disc diffusion method[20]. The medium used for antibacterial study was Muller-Hinton agar (Hi-media Pvt. Ltd, India)

Composition of Muller-Hinton agar

Beef infusion from	300.00g
Casein acid hydrolysate	17.500g
Starch	1.50g
Agar	17.0g
Distilled water	1000mL
pH	7.3

Medium used for antigungal activities was Potato dextrose agar (Hi-media Pvt. Ltd, India).

Composition of Potato dextrose agar

Potato infusion from	200 g/lit
Dextrose	20 g/lit
Agar power	15 g/lit
pH	5.6±0.2

Test Procedure:

The media was prepared by dissolving weighed ingredients and was sterilized at 121°C and 15lbs/$inch^2$ pressure for 15 min. After sterilization it was cooled down at about 50°C and poured into sterile Petri plates and allowed to solidify. The plates were seeded with 24 hr old active nutrient broth culture of the test organism in order to obtain lawn culture.

All the compounds have been screened for both antimicrobial and antifungal activity by using disc diffusion assay[21-22]. For this sterile filter paper disc (6mm) impregnated with fixed doses of compounds were placed on pre-inoculated Mullar-Hilton plate. The disc bearing plates were incubated at 37^{0}C for 24 hrs. Inhibition zones read after incubation at 37^{0}C for 24 hrs. for bacterial strains and for fungal strains inhibition zones read after incubation at 35^{0}C for 48 hrs. The compounds were taken at a concentration or 1mg/ml using dimethyl sulphoxide as a solvent .Amikacin (100 ug/ml) was used as standard for antibacterial and Fluconazole (100ug/ml) as a standard for antifungal activity. The compound were screened for antibacterial activity against *Escherichia coli, Proteus vulgaris , Staphylococcus aureus , Salmonella typhi , Klebsiella pneumoniae , Psudomonas aeruginosa in* Mullar-Hilton medium *Aspergillus niger* and *Candida albicans* in potato dextrose agar medium. The zone of inhibition observed and interpreted by using antibiotic zone reader. The results were cited in Table 2

V. RESULTS AND DISCUSSIONS

3.3Table 2: Zone size of 3-thio-4-aryl-5-tolyl-[1, 2, 4]-dithiazolidine [hydrochloride] (Scheme 1-3)(a-f)

Comp ounds	*E. Coli*	*S. aureus*	*P. vulgaris*	*P. aeruginosa*	*S. typhi*	*K. pneumonie*	*A. niger*	*C. albicance*
IIIa	++	+++	--	++	+++	++	+++	+++
IIIb	++	++++	--	--	++	--	++	++
IIIc	--	++	++	++	--	--	--	++++
IIId	++	++	+++	--	+++	--	++	--
IIIe	--	++	++	++	--	++	+++	++
IIIf	+++	+++	+++	--	++++	++	+++	+++

++++	Strong activity (above 18mm)
+++	Moderate activity (above 14 to 18mm)
++	Weak activity (above 8-14mm)
--	Inactive (below 8mm)

The compounds **IIIa** and **IIIb** exhibited moderate to weak inhibitory activity against *E. coli, S. typhi, P. aeruginosa*, *A. niger* and *C. albicans* while it had no inhibitory activity against *P. vulgaris* and *K. pneumonie.* **IIIb** exhibited good activity against S. aureus.

The compounds **IIIc** and **IIId** exhibited moderate to weak inhibitory activity against *P. vulgaris, S. aureus*. While for other bacteria and fungi it showed weak to no activity. The compound **IIIc** showed strong inhibition against *C. albicans*.

The compound **IIIe** and **IIIf** exhibited moderate to weak inhibitory activity against *S. aureus, P. vulgaris, K. pneumonie, A. niger* and *C. albicans*. **IIIf** showed promising activity against *S. typhi*.

Amikacin (100µg/mL) was used as a standard for antibacterial activity and Fluconazole (100µg/mL) was used as a standard for antifungal activity.

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