SYNTHESIS AND POTENTIAL ANTIMICROBIAL ACTIVITY OF SOME BIOLOGICALLY ACTIVE 4-ARYL-3, 5-DI-(*O*-TOLYLIMINO)-1, 2, 4-DITHIAZOLIDINES (HYDROCHLORIDE)

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Abstract: A series of novel 4-aryl-3, 5-di-(*o*-tolylimino)-1, 2, 4-dithiazolidines (Hydrochloride)was synthesized by the interaction of 1-*o*-tolyl-3-aryl thiocarbamides with *N*-phenyl-*S*-chloro-isothiocarbamoyl chloride. Initially 1-*o*-tolyl-3-aryl thiocarbamides were prepared by the interaction of *o*-tolylisothiocyanate with different aryl amine in benzene medium. The structures of newly synthesized 1, 2, 4-dithiazolidines (Hydrochloride)have been established on the basis of chemical transformation, elemental analysis, and IR, ^{1}H NMR, and Mass spectral studies. The title compounds have been assayed for their biological activity against gram-positive as well as gram-negative microorganisms namely *Escherichia coli, Staphylococcus aureus, Penicillum notatum*and *Aspergillus niger*. The title compounds showed most significant activity against the microbial strains used.

Keywords: *o*-tolylisothiocyanate, aryl amine,thiocarbamides, isothiocarbamoyl chloride, 1, 2, 4-dithiazolidines (Hydrochloride), Antimicrobial activity.

Introduction:

Heterocyclic compounds have gained immense importance in human life. The structural diversity and biological importance of nitrogen-sulphur heteroatom containing compounds have made heterocyclic compounds attractive synthetic targets over many years. They are found in bioorganic and medicinal chemistry with applications in drug discovery. Most of the modern drug contain heterocyclic pharmacophore shows diverse pharmacological activities like antimicrobial, cardiovascular, and anesthetic agents and also in agricultural as herbicides, insecticides, pesticides, and fungicides.

Dithiazolidines constitutes a major role in the synthesis of various heterocyclic moieties. Thiocarbamides and their heterocyclic derivatives have gained recently much interest as inhibitors of Human Immunodeficiency Virus (HIV)[1] and Therapeutic agents[2].

Synthesis and biological evaluation of various pharmacologically important [1,2,4]-dithiazolidines have been reported earlier[3-8]. The literature has been enriched with progressive findings about the synthesis of [1,2,4]-dithiazolidines by using the reagent *N*-phenyl-*S*-chloro isothiocarbamoyl chloride and by oxidative cyclisation using bromine and iodine[9-10]. [1,2,4]-dithiazolidines have been found to have potent anti-inflammatory and antitumor properties as they down regulate the NF-kB transcription factor[11]. Various substituted [1,2,4]-dithiazolidines are known for their medicinal activities particularly as antibacterial and antifungal agents[12-14].

In view of the utility of *N*-aryl/alkyl-*S*-chloro isothiocarbamoyl chloride in the synthesis of nitrogen and sulphur containing heterocyclic compounds and as a part of our research towards the development of efficient methodologies for the synthesis of heterocyclic compounds, we report here in the synthesis, characterization and antimicrobial potential study of substituted [1,2,4]-dithiazolidine derivatives.

Results and discussion:

To a well cooled chloroformic solution of 1-*o*-tolyl-3- phenyl-thiocarbamides **(1a)** (0.5 g, 0.002 M in 20 ml) was added a cool chloroformic solution of *N*-phenyl-*S*-chloro isothiocarbamoyl chloride **(2)** (0.43 g, 0.002 M in 10 ml) and the mixture was shaken for few min. A brisk reaction with evolution of hydrogen chloride was noticed. The reaction mixture was refluxed over boiling water bath for 3 hrs. Afterwards the solvent chloroform was distilled off and the resultant syrupy mass triturated several times with petroleum ether (60-80˚C), a solid was obtained. It was crystallized from ethanol.

The IR, 1H NMR, Mass spectral analysis[15-18] and elemental analysis (**Table-1**) clearly indicated the product and assign the structure 4-phenyl-3, 5-di-(*o*-tolylimino)-1,2,4-dithiazolidines (Hydrochloride)**(3a)**.

Similarly, when the reactions were extended to other 1-*o*-tolyl-3-aryl-thiocarbamides **(1b-h)** the corresponding 1,2,4-dithiazolidines (Hydrochloride) **(3b-h)** was obtained **(Scheme)**.

Experimental Section:

Melting points of the synthesized compounds were recorded on electro thermal melting point apparatus are uncorrected. Specific rotations of the newly synthesized compounds were measured on Equip-Tronic digital polarimeter model no. EQ 800 at 30^0 C in $CHCl_3$. IR spectra were recorded on a Model - Agilent Cary 630 FTIR spectrometer. ^{1}HNMR were obtained on a Bruker DRX-300 (300 MHz FT NMR) NMR spectrometer in $CDCl_3$ solution with TMS as an internal reference. The MS spectra were recorded on a Agilent 6520 Q-TOF (ESI-HRMS) mass spectrometer. Purity of the compounds was checked by thin layer chromatography using Merck silica gel coated aluminum plates and petroleum ether: ethyl acetate as eluent.

Procedure:

1) Preparation of *o*-tolyl isothiocyanate

To an ice cold mixture of ammonia and pure carbon disulphide, *o*-toluidine was added with constant stirring for 30 min. A heavy precipitate of ammonium *o*-tolyl dithiocarbamate separates out. It is filtered, washed with acetone and dried. The oxidative decomposition of ammonium aryl dithiacarbamate with lead nitrate yields *o*-tolyl isothiocyanate.

2) Preparation of 1-*o*-tolyl-3-aryl thiocarbamides (1a-h)

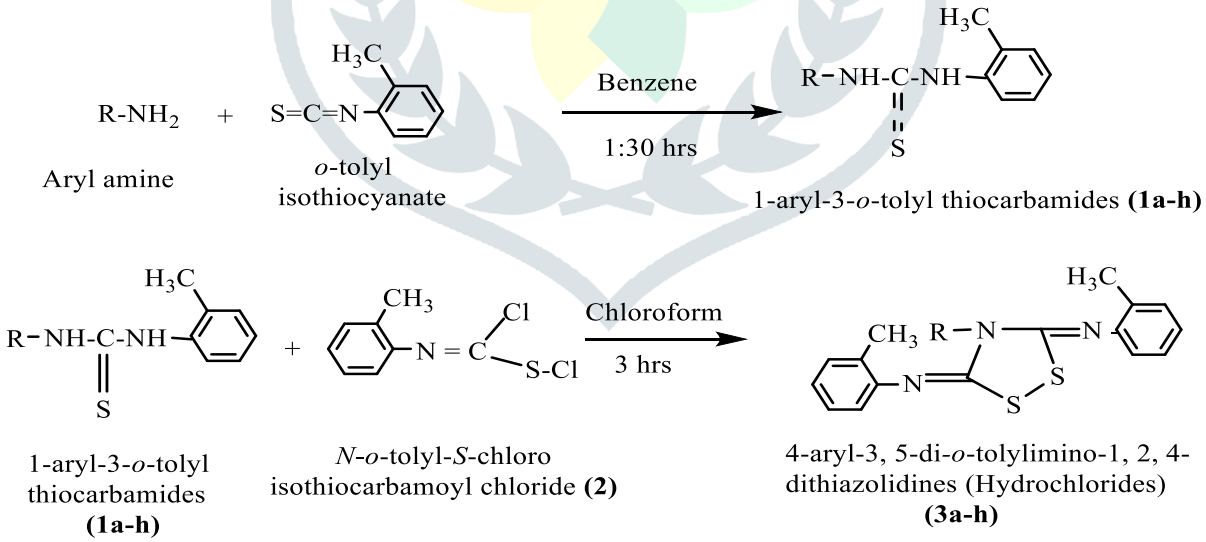
To a benzene solution of *o*-tolyl isothiocyanate, aryl amine was added. The resultant reaction mixture was then refluxed over boiling water bath for about 1:30 hrs. It was cooled. The solvent was distilled of, a white solid product obtained. It was purified by ethanol.

Spectral Analysis:

4-phenyl-3, 5-(di-*o*-tolylimino)-1, 2, 4-dithiazolidin (Hydrochloride) (3a):
IR(KBr cm^{-1}): ν 3140 (N-H), 3140 (Aromatic C-H), 2951 (Aliphatic C-H), 1531 (C=N), 1446 (C=C), 1265 (C-N), 705 (C-S); **^{1}H NMR ($CDCl_3$)**: δ 7.1-6.4 (13H, m, Ar-H), 2.35 (6H, s, methyl protons; **Mass (*m/z*)**: 389 (M^+),130, 91, 77.

4-*o*-chlorophenyl-3, 5-di-(*o*-tolylimino)-1, 2, 4-dithiazolidines (Hydrochloride) (3d)
IR(KBr cm^{-1}): ν 3111 (Aromatic C-H), 2945 (Aliphatic C-H), 1531 (C=N), 1442 (C=C), 1327 (C-N), 790 (C-S); **^{1}H NMR ($CDCl_3$)**: δ 7.1-6.5 (12H, m, Ar-H), 2.35 (6H, s, methyl protons; **Mass (*m/z*)**: 423 (M^+),130,112, 91.

4-*p*-methoxyphenyl-3, 5-di-(*o*-tolylimino)-1, 2, 4-dithiazolidine (Hydrochloride) (3h) IR(KBr cm^{-1}): ν 3008 (Aromatic C-H), 2902 (Aliphatic C-H), 1510 (C=N), 1417 (C=C), 1298 (C-N), 786 (C-S); **^{1}H NMR ($CDCl_3$)**: δ 7.1-6.3 (12H, m, Ar-H), 3.75 (3H, s, methoxy protons), 2.35 (6H, s, methyl protons; **Mass (*m/z*)**: 419 (M^+),130,96, 91.



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

Scheme

Table-1: Physical Characterization of 4-aryl-3,5-di-(o-tolylimino)-1, 2, 4-dithiazolidines (Hydrochlorides) (3a-h)

Sr.no	4-aryl-3,5-di-(o-tolylimino)-1, 2, 4-dithiazolidines (Hydrochlorides) (3a-h)	M.P. ^{O}C	% Yield	Rf value 7: 3 EtOAc: Pet ether	Elemental Analysis Found (Required)	
					N	S
1.	**3a**	102	80	0.65	10.75 (10.79)	16.39 (16.45)
2.	**3b**	185	83	0.68	10.25 (11.08)	16.89 (16.89)
3.	**3c**	196	85	0.86.	10.25 (11.08)	16.89 (16.89)
4.	**3d**	110	72	0.78	9.85 (9.92)	15.08 (15.13)
5.	**3e**	172	78	0.88.	9.28 (9.63)	13.99 (14.02)
6.	**3f**	130.	86	0.78	9.28 (9.63)	13.99 (14.02)
7.	**3g**	161	75	0.75	9.58 (9.73)	14.58 (14.83)
8.	**3h**	144	79	0.70	9.18 (9.25)	15.20 (15.27)

Biological Evaluation:

All the compounds have been screened for both antimicrobial and antifungal activity using cup plate agar diffusion method[19-20] by measuring the inhibition zone in mm. the compounds were taken at a concentration of 1 mg/ml using dimethyl sulphoxide (DMSO) as solvent.(**Table-2**)

Antibacterial activity

Gentamycin (100 µg/ml) was used as standard for antibacterial activity. The compounds were screen for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* species by using Nutrient Agar medium.

Antibacterial studies of these compounds indicated that compounds **3a**, **3d**, **3e** and **3g** show appreciable activity towards *E. coli*. Compounds **3c, 3f, 3g** and **3h** shows good activity against *S. aureus*. All the other compounds exhibited low to moderate activity.

Antifungal activity

The compounds were screen for antifungal activity against *Pinicillium notatum* and *Aspergillus niger* species was determined by using Potato Dextrose Agar medium. Fluconazole (100 µg/ml) as standard for antifungal activity

All compounds displayed promising activity against *P. notatum* and are effective towards *A. niger*. While other compounds inhibited moderate to low activity.

Table-2: Antimicrobial activity of 4-aryl-3,5-di-(o-tolylimino)-1, 2, 4-dithiazolidines (Hydrochlorides) (3a-h)

4-aryl-3,5-di-(o-tolylimino)-1, 2, 4-dithiazolidines (Hydrochlorides) (3a-h)	Antibacterial**		Antifungal**	
	E. coli	*S. aureus*	*P. notatum*	*A. niger*
3a	20	17	21	20
3b	18	17	24	20
3c	19	19	23	22
3d	22	18	23	18
3e	20	18	22	18
3f	18	22	20	19
3g	20	21	23	17
3h	18	20	24	22
Gentamycin	24	22	-	-
Fluconazole	-	-	26	24

Conclusion:

1, 2, 4-dithiazolidines (Hydrochlorides derivatives were synthesized and characterized for their structure elucidation. Various chemical and spectral data supported the structures. The method adopted in this investigation is simple, efficient and inexpensive and is useful in synthesizing pharmacologically important molecules.

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