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SYNTHESIS AND BIOLOGICAL EVALUATION OF 2-ARYLIMINO-5-HYDRAZINO-1,3,4-THIADIAZOLIDINES

Reshal Deshmukh
Department of Chemistry, Shivaji Science College,
Congress Nagar, Nagpur-12 (MS), India.

ABSTRACT:
Distinctive synthesis of some new 1,3,4 thiadiazolidines(IV) were produced by the reaction of Thiocarbohydrazide(I) and N-aryl isocynodichloride(II) by refluxing in chloroform medium. The reaction gave 2-aryl-5-hydrazino-1,3,4-thiadiazolidine hydrochloride(III), which on basification with dilute ammonium hydroxide solution gave compound(IV). The compound (IV) thus prepared, were acetylated by acetic anhydride and glacial acetic acid. The structure of acetyl derivatives(V) were established on the basis of elemental analysis, equivalent weight determination, IR and PMR spectral studies.

Keyword: 5-membered ring system with N & S, Diaza derivative of thiophene, vital important Antibacterial drug

INTRODUCTION:

Five-membered ring compounds such as imidazole, oxazole, thiazole, oxadiazole and thiadiazole, generally have biological activities. The synthesis of substituted thiadiazoles and related compounds have attracted the attention as these compounds constitute the structural frameworks of several naturally occurring compounds. In recent decades, research it has shown that the thiadiazole ring is an important framework with broad-spectrum biological activity. 1,3,4 Thiadiazolidine moiety show a wide spectrum of biological activities such as carbonic anhydrase inhibitors, analgesic, anti-inflammatory, anti-bacterial etc. Thiadiazolidines are of vital importance as drugs. These ring systems have been successfully incorporated in commercial drugs and pesticides in past and still offers chances to various new types to increase their activities.

Thiadiazolidines are the five membered heterocyclic systems in which two Nitrogen and sulphur atoms are incorporated .For the synthesis of 1,3,4 thiadiazolidines the cyclisation and other routes have been employed earlier[1-8]. In view to explore the new route for synthesis of1,3,4- thiadiazolidines, N-aryl isocynodichloride was treated with thiocarbohydrazide in equimolar proportion. Therefore, it appeared sufficiently interesting to prepare aryl isocynodichloride and use these reagents as intermediate in the synthesis of nitrogen and sulphur containing heterocyclic

compound. With this aim in mind, N-phenylisocynodichloride was prepared and its reaction with substituted thiosemicarbazides have been carried out and 1, 3, 4,-thiadiazolidines have been isolated in good yield.

MATERIAL AND METHOD:

The present paper is an account of N-aryl isocynodichloride(II) and thiocarbohydrazide(I) for the synthesis of 2 -arylimino-5-hydrazino-1,3,4-thiadiazolidine which were further converted into their acetyl derivative

1 Preparation of Thiocarbohydrazide[13,14] (I)-
24 ml hydrazine hydrate was mixed with 50ml of hot distill water. To this solution was added 40ml of carbon disulphide drop by drop in water bath at 50-60^{o}C .After the addition it was refluxed for 1-2 hour in water bath, when the solution changes to dark brown. The solution was allowed to stand overnight when the crystals of thiocarbo hydrazide separates' out.

2 Preparation of N-aryl isocyanodichloride[15] (II)-
To a chloroformic solution of aryl isothiocyanate (0.01mole) gaseous chlorine (0.2mole) was passed at the rate of 7g/hr at 10-15^{0}C and the required N-aryl isocynodichloride was obtained. The

different isocynodichloride(IIa-IId)were prepared by extending the above reaction of excess chlorine gas with corresponding isothiocyanates and related isocynodichlorides were isolated.

3 Interaction of thiocarbohydrazide (I) and N-aryl isocynodichloride (II)

Formatation of 2-arylimino-5-hydrazino-1,3,4-thiadiazolidine hydrochloride (III) and their free bases (IVa-d)-

Synthesis of 2-phenylimino-5-hydrazino-1,3,4-thiadiazolidines(IVa) -

To a solution of thiocarbohydrazide in chloroform(I) (0.01mole in 20ml)was mixed phenyl isocyanodichloride(IIa)(0.01mole). The resultant mixture was refluxed for 3hrs over a boiling water bath. During the reaction evolution of hydrogen chloride gas was noticed .After completion of reaction the solvent was distill off and semisolid sticky mass was obtained,it was washed with petroleum ether (60-80^{o}C) which afforded a yellowish granular solid (IIIa). The compound (IIIa) was detected to be acidic to litmus. On determination of Eq.wt it was found to be monohydrochloride of 2-phenylimino-5-hydrazino-1,3,4-thiadiazolidine(Eq.Wt found 195,requires 207).On basification of (IIIa) with dilute amm.hydroxide afforded a free base(IVa) m.p 158^{0}C .

Synthesis of 2-o-tolylimino-5-hydrazino-1,3,4-thiadiazolidines(IVb) -

The chloroformic solution of thiocarbohydrazide (I)(0.01molein 20ml) was mixed with (0.01mole) N-o-tolyl isocyanodichloride(IIb) in chloroform. The reaction mixture was refluxed for 3 hrs, over a boiling water bath. The evolution of hydrogen chloride gas was noticed. After completion of reaction the solvent was distill off and semi solid sticky mass was obtained,it was washed with petroleum ether (60-80^{o}C) which afforded a yellowish granular solid (IIIb). The compound (IIIb) was detected as acidic to litmus. On determination of Eq.wt it was found to be monohydrochloride of 2-o-tolylimino-5-hydrazino-1,3,4-thiadiazolidine (Eq.Wt found 212,requires 221).On basification of (IIIb) with dilute ammo. Hydroxide afforded a free base(IVb) m.p 166^{0}C .

Synthesis of 2-m-tolylimino-5-hydrazino-1,3,4-thiadiazolidines(IVc) -

The chloroformic solution of thiocarbohydrazide (I)(0.01mole in 20ml) was mixed with (0.01mole) N-m-tolyl isocyanodichloride(IIc) in chloroform. The reaction mixture was refluxed for 3hrs, over a boiling water bath. The evolution of hydrogen chloride gas was noticed. After completion of reaction the solvent was distill off and semisolid sticky mass was obtained,it was washed with petroleum ether (60-80^{o}C) which afforded a yellowish granular solid (IIIc). The compound (IIIc) was detected to be acidic to litmus.On determination of Eq.wt it was found to be monohydrochloride of 2-m-tolylimino-5-hydrazino-1,3,4-thiadiazolidine(Eq.Wt found 278,requires 221).On basification of (IIIc) with dilute amm.hydroxide afforded a free base(IVc) m.p 210^{0}C .

Synthesis of 2-p-tolylimino-5-hydrazino-1,3,4-thiadiazolidines(IVd) -

The chloroformic solution of thiocarbohydrazide (I)(0.01molein 20ml) was mixed with (0.01mole) N-p-toly lisocyanodichloride(IId) in chloroform. The reaction mixture was refluxed for 3hrs, over a boiling water bath. The evolution of hydrogen chloride gas was noticed. After completion of reaction the solvent was distill off and semisolid sticky mass was obtained,it was washed with petroleum ether (60-80^{o}C) which afforded a yellowish granular solid (IIId). The compound (IIId) was detected to be acidic to litmus. On determination of Eq.wt it was found to be monohydrochloride of 2-p-tolylimino-5-hydrazino-1,3,4-thiadiazolidine(Eq.Wt found 210,requires 221).On basification of (IIId) with dilute amm.hydroxide afforded a free base(IVd) m.p 190^{0}C .

4 Interaction of 2-aryl imino-5- hydrazino-1,3,4-thiadiazolidines(IV) and acetic anhydride with glacial . acetic acid :

Formatation of 2-arylimino-5- acetyl hydrazino-1,3,4-thiadiazolidine (Va-d) - The compound IVa,IVb, IVc and IVd (0.01mole) was treated with acetic anhydride(0.01mole) and acetic acid as solvent(10ml).The mixture was refluxed over boiling water bath for 1hr followed by dilution with water,afforded (Va, Vb,Vc andVd). It was crystallized from ethanol.

REACTION SCHEME:

The formation of 2-Arylimino-5-hydrazino -1,3,4-thiadiazolidine (IV) and their acetyl derivative (V) can be stated as-

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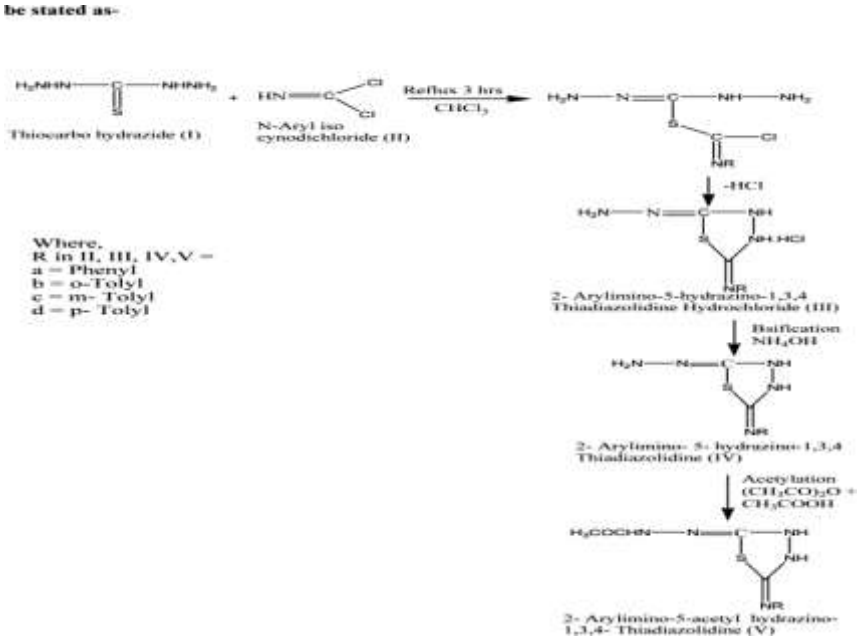

Table 1: Formation of 2- phenylimino-5-hydrazino- 1,3,4-thiadiazolidines(IV)

Sr. no	**N-aryl isocynodichloride (II)**	**2-phenylimino-5-hydrazino-1,3,4-thiadiazolidine hydrochloride (III)**	**2,phenylimino-5- hydrazino 1,3,4-thiadiazolidines (IV)**	**M P ^{o}C (I)**	**Mol.Formla**	Elemental analysis(%) Found(Calculated)			
						C	**H**	**N**	S
1	N-phenyl-isocyano dichloride(**IIa)**	2-phenylimino-5-hydrazino 1,3,4-thiadiazolidine hydrochloride **(IIIa)**	2-phenylimino-5-hydrazino 1,3,4-thiadiazolidine **(IVa)**	135	$C_{10}H_{11}N_5SO$	47.86 (48.19)	4.33 (4.41)	27.91 (28.11)	12.66 (12.85)
2	N-o-tolyl isocyano dichloride**(IIb**)	2-o-tolylimino-5-hydrazino 1,3,4-thiadiazolidine hydrochloride **(IIIb**)	2-o-tolylimino-5-hydrazino 1,3,4-thiadiazolidine **(IVb)**	164	$C_{11}H_{13}N_5SO$	49.88 (50.19)	4.68 (4.94)	26.46 (26.61)	11.68 (12.16)
3	N-m-tolyl isocyano dichloride**(IIc)**	2-m-tolylimino-5-hydrazino 1,3,4-thiadiazolidine hydrochloride **(IIIc)**	2-m-tolylimino-5-hydrazino 1,3,4-thiadiazolidine **(IVc)**	180	$C_{11}H_{13}N_5SO$	49.85 (50.19)	4.76 (4.94)	26.49 (26.61)	12.01 (12.16)
4	N-p-tolyl isocyano dichloride**(IId)**	2-p-tolylimino-5-hydrazino 1,3,4-thiadiazolidine hydrochloride **(IIId)**	2-p-tolylimino-5-hydrazino 1,3,4-thiadiazolidine **(IVd)**	194	$C_{11}H_{13}N_5SO$	49.76 (50.19)	4.76 (4.94)	26.42 (26.61)	11.93 (12.16)

Reactants: *Thiocarbohydrazide (I) and N-arylisocynodichloride (II)*

Table 2: Formation of 2-phenylimino-5- acetyl hydrazino-1, 3, 4-thiadiazolidines (V)

Sr. no	2-phenylimino-5-hydrazino1,3,4-thiadiazolidine (IV)	2,phenylimino-5-acetyl hydrazino 1,3,4-thiadiazolidines (V)	M.P ^{0}C	Mol.Formula	Elemental analysis(%) Found(Calculated)			
					C	H	N	S
1	2-phenylimino-5-hydrazino 1,3,4-thiadiazolidine **(IVa)**	2-phenylimino-5-acetyl hydrazino 1,3,4- thiadiazolidine **(Va)**	135	$C_{10}H_{11}N_5SO$	47.86 (48.19)	4.33 (4.41)	27.91 (28.11)	12.66 (12.85)
2	2-o-tolylimino-5-hydrazino 1,3,4-thiadiazolidine **(IVb)**	2-o-tolylimino-5-acetyl hydrazino 1,3,4- thiadiazolidine **(Vb)**	164	$C_{11}H_{13}N_5SO$	49.88 (50.19)	4.68 (4.94)	26.46 (26.61)	11.68 (12.16)
3	2-m-tolylimino-5-hydrazino 1,3,4-thiadiazolidine **(IVc)**	2-m-tolylimino-5-acetyl hydrazino 1,3,4- thiadiazolidine **(Vc)**	180	$C_{11}H_{13}N_5SO$	49.85 (50.19)	4.76 (4.94)	26.49 (26.61)	12.01 (12.16)
4	2-p-tolylimino-5-hydrazino 1,3,4-thiadiazolidine **(IVd)**	2-p-tolylimino-5-acetyl hydrazino 1,3,4- thiadiazolidine **(Vd)**	194	$C_{11}H_{13}N_5SO$	49.76 (50.19)	4.76 (4.94)	26.42 (26.61)	11.93 (12.16)

Reactants: *2- phenylimino-5-hydrazino- 1,3,4-thiadiazolidines(IV) and acetic anhydride*

Antimicrobial Activity 2-Arylimino-5-hydrazino -1,3,4-thiadiazolidine (IV)

The 1, 3, 4-thiadiazolidines (IV) when screened in vitro against some common bacteria viz. E. coli,S. aureaus, B. subtilis, P. Argenosa[17,18] it was noticed that most of all these compounds have shown remarkable inhibitory activity.

An assay of newly synthesized 1, 3, 4-thiadiazolidines (IV) revels that, almost all the compounds were strongly active against all the test pathogens E .coli , S. aureus , B. Subtilis and P. Argenosa. Their inhibitory impact on the bacterial growth is remarkable The minium inhibitory concentration (MIC) values were determined by serial dilution method[22-23] by dissolving compounds in methanol.

1 Preparation of sample: 0.001 g/ 1 mg was taken and dissolved in 1 ml of methanol

2 Preparation of inoculums: Stock cultures were maintained at 4 ^{0}C on slants of nutrient agar. Active cultures of experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated for 24 hrs at 37^0 C .

Table-3: Results of Antimicrobial Testing for 2-Arylimino-5-hydrazin-1,3,4-thiadiazolidine IVa to Ivd

Organisms	Compound (Concentration 100ug /ml)			
	IVa	**IVb**	**IVc**	**Ivd**
E coli	15mm	10mm	14mm	12mm
S aureus	18mm	14mm	20mm	14mm
P vulgaris	18mm	14mm	20mm	35mm
B subtilis	-----	14mm	----	14mm

Streptomycin was used as standard

(Less than 12mm) Inactive

(12-16 mm) Weakly active

(17-20mm)Moderately active

(21-37mm) Highly active

3 Screening of Bacteria: The disk diffusion method was used for antimicrobial activity. The nutrient agar were poured in Petri plates and allowed it to solidify. The above prepared microbial cultures were spread uniformly on the surface of the agar. The diffused disks of each sample are placed on the agar. Plates were then incubated at 37°C for 24hrs. Antimicrobial results are shown in Table 3.

RESULT:

Thiocarbohydrazide (I) and N-phenyl isocynodichloride (IIa) were mixed in same proportion and refluxed in chloroform medium for 3 hrs. The evolution of hydrochloride gas was noticed .After completion of reaction and distilling

off solvent afforded sticky mass, which on washing with petroleum ether (60-80^0) followed by addition of little amount of ethanol gave pale yellow solid(IIIa). It was crystallized from ethanol, mp 180^{0}C . It was found to be monohydrochloride of 2-phenylimino-5-hydrazino-1,3,4-thiadiazolidine on the basis of equivalent weight determination (Eq wt. Found 198. $C_8H_9N_5S$ requires 207) Basification **of** (IIIa) with ammonium hydroxide afforded free base (IVa). It was crystallized from ethanol mp. 158^{0}C**.** On the basis of elemental analysis of product with mp 158^{0}C the molecular formula was(Found C=46.12, H=4.18, N=33.69 and S=15.45% $C_8H_9N_5$requires C=46.37, H=4.34, N=33.81 and S=15.45%)

The compound (IVa) gave positive test for N and S elements. It was found non desulphurisable, when boiled with alkaline plum bite solution. On pyrolysis characteristic smell of phenyl isothiocyanate was quite perceptible.

The Infrared analysis[10] of (IVa) showed the presence of ᵘNH **s**tretching at 3400-3100cm^{-1}**,** ᵘC=N stretching at 1689-1471cm^{-1}**,** ᵘC-S stretching at 800-600cm^{-1}, ᵘN-N stretching at1202cm^{-1} and C-N stretching at 1350-1280cm^{-1}

The PMR spectrum of (IVa) distinctly displayed the signals due to aromatic protons(δ 6.9 to 7.2), NH protons(δ 7.4ppm) and NH_2 protons at (δ5.7ppm)**.**

On the basis of above facts the compound (IVa) was assigned the structure 2-phenylimino-5-hydrazino-1,3,4-thiadiazolidine. Compound (IVb-IVd) were synthesized by extending the reaction of Thiocarbohydrazide (I) and N-phenyl isocynodichloride (IIb-IId) the related 1,3,4,thiadiazolidine (IVb-IVd) were Isolated in Good Yield. 2-phenylimino-5-hydrazino-1,3,4-thiadiazolidine (IVa) on Refluxing with Glacial acetic acid and acetic anhydride mixture in 1:1 ratio for one hour followed by distillation with water afforded a solid (Va). 2-Phenylimino-5-acetyl hydrazino-1,3,4-Thiadiazolidine (Va) crystallized with ethanol M.P.135^0 C .The (Va) gave positive test for N,S element and for –COCH3 group. The elemental analysis of the product indicated its Molecular formula $C_{10}H_{11}N_5SO$ (Found C=47.86%, H=4.33, %, N=27.9 %, S=12.66%) calculated for ($C1_0H_{11}N_5SO$ C=48.19%, H=4.41%, N=28.11%, S=18532%)

The IR analysis showed the presence of following absorption band due to ᵘNH(3267 cm-1),ᵘC=N(1593 cm-1), ᵘC-N(1329 cm-1) ,ᵘN-N(1190cm-1), ᵘC-S(693 cm-1), ,ᵘC=O(1643 cm-1), On the basis of above facts the compound (Va) has been assigned the structure as 2-Phenylimino -5 -acetyl hydrazino -1,3,4-Thiadiazolidine (Va) . The other related acetyl derivatives (Vb-Vd) were prepared by extending the above reaction.

An assay of newly synthesized 1, 3, 4-thiadiazolidines (IV) revels that, almost all the compounds were strongly active against all the test pathogens E .coli , S. aureus , B. Subtilis and P. Argenosa. Their inhibitory impact on the bacterial growth is remarkable The minimum inhibitory concentration (MIC) values were determined by serial dilution method by dissolving compounds in methanol.

CONCLUSION:

The thiadiazolidine ring is an important framework with broad-spectrum biological activity. 1,3,4 Thiadiazolidine containing compounds show wide spectrum of biological activities such as carbonic anhydrase inhibitors, analgesic, anti-inflammatory, anti-bacterial etc. Thiadiazolidines are of vital importance as drugs. These ring systems have been successfully incorporated in commercial drugs and pesticides in past and still offers chances to various new types to increase their activities.

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