SYNTHESIS AND BIOLOGICAL ACTIVITY OF PYRAZOLO[3,4-D]THIAZOLE DERIVATIVES OF 2-((1H-BENZO[D]IMIDAZOL-2-YL)THIO) ACETO HYDRAZIDE

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Abstract:
4-Thiazolidinone derivatives of 2-((1H-benzo[d]imidazol-2-yl) thio) aceto hydrazide undergo condensation with benzaldehyde to give Arylidine derivatives (2a-e) in good yields. The cyclo condensation of compounds (2a-e) with Phenyl hydrazine yields Pyrazolo [3,4-d]thiazole derivatives (3a-e). The structures of all compounds were confirmed on the basis of analytical and spectral data. All the newly synthesized compounds were evaluated for their antibacterial and antifungal activities.

Keywords: 2-((1H-benzo[d]imidazol-2-yl) thio) aceto hydrazide, 4-Thiazolidine, Arylidine, Pyrazolo [3,4-d] thiazole, analytical and spectral data, antibacterial and antifungal activity.

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Introduction:

Benzimidazole is a heterocyclic aromatic organic compound which enjoys the attention as a versatile Pharmacophore in medicinal chemistry. The benzimidazole ring is one of the privileged scaffolds for the development and synthesis of novel molecules of therapeutic value [1]. Number of approved common used medicines having benzimidazole moiety [2-5].

Benzimidazole (BMZ) family of drugs has been now repurposed as anti-cancer drugs. However, offering a general reformulation method for these drugs is essential due to their hydrobhobicity and low aqueous solubility [6]. Diabetes mellitus (DM) is a common chronic disease in constant growth that is taking on epidemic proportions especially in developing countries. More than 220 million people worldwide suffer from DM and this figure is expected to increase to 400 million cases by 2030 [7]. The 5-Arylidine-4-thiazolidinone derivatives are reported well for treatment of diabetic complications [8]. Recently 5-Arylidene-2-oxo-4-thiazolidinones and 2-phenylimino analogues were evaluated for their anti degenerative activity on human chondrocyte cultures and results indicated that 5-arylidene-4-thiazolidinone derivatives exhibit anti degenerative activity and could block multiple cartilage destruction during the osteoarthritis process [9]. Initially pyrazolo [3,4-d] thiazole derivatives were prepared by dye research laboratory [10]. Pyrazolo [3,4-d] thiazole derivatives reported as a potential anti-HIV Inhibitors [11,12]. C.N. Khobragade and his Team [13] reported synthesis and biological activity of pyrazolo [3,4-d] thiazolo[3,2-a] pyrimidin-4-one derivatives. Poonam Gautam and R. P. Chaudhary [14] reported facile synthesis of substituted dihydro-1H-pyrazolo [3,4-d] thiazoles through enaminones of 4-thiazolidiones.

In view of the above biological importance and in continuation of our studies on the synthesis and characterization of novel pyrazolo [3,4-d] thiazole [11–14], here we report facile synthesis of fused pyrazolo[3,4-d]thiazoles from 4-thiazolidinones through arylidine intermediates.

Material and methods:

The chemicals used to synthesize the compounds were of laboratory grade. The melting points of synthesized compounds were measured by open capillary method and they were uncorrected. The purity of compounds was observed by TLC using silica gel coated aluminium plates (Merck) and spots were visualized under UV light. The IR spectra were recorded in KBr pellets on a Nicolet 400D spectrometer. Mass spectra were recorded on LC-MSD-Trap-SL_01046 and NMR analysis was carried out on Bruker spectrometer at 400 MHz using DMSO as solvent and TMS as an internal standard. Elemental content of all compounds determined by Thermofinigan Flash EA (Italy). The sulfur and halogen determined by carious method.

General procedure:

Step-I: Synthesis of (Z)-2-((1H-benzo[d] imidazol-2-yl)thio)-N-(5-benzylidene-4-oxo-2-(5 -substituted phenylfuran-2-yl)thiazolidin-3-yl) acetamide (5a-e):

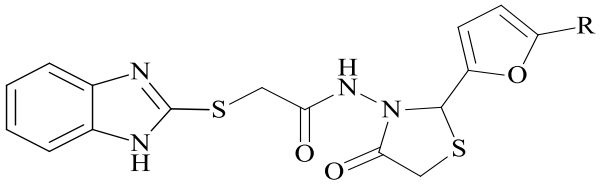
The 5-arylidine derivatives were prepared by reported process in literature [15-17].

A mixture of 4-Thiazolidinone derivatives (3a-e) (0.01 mol), benzaldehyde (0.01 mol) and piperidine (0.08 mol) in Ethanol (50 mL) was refluxed for 24 hrs. The reaction mixture was poured into water and acidified with glacial Acetic acid until pH 3-4 to give a crude solid. The product was crystallized from methanol providing pure compounds (5a-e). The yields, melting points and other characterization data of these compounds are given in Table-1.

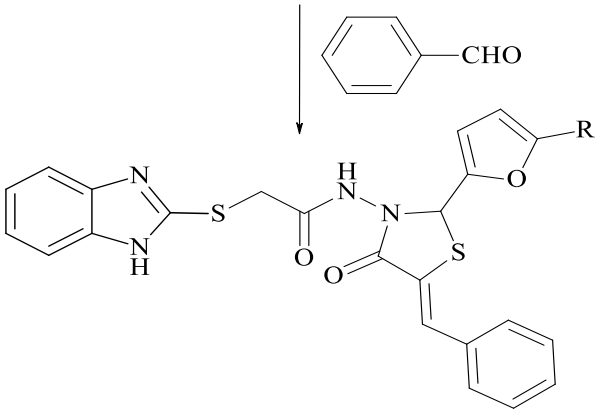
Step-II: Synthesis of 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(2,3-diphenyl-5-(5-substitutedphenylfuran-2-yl)-3,3a-dihydro-2H-pyrazolo[3,4-d]thiazol-6(5H)-yl) acetamide (6a-e):

The pyrazolo[3,4-d]thiazole derivatives were synthesized by reported process in literature [18-20].

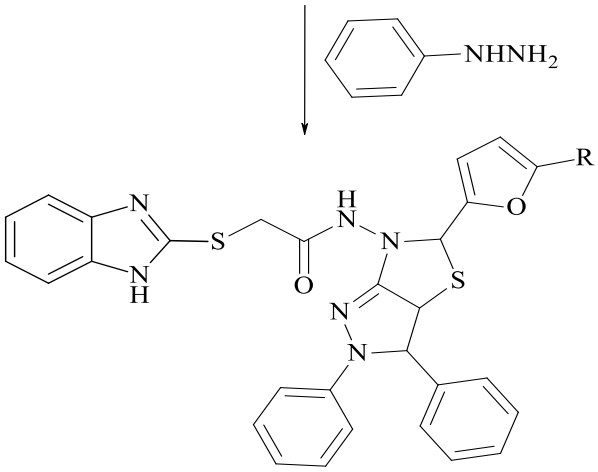
A mixture of 5-arylidine derivatives (5a-e) (0.01 mol), phenyl hydrazine (0.015 mol), and anhydrous sodium acetate (0.02 mol) in ethanol (25 mL) was heated under reflux for 20–24 hrs. The progress of the reaction was monitored by TLC. The solvent was removed by distillation. Finally the inorganic salts were removed by adding water into reaction mass and product were extracted using methylene dichloride. The organic layer was distilled of to get crude product (6a-e). The crude product was crystallized in methanol providing pure compounds (6a-e). The yields, melting points and other characterization data of these compounds are given in Table-2. The whole synthetic route was given in scheme1.



4-Thiazolidinone derivatives of 2a-e **[3a-e]**



Arylidine derivatives of 3a-e **[5a-e]**



Pyrazolo [3,4-d] thiazole derivatives **[6a-e]**

Where, R = C_6H_5, 3-NO_2-C_6H_4, 4-Cl-C_6H_4, 4-Br-C_6H_4, 2,4-Cl_2-C_6H_3

Scheme 1

Table-1: Yield, melting point and elemental analysis of compounds (5a-e)

Compd.	Molecular formula (Mol. Wt.)	Yield	M.P.°	Elemental analysis							
				%C		%H		%N		%S	
				Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
5a	$C_{29}H_{22}N_4O_3S_2$ (538.64)	87	120-122	64.66	65.00	4.12	4.10	10.40	10.40	11.91	12.00
5b	$C_{29}H_{21}N_5O_5S_2$ (583.64)	89	107-110	59.68	59.70	3.63	3.60	12.00	12.00	10.99	11.00
5c	$C_{29}H_{21}ClN_4O_3S_2$ (573.09)	92	110-112	60.78	60.70	3.69	3.70	9.78	9.80	11.19	11.20
5d	$C_{29}H_{21}BrN_4O_3S_2$ (617.54)	88	114-115	56.40	56.40	3.43	3.40	9.07	9.10	10.38	10.40
5e	$C_{29}H_{20}Cl_2N_4O_3S_2$ (607.53)	87	127-128	57.33	57.30	3.32	3.30	9.22	9.20	10.56	10.60

For, Compound 5c: % Cl = 6.19% (Calculated) & 6.20% (Found); Compound 5d: % Br = 12.94% (Calculated) & 12.90% (Found); Compound 5e: % Cl = 11.67% (Calculated) & 11.70% (Found).

Table-2: Yield, melting point and elemental analysis of compounds (6a-e)

Compd.	Molecular formula (Mol. Wt.)	Yield	M.P.°	Elemental analysis							
				%C		%H		%N		%S	
				Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
6a	$C_{35}H_{28}N_6O_2S_2$ (628.77)	55	145-147	66.86	66.90	4.49	4.50	13.37	13.30	10.20	10.20
6b	$C_{35}H_{27}N_7O_4S_2$ (673.76)	58	126-128	62.39	62.40	4.04	4.00	14.55	14.50	9.52	9.50
6c	$C_{35}H_{27}ClN_6O_2S_2$ (663.21)	60	140-142	63.38	63.40	4.10	4.10	12.67	12.60	9.67	9.60
6d	$C_{35}H_{27}BrN_6O_2S_2$ (707.66)	62	132-134	59.40	59.40	3.85	3.80	11.88	11.90	9.06	9.10
6e	$C_{35}H_{26}Cl_2N_6O_2S_2$ (697.66)	63	128-130	60.26	60.20	3.76	3.80	12.05	12.00	9.19	9.20

For, Compound 6c: % Cl = 5.35% (Calculated) & 5.30% (Found); Compound 6d: % Br = 11.29% (Calculated) & 11.30% (Found); Compound 6e: % Cl = 10.16% (Calculated) & 10.10% (Found).

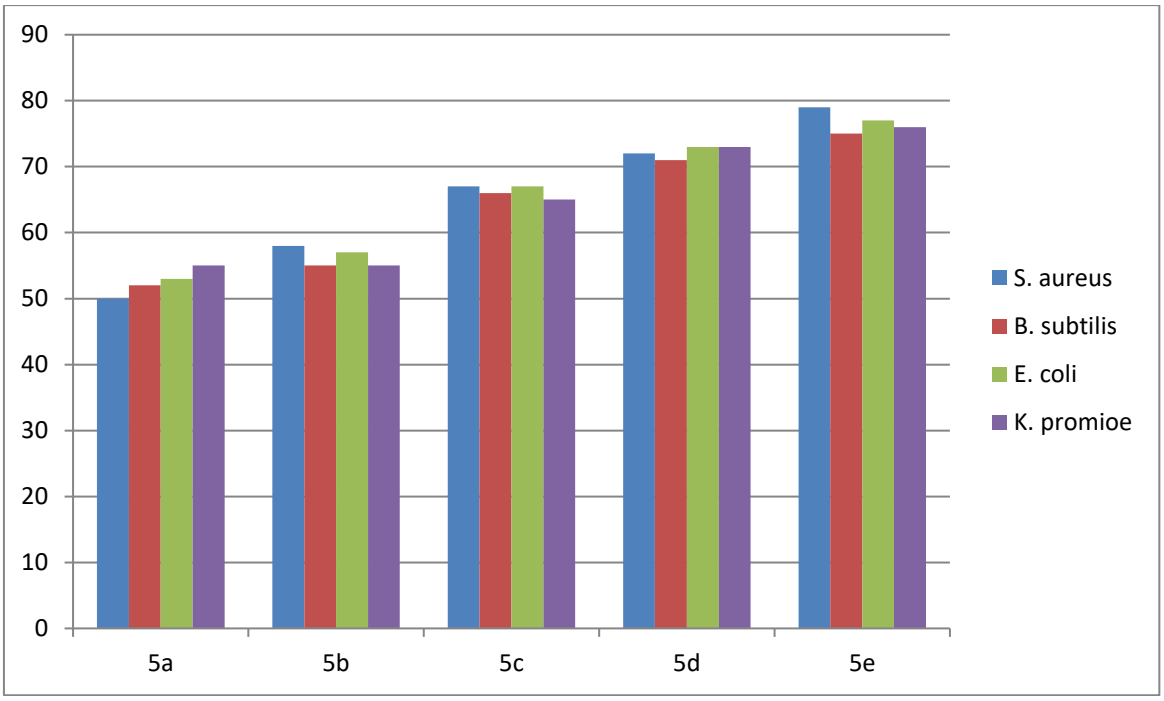
Biological screening:

Antibacterial activities

The antibacterial activities of all the compounds were studied against gram-positive bacteria (*Staphylococcus aureus and Bacillus subtilis*) and gram-negative bacteria (*E.coli, and klebsiella promioe*) at a concentration of 50µg/ML by agar cup plate method. A methanol system was used as control in this method. Similar conditions using tetracycline as a control was used standard for comparison [21,22]. The % area of inhibition of zone measured in mm. Compounds 5e, 5d and 5c were found to be toxic for microbes.

Table-3: Antibacterial activity of compounds (5a-e & 6a-e)

Compounds	Gram +Ve		Gram –Ve	
	Staphylococcus aureus	*Bacillus subtilis*	*E.coli*	*Klebsiella promioe*
5a	50	52	53	55
5b	58	55	57	55
5c	67	66	67	65
5d	72	71	73	73
5e	79	75	77	76
6a	52	55	54	57
6b	52	54	56	54
6c	66	67	68	66
6d	73	72	72	74
6e	78	76	76	78



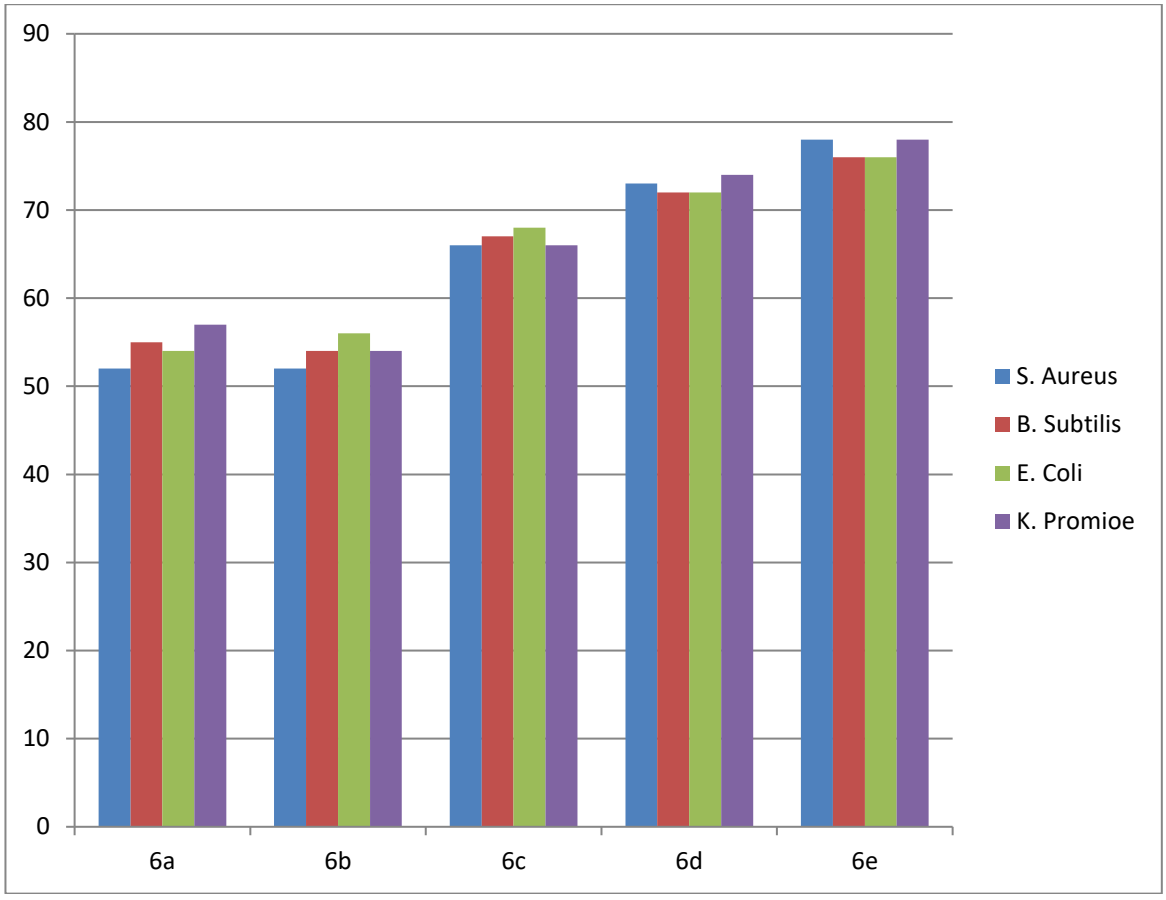


Figure 1: Antibacterial activities of compounds (5a-e & 6a-e)

Antifungal activities

The fungicidal activity of all the compounds was studied at 1000 ppm concentration in vitro. Plant pathogenic organisms used were *Nigrospora Sp, Aspergillus niger, Botrydepladia thiobromine, and Rhizopus nigricum, Fusarium oxyporium*. The antifungal activity of all the compounds was measured on each of these plant pathogenic strains on a potato dextrose agar (PDA) medium. Such a PDA medium contained potato 200g, dextrose 20g, agar 20g and water. Five days old cultures were employed. The compounds to be tested were suspended (1000 ppm) in a PDA medium and autoclaved at 120^{o} C for 15 minute at 15 atmosphere pressure. These media were poured into sterile Petri plates and the organisms were inoculated after cooling the Petri plates. The fungicidal activity displayed by various compounds is shown in Table-4.

Table-4: Antifungal activity of compounds (5a-e & 6a-e)

	Zone of Inhibition at 1000 ppm (%)				
Compounds	**Nigrospora Sp.**	**Aspergillus Niger**	**Botrydepladia Thiobromine**	**Rhizopus Nigricum**	**Fusarium oxyporium**
5a	60	58	62	61	60
5b	62	63	64	63	61
5c	69	70	67	68	67
5d	67	68	66	65	68
5e	70	72	71	75	73
6a	61	59	63	60	62
6b	61	62	63	64	62
6c	68	71	66	67	66
6d	68	67	67	66	69
6e	72	73	70	74	76

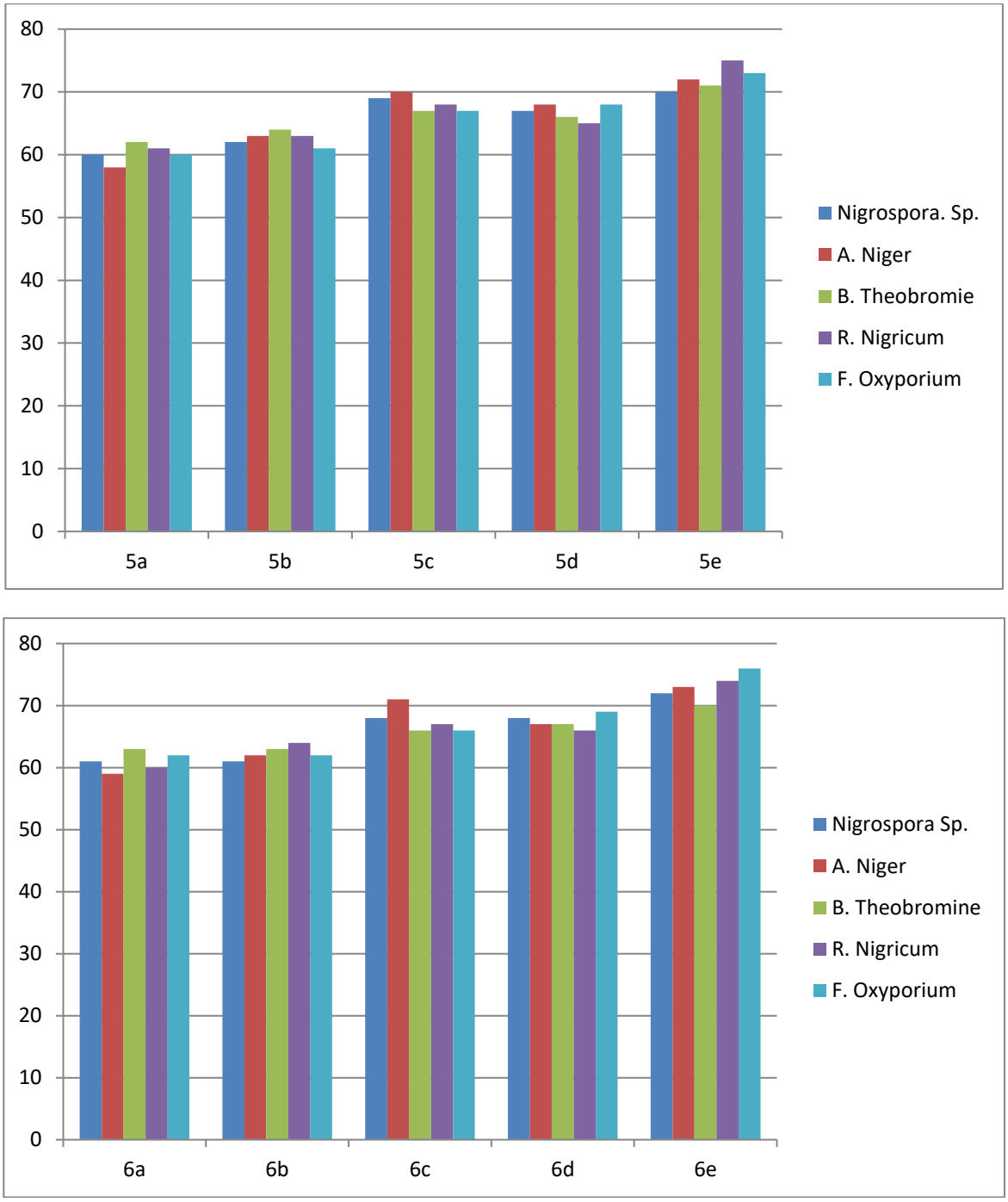


Figure 2: Antifungal activities of compounds (5a-e & 6a-e)

Results and discussion:

It was observed that 4-thiazolidione derivatives (3a-e) reacted with benzaldehyde to give (Z)-2-((1H-benzo[d]imidazol-2-yl) thio)-N-(5-benzylidene-4-oxo-2-(5-substituted-phenylfuran-2-yl) thiazolidin-3-yl) acetamide (5a-e). The structures of 5a-e were confirmed by elemental analysis (results given in Table-1) and IR spectra showing an absorption band at FT-IR (KBr): 1560 cm^{-1} (C=C), 1720-1660 cm^{-1} (C=O); ^1H NMR (300 MHz, DMSO-d_6): δ 7.35 (s, 1H, -$CH_{=}$C), 8.50 (s 1H, NH) & 7.0-8.0 (m, 10H, Aromatic & Furan). Finally, the compounds were characterized based of mass analysis data. The result of mass analysis match with the theoretical molecular weight of compound 5a-e.

The 5-Arylidine-4-thiazolidione derivatives (5a-e) further cyclized with phenyl hydrazine to give pyrazolo [3,4-d] thiazole derivatives (6a-e). The structures of 6a-e were confirmed by elemental analysis (results given in Table-2) and IR spectra showing an absorption band at FT-IR (KBr): 1584 cm^{-1} (C=N), 1650-1630 cm^{-1} (C=O); ^1H NMR (300 MHz, DMSO-d_6): δ 1.97 (s, 2H, -CH_2), 4.5 (s 1H, N-CH-S), 6.2 (s 1H, pyrazolo thiazole) & 7.1-

7.9 (m, 16H, Aromatic & Furan). Finally, the compounds were characterized based of mass analysis data. The result of mass analysis match with the theoretical molecular weight of compound 6a-e.

Conclusion: The Arylidine thiazolidinone derivatives (5a-e) and pyrazolo [3,4-d] thiazole derivatives (6a-e) were synthesized and characterized through various spectroscopy techniques. The biological potential of prepared derivatives was evaluated as effective antibacterial and antifungal agent.

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