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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NEW 2,5-DISUBSTITUTED 1,3,4-THIADIAZOLE DERIVATIVES.

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ABSTRACT

A series of [2-(substituted aryl)-3-{5-(substituted phenyl)-1,3,4-thiadiazole}] - 4-oxo-thiazolidines ***(3a-o)*** *derivatives were synthesized by the reaction of the substituted Schiff bases* ***(2a-o)*** *with thioglycollic acid in ethanol. Structure of the synthesized compounds were confirmed on the basis of physicochemical and spectral data (IR, [1]HNMR, [13]CNMR and Mass). All the synthesized compounds were screened of gram positive, gram negative bacteria using cup-plate-agar diffusion method. Among the series, compounds* ***3a, 3c, 3d, 3f, 3g and 3h*** *showed significant activity against Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis respectively as compared with standard cloxacillin.*

Key Words: Antibacterial activity, Schiff bases, 1,3,4-Thiadiazoles

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INTRODUCTION

Although mankind has developed a large arsenal of antibacterial agents, lethality in infectious diseases still is the 2nd leading cause of death worldwide.[1] Another worrisome fact is the constantly increasing number of microbial pathogens that develop or acquire resistance against antibiotics currently in use.[2,3] To combat resistant bacteria, there is an obvious need for effective strategies such as development of new antimicrobial drugs against not yet exploited targets.[4-6] Traditionally small molecules have been a reliable source for discovering novel biologically active compounds.[7,8]Azoles occupy a unique place in the realm of natural and synthetic organic chemistry. These compounds have intrinsic biological activities and constitute the structural feature of many bioactive compounds.

Heterocycles bearing a 1,3,4-thiadiazole moieties are reported to show a broad spectrum of pharmacological properties such as anti-inflammatory[9,10], antiviral[11], antibacterial[12,] 4-thiazolidinone is an imperative scaffold that not only synthetically important but also possesses a wide range of promising biological activities. 4-Thiazolidinone derivatives are known to possess antibacterial[13], antifungal[14], antiviral[15] and antituberculosis[16] properties.

Owing to the above facts and in continuation of our research work on novel heterocyclic compounds, herein we report the synthesis of some new 2,5-disubstituted-1,3,4-thiadiazole substituted thiazolidinone derivatives and evaluated for their antibacterial activity.

MATERIALS AND METHODS

All research chemicals were purchased from Sigma-Aldrich (St.Louis, Missouri, USA) used as such for the reactions. Solvents expect laboratory reagent grade were dried and purified according to the literature when necessary. Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel plates from E. Merk. (Mumbai). Melting points of synthesized compounds were determined in Thermonik (Mumbai, India) melting point apparatus and IR spectra were recorded on Thermo Nicolet IR200 FT-IR Spectrometer (Madison WI, USA) by using KBr pellets. The ^{1}HNMR were recorded on Bruker AVANCE 400 (Bruker, Germany) using DMSO-d_6 as solvent. Chemical shifts are reported in δ [ppm] units with respect to TMS as internal standard. The purity of all the compounds was examined by TLC on pre-coated silica gel plates from E. Merk. (Mumbai). using n-Hexane and Ethyl acetate (50:50) as a mobile phase and iodine vapors as visualizing agent.

Synthesis

General procedure for preparation of 2-amino-5-[substituted phenyl]-1,3,4-thiadiazole (1a-f):

To a mixture of substituted benzoic acid (0.1mole) and thiosemicarbazide (0.1mole), phosphorus oxy chloride (30 ml) added drop wise with constant stirring. It was refluxed gently for 1-2 h. The reaction mixture cooled to 5 ^{O}C. To this crushed ice (~90 gm) was added very carefully (exothermic reaction) and reaction mixture was further refluxed for 4 h, cooled and filtered. The filtrate was neutralized with saturated solution of KOH. The precipitated solid was filtered, washed with cold water, dried and recrystallised from absolute ethanol.

Procedure of Schiff bases {5-(substituted phenyl)-2-(2-substituted benzylideneamino) -1,3,4-thiadizole (2a-o):

A mixture of above synthesized 2-amino-5-[substituted phenyl]-1,3,4-thiadiazole (1a-f) (0.01mole) and different aromatic aldehydes (0.01 mole) in ethanol (35 ml) was refluxed on a water bath for about 5 h and cooled. The solid thus obtained was separated out filtered and recrystallised from chloroform to yielded the Schiff bases.

Procedure of [2-(substituted aryl)-3-{5-(substituted phenyl)-1,3,4-thiadiazole}]-4-oxo-thiazolidines (3a-o):

A mixture of above prepared Schiff bases i.e. {5-(substituted phenyl)-2-(2-substituted benzylidene amino)-1,3,4-thiadizole (2a-o) (0.01mole) and thioglycollic acid (0.01 mole) in ethanol (25 ml) were refluxed on a water bath for about 6 h. and cooled. The solid thus obtained was separated out , recrystallised from methanol.

Antibacterial activity

Test microorganisms

The antibacterial activity of synthetzed compounds was determined against standard strains of the Gram-positive bacteria *Staphylococcus aureus* (737), *Bacillus subtilis* (1133), Gram-negative *Pseudomonas aeruginosa* (1036), *Escherichia coli* (MTCC 1554) were used for the biological assays.

Antibacterial activity

For the antibacterial activity of compounds (3a-o) the test organism were revived by inoculating in broth media and grown at 37 ^{0}C for 18 h. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (200 µl) and spread evenly on the plate. After 20 min, the

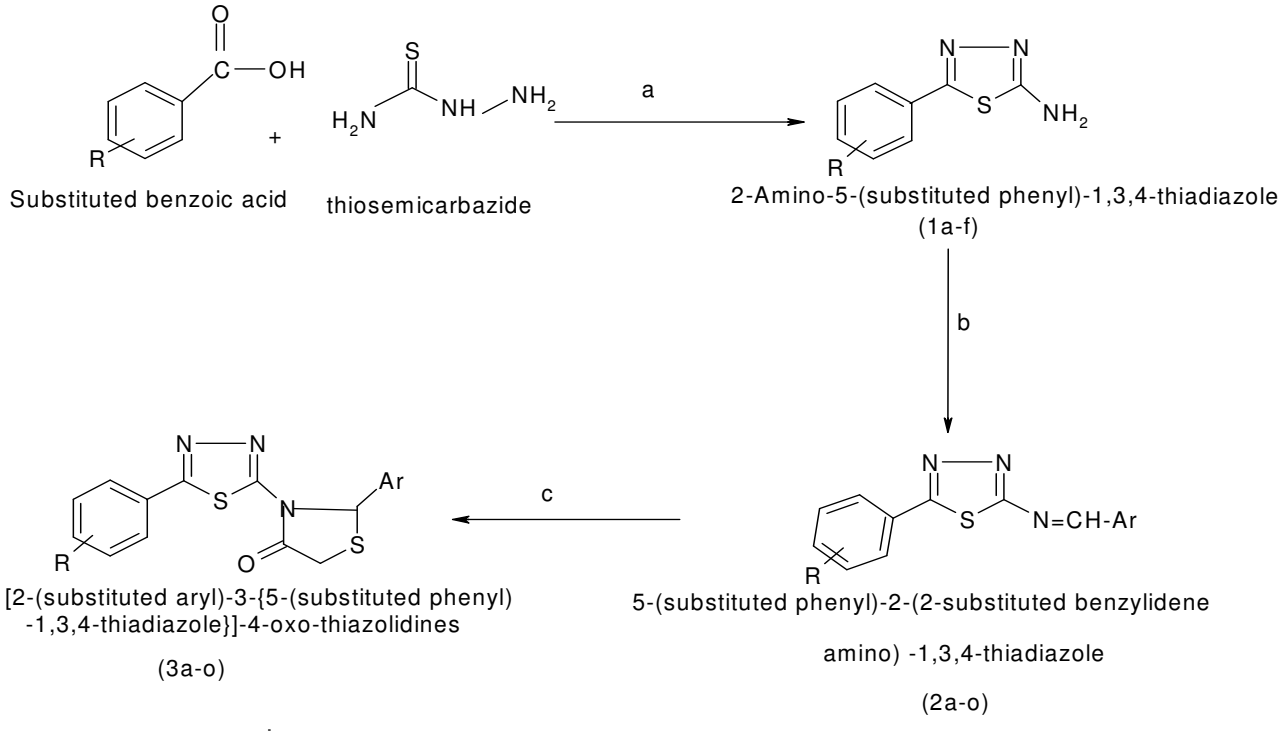
wells were filled with 100 µg of each compound (10mg/ml stock in DMSO). The control plates with antibiotic (10mg/ml stock) and DMSO were also prepared. All the plates were incubated at 37^0C for 24 h and the diameter of inhibition zone were noted. The compounds with significant activity were selected for the determination of minimum inhibitory concentration (MIC).The tubes containing the above media (5 ml, without agar) were autoclaved at 121^0C, 15 lbs. Each tube was added with the required volume of the compound, enough for concentrations varying from 100, 50, 25, 12.5, 6.25, 3.125 µg/ml and inoculated with 18 h old cultures (50µl) and mixed gently. The control tubes were added with antibiotic and DMSO was also prepared. All the tubes were incubated at 37^0C for 24 h and the absorbance of the biomass was read at 660 nm, against autoclaved media as blank along with the compound, without inoculums.[18] Minimum inhibitory concentration (MIC) of the synthesized compounds were determined against *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa* by serial dilution technique.

RESULT AND DISCUSSION

Synthetic

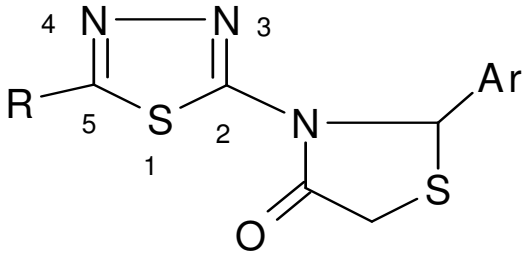
A new series of [2-(substituted phenyl)-3-{5-(substituted phenyl)-1,3,4-thiadiazole}]-4-oxo-thiazolidines (3a-o) was achieved through the versatile and efficient synthetic route[19] outlined in Scheme-1. 2-amino-5-(substituted phenyl)-1,3,4-thiadiazole(1a-f) were obtained by condensation of appropriate substituted benzoic acids and thiosemicarbazide in the presence of phosphorus oxy chloride ($POCl_3$), which on further condensation with different aromatic aldehydes ethanol as the solvent yielded the corresponding Schiff bases i.e.{5-(substituted phenyl)-2-(2-substituted benzylidene amino-1,3,4-thiadizole (2a-o). The titled compounds [2-(substituted aryl)-3-{5-(substitutedphenyl)-1,3,4-thiadiazole}]-4-oxo-thiazolidines (3a-o) were synthesized by reacting Schiff bases with thioglycollic acid in ethanol. The physico-chemical and spectral data of synthesized compounds (3a-o) are summarized in Table No.1,2 respectively.

Scheme1. Synthesis of [2-(substituted aryl)-3-{5-(substituted phenyl)- 1,3,4-thiadiazole}-4-oxo-thiazolidines (3a-o)*:



3a: **R** = 4Cl-C_6H_4**, Ar =** 3NO_2 -C_6H_4; **3b:** **R =** 4Cl-C_6H_4**, Ar =**2,4Cl-C_6H_4**;**
3c: R= 4OCH_3-C_6H_4, **Ar** = C_6H_5; **3d:** **R=** 4OCH_3-C_6H_4**, Ar=**4OCH_3-C_6H_4**;**
3e: R= 4OCH_3-C_6H_4, **Ar**=3NO_2-C_6H_4; **3f: R**= 4OCH_3-C_6H_4, **Ar=**2,4Cl-C_6H_4;
3g: R= 4F-C_6H_4, **Ar**=C_6H_5; **3h: R=**4F-C_6H_4, **Ar** = 2,4Cl-C_6H_4;
3i: **R=**4F-C_6H_4, **Ar**= 4Cl-C_6H_4; **3j: R=**C_6H_5**, Ar =** C_6H_5**;**
3k: R= C_6H_5**, Ar=**3NO_2-C_6H_4;**3l: R=** C_6H_5**, Ar=**2,4Cl-C_6H_4;
3m: R=C_6H_5**, Ar=**4Cl-C_6H_4; **3n: R=** 4NO_2-C_6H_4**, Ar**=4OCH_3-C_6H_4;
3o: R=4NO_2-C_6H_4**, Ar=**2,4Cl-C_6H_4
***Reaction and condition**: (a) $POCl_3$, reflux, 4hr; (b) Ar-CHO, ethanol, reflux, 5hr; (C) $SHCH_2COOH$, ethanol, 6hr.

Table 1: Physico-chemical data of [2-(substituted aryl)-3-{5-(substituted phenyl) -1, 3, 4-thiadiazole}]-4-oxo-thiazolidines (3a-o):



Compounds	R	Ar	Yield (%)	M. P. (°C)	Rf*	Molecular formula
3a	4-Cl, C_6H_4	3 -NO_2,C_6H_4	75	205	0.92	$C_{17}H_{11}O_3S_2ClN_4$
3b	4-Cl ,C_6H_4	2,4-Cl,C_6H_4	55.5	150	0.24	$C_{17}H_{10}OS_2Cl_3$
3c	4-OCH_3,C_6H_4	C_6H_5	80	168	0.31	$C_{18}H_{15}O_2S_2N_3$
3d	4-OCH_3,C_6H_4	4-OCH_3,C_6H_4	70	170	0.86	$C_{18}H_{17}O_3S_2N_3$
3e	4-OCH_3,C_6H_4	3 -NO_2,C_6H_4	75	135	0.61	$C_{18}H_{14}O_4S_2N_4$
3f	4-OCH_3,C_6H_4	2,4-Cl,C_6H_4	62	155	0.88	$C_{18}H_{13}O_2S_2ClN_3$
3g	4-F, C_6H_4	C_6H_5	80	175	0.73	$C_{17}H_{12}OS_2FN_3$
3h	4-F, C_6H_4	2,4-Cl,C_6H_4	66.6	206	0.27	$C_{17}H_{10}OS_2FCl_2N_3$
3i	4-F,C_6H_4	4-Cl,C_6H_4	83.3	235	0.49	$C_{17}H_{11}OS_2ClFN_3$
3j	C_6H_5	C_6H_5	75	130	0.98	$C_{17}H_{13}OS_2N_3$
3k	C_6H_5	3 -NO_2,C_6H_4	77.5	120	0.84	$C_{17}H_{12}O_3S_2N_4$
3l	C_6H_5	2,4-Cl,C_6H_4	65	185	0.29	$C_{17}H_{11}OS_2Cl_2N_3$
3m	C_6H_5	4-Cl,C_6H_4	60	170	0.56	$C_{17}H_{12}OS_2ClN_3$
3n	4-NO_2,C_6H_4	4-OCH_3,C_6H_4	76.2	178	0.28	$C_{18}H_{14}O_4S_2N_4$
3o	4-NO_2,C_6H_4	2,4-Cl-C_6H_4	57.5	165	0.71	$C_{17}H_{10}O_3S_2Cl_2N_3$

*n-Hexane and Ethyl acetate (50:50) as a mobile phase and iodine vapors as visualizing agent and pet ether: ethyl acetate (80:20).

Table 2: Spectral data of [2-(substituted aryl)-3-{5-(substituted phenyl)-1,3,4- thiadiazole}]-4-oxo-thiazolidines:

Compounds	IR (KBr, cm^{-1})	^1H NMR spectra (δ,ppm)	^{13}C NMR spectra (δ,ppm)	Mass (m/e value)
3a	1690.08 (C=O), 1533.90 (C=C), 1589.03(C=N)	3.87 (s, 2H,CH_2), 6.92(d,2H,Ar-H), 6.99(d,2H,ArH), 7.67(d,2H,Ar-H) 7.96(d,2H,Ar-H)	123.9 (s,2C,Ar-C), 134.3 (s,1C,Ar-C), 45.8 (s,2C, CH_2), 207.1 (s,1C, C=O).	413 (M^+, 5.38%), Base peak 212.0 (100%), Fragment ion peak , 387.3(5.9%), 381.3(26.9%), 353.5(25%), 343.2(9.5%), 301.1(7.7%), 278.9 (5.32%).
3c	1686.23(C=O), 1623.01(C=N), 1505.29(C=C).	3.59(s,2H,CH_2), 7.43(d,2H,ArH), 7.95(d,2H,Ar-H)	55.8 (s,1C,OCH_3), 114.3 (s,2C,Ar-C), 129.2 (s,6C,Ar-C), 210 (s,1C,C=O) 47.0 (s,1C,CH_2)	364.1(M^+, 2.9%), Base peak 208.1(100%), Fragment ion peak 273.1(2.9%), 261(5.38%), 230(11.3%), 210(5.9%), 209.1(13.7%)
3e	1689.65 (C=O), 1612.08(C=N), 1506.47 (C=C)	3.86(s,2H,CH_2), 6.93(d,2H,ArH), 6.97(d,2H,ArH), 7.7(d,2H,ArH), 7.94(d,2H,Ar-H)	55.56 (s,1C,OCH_3), 113,114,121,128,131 , 161(s,6C,Ar-C), 167 (s, 1C, C=O)	-
3g	1715.61(C=O), 1630.21(C=N), 1506.21(C=C).	3.61(S,2H,CH_2), 7.60(s,5H,Ar-H), 7.23(d,2H,Ar-H), 7.7(d,2H,Ar-H).	50(s,1C,CH_2), 115.5(s,2C,Ar-C), 130.3(s,2C,Ar-C), 131.2(s,6C,Ar-C), 162.9(s,1C,Ar-C), 202(s,1C,Ar-C).	-
3j	1723.70(C=O), 1627.85(C=N)	3.60(s,2H,CH_2), 7.49(d,2HArH), 7.53(d,2H,Ar-H), 7.74(d,2H,Ar-H)	126.75,128.66,129.38,131.65,156.20 (s,5C,Ar-C), 169.38(s,1C,C=O)	-

Antibacterial activity

All the newly synthesized compounds were screened for the antibacterial by cup-plate method using Muller Hinton agar. cloxacillin was used as a standard drug. Table No.3 revealed the zone of inhibition of [2-(substituted aryl)-3-{5-(substituted phenyl)-1,3,4-thiadiazole}]-4-oxo-thiazolidines (3a-o) compounds . Among the series, compounds **3a**, **3c**, **3d**, **3f** and **3g, 3h** exhibited moderate to good antibacterial activity with zone of inhibition(in cm) 1.1, 1.0, 0.8, 0.9, 1.3 and 1.1 respectively as compared with standard cloxacillin(1.4 cm). MICs were recorded as the minimum concentration of a compound that inhibits the growth of tested microorganisms reaveled in the table No.4

Table 3: Screening of Compounds (3a-o) at Concentration at 100 µg by cup-plate method (values are in cm)

Compounds Name	*E. coli*	*S. aureus*	*P. aeruginosa*	*B. subtilis*
standard	3.4	1.4	2.6	2.5
DMSO	0	0	0	0
3m	0.4	0.6	0	0.7
3f	0.7	0.9	0.5	0.9
3c	0	1.0	0.9	0.8
3g	0	1.3	0.8	0.7
3j	0	0.3	0.4	0.4
3k	0	0.6	0.2	0.8
3b	0	0.4	0.5	0.5
3i	0	0.5	0	0.5
3o	0	0.8	0.9	0.9
3e	0	0.2	0.5	0.2
3l	0	0	0.5	0
3h	0	1.1	0.8	0.8
3n	0	0.6	0.9	0.8
3d	0.9	0.8	0.9	0.9
3a	0.7	1.1	0.8	0.5

Table 4: Minimum Inhibitory Concentration (MIC) (Concentration µg/ml) of synthesized compound (3d)

***S. aureus* (Gram positive bacteria)**

Compound Name	OD at 100 µg	% growth	OD at 50 µg	% growth	OD at 25 µg	% growth	OD at 12.5 µg	% growth	OD at 6.25 µg	% growth	OD at 3.125 µg	% growth	OD at 1.5 µg	% growth
Control	0.56	100	0.56	100	0.56	100	0.56	100	0.56	100	0.56	100	0.56	100
Antibiotic	0	0	0	0	0.04	7.14	0.09	06.07	0.13	23.21	0.19	33.92	0.25	44.64
DMSO	0.56	100	0.56	100	0.56	100	0.56	100	0.56	100	0.56	100	0.56	100
3d	0.27	48.21	0.33	58.92	0.42	75.0	0.48	85.71	0.54	96.42	0.56	100	0.56	100

***B. subtilis* (Gram positive bacteria)**

Compound Name	OD at 100 µg	% growth	OD at 50 µg	% growth	OD at 25 µg	% growth	OD at 12.5 µg	% growth	OD at 6.25 µg	% growth	OD at 3.125 µg	% growth	OD at 1.5 µg	% growth
Control	0.66	100	0.66	100	0.66	100	0.66	100	0.66	100	0.66	100	0.66	100
Antibiotic	0	0	0	0	0.11	16.66	0.26	39.39	0.39	59.09	0.52	78.78	0.62	93.93
DMSO	0.66	100	0.66	100	0.66	100	0.66	100	0.66	100	0.66	100	0.66	100
3d	0.22	33.33	0.28	42.42	0.33	50.0	0.4	60.60	0.48	72.72	0.58	87.87	0.65	98.48

E. coli **(Gram negative bacteria)**

Compound Name	OD at 100 µg	% growth	OD at 50 µg	% growth	OD at 25 µg	% growth	OD at 12.5 µg	% growth	OD at 6.25 µg	% growth	OD at 3.125 µg	% growth	OD at 1.5 µg	% growth
Control	0.64	100	0.64	100	0.64	100	0.64	100	0.64	100	0.64	100	0.64	100
Antibiotic	0	0	0	0	0	0	0.06	9.37	0.13	20.31	0.22	34.37	0.36	56.25
DMSO	0.64	100	0.64	100	0.64	100	0.64	100	0.64	100	0.64	100	0.64	100
3d	0.24	37.5	0.31	48.43	0.35	54.68	0.44	68.75	0.49	76.56	0.56	87.5	0.61	95.31

P. aeruginosa **(Gram negative bacteria)**

Compound Name	OD at 100 µg	% growth	OD at 50 µg	% growth	OD at 25 µg	% growth	OD at 12.5 µg	% growth	OD at 6.25 µg	% growth	OD at 3.125 µg	% growth	OD at 1.5 µg	% growth
Control	0.78	100	0.78	100	0.78	100	0.78	100	0.78	100	0.78	100	0.78	100
Antibiotic	0	0	0	0	0.09	11.53	0.19	24.35	0.26	33.33	0.39	50.0	0.59	75.64
DMSO	0.78	100	0.78	100	0.78	100	0.78	100	0.78	100	0.78	100	0.78	100
3d	0.25	32.05	0.33	42.3	0.39	50.0	0.46	58.97	0.52	66.66	0.59	75.64	0.68	87.17

CONCLUSION

This study reports the successful synthesis of the [2-(substituted aryl)-3-{5-(substituted phenyl)-1,3,4- thiadiazole}]-4-oxo-thiazolidines (3a-o) title compounds in good yields and evaluation of antibacterial activity by cup-plate method. The investigation of antibacterial activity revealed that **3a**, **3c**, **3d**, **3f** and **3g, 3h** compounds showed moderate to good level of antibacterial activity.

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