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Original Research Article

Synthesis Characterisation and *In-Vitro* Anti-microbial Activity of Some Novel 1, 3, 4-Thiadiazole Derivatives

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ABSTRACT

The various derivatives of thiadiazole were prepared by the reaction of 2-chloro-*N*-[5-(2-hydroxyphenyl)-1, 3, 4-thiadiazol-2-yl]-acetamide, 2-chloro-*N*-[5-(4-chlorophenyl)-1, 3, 4-thiadiazol-2-yl]-acetamide, 2-chloro-*N*-[5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl]-acetamide with thiourea /hydrazine hydrate / piperidine in dry alcohol. Elemental analysis, IR, ^{1}H NMR, mass spectral data confirmed the structure of the newly synthesized compounds. All the derivatives of these moieties were evaluated for *in-vitro* antimicrobial activity. Most of the synthesized compounds showed potent antimicrobial activity at 100 and 50 µg/ml. Compounds showed most significant antibacterial activity against gram positive test organism *B. subtilis* and most significant antifungal activity against test organisms *C. albicans.*

Keyword: Synthesis; Aryl carboxylic acid; Thiosemicarbazide; 1, 3, 4-Thiadiazole; Chloroacetylation; Antimicrobial activity

INTRODUCTION

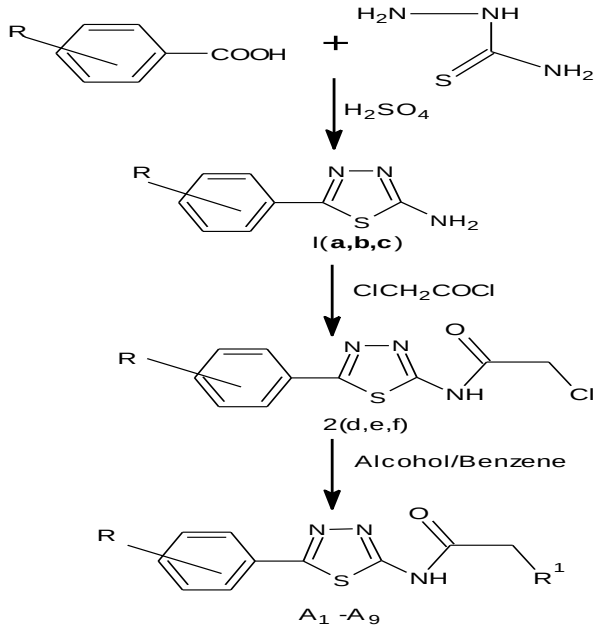
Microbial diseases are very common all over the world. Currently, used anti-microbial agents are not effective due to development of the resistance against them and therefore, it is an on-going effort for the synthesis of the new antimicrobial agents. Antimicrobial agents reduces or completely block the growth and multiplication of bacteria and are helpful in the treatment of various infectious diseases like meningitis, malaria, Tuberculosis, pneumonia, AIDS, *etc*. Heterocyclic Compounds are the cyclic compounds having as ring members atoms of at least two different elements, e.g. quinolone, 1, 2-thiazole, bicycle [3.3.1] tetrasiloxane. Usually they are indicated as counterparts of carbocyclic compounds, which have only ring atoms from the same element. Another classical reference book, the *Encyclopaedia Britannica*, describes a heterocyclic compound, also

called a heterocycle, as: any of a class of organic compounds whose molecules contain one or more rings of atoms with at least one atom (the heteroatom) being an element other than carbon, most frequently oxygen, nitrogen, or sulfur [2]. Although heterocyclic compounds may be inorganic, most contain within the ring structure at least one atom of carbon, and one or more elements such as sulfur, oxygen, or nitrogen [3]. Since non-carbons are usually considered to have replaced carbon atoms, they are called heteroatoms. The structures may consist of either aromatic or non-aromatic rings. There are number of thiadiazoles which contain the nitrogen in different positions such as 1, 2, 3-thiadiazoles [1] and their benzo derivatives [2], the 1, 2, 4-thiadiazoles [3], the 1,3, 4-thiadiazoles [4] and the 1,2,5-thiadiazles (5) and their benzo derivatives [6] etc. The biological activity of the compounds is mainly dependent on their molecular structures [4]. A recent literature survey revealed that the 1, 3, 4-thiadiazole moiety have been widely used by the medicinal chemist in the past to explore its biological activities. The Development of 1, 3, 4-thiadiazole chemistry is linked to the discovery of Phenylhydrazines and hydrazine in the late nineteenth century. It is also well established that various derivatives of 1, 3, 4 - thiadiazoles exhibit broad spectrum of pharmacological properties such as anti-inflammatory [5], antiviral [6], anticonvulsant [7, 8], antifungal [9], anthelmintic [10] CNS depressant [11] and diuretic [12] activities, when properly substituted in the 2-and 5-positions [13]. In the medical field one of the best known drugs having a 1, 3, 4-thiadiazole moiety is acetazolamide, which is a carbonic anhydrase inhibitor launched in 1954. Its indications and usage are many including the treatment of glaucoma, epilepsy and congestive heart failure [14]. Natural and synthetic azetidinone derivatives occupy a central place among medicinally important compounds due to their diverse and interesting antibiotic activities.

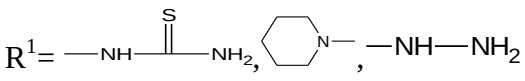
MATERIAL AND METHOD

Synthetic Procedure

Melting points of all synthesized compounds were determined by open capillary tube method and were uncorrected. Purity of all synthesized compounds was checked by thin layer chromatography technique (0.2 mm thickness of silica gel GF plates) and iodine was used as visualizing agent. IR spectra were recorded on THERMO NICOLET iS10 FT-IR spectrometer using KBr disc method. Elemental analysis was performed using a Euro EA Elemental Analyser. Spectral and Elemental analysis was carried out at Central Analytical Instrument Facility (CAIF), Guwahati biotech park, spectra were recorded on 400-MHz BRUKER spectrometer in dimethylsulfoxide-d_6 as solvent and tetramethylsilane (TMS) as internal standard and chemical shift was expressed in δ or ppm.



R=2OH, 4Cl, $2NO_2$



Scheme 1: Schematic representation of 1, 3, 4-thiadiazole derivatives.

EXPERIMENTAL

Synthesis of 5-(2-hydroxyphenyl)-2-amino -[1, 3, 4]-thiadiazole 1(a) [15, 16]

A mixture of thiosemicarbazide (0.1mole), 2-hydroxybenzoic acid (0.1mole) and conc. Sulphuric acid (5ml) in 50 ml of ethanol was refluxed for 2-3hour.Reaction was monitored by TLC using mobile phase Chloroform: methanol (4:1).After completion of the reaction the reaction mixture was poured on to crushed ice. The solid separated out was filtered, washed with cold water and recrystallized from ethanol to give colourless crystals, Yield 90 %, m.p.183-186^{o}C.

IR (KBr) *v*/(cm^{-1}): 3514.22 (O-H, st.), 663.92, 688.65 (C-S-C, st.), 3428.26 (NH_2,N-H,st.), 1616.23 (C=N, st.), 1425.76 (Aryl C=C, st.), 3018.75(Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 6.89-7.20(m, 4H, ArH), 10.32 (s, 1H, OH), 2.60-2.65 (bs, 2H, NH_2), Mass spectrum m/z: 193 (M^+).

Synthesis of 5-(4-chlorophenyl) -2-amino - [1, 3, 4]-thiadiazole (1b) [15, 16]

A mixture of thiosemicarbazide (0.1mole), 4-chlorobenzoic acid (0.1mole) and conc. sulphuric acid (5ml) in 50 ml of ethanol was refluxed for 2-3hour.Reaction was monitored by TLC using mobile phase Chloroform: methanol (4:1). After completion of the reaction the reaction mixture was poured on to crushed ice. The solid separated out was filtered, washed with cold water and recrystallized from ethanol to give colourless crystals,Yield 79%, m.p.165-167^0 C ; IR (KBr) *v*/(cm^{-1}): 762.35 (C-Cl, st.), 682.35 (C-S-C, st.), 3447.21 (NH_2, N-H,st.), 1647.11 (C=N, st.),1491.70(C-N, st.), 1425.79 (Aryl C=C, st.),3025.25(Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 6.95-7.35 (m, 4H, ArH), 2.48 (bs, 2H, NH_2), Mas spectrum m/z :211(M^+).

Synthesis of 5-(2-nitrophenyl) -2-amino - [1, 3, 4]-thiadiazole (1c) [15, 16]

A mixture of thiosemicarbazide (0.1mole), 2-notrobenzoic acid (0.1mole) and conc. sulphuric acid (5ml) in 50 ml of ethanol was refluxed for 2-3hour.Reaction was monitored by TLC using mobile phase Chloroform: methanol (4:1). After completion of the reaction the reaction mixture was poured on to crushed ice. The solid separated out was filtered, washed with cold water and recrystallized from ethanol to give light yellow colour crystals, Yield 88%, m.p.225-227^{0}C ; IR (KBr) *v*/(cm^{-1}): 1375.53 , 1545.11, (NO_2, st.), 687.73 (C-S-C, st.), 34.50.78 (NH_2, N-H,st.), 1649.95 (C=N, st.), 1416.45 (Aryl C=C, st.),3087.72(Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.30-7.73(m, 4H, ArH), 2.59 (bs, 2H, NH_2), Mass spectrum m/z: 222 (M^+).

Synthesis of 2-Chloro-*N*-[5-(Substituted-phenyl) - [1, 3, 4]-thiadiazol-2-yl]-acetamide 2 (d, e, f) [17, 18]

To the mixture of appropriately substituted compound **I (a, b, c)** (10mmol) in dry benzene (15ml) and 2 ml of dry pyridine, was cooled to 0-5^{0}C.Chloro-acetyl chloride (20mmole) dissolved in dry benzene (10ml) was added drop wise to the solution with constant stirring at room temperature. After complete addition, the reaction mixture was refluxed for about 6-8h. Benzene was removed *in vacuo*. The residue was poured over crushed ice. The precipitate was filtered, washed with water. The crude product was dried and crystallized from 1,4-dioxane to yield compound **(IV d, e, f)**; the purity of compounds was analyzed by TLC using benzene: acetone (9:1) as mobile phase. Yield 64.4%, m. p 210 - 212^{0}C.

2-(Substituted-amino)-*N*-[5-(Substituted-phenyl)-1, 3, 4-thiadiazol-2-yl]-acetamide (A1-A9)

The compound **IV (d, e, f)** 2-Chloro-*N*-[5-(Substituted-phenyl) - [1, 3, 4]-thiadiazol-2-yl]-acetamide (0.01 mol) was taken in about 25 ml of dry alcohol and 0.01 mol of thiourea /hydrazine hydrate / piperidine was added to it and the mixture was heated on water bath for 9 h. The content was

cooled under tap water, filter, dried and recrystalized from alcohol. Purity of the compounds was analyzed by petroleum ether: acetone (9:1) as mobile phase.

The structures of synthesized compounds under investigation were supported by the Physical parameter, ^{1}H-NMR, FTIR and MASS spectral measurement. The Physical parameter, ^{1}H-NMR, FTIR and Mass spectral data of the synthesized compounds spectra were recorded and assigned in Table 1 and 2.

ASSESSMENT OF IN-VITRO ANTIMICROBIAL ACTIVITY

Microbial culture maintenance

The microbial cultures were procured from Dept. of Microbiology, Jadavpur University. The bacterial cultures were cultured in nutrient broth prior to the screening of antimicrobial activity each isolate was checked for its purity and several colonies were emulsified into 50 ml nutrient broth. The inoculated flasks were incubated at 37^{0}C for 18 h on a rotary shaker at 150 rpm. The bacteria were subcultured on agar slants and maintained at 4^{0}C until further use. The fungal cultures were grown on Sabouraud's dextrose agar medium containing streptomycin. The plates were incubated in an environmental chamber set at 25^{0}C±2^{0}C, 90 % relative humidity (RH), and

Zone of inhibition [19, 20, 21]

All synthesized compounds were screened for their The *in vitro* antimicrobial activity of all the compounds and standard drugs were assessed against two representatives of gram-positive bacteria *viz Bacillus subtilis* (ATCC11774), *Staphylococcus aureus* (NTCC-6571), two gram-negaitive bacteria *viz Escherichia coli* (TG14) , *Pseudomonas aeruginosa* (ATCC9027) and two fungal strains *viz*. *Aspergillus niger* and *Candida albicans* by cup plate method. The inhibition zone was measured in mm using Amoxycillin,Ofloxacin and Amphoteracine B as standards in dimethyl sulphoxide (DMSO). DMSO showed no inhibition zone. Each compound and standard drugs were diluted obtaining 1000µg/ml concentration, as a stock solution. All the compounds were tested at a concentration of 50µg/ml and 100µg/ml. Each experiment was repeated twice and the average of the two determinations was recorded. Then the potency of the compounds was calculated by using the following formula as described by Edwin and Marion [22].

$$Potence = Antilog[2 + d\left(\frac{(U2+U1)-(S2+S1)}{(U2-U1)+(S2-S1)}\right)]$$

In the above equation, 2 is the factor for converting to percent and *d* is the log of the ratio of the

16:8hrs light: dark regime. An aqueous conidial suspension of 10^7 conidia/ml containing 105mg/ml Tween 80 was prepared from 14 day old culture. The conidial viability of the fungi was tested and inoculated with more than 95% germination.

Evaluation of antimicrobial activity

Evaluation of antimicrobial activities of the test compounds was performed by Cup plate method using muller –Hinton agar medium. The cups of 9 mm diameter were made by scooping out medium with a sterilized cork borer in Petridis which was streaked with the organisms and The solutions of each test compound (0.05 ml, 50 µg/ml & 0.1 ml, 100 µg/ml) were added separately in the cups using a micropipette. The plates were incubated at 37^{0}C for bacteria and 26^{0}C for fungi. Following incubation the plates were observed for zones of inhibition. The inhibition zone around the cup as calculated edge to edge zone of confluent growth which is usually corresponds to the sharpest edge of the zone and to be measured diameter in millimeter.

stronger concentration to the weaker. This ratio between dilutions must be the same for both the standard and the compound being assayed.
U2 is zone of inhibition of compound at 100µg/ml,
U1 is zone of inhibition of compound at 50µg/ml,
S2 is zone of inhibition of standard at 100µg/ml,
S1 is zone of inhibition of standard at 50µg/ml.
The results were reported in Table 3, 4 and 5.

Minimum inhibitory concentration (MIC) [23]:

The lowest concentration (highest dilution) of compound preventing the appearance of turbidity is considered to be the minimal inhibitory concentration (MIC). At this dilution the compound is known to be bacteriostatic. Bacterial strains were primarily inoculated into Mueller-Hinton agar for overnight growth. A number of colonies were directly suspended in saline solution until the turbidity matched the turbidity of the McFarland standard (approximately10^8 CFU ml^{-1}), i.e., inoculum size for test strain was adjusted to10^8 CFU ml^{-1} (Colony Forming Unit per milliliter) per well by comparing the turbidity (turbidimetric method). Similarly, fungi were inoculated on Sabouraud Dextrose broth and the procedures of inoculum standardization were similar. DMSO was used as diluents/vehicle to get desired concentration of the synthesized compounds and standard drugs to test upon standard microbial strains, i.e., the compounds were dissolved in DMSO and the solutions were diluted with a culture medium.

Each compound and standard drugs were diluted obtaining 2000µg/ml concentration, as a stock solution. By further progressive dilutions with the test medium, the required concentrations were obtained for primary and secondary screening. In primary screening, 0.1ml of culture (bacterial and fungal) was added to the solution of 1000, 500 and 250µg/ml concentrations of the synthesized compounds were tested.

The active compounds found in this primary screening were further diluted to obtain 200, 100, 62.5, 50, 25, 12.5 and 6.250µg/ml concentrations for secondary screening to test in a second set of dilution against all microorganisms. Eight MIC tubes were taken and labeled it as number 1-8. Briefly, the control tube (8^{th} tube) containing no antibiotic is immediately sub cultured [before inoculation] by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the tested organism.The tubes are then put for incubation at 37 ^{0}C for 24 h for bacteria and 48 h for fungi. Growth or a lack of growth in the tubes containing the antimicrobial agent was determined by comparison with the growth control, indicated by turbidity. The lowest concentration that completely inhibited visible growth of the organism was recorded as the minimal inhibitory concentration (MIC, µg/ml), i.e., the amount of growth from the control tube before incubation (which represents the original inoculum) is compared. A set of tubes containing only seeded broth and the solvent controls were maintained under identical conditions so as to make sure that the solvent had no influence on strain growth. The result of this is much affected by the size of the inoculums. The test mixture should contain 10^8 CFU ml^{-1} organisms. The interpretation of the results was based on Amphotericin B break points for the fungi and also on amoxicillin and ofloxacillin for bacterial pathogens. The protocols were summarized in Table 6 as the minimal inhibitory concentration (MIC, µg/ml).

RESULTS AND DISCUSSION

Chemistry

Treatment of aryl carboxylic acid in absolute ethanol with thiosemicarbazide afforded the corresponding 2-amino-5(substituted phenyl)-1, 3, 4-thiadiazole **I** (a,b and c). Molecular formula of the compounds (Table 1) derived from elemental analyses data are supported by their molecular weight.

The IR spectrum of **1a** showed characteristic absorption bands at 3428 cm^{-1} characteristic due to

NH_2 functions in addition to the -OH absorption band at 3514 cm^{-1}, C-S-C absorption band at 688 cm^{-1}. Its ^1H NMR spectrum revealed the characteristic signal at δ 10.32 assigned to OH protons, two characteristic signals at δ 2.60 and 2.65 assigned to NH_2 protons which is exchangeable with D_2O, confirming the formation of thiadiazole. Also, its mass spectrum showed the molecular ion peak at *m/z* 193 [M^+] and the base peak at *m/z* 94.

The IR spectrum of **1b** showed characteristic absorption bands at 3447 cm^{-1} characteristic which is due to NH_2 functions in addition to the C-Cl absorption band at 762 cm^{-1}, C-S-C absorption band at 682 cm^{-1} and C=C (aromatic)absorption band at 1425 cm^{-1}. Its ^1H NMR spectrum revealed the characteristic signal at δ 2.48 assigned to NH_2 protons which is exchangeable with D_2O, confirming the formation of thiadiazole. The mass spectrum showed the molecular ion peak at *m/z* 211 [M^+] and the base peak at *m/z* 42.

The IR spectrum of **1c** has exhibited characteristic absorption bands at 3450 , 682 and 1416 cm^{-1} due to NH_2 , C-S-C and C=C (aromatic) functions respectively .Two characteristic absorption band at 1375, 1545 cm^{-1} which are due to NO_2 function. It was also showed proton signals at: δ 2.59 (NH_2) and δ 7.30-7.73 (Ar-H), respectively. Mass spectrum **(1c)** of the compound exhibited its molecular ion (M+) at m/z 222 and the base peak at *m/z* 206.

To the mixture of compounds **I (a, b, c)** added solution of chloro-acetyl chloride with constant stirring at room temperature. After complete addition, the reaction mixture was refluxed for about 6-8h. The precipitate was filtered, washed with water to yield compound **2 (a, b, c)**; Yield 64.4%, m. p 210 - 212^{0}C.

Compound **2 (a, b, c)** was refluxed for 9h with thiourea /hydrazine hydrate / piperidine in alcohol, to yield **V (A13-A21).**

The FTIR spectrum of compound **A1, A2** and **A3** showed a medium intensity band at 1622, 1612 and 1624 cm^{-1} that could correspond with (C=N) stretching in the vicinity of 1,3,4-thiadiazole ring[1]. In this spectrum there are two other characteristic bands at 3559, 3518, 3521 and 1689, 1655, 1640 cm^{-1} due to (O-H) and (C=O) stretching vibrations, respectively. Whereas the compound **A1**, showed two absorption band at 1122 and 3490 cm^{-1} for (C=S) and (NH_2) stretching vibrations, respectively and in the compound **A2** two absorption band was appeared at 1324 and 2860 cm^{-1} for (C-N, st. piperidine) and (CH_2, st. piperidine) stretching vibrations, respectively. Two characteristic band was found to be at 3453 and 3088 cm^{-1} stretching vibrations, respectively, indicated the presence of (N-H, NH_2, st.) and (C-H, CH_2, st.) functions in compound **A3.** The ^{1}HNMR spectra of these compounds **A1, A2** and **A3** showed the signal for the (O-H) group in the δ 10.12, 10.15 and 9.64 and those for the NH (amide) group at δ 9.22, 8.69 and 8.85, respectively. The mass spectrum were showed the molecular ion peak at *m/z* 309 [M^+], 318 [M^+], 265 [M^+], respectively, and the base peak at *m/z* 73, 128 and 127 respectively.

The structures of compounds **A4, A5 and A6** were assigned by IR and ^1H NMR spectroscopic data, which are consistent with the proposed molecular structures. IR spectra of compound **A4, A5** and **A6** showed characteristic bands for NH, CH–aliphatic, C-Cl and C=O groups. ^{1}H-NMR spectrum of compound **A4, A5** and **A6** showed signals for CONH at δ 9.31, 8.85 and 8.81, respectively, for CH_2 at δ 3.72, 4.27 and 3.55, respectively. The primary amino group in compound **A4** and **A6** were depicted by the presence of NH function at δ 2.60 and 1.99, respectively. The appearance of multiplate at range δ 1.78-2.69 confirmed the presence of the pyrrolidine ring system in compound **A5**. Mass spectrum of compounds **A4**, **A5** and **A6** were showed the molecular ion peak at *m*/*z*: 328 [M+1]$^+$, 336 and 282 [M-1]$^+$, with a base peak at *m*/*z*: 100, 126 and 157 respectively.

The structures of compounds **A7**, **A8** and **A9** were assigned by IR and ^1H NMR spectroscopic data,

which are consistent with the proposed molecular structures. The primary amino group in compounds **A7** and **A8** was depicted by the presence of NH asymmetric stretch at 3400 and 3434 cm^{-1}. The IR bending vibration corresponding to C=S of compound **A7** appeared at 1202 cm^{-1}. The presence of heterocyclic pyrrolidine moiety in compound **A8** was demonstrated by the presence of C-N at 1345 cm^{-1}. The appearance of C=O stretch in the range of 1626 -1725 cm^{-1} indicated the formation of secondary amides (**A7 – A9**) by the reaction of hydrazine/ thiourea/ piperidine with the 2-chloro-*N*-[5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl]acetamide.

The appearance of singlet at δ 8.81, 8.95 and 9.10, respectively, corresponds to the proton of CONH in the NMR of all the compounds indicated the presence of secondary amide to the 2nd position of synthesized 1, 3, 4-thiadiazoles moiety (**A7- A9**). Mass spectrum of compounds **A7**, **A8** and **A9** were showed the molecular ion peak at *m*/*z*: 338 [M^+], 347 [M^+] and 294 [M^+], respectively, with a base peak at *m*/*z*: 181, 225 and 88 respectively.

Table 1: Physical parameter of the synthesized compounds

Compd.	Mol. formula	Mol.Wt	m.p. ^{0}C	Yield %	Rf	Elemental analysis					
						Calculated			Found		
						C	H	N	C	H	N
1a	$C_8H_7N_3OS$	193.22	185-186	90	0.71	49.73	3.65	21.75	49.63	3.85	21.71
1b	$C_8H_6ClN_3S$	211.67	165-167	79	0.72	45.39	2.86	19.85	45.12	2.67	19.80
1c	$C_8H_6N_4O_2S$	222.22	225-227	88	0.70	43.24	2.72	25.21	43.20	2.67	25.20
2a	$C_{10}H_8ClN_3O_2S$	269.70	210-212	61	0.78	44.53	2.99	15.58	44.55	3.09	15.51
2b	$C_{10}H_7Cl_2N_3OS$	288.15	190-193	67	0.69	41.68	2.45	14.58	40.98	2.45	14.58
2c	$C_{10}H_7ClN_4O_3S$	298.70	181-183	72	0.73	40.21	2.36	18.76	39.91	2.40	19.01
A1	$C_{11}H_{11}N_5O_2S_2$	309.36	169-172	45	0.76	42.71	3.58	22.64	42.45	3.50	22.81
A2	$C_{15}H_{18}N_4O_2S$	318.39	173-175	52	0.75	56.58	5.70	17.60	57.08	5.60	17.76
A3	$C_{10}H_{11}N_5O_2S$	265.29	145-147	47	0.79	45.27	4.18	26.40	45.20	4.10	25.89
A4	$C_{11}H_{10}ClN_5OS_2$	327.81	198-200	40	0.78	40.30	3.07	21.36	40.30	3.07	21.36
A5	$C_{15}H_{17}ClN_4OS$	336.83	135-138	65	0.81	53.49	5.09	16.63	53.59	5.20	15.93
A6	$C_{10}H_{10}ClN_5OS$	283.73	149-152	79	0.72	42.33	3.55	24.68	42.63	3.78	24.99
A7	$C_{11}H_{10}N_6O_3S_2$	338.36	215-217	48	0.73	39.05	2.98	24.84	39.05	2.98	24.84
A8	$C_{15}H_{17}N_5O_3S$	347.39	220-222	57	0.77	51.86	4.93	20.16	51.96	4.63	20.06
A9	$C_{10}H_{10}N_6O_3S$	294.28	228-230	43	0.72	40.81	3.42	28.56	40.81	3.42	28.56

***In-Vitro* Antimicrobial Activity**

Antimicrobial Activity (In-Vitro)

All the compounds synthesized were assayed for their antibacterial activity against two gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and two gram negative bacteria *(Escherichia coli*,*Pseudomonas aeruginosa*) and for antifungal activities against *Aspergillus niger*, *Candida albicans* by cup plate method.

Further, their minimum inhibitory concentration (MIC) values against these microorganisms were determined by serial dilution method.

Mullar-Hinton agar plates (37^{0}C, 24h) and Sabourand dextrose agar plates (26^{0}C, 72 h) were used for the cultivation of bacteria and fungi, respectively. The results of antibacterial and antifungal activity tests are summarized in Table 1, Table 2, Table 3 and Table 4 of inhibition zones

measurements at conc. 50 and 100 µg/ml and MIC at 200, 100, 62.5, 50, 25, 12.5 and 6.250 µg/ml concentrations for secondary screening to test in a second set of dilution against standard drugs *viz*. amoxicillin, ofloxacin and amphotericin B for comparison. Most of the synthesized compounds were found to possess varied degree of bacterial activities as evident from their minimal inhibitory concentration (MIC).

Table 2: ^{1}H- NMR, FT-IR and Mass data of compounds (A1-A9)

Copd. No.	1H-NMR	IR (KBr) *v*/(cm^{-1})	Mass (m/z)
A1	^{1}H NMR (400MHz, DMSO-*d*6) δ 6.90-7.52 (m, 4H, ArH); 10.12 (s, 1H, OH); 3.45 (d, 2H, CH_2); 6.16 (t, 1H, CH_2-N<u>H</u>); 9.22 (s, 1H, CONH); 2.87, 2.90 (d, 2H, NH_2)	3559.56 (O-H), 688.51 (C-S-C, st.), 3411.10 (N-H, st.), 1689.70 (C=O, st.), 3119.59 (CH_2, C-H, st.), 1122.86 (C=S) 1426.53 (Aryl C=C, st.), 3080.72 (Aryl C-H, st.), 3490.53(NH_2, st.)	**309 (M^+)**
A2	^{1}HNMR (400MHz, DMSO-*d*6) δ 6.88-7.51(m, 4H, ArH);10.15 (s, 1H, OH); 3.12 (s, 2H, CH_2); 8.69 (s,1H,CONH); 1.51-1.69 (m, 6H, piperidine), 2.60, 2.71 (t, 4H, piperidine)	3518.34 (O-H), 697.28, 659.15 (C-S-C, st.), 3237.91 (N-H, st.), 1655.19 (C=O, st.), 3040.95 (CH_2, C-H, st.), 1612.63 (C=N), 1324.90 (C-N,st.piperidine), 2860.33 (CH_2, C-H, st. piperidine), 1445.93 (Aryl C=C, st.), 2998.01 (Aryl C-H, st.).	**318 (M^+)**
A3	^{1}H NMR (400MHz, DMSO-*d*6) δ 6.28-7.94(m, 4H, ArH); 9.64 (s, 1H, OH); 3.91 (d, 2H, CH_2); 2.54 (m, 1H, CH_2-N<u>H</u>); 8.85 (s, 1H, CON<u>H</u>); 2.45 (d, 2H, NH_2)	3521.96 (O-H), 680.47 (C-S-C, st.), 3420.28 (N-H, st.), 1640.57 (C=O, st.), 3088.23 (CH_2, C-H, st.), 1621.31 (C=N), 1464.43(C-N), 1449.63 (Aryl C=C, st.), 3014.04 (Aryl C-H, st.), 3453.36(NH_2, st.).	**265 (M^+)**
A4	^{1}H NMR (400MHz, DMSO-*d*6) δ 7.45-7.94(m, 4H, ArH); 3.55 (d, 2H, CH_2); 2.81 (m, 1H, CH_2-N<u>H</u>); 8.83 (s, 1H, CON<u>H</u>); 2. 60 (s, 2H, NH_2)	762.38 (C-Cl), 682.01 (C-S-C, st.), 3410.65 (N-H, st.), 1681.41 (C=O, st.), 2991.53 (CH_2, C-H, st.), 1591.77 (C=N), 1491.66 (C-N), 1128.66, 1175.69 (C=S), 1424.68 (Aryl C=C, st.), 2836.65 (Aryl C-H, st.), 3289.64 (NH_2, st.).	**328(M^++1$^+$**
A5	^{1}HNMR (400MHz, DMSO-*d*6) δ 7.33-7.43 (m, 4H, ArH); 3.72 (s, 2H, CH_2); 9.31 (s, 1H, CONH); 1.78-1.98 (m, 6H, piperidine), 2.57-2.69 (t, 4H, piperidine)	762.40 (C-Cl), 650.06, 681.85 (C-S-C, st.), 3410.63 (N-H, st.), 1655.19, 1700.08 (C=O, st.), 3083.19 (CH_2, C-H, st.), 1593.13 (C=N), 1491.75(C-N), 1423.33 (Aryl C=C, st.), 2991.53 (Aryl C-H, st.).	**336 (M^+)**
A6	^{1}H NMR (400MHz, DMSO-*d*6) δ 7.33-8.33(m, 4H, ArH); 4.27 (d, 2H, CH_2); 2.49 (m, 1H, CH_2-N<u>H</u>); 8.85 (s,1H,CON<u>H</u>); 1.99 (d, 2H, NH_2)	703.34 (C-Cl), 653.01 (C-S-C, st.), 3310.44 (N-H, st.), 1688.21 (C=O, st.), 3104.36 (CH_2, C-H, st.), 1624.25 (C=N), 1443.31 (Aryl C=C, st.), 3091.58 (Aryl C-H, st.).	**282 (M^+1)$^+$**
A7	^{1}H NMR (400MHz, DMSO-*d*6) δ 7.01-8.45 (m, 4H, ArH); 3.55(d, 2H, CH_2); 2.49 (t, 1H, CH_2-N<u>H</u>); 8.81 (s, 1H, CON<u>H</u>); 1. 98 (d, 2H, NH_2)	1345.95,1392.86 (NO_2), 670.11 (C-S-C, st.), 3340.70 (N-H, st.), 1632.11,1731.44 (C=O, st.), 2770.99 (CH_2, C-H, st.), 1650.21 (C=N), 1345.95, 1392.86 (C-N), 1202.56, 1077.95, 1054.39 (C=S), 1441.47 (Aryl C=C, st.), 3039.76 (Aryl C-H, st.), 3400.35 (NH_2, st.).	**338 (M^+)**
A8	^{1}HNMR (400MHz, DMSO-*d*6) δ 7.76-8.15 (m, 4H, ArH); 3.31 (s, 2H, CH_2); 8.95 (s, 1H, CONH); 1.59-1.63 (m, 6H, piperidine),	1398.11, 1515.18 (NO_2), 681.85 (C-S-C, st.), 1725.98 (C=O, st.), 3116.52 (CH_2, C-H, st.), 1653.50 (C=N), 1490.83 (Aryl C=C, st.), 3055.76	**347(M^+)**

	2.51-2.62 (t, 4H, piperidine)	(Aryl C-H, st.).	
A9	^{1}H NMR (400MHz, DMSO-*d*6) δ 7.43-7.91 (m, 4H, ArH); 3.68 (d, 2H, CH_2); 2.90 (m, 1H, CH_2-NH); 9.10 (s, 1H, CONH); 2.89 (d, 2H, NH_2)	1521.33 (NO_2), 650.50 (C-S-C, st.), 3450.05 (N-H, st.), 1626.76 (C=O, st.), 1570.14, 1606.78 (C=N), 1443.31 (Aryl C=C, st.), 3096.47 (Aryl C-H, st.), 3190.43 (CH_2 N-H), 3434.03 (NH_2, st.).	**294(M^+)**

Zone of Inhibition

The results of antibacterial activity are shown in the Table 1 and Table 2 of inhibition zones measurements at conc.50 µg/ml and 100µg/ml. The investigation of the structure–activity relationship of antibacterial screening revealed that the compounds **A2** and **A5** (30.47, 35.58 and 33.26, 37.49% , respectively), with *o*-hydroxphenyl and *p*-chloro phenyl substituent at the 5-position of the nucleus were found to be highly active against *Staphylococcus aureus* and *Bacillus subtilis*. But against *Bacillus subtilis* compound **A5** was more potent as compare to *Staphylococcus aureus* due to presence of halogen group.

In addition compound **A8** exhibited good inhibitory activities (42.85%) with electron withdrowing group (eg. nitro) substituted phenyl ring on C5 position of the thiadiazole have exhibited better activity against gram negative bacteria *E. coli*. This finding suggests that the presence of a piperidinyl ring at linked to the (C2) 1, 3, 4-thiadiazole moiety promotes increased biological activity. But it was found that compound **A6** and **A7** were inactive towards *E. coli.* Whereas compounds **A1-A9** showed moderate to poor activities against *P.aeruginosa* as compared to ofloxacilin.

The results of antifungal activity are shown in the Table 5 of inhibition zones measurements at conc. 50µg/ml and 100µg/ml. Compound **A5** showed the highest activity (30.45%). Whereas rest of the compounds were exhibited moderate activities, while compound **A3 and A6** did not show any antifungal activity against *A .niger* as compared to Amphotericin B (Table5). Tested compounds **A2** possess promising antifungal activity (39.17%) but the remaining compounds possess poor activities against *C. albicans.*

Thus, it is concluded from the antimicrobial screening results that the most of 1, 3, 4-thiadiazole derivatives have good antibacterial activity compare to the standard against all bacteria and fungi at a concentration of 100µg/ml.

Table 3: In *vitro* antibacterial (gram +ve) activities of the prepared compounds: Zone of inhibition in (mm)

Compound	*S. aureus*		Amoxycilline		%Potency	*B. subtilis*		Amoxycilline		%Potency
	U2	U1	S2	S1		U2	U1	S2	S1	
A1	13	12	29	23	26.30	10	5	27	22	29.37
A2	15	14	29	23	30.47	12	6	27	22	35.48
A3	11	10	29	23	22.54	11	9	27	22	24.09
A4	13	11	29	23	28.51	12	8	27	22	30.97
A5	16	15	29	23	33.26	12	5	27	22	37.49
A6	12	11	29	23	24.09	11	8	27	22	26.60
A7	14	13	29	23	27.98	10	6	27	22	27.28
A8	16	16	29	23	29.99	11	5	27	22	33.26
A9	12	12	29	23	21.41	9	7	27	22	21.23
DMSO	----	----	----	----	----	----	----	----	----	----

U2: Zone of inhibition of compound at 100 µg/ml; U1: Zone of inhibition of compound at 50 µg/ml; S2: Zone of inhibition of standard at 100 µg/ml; S1: Zone of inhibition of standard at 50 µg/ml.
Zone diameter of growth inhibition: (------) no inhibition.

Table 4: In *vitro* antibacterial (gram -ve) activity of the synthesized compounds : Zone of inhibition in (mm)

Compound	*E . coli*		Ofloxacin		%Potency	*P. aeruginosa*		Ofloxacin		%Potency
	U2	U1	S2	S1		U2	U1	S2	S1	
A1	4	3	17	13	22.69	4	3	19	14	21.54
A2	4	3	17	13	24.94	5	4	19	14	24.94
A3	------	------	17	13	------	3	2	19	14	15.23
A4	3	2	17	13	18.66	4	3	19	14	23.06
A5	6	5	17	13	24.32	5	4	19	14	27.03
A6	0	0	17	13	0.00	3	2	19	14	17.10
A7	0	0	17	13	0.00	4	3	19	14	20.88
A8	9	7	17	13	42.85	5	4	19	14	24.54
A9	6	5.5	17	13	24.32	-----	-----	19	14	-----
DMSO	----	----	----	----	----	----	----	----	----	----

U2: Zone of inhibition of compound at 100 µg/ml; U1: Zone of inhibition of compound at 50 µg/ml; S2: Zone of inhibition of standard at 100 µg/ml; S1: Zone of inhibition of standard at 50 µg/ml.
Zone diameter of growth inhibition: (------) no inhibition.

Table 5: In *vitro* antifungal activities of the prepared compounds: Zone of inhibition in (mm)

Compound	*A .niger*		Amphotericin B		%Potency	*C. albicans*		Amphotericin B		%Potency
	U2	U1	S2	S1		U2	U1	S2	S1	
A1	3	3	18	12	24.94	5	4.6	16	11	30.97
A2	3	3	18	12	28.84	3.5	2.7	16	11	39.17
A3	0	0	18	12	0.00	4.1	3.4	16	11	29.17
A4	3	2	18	12	25.62	4.2	3.1	16	11	30.90
A5	4	3	18	12	30.48	6	5.3	16	11	36.30
A6	0	0	18	12	0.00	3.1	2.3	16	11	27.28
A7	3	2	18	12	24.54	4.1	3.4	16	11	30.19
A8	3	2	18	12	27.98	5.4	4.1	16	11	36.05
A9	3	2	18	12	24.80	------	------	16	11	-------
DMSO	----	----	----	----	----	----	----	----	----	----

U2: Zone of inhibition of compound at 100µg/ml; U1: Zone of inhibition of compound at 50µg/ml; S2: Zone of inhibition of standard at 100µg/ml; S1: Zone of inhibition of standard at 50µg/ml.
Zone diameter of growth inhibition: (------) no inhibition.

Minimum Inhibitory Concentration (MIC)

The examination of the data (Table 6) reveals that most of the compounds showed antibacterial and antifungal activity when compared with standard drugs amoxicillin, ofloxacin and amphotericin B. Compounds **A5** was found to be active against the employed strain to inhibit the growth of organism in comparisons to amoxicillin against *Staphylococcus aureus* (MIC =200µg/ml) and rest of the compounds were showed moderate to poor activity. In

particularly it was found to be compounds **A2**, **A5 and A8** possess the significance activity against *B. subtilis* (MIC =500µg/ml). It was revealed that the presence of electron withdrawing group, especially halogen and electron donating group in the phenyl ring linked to the 1, 3, 4-thiadiazole moiety play a vital role to increase the biological activity. Towards Gram-negative strain compounds **A2**, **A5** and **A8** showed significant activity (MIC =500µg/ ml), whereas **2b** was found to be equally potent, to ofloxacin, *Escherichia coli* (MIC= 100 µg/ml). Against fungal pathogen *Aspergillus niger,* compounds **A8** was found to be highly potent (MIC =250µg/ml) whereas rest were found to exhibit comparable activity to amphotericin B towards *Aspergillus niger.* Compounds **A2, A5, A8** and **A9** were exhibited comparable activity to amphotericin B against *Candida albicans* (MIC=500µg/ml). The remaining compounds showed moderate to poor activity to inhibit the growth of microbial pathogens and are all less effective than standard drugs. Compounds **A1-A9**, some of them are essentially possess a tertiary alkylamine moiety which might be rapidly converts the "base" into the corresponding water soluble salts with the various mineral acids. Obviously, the basic entity is frequently regarded as the hydrophilic entity of the tested compound. It has been scientifically proved that the onium ions generated by protonation of the tertiary amine function were urgently required in carrying out the binding phenomenon with the receptors. From antimicrobial screening results, it is interesting to note that a minor alteration in the molecular configuration of the investigated compounds may have a pronounced effect on antimicrobial activity.

Table 6: Antimicrobial activity of the synthesized compounds

Compound	Minimum inhibitory concentration (MIC, µg/mL)					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	S. aureus	*B.subtilis*	*E. coli*	*P . aeruginosa*	*A . niger*	*C. albican*
A1	1000	1000	1000	1000	1000	1000
A2	250	500	500	500	1000	500
A3	1000	1000	1000	1000	1000	1000
A4	1000	500	1000	1000	1000	1000
A5	200	500	500	500	500	500
A6	1000	1000	1000	1000	1000	1000
A7	250	1000	1000	1000	1000	1000
A8	500	500	500	1000	250	500
A9	1000	1000	1000	1000	1000	500
Amoxicillin	50	250	----	----	----	----
AmphotericinB	----	----	----	----	250	100
Ofloxacin	----	----	100	250	----	----

CONCLUSION

The preparation procedures follow in this work offers reduction in the reaction time, operation simplicity, cleaner reaction and easy work-up. Thiadiazoles are mesoionic system, a poly-heteroatomic system containing a five-membered heterocycle associated with a conjugation of p and π electrons and distinct regions of positive and negative charges leading to highly polarizable derivatives. This distinctive characteristic allows mesoionic compounds to effectively cross-cellular membranes and interacts with biological molecules in unique ways. The good liposolubility of the sulphur atom in the heterocycle might also have a

positive effect on the biological activity and pharmacokinetic properties of thiadiazole-containing compounds. The antimicrobial data given for the compounds allowed us to state that the variation of antimicrobial activity may be associated with the nature of tested microorganisms and also is due to the chemical structure of the tested compounds. Performed SAR observation has showed the importance of electronic environment on antimicrobial activity. The presence of halogens (especially chloro) substituents on the aromatic ring have increased the activity of the compounds compared to those with other substituent which may be due to the presence of the versatile pharmacophore which might increase the lipophilic character of the molecules and thus facilitate the crossing through the biological membrane of the microorganisms and thereby inhibit their growth.

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