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SYNTHESIS, SPECTRAL STUDIES OF SOME IMIDAZO [2, 1-b][1,3,4]THIADIAZOLE DERIVATIVES AS A POTENT ANTIMICROBIAL AGENTS

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ABSTRACT

Heterocyclic compounds occupy a central position among those molecules that makes life possible. The chemistry of heterocyclic compounds has been an interesting field of study for a long time. Heterocyclic nucleus 1, 3, 4 thiadiazole constitutes an important class of compounds for new drug development. The synthesis of novel thiadiazole derivatives and investigation of their chemical and biological behaviour have gained more importance in recent grades. During the recent years there has been intensive investigation of different class of thiadiazole compound many of which possess pharmacological activities. Among of these compounds having 1,3,4-thiadiazole nucleus are known to exhibit unique anti-inflammatory, anti-analgesics, antimicrobial, anti-tumour, antifungal, antimicrobacterial, anticonvulsant, antidiabetic, antiviral activities. So, far modification of thiadiazole ring have proven highly effective with improved potency and lesser toxicity. The present review highlights the recently synthesized thiadiazole possess important biological activities.

KEYWORDS: Antimicrobial agents, 1, 3, 4- Thiadiazole, Imidazo thiadiazoles.

1. INTRODUCTION

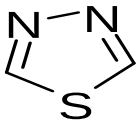
1.1 Antimicrobial Agents

Antimicrobial drugs are the greatest contribution of the 20th century to therapeutics. Infectious diseases are the leading cause of death. Microbial infections caused by various types of bacteria and fungi. The treatment of infectious diseases still remains an important and challenging problem because of a combination factors including emerging infectious

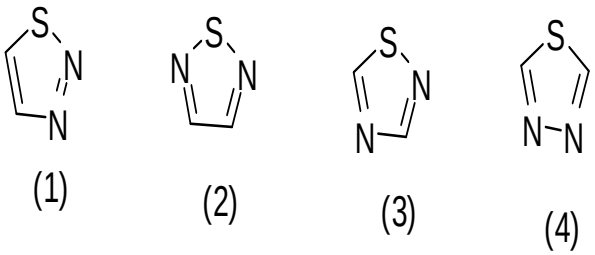
diseases and increasing numbers of multi drug resistant pathogens and to treat infections caused by multi drug bacteria,[1-6] Any antimicrobial is any substance of natural, semisynthetic or synthetic origin that kills or inhibit the growth of microorganisms but causes little or no damage to host. All antibiotics are antimicrobials but not all antimicrobials are antibiotics.[7] Antimicrobials that kill microbes are called microbiocidal and those inhibit their growth called microbiostatic. Antimicrobial medicines can be classified according to microorganisms to whom they act against.

1.2 Thiadiazoles

Thiadiazole is five membered system containing hydrogen binding domain. Thiadiazole is heterocyclic compound bearing two nitrogen atom and one sulphur atom as part of aromatic five membered ring.[8] Sulphur atom and two electron donar nitrogen system exhibit a wide variety of biological activity.

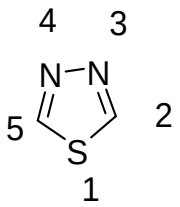


Thiadiazole and related compounds are called 1,3,4- Thiadiazole (two nitrogen and one other heteroatom in a five membered ring.) They occur in nature in four isomeric forms: 1,2,3-thiadiazole, 1,2,5-thiadiazole, 1,2,4-thiadiazole, 1,3,4-thiadiazole.[9]



(1) (2) (3) (4)

Thiadiazole can act as the bio-isosteric replacement of thiazole moiety. This nucleus is known to have unique antibacterial and anti-inflammatory activities. The numbering of monocyclic azole system begins with heteroatom that is in the highest group in the periodic table and with the element of lowest atomic weight in that group. Hence the numbering of 1,3,4-thiadiazole is done in this way. This designated that one sulphur group is present in ring.[10]



3 1,3,4- Thiadiazoles

1,3,4- Thiadiazoles have become an important class of heterocycles and a great interest of researchers because of their broad types of biological activity. 1,3,4- Thiadiazole nucleus is one of the most important and well known heterocyclic nuclei which is a common and integral feature of a variety of natural products and medicinal agents. It represents an important heterocyclic system due to their pharmacological activities and are associated with diverse biocidal activities by virtue of toxophoric –N=C-S grouping. The 1,3,4- Thiadiazole isomer of thiadiazole series and its dihydro-derivatives provide a bulk of literature on thiadiazole. 1,3,4-thiadiazole derivatives possess interesting biological activity probably conferred to them due to strong aromaticity of the ring system which leads to great *in vivo* stability and generally lack of toxicity for higher vertebrates including humans when diverse functional group that interact with biological receptor are attached to aromatic ring,[11] Thiadiazole is also bioisosteres of oxadiazole, oxazole and benzene. Substitution of these heterocycles with a thiadiazole typically leads to analogues with improved activities because sulfur atom imparts improved liposolubility.[12] The literature review showed that the thiadiazole nuclei have antimicrobial, antiinflammatory, anticancer, anticonvulsant, antidepressant, antioxidant, radioprotective and anti-leishmanial activities.[13] The biological activity of the compounds is mainly dependent on their molecular structures. [1,3,4]thiadiazoles are very interesting compounds due to their important applications in many pharmaceutical, biological and analytical field.[14]

2. MATERIALS AND METHOD

Chemicals used were of LR grade and purchased from Spectrochem, Sigma-Aldrich, Merck India, Loba chemical. All solvents were used after purification, distillation and drying. Silica gel pre-coated plates from e-Merck and Co., were used for TLC and spots located either by UV or by visualizing reagents. The identification and characterization of the compounds were carried out by the following procedure to ascertain that all the prepared compounds were of different chemical nature than the respective parent compound.

Melting point

Solubility

Thin layer chromatography

Infrared Spectroscopy

Proton nuclear magnetic resonance

Mass spectroscopy

Solvent systems used for developing the chromatograms are:

- Methanol: Chloroform (1%)

Instruments used for characterization

All the melting points are determined in open-capillary tubes by heating in paraffin bath and were uncorrected. The IR spectra were recorded by placing the solid sample on ATR crystal by using Bruker's FTIR and the transmittance spectra were obtained by using OPUS 6.5 software. ^{1}H NMR and ^{13}C NMR spectra of the synthesized compounds were recorded in $CDCl_3$/DMSO solution on a Bruker Avance. II 400 MHz NMR spectrometer at SAIF, Punjab University (Chandigarh). Proton chemical shift are relative to tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on MAT 120 at SAIF, Punjab University (Chandigarh)

3. CHEMISTRY

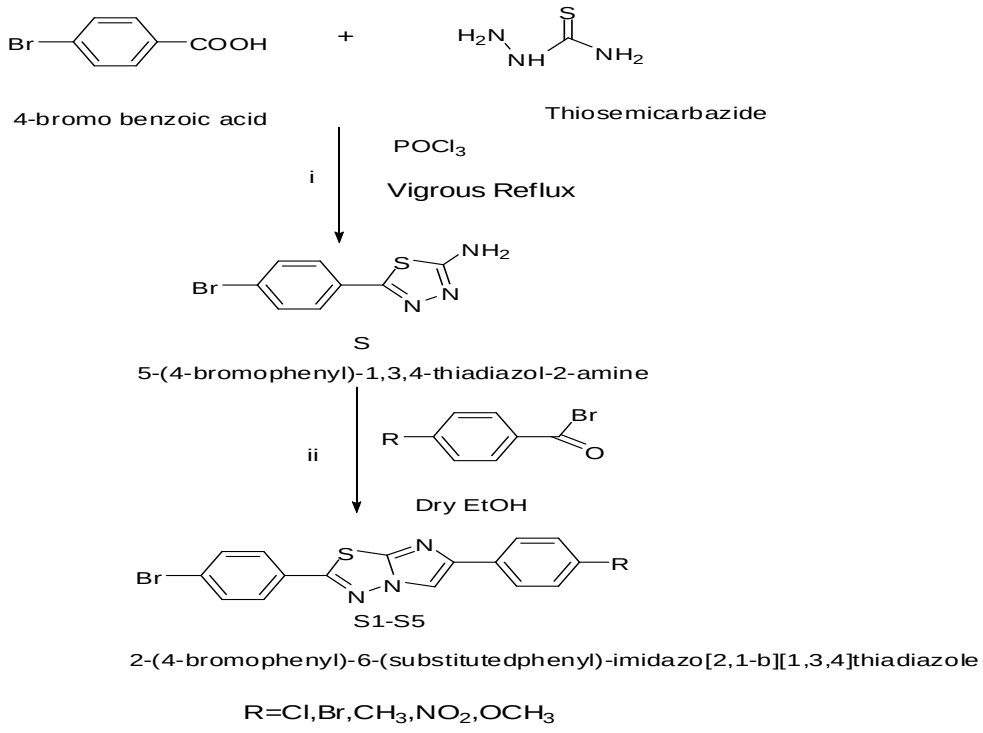


Figure 1: General scheme for synthesis of Imidazo[2,1-b][1,3,4]Thiadiazole derivatives

3.1 General procedure for synthesis of Imidazo[2,1-b][1,3,4]Thiadiazole derivatives

3.1.1 Step -1 Synthesis of 5-(4-bromophenyl)-1,3,4-thiadiazol-2-amine (S)

The mixture of 4-bromobenzoic acid (50mmol), thiosemicarbazide (50mmol) and $POCl_3$ (25ml) was refluxed at 75°C for 2 hours. After cooling down to room temperature, cold water was added. The mixture was again refluxed for 5 hrs. After cooling, the mixture was alkalined to PH 8 by the dropwise addition of 50% NAOH solution under stirring. The precipitates were filtered and recrystallized from ethanol to obtain compound **(S).**

3.1.2 Step -2 Synthesis of 2-(4-bromophenyl)-6-substitutedphenyl)Imidazo[2,1-b][1,3,4] Thiadiazole derivatives (S1-S5)

A mixture of equimolar quantities of 5-(4-bromophenyl)-1,3,4-thiadiazol-2-amine**(S)** and substituted bromoacetyl compound was refluxed in dry ethanol for 46 hrs. The excess of solvent was distilled off and the solid hydrobromide separated out was collected by filteration then suspended in water and neutralized by aqueous sodium carbonate solution to get free base **(S1-S5).** It was filtered washed with water, dried and recrystallized from suitable solvent like ethanol, methanol and acetone etc.

4. BIOLOGICAL EVALUATION

4.1 Antimicrobial activity

Antibacterial and antifungal activities were measured by disc- diffusion method (for zone of inhibition) and microdilution method (for minimum inhibitory concentration). The activity of compounds are prepared with ciprofloxacin and fluconazole as standard drugs for antibacterial and antifungal evaluation.

The synthesized compounds were tested for antibacterial and antifungal activity by method named as disc diffusion method.

In this method the given compounds were dissolved in the extraction solvent di methyl sulfoxide (DMSO) to a final concentration. Antimicrobial tests were then carried out using the disc diffusion method with a suspension containing 10^8 colony forming units per ml of bacteria and 10^6 colony forming units per ml of yeast/fungi spread on nutrient agar (HMEDIA) and Saboured Dextrose Agar (HIMEDIA) resp. Compounds were applied to the disc (6mm diameter) and allow to soak in and were then placed on the inoculated media. Negative controls were prepared using the same solvents as employed to obtain the extracts. As positive controls ciprofloxacin (10mcg CDRI) used for bacterial strains and fluconazole

(10 mcg CDRI) for fungal strains. The inoculated plates were incubated at 37°C for 24 hrs for bacterial strains and at 25°C for 48 hrs to 120 hrs for yeast/fungal strains. Antimicrobial activity was evaluated by measuring the inhibition zone against test microorganisms that was sensitive to given compounds in the disc diffusion assay.

5. RESULTS AND DISCUSSION

5.1 Physical Characterization

Melting points of the synthesized compounds was taken in open capillary tubes and was uncorrected and were found to be in the range of 110-160°C. Thin layer chromatography was performed using silica gel coated plates of 0.25 mm thickness. Eluents used were methanol: chloroform (1%). Spots were observed and the Rf value was calculated. IR spectra were recorded on FT-IR spectrometer. ^{1}H NMR DMSO/CDCL$_3$ spectra were measured on a Bruker Avance II MHZ. Mass spectra were recorded on MAT 120. The starting materials were either commercially available or synthesized according to the references cited.

All the synthesized compounds were found to be freely soluble in Methanol, Chloroform, DMSO and water.

General Products

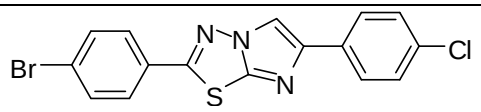
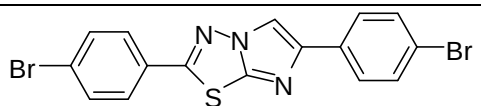
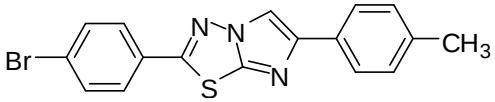
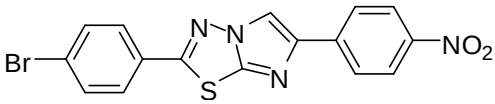
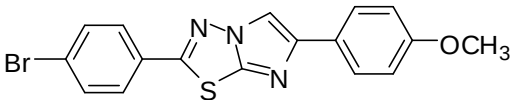
Compd.	Bromoacetyl compounds	Name	Structure
S1	4-chloro phenacyl bromide	2-(4-bromophenyl)-6-(4-chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazole	
S2	4-bromo phenacyl bromide	2,6-bis(4-bromophenyl)imidazo [2,1-b][1,3,4]thiadiazole	
S3	4-methyl phenacyl bromide	2-(4-bromophenyl)-6p-tolyl imidazo[2,1-b][1,3,4]thiadiazole	
S4	4-nitrophenacyl bromide	2-(4-bromophenyl)-6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole	
S5	4-methoxy phenacyl bromide	2-(4-bromophenyl)-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazole	

Table 1. Physical Characterization Of Synthesized Compounds (S1-S5)

S.No	Compounds	% Yield	MeltingPoint (°C)	Molecular Formula	Molecular Weight	Rf Value
1.	S1	28	140-160	$C_{16}H_9BrClN_3S$	390.6	0.5
2.	S2	84	110-120	$C_{16}H_9Br_2N_3S$	435.1	0.6
3.	S3	61	130-140	$C_{17}H_{12}BrN_3S$	368.9	0.6
4.	S4	68	130-135	$C_{16}H_9BrN_4O_2S$	399.6	0.6
5.	S5	74	140-145	$C_{17}H_{12}BrN_3OS$	384.9	0.5

5.2 Spectral analysis

The infra red spectroscopy was performed on FT-IR instrument. Presence of stretching in the range 3150-3050 Cm^{-1} indicates the presence of CH functional group. Stretching between 1550 & 1350 Cm^{-1} indicates the presence of N=O group. Presence of stretching range between 1600&1475 Cm^{-1} indicates the presence of C =C group. Stretching range between 1690-1640 Cm^{-1} indicates the presence of C=N functional group. Presence of stretching ranges between 1350-1000, 785-540, <667, 1450&1375 Cm^{-1} indicates the presence of functional group C-N, Chlorine, bromine and methyl respectively.

^1H Nuclear magnetic resonance spectroscopy was recorded on Bruker Avance II 400 MHZ . ^1H NMR the chemical shifts were reported as parts per million downfield from tetramethylsilane, solvent used as DMSO. ^1H NMR of compounds shows downfield at 4.8-5.2 that confirms the presence of $–NH_2$ group in the structures. All aromatic protons showed characterstic downfield. All compounds showed respective downfields that supported the structure of various synthesized thiadiazoles.

Mass spectroscopy was performed on MAT 120 using DMSO as solvent. All the compounds showed characterstic molecular ion (m+2, m-2, m-3, m+3) peaks and base peak that further confirms the structure of the synthesized compounds.

5.2.1 2-(4-bromophenyl)-6-(4-chlorophenyl)Imidazo[2,1-b][1,3,4]Thiadiazole (S1)

m/z: 394.9[m+4] ; IR cm^{-1}: 1674.6(Arom, -C=C), 3081.0(Arom, -C-H), 1583.7(-C=N), 1394.8(-C-N), 728.3(-C-Cl) ; ^1H NMR: 8.7(s, Ar-H,imidazole), 7.4-8.4(m, 8H, Ar-H).

5.2.2 2,6-bis(4-bromophenyl)Imidazo[2,1-b][1,3,4]Thiadiazole (S2)

m/z: 437.8[m+2] ; IR cm^{-1}: 1389.02(Arom, -C=C), 3047.6(Arom, -C-H), 1666.04(-C=N), 1389.2(-C-N), 593.09(-C-Br) ; ^1H NMR: 8.2(s, Ar-H,imidazole), 7.4-8.1(m, 8H, Ar-H).

5.2.3 2-(4-bromophenyl)-6-p-tolylimidazo[2,1-b][1,3,4]Thiadiazole (S3)

m/z: 365.2[m-3] ; IR cm^{-1}: 1462.7(Arom, -C=C), 3004.4(Arom, -C-H), 1668.6(-C=N), 1392.2 (-C-N), 1392.2(-C-CH_3) ; ^{1}H NMR: 8.2(s, Ar-H,imidazole), 7.6-8.1(m, 8H, Ar-H), 3.83(s, 3H, -OCH_3).

5.2.4 2-(4-bromophenyl)-6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]Thiadiazole (S4)

m/z: 397.05[m-2] ; IR cm^{-1}: 1591.5(Arom, -C=C), 3106.3(Arom, -C-H), 1700.1(-C=N), 1003.1(-C-N), 1591.5(-C-NO_2) ; ^{1}H NMR: 8.2(s, Ar-H,imidazole), 7.1-8.0(m, 8H, Ar-H).

5.2.5 2-(4-bromophenyl)-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]Thiadiazole (S5)

m/z: 385[m+1] ; ^{1}H NMR: 2.5(s, 3H, -CH_3), 8.4(s, Ar-H, imidazole), 7.6-8.5(m, 8H, Ar-H).

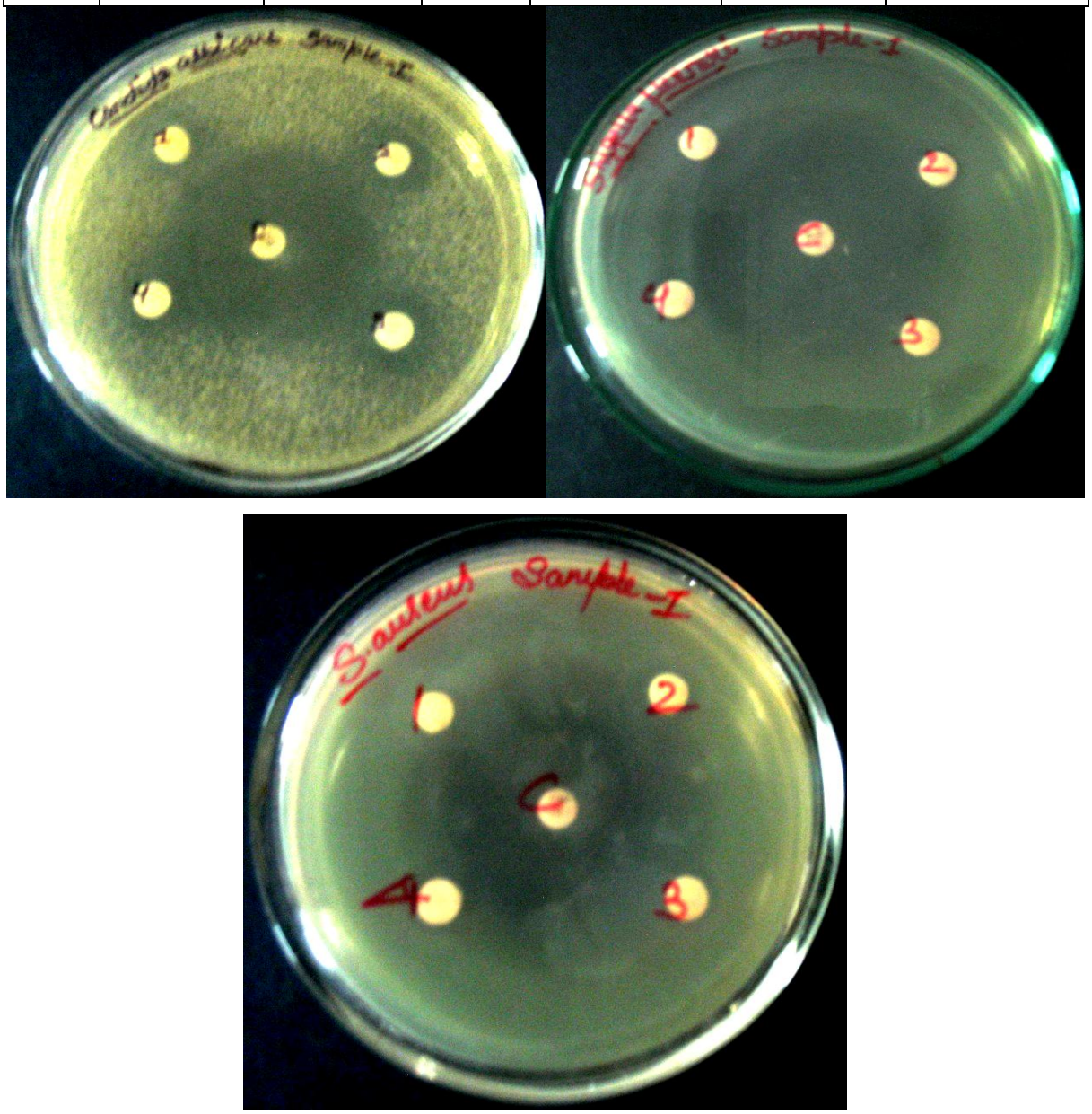
5.3 Antimicrobial activity

Antimicrobial activity was measured by disc-diffusion method. All the synthesized compounds were subjected to biological evaluation for antimicrobial activities. In this standard drugs used are fluconazole and ciprofloxacin and strains used are Stayphylococcus aureus(MTCC 3160), Shigella flexneri(MTCC 1457), Candida albicans(MTCC 227). Compounds S1-S5 possess god activity against Candida albicans as compared to Stayphylococcus aureus. All the compounds showed variability in activity against Shigella flexneri at various sample concentrations.

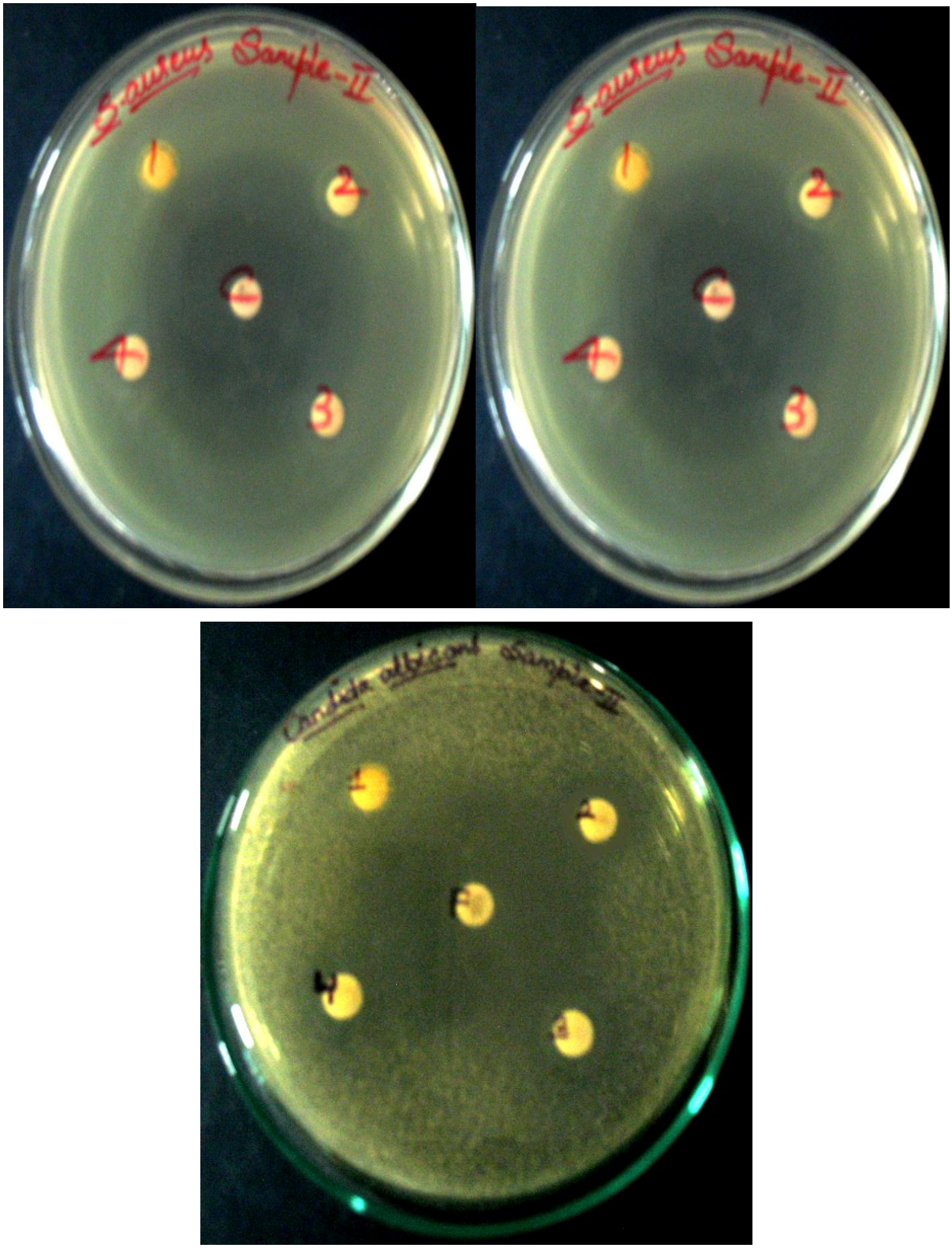
Table 2: Zone of inhibition produced by compounds and standard drugs

Sr. No.	Compound	Conc. µg/ml	Disc size	Zone of inhibition in mm		
				S.aureus MTCC 3160	C. albicans MTCC 227	Shigella flexneri MTCC 1457
1.	Standard	10 mcg/ml	6mm	33.97 mm	30.45 mm	43.90 mm
	4-Cl (S1)	1000mg/ml	6mm	14.7 mm	12.46 mm	9.76 mm
		500mg/ml	6mm	11.17 mm	11.696 mm	9.23 mm
		250mg/ml	6mm	10.25 mm	11.54 mm	8.92 mm
		125mg/ml	6mm	9.12 mm	10.33 mm	6.54 mm
2.	Standard	10 mcg/ml	6mm	31.77 mm	26.46 mm	44.47 mm
		1000mg/ml	6mm	NIL	10.45 mm	NIL
	4-Br (S2)	500mg/ml	6mm	9.51 mm	12.46 mm	8.07 mm
		250mg/ml	6mm	8.93 mm	12.26 mm	7.16 mm
		125mg/ml	6mm	8.05 mm	11.71mm	6.20 mm
3.	Standard	10 mcg/ml	6mm	36.84 mm	34.79 mm	42.86 mm
	4-CH_3	1000mg/ml	6mm	NIL	16.98 mm	NIL
		500mg/ml	6mm	NIL	16.88mm	8.9 mm
		250mg/ml	6mm	8.02 mm	13.06mm	NIL

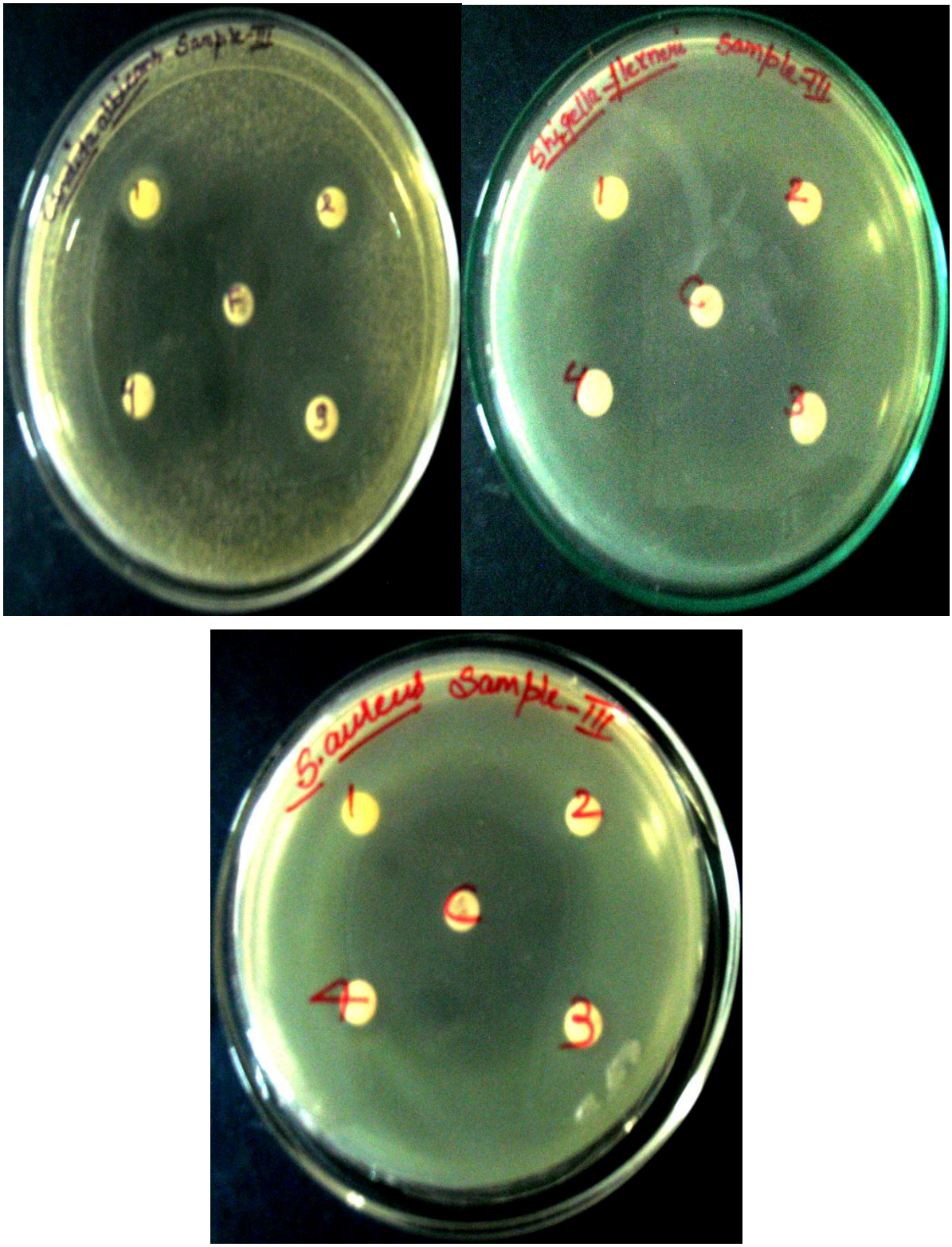
	(S3)	125mg/ml	6mm	7.12 mm	13.05mm	7.9 mm
4.	Standard	10 mcg/ml	6mm	33.40 mm	32.60 mm	44.26 mm
	4-NO_2 (S4)	1000mg/ml	6mm	6.79 mm	14.25 mm	12.97 mm
		500mg/ml	6mm	6.42 mm	14.12 mm	11.72 mm
		250mg/ml	6mm	6.10 mm	12.17 mm	10.69 mm
		125mg/ml	6mm	NIL	11.16 mm	12.64 mm
5.	Standard	10mcg/ml	6mm	35.10 mm	36.23 mm	44.42 mm
	4- OCH_3 (S5)	1000mg/ml	6mm	NIL	14.10 mm	NIL
		500mg/ml	6mm	7.12 mm	12.53mm	10.30 mm
		250mg/ml	6mm	NIL	11.83 mm	10.13 mm
		125mg/ml	6mm	6.6 mm	11.14 mm	6.92 mm



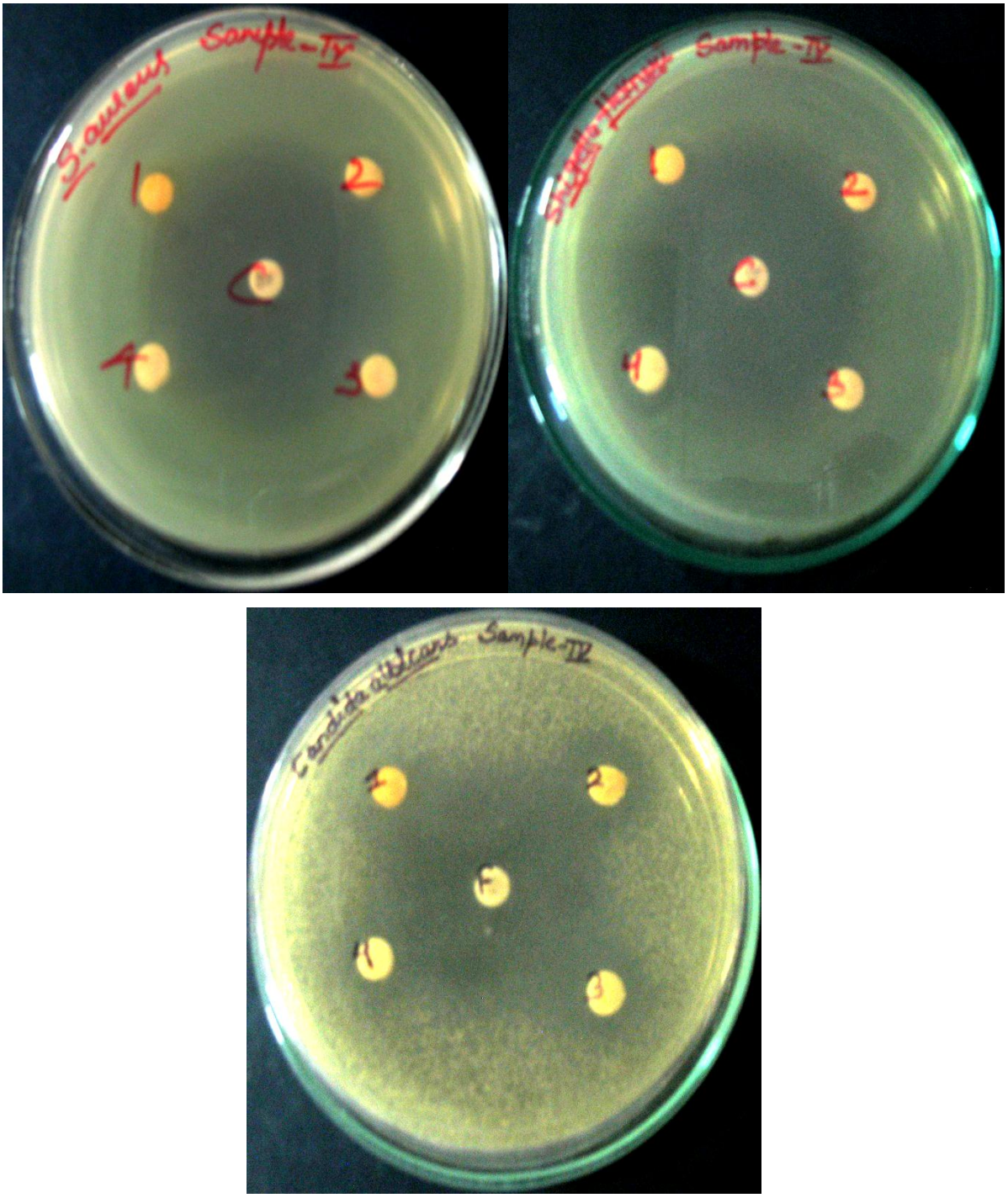
Photograph 1. Plates showing inhibition of bacterial strains



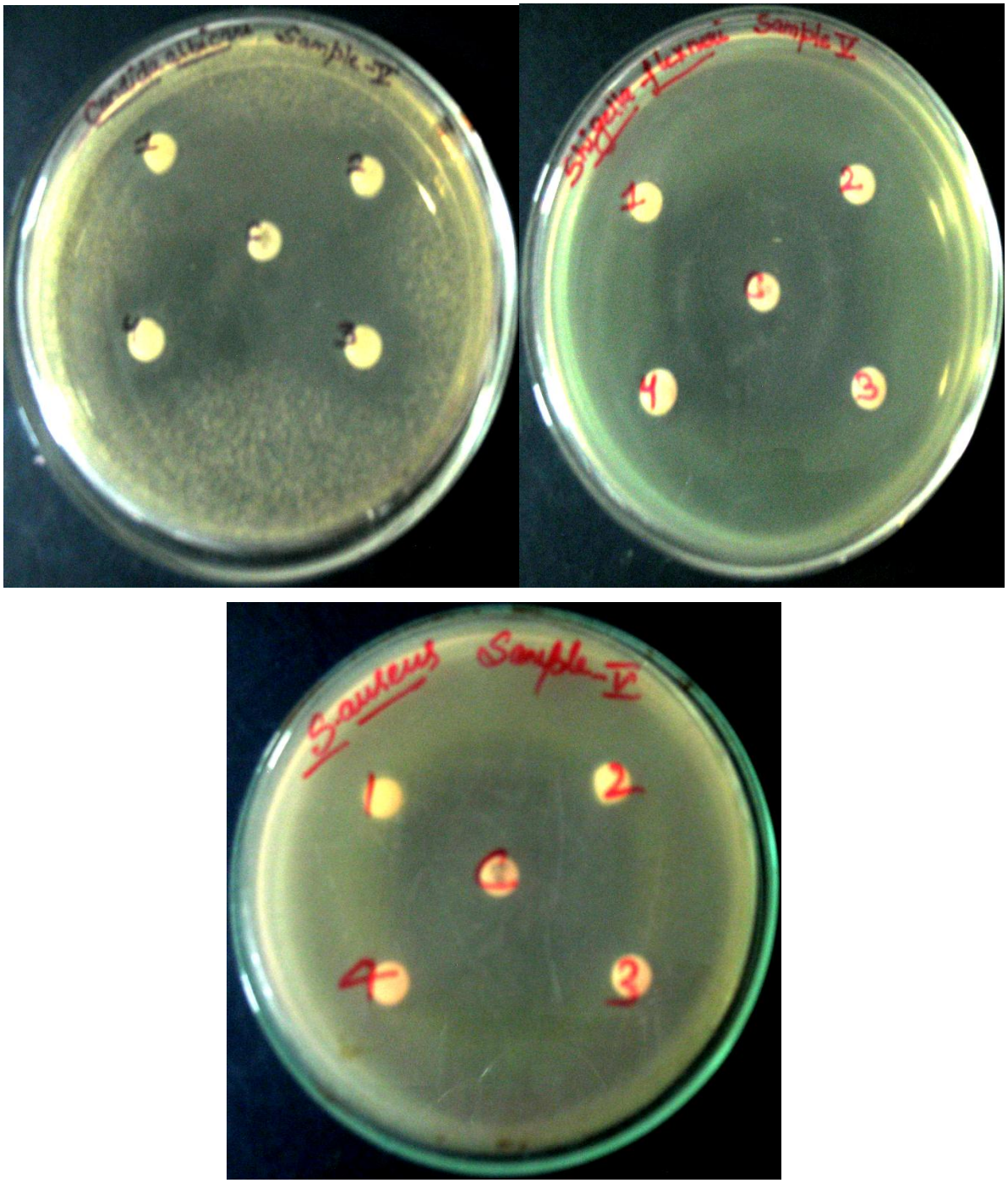
Photograph 2. Plates showing inhibition of bacterial strains



Photograph 3. Plate showing inhibition of bacterial strains



Photograph 4. Plates showing inhibition of bacterial strains



Photograph 5. Plates showing inhibition of bacterial strain

DISCUSSION

We tried to synthesized various derivatives of 5-(4-bromophenyl)-1,3,4-thiadiazol-2-amine **(S)** using 4-bromo benzoic acid as starting material . Synthesis was carried according to reaction shown in scheme. The compound**(S)** was prepared according to literature method using 4-bromo benzoic acid and thiosemicarbazide and heating in phosphorous oxychloride at 75˚C followed by vigorous refluxing with water for 5 hours. This 5-(4-bromophenyl)-1,3,4-

thiadiazol-2-amine**(S)** was further condensed with various substituted bromoacetyl compounds in the presence of dry ethanol and refluxed for the duration of 46 hours to get preparation of 2-(4-bromophenyl)-6-(substitutedphenyl)imidazo[2,1-b][1,3,4]thiadiazole**(S1-S5).** The reaction was monitored by Thin-Layer Chromatography using suitable mobile phase such as Chloroform: Methanol. The Rf values were compared and found that they were different from each others. After the completion of reaction the product were purified by appropriate method. The melting point of the derivatives was determined. The melting point obtained was different from each other which confirmed the formation of new derivatives. These synthesized compounds were then further evaluate for their antimicrobial activities against bacterial and fungal strains as shown in the results.

6. CONCLUSION

All the newly synthesized Imidazo [2,1-b][1,3,4]thiadiazole derivatives were analysed with different spectral techniques and screened invitro for their antimicrobial activity against both gram-positive and gram-negative strains of bacteria and also subjected for antifungal activities. The observed zone of inhibition was observed with the help of Vernier Calliper in mm.

The zone of inhibition produced by different compounds shows that,

1. In Staphylococcus aureus chlorinated compound shows good result for antimicrobial activity than other synthesized derivatives. Substitution by other halogens such as bromine, methyl, methoxy, nitro decreases the activity.
2. In Candida albicans methylated compound is better than other synthesized derivatives. Substitution by other halogens decreases activity.
3. In Shigella flexneri nito compound is better than other synthesized compounds. Substitution by other halogens decreases activity.

The Candida albicans shows good activity against Stayphylococcus aureus. For future perspective more structural variations are needed to explore and optimize the pharmacophore for its antibacterial activity.

7. ACKNOWLEDGMENT

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