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Synthesis, spectral studies and antimicrobial activity of some imidazo [2,1-b] [1,3,4] thiadiazole derivatives

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ABSTRACT

In the present study, we have reported the synthesis of various Imidazo [2, 1-b] [1,3,4] thiadiazole derivatives. These derivatives are of interest because they exhibit a wide range of pharmacological activities. In the reactions we have reacted various substituted benzoic acids with thiosemicarbazide in the presence of Phosphorous Oxychloride to yield the substituted 1, 3, 4-thiadiazole nucleus. Further this was treated with various substituted bromoacetyl compounds to synthesize various derivatives. These derivatives were further characterized by various spectral techniques and were screened for the antimicrobial activity against Bacterial strains of Shigella flexneri, Staphylococcus aureus and Candida albicans.

INTRODUCTION

Antimicrobial drugs are the greatest contribution of the 20th century to therapeutics. Their advent changed the outlook of the physician about the power of drugs can have on diseases [1]. Thiadiazole is a versatile moiety that exhibits a wide variety of biological activities. It acts as “hydrogen binding domain” and “two electron donor system” with a constrained pharmacophore [2]. Thiadiazole can act as the bio-isosteric replacement of thiazole moiety. So it act like third and fourth generation cephaelosporins. Hence it can be used in antibiotic preparations. Thiadiazole is 5-membered ring system containing two nitrogen and one sulphar atom [3]. The 1, 3, 4-thiadiazole isomer of thiadiazole series and its dihydro-derivatives provide a bulk of literature on thiadiazole. The literature review showed that the thiadiazole nuclei have antimicrobial [7-9]. Antitubercular [10], anticancer [11-12], anti-inflammatory [13], anticonvulsant, antidepressant, antioxidant, radioprotective and anti-leishmanial activities [4], Many drugs containing thiadiazole nucleus are available in the market such as acetazolamide, methazolamide, sulfamethazole etc. The fusion of a imidazole ring with a [1,3,4]thiadiazole nucleus give rise to a class of heterocyclic systems containing a bridgehead nitrogen atom known as imidazo- thiadiazoles. These may be of two types, the imidazo[2,1-b][1,3,4]thiadiazoles and the imidazo[5,1-b][1,3,4]thiadiazoles [5], Imidazo[2,1-b][1,3,4]thiadiazole derivatives were first discovered in the early 1950s and, since then, the research work on this heterocyclic system has led to significant developments in their chemistry and biology. The structures of imidazo[2,1-b][1,3,4] thiadiazoles are closely related to the biologically vibrant imidazo[1,3,4]thiazole heterocycles, in which the -CH group in the thiazole ring is substituted by the isosteric nitrogen atom, but their properties often possess marked differences [6].

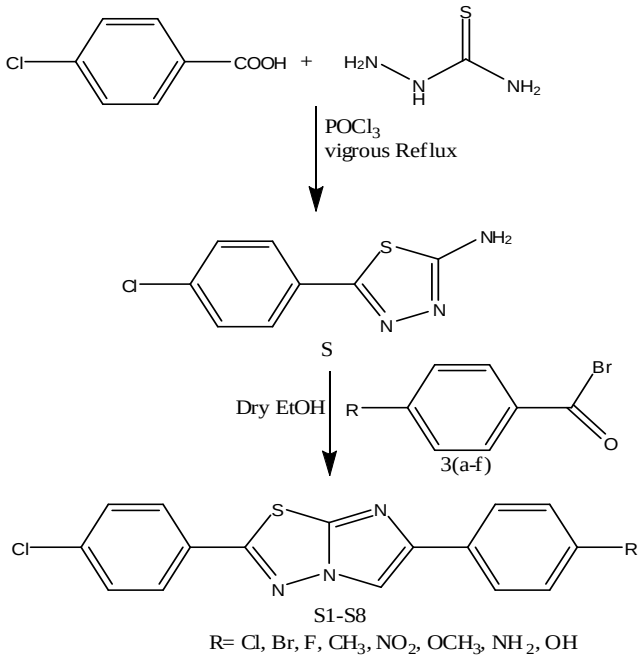
MATERIALS AND METHODS

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. Various solvent systems used for developing the chromatograms are:

- Hexane:Ethylacetate (40%)
- Methanol:chloroform (1%)

All the melting points are determined in open-capillary tubes by heating in paraffin bath and were uncorrected. ^{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 shifts are relative to Tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on MAT 120 at SAIF, Punjab University (Chandigarh).

2.2 SCHEME

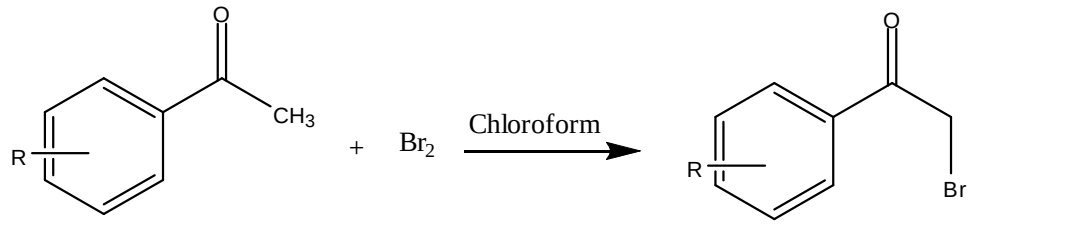


2.3 PREPARATION OF 5-(4-CHLOROPHENYL)-1, 3, 4-THIADIAZOL-2-AMINE (S)

The mixture of 4-chlorobenzoic acid (50mmol), thiosemicarbazide (50mmol) and $POCl_3$ (20ml) was refluxed at 75°C for 2 hrs. After cooling down to room temperature, cold water was added. The mixture was again refluxed for 4 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)**.

2.4 PREPARATION OF SUBSTITUTED BROMOACETYL COMPOUNDS 3(a-h)

General Reaction



Substituted Acetophenone — Substituted Bromoacetyl Compounds 3(a-h)

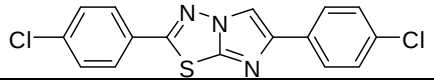
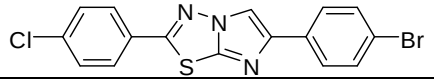
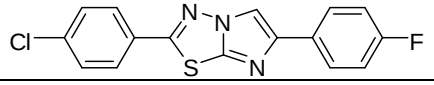
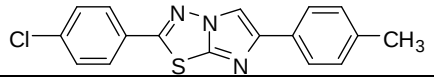
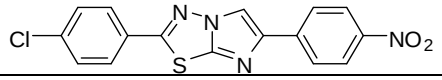
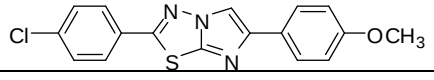
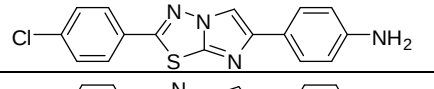
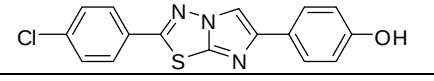
General Procedure

Substituted Acetophenone (25 mmol) was dissolved in chloroform (10ml). To the mixture, bromine (25mmol) was added at once. The flask was then immediately attached to a reflux condensor suitably for the absorption of hydrogen bromide, then it was shaked to make the mixture homogeneous and then allowed to stand. After a few minutes a vigorous reaction took place with the evolution of hydrogen bromide. The reaction was settled down for some time and solid was obtained.

2.5 PREPARATION OF 2-(4-CHLOROPHENYL)-6-(SUBSTITUTED PHENYL) IMIDAZO[2,1-b][1,3,4] THIADIAZOLE (S1-S8)

A mixture of equimolar quantities of 5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine **(S)** and substituted bromoacetyl compound **(3a-3h)** was refluxed in dry ethanol for 46 hrs. The excess of solvent was distilled off and the solid hydrobromide that separated was collected by filteration, suspended in water, and neutralized by aqueous sodium carbonate solution to get free base **(S1-S8)**. It was filtered washed with water, dried, and recrystallized from suitable solvent like ethanol, methanol, and acetone etc.

General products

Compound	Bromoacetyl compounds	Name	Structure
S1	4-chloro phenacyl bromide	2,6-bis(4-chlorophenyl)imidazo [2,1-b] [1,3,4] thiadiazole	
S2	4-bromo phenacyl bromide	6-(4-bromophenyl)-2-(4-chlorophenyl) imidazo [2,1-b][1,3,4]thiadiazole	
S3	4-fluoro phenacyl bromide	2-(4-chlorophenyl)-6-(4-fluorophenyl) imidazo [2,1-b][1,3,4]thiadiazole	
S4	4-methyl phenacyl bromide	2-(4-chlorophenyl)-6p-tolyl imidazo [2,1-b] [1,3,4]thiadiazole	
S5	4-nitro phenacyl bromide	2-(4-chlorophenyl)-6-(4-nitrophenyl) imidazo [2,1-b][1,3,4]thiadiazole	
S6	4-methoxy phenacyl bromide	2-(4-chlorophenyl)-6-(4-methoxyphenyl) imidazo [2,1-b][1,3,4]thiadiazole	
S7	4-amino phenacyl bromide	2-(4-chlorophenyl)-6-(4-aminophenyl) imidazo [2,1-b][1,3,4]thiadiazole	
S8	4-hydroxy phenacyl bromide	2-(4-chlorophenyl)-6-(4-hydroxyphenyl) imidazo[2,1-b][1,3,4]thiadiazole	

2.6 DETERMINATION OF ANTIMICROBIAL ACTIVITIES OF SYNTHESIZED COMPOUNDS

Disc diffusion Susceptibility Testing was performed to carry out the initial screening of relative antimicrobial effects of the synthesized compounds. This method is a simple way of determining the susceptibility of a microorganism to an antimicrobial agent by inoculating an agar plate with the culture and allowing the antimicrobial agent to diffuse into the agar medium. A filter disc impregnated with the agent is applied to the surface of an agar plate containing the microorganism to be tested. The effectiveness of a particular antimicrobial agent is shown by the presence of growth inhibition zones. The zones of inhibition appear as clear areas surrounding the disc from which the substances with antimicrobial activity diffused. In the disc diffusion assays, Tests were carried out against bacterial and fungal strains. The synthesized compounds were tested for activity against *Candida albicans*, *Staphylococcus aureus* and *Shigella flexneri.*

2.6.1 STANDARD PROCEDURE

1. Fresh cultures were prepared daily in 10 ml broth by transferring one loop of stock bacteria which are kept in –80°C.
2. These cultures incubated for 18 h and subcultures were obtained by transferring 30 µl from this 18 h incubated cultures to fresh broth (10 ml).
3. Experiments were performed with daily prepared subcultures which are standardized for inoculation on agar surface corresponding to certain numbers of CFU/ ml. Log phases of growth curves were taken into account to reach approximate inoculation numbers also the standardized inoculums were confirmed by measuring OD values.

4. 100 µl of bacteria culture during the lag phase were inoculated on to agar surface. The agar dept adjusted to 25ml for each plate.
5. Inoculated culture was dispersed by streaking the sterile swab over the entire sterile agar surface by rotating the plate 60°C each time to ensure the inoculums uniformly spread.
6. The inoculated plates were allowed to sit for 5-10 minutes to let the broth absorb into agar.
7. Sterile blank discs were applied by soaking them into sterilized extracts by using 0.45 µm filters.
8. The concentration of extract solutions was determined as direct 10 µl for plant extracts.
9. *D*isc was placed on each plate and then gently pressed to ensure contact with the agar surface. The standard disc is placed in centre of plate for comparison.
10. Plates were incubated for 24h at 37°C.
11. After 24h the zone diameters were measured by using a varnier caliper and results were expressed as mm.
12. Assays for disc diffusion were performed twice.
13. After all steps plates were sterilized in Autoclave at 121°C for half an hour and discarded properly as biohazard material.

2.6.2 DISC DIFFUSION ASSAY

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/fungus spread on nutrient agar (HIMEDIA) and Saboured Dextrose Agar (HIMEDIA) resp. Compounds were applied to the disc (6 mm diameter) and allow to soak in, and were than placed on the inoculated Media. Negative controls were prepared using the same solvents as employed to obtain the extracts. As positive controls ciprofloxacin (10 mcg, 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 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.

RESULTS AND DISCUSSION

We tried to synthesized various derivatives of 5-(4-chlorophenyl)-1, 3, 4-thiadiazol-2-amine **(S)** using 4-chloro benzoic acid as starting material. Synthesis was carried according to reaction shown in Scheme. The compound **(S)** was prepared according to literature methods using 4-chloro benzoic acid and thiosemicarbazide and heating in phosphorous oxychloride at 75°C followed by vigorous refluxing with water for 4 hr. This 5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine **(S)** was further condensed with various substituted Bromoacetyl compounds **3(a-f)** in the presence of dry ethanol and refluxed for the duration of 46 hr to get preparation of 2-(4-chlorophenyl)-6-(substitutedphenyl) imidazo[2,1-b][1,3,4]thiadiazole (S1-S8).

3.1. ANALYTICAL DATA OF SYNTHESIZED COMPOUNDS

Compound	Mol. Formula	Mol. Weight	Melting Point	R_f	Yield
S1	$C_{16}H_9Cl_2N_3S$	346.23	158-160°C	0.6	67%
S2	$C_{16}H_9BrClN_3S$	390.68	166-168°C	0.5	74%
S3	$C_{16}H_9ClFN_3S$	329.78	192-194°C	0.7	70%
S4	$C_{17}H_{12}ClN_3S$	325.82	162-164°C	0.6	62%
S5	$C_{16}H_9ClN_4O_2S$	356.79	172-174°C	0.5	69%
S6	$C_{17}H_{12}ClN_3OS$	341.81	188-190°C	0.8	70%
S7	$C_{16}H_{11}ClN_4S$	326.80	178-180°C	0.7	58%
S8	$C_{16}H_{10}ClN_3OS$	327.02	184-186°C	0.6	68%

3.2. SPECTRAL DATA OF SYNTHESIZED COMPOUNDS

S1: IR Bands (KBr cm^{-1}): 1677.24 (Ar, -C=C str), 3000.17 (Ar, -C-H str), 1541.14 (-C=N str), 1341.8 (-C- N str), 793.15 (-C-Cl); **^{1}H NMR (DMSO δppm):** 8.6 (s, Ar-H, imidazole); 7.1-8.2 (m,8H, Ar-H).

S2: ^{1}H NMR (DMSO δppm): 8.6 (s,Ar-H, imidazole); 7.1-8.2 (m,8H, Ar-H), **MS, m/z :** [m^+] 390.2; [m+1] 391.2; [m+2] 392.2

S3: IR Bands (KBr cm^{-1}): 1677.24 (Ar, -C=C str), 3099.17 (Ar, -C-H str), 1564.22 (-C=N), 1405.12 (-C-N), 962.12 (-C-F); **^{1}H NMR (DMSO δppm):** 8.6 (s,Ar-H, imidazole); 7.1-8.2 (m, 8H, Ar-H).

S4: ^{1}H NMR (DMSO δppm): 2.35 (s, 3H, $-CH_3$); 8.44 (s, Ar-H, imidazole); 7.1-8.1 (m,8H, Ar-H); **MS, m/z :** [m+1] 326.2, [m+2] 327.2

S5: IR Bands (KBr cm^{-1}): 1677.24 (Ar, -C=C str), 3099.17 (Ar, -C-H str), 1593.18 (-C=N), 1465.18 (-C-N), 1564.22 (N=O); **^{1}H NMR (DMSO δppm):** 8.8 (s,Ar-H imidazole); 7.5-8.2 (m, 8H, Ar-H).

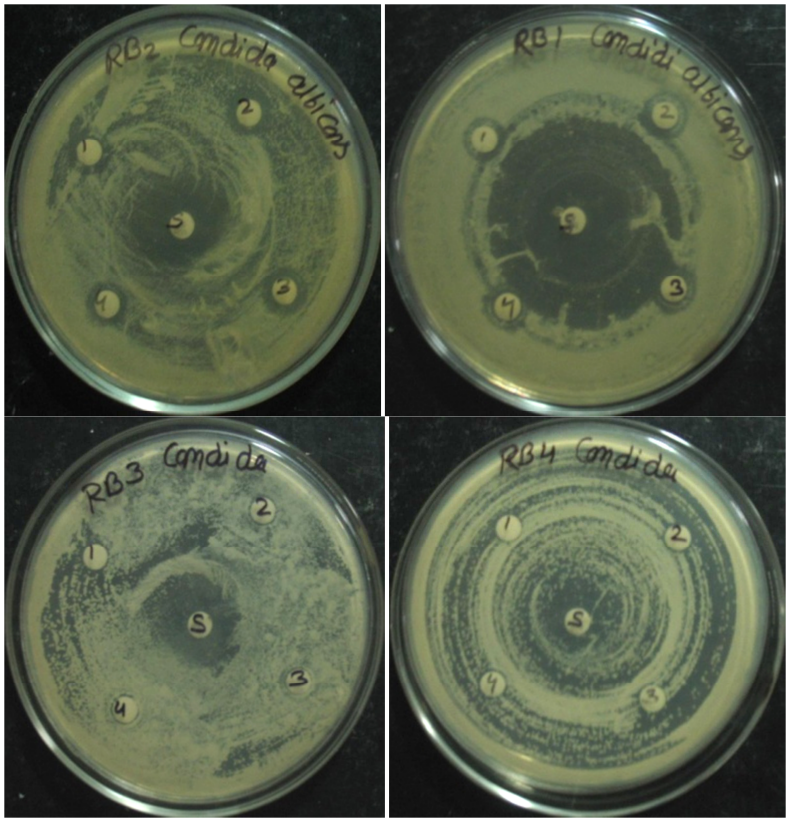
S6: ^{1}H NMR (DMSO δppm): 3.90 (s, 3H, -CH_3); 8.49 (s, Ar-H, imidazole); 7.7-8.1 (m,8H, Ar-H); **^{13}CNMR ($CdCl_3$, δppm):** 66.31, 100.78, 113.20, 124.75, 129.41, 137.98, 151.57, 154.07, 159.99, 160.18, 164.33

S7: IR Bands (KBr cm^{-1}): 1677.6 (Ar, -C=C str), 3085.7 (Ar, -C-H str), 1520.4 (-C=N), 1324.1 (-C-N), 3319.4 (-N-H); **^{1}H NMR (DMSO δppm):** 8.4 (s, Ar-H, imidazole); 3.2(s, 2H,-NH_2); 6.9-8.2(m, 8H, Ar-H).

S8: IR Bands (KBr cm^{-1}): 1663.28 (Ar, -C=C str), 3087.17 (Ar, -C-H str), 1567.18 (-C=N), 1493.18 (-C-N), 3318.5 (O-H); **MS, m/z:** [m^+] 327.02; [m+2] 329.02

3.3 ANTIMICROBIAL ACTIVITY OF SYNTHESIZED COMPOUNDS

The antimicrobial activity of synthesized compounds was evaluated at M.Tech Labs, Panchkula. All the synthesized compounds were subjected to antimicrobial activity against Gram +ve bacteria: *Staphylococcus aureus* (MTCC3160), Gram –ve bacteria: *Shigella flexneri* (MTCC1457) and fungal strain *Candida albicans (MTCC 227).* The following pictures show zone of inhibition produced in the culture media by the synthesized compounds and standard antifungal drug Fluconazole (10µg/ml) for *Candida albicans.*



In the same manner, zones of inhibition were observed for *Staphylococcus aureus* and *Shigella flexneri* and measured in mm. The standard drug used for these strains was Ciprofloxacin (10µg/ml).

Table No. 8 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	*Candida albicans* MTCC 227	*Shigella flexneri* MTCC 1457
1.	Standard	10	6mm	26.90	22.70	28.71
	RB1 (S1)	100	6mm	11.25	12.23	14.18
		50	6mm	10.55	14.17	10.76
		25	6mm	10.59	12.65	10.35
		12.5	6mm	12.88	11.12	13.12
2.	Standard	10	6mm	27.13	20.12	24.99
	RB2 (S2)	100	6mm	16.19	10.95	11.23
		50	6mm	13.25	11.94	14.27
		25	6mm	12.95	10.20	12.52
		12.5	6mm	10.11	11.02	10.72
3.	Standard	10	6mm	22.12	20.08	26.68
	RB3 (S3)	100	6mm	11.88	16.89	14.69
		50	6mm	11.18	11.95	13.14
		25	6mm	10.62	13.28	11.48
		12.5	6mm	10.29	10.29	10.46
4.	Standard	10	6mm	28.94	20.91	28.40
	RB4 (S4)	100	6mm	8.12	7.81	7.74
		50	6mm	7.18	7.45	7.48
		25	6mm	9.95	6.86	7.39
		12.5	6mm	8.15	7.42	7.60
5.	Standard	10	6mm	26.84	21.24	29.33
	RB5 (S5)	100	6mm	12.08	8.42	10.62
		50	6mm	11.06	10.46	10.35
		25	6mm	9.04	12.42	10.11
		12.5	6mm	10.06	7.21	7.35
6.	Standard	10	6mm	22.44	20.42	29.79
	RB6 (S6)	100	6mm	12.18	7.66	9.57
		50	6mm	9.12	11.16	7.69
		25	6mm	12.32	8.40	8.52
		12.5	6mm	8.06	10.04	9.82

From the above observations, it was found that all the synthesized compounds possess moderate antibacterial and antifungal activity. The antimicrobial activities were less than the standard drugs: Ciprofloxacin and Fluconazole. Compounds S1-S6 possess gud activity against *Staphylococcus aureus* as compared to against *Candida albicans.* All the compounds showed variability in activity against *Shigella flexneri* at various sample concentrations.

CONCLUSION

The main focus of this research work was to synthesize, purify, characterize and evaluate the antimicrobial activity of newly synthesized 1, 3, 4-thiadiazole analogs. A two step reaction was used to synthesize Imidazo thiadiazole derivatives. The desired purity was achieved by re-crystallisation using suitable solvents. Structural elucidation of synthesized compounds was done by using various analytical techniques like IR, NMR & Mass Spectroscopy. The physical properties like melting point, solubility etc. were also evaluated to support the identity of the compounds. Further to evaluate the antimicrobial activity of newly synthesized compounds these compounds were assessed against Gram +ve bacteria *Staphylococcus aureus* (MTCC 3160), Gram –ve bacteria *Shigella flexneri* (MTCC 1457), fungal strain *Candida albicans* (MTCC 227). Compounds S1-S3 showed good result for antimicrobial activity which gave the basis of conclusion that the halogen (-Cl,-F,-Br), substituted thiadiazoles were found to possess higher antimicrobial activity than $–CH_3$ and $-OCH_3$ substituted derivatives.

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