ORIGINAL RESEARCH ARTICLES

SYNTHESIS, SPECTRAL STUDIES AND BIOLOGICAL ACTIVITY OF SOME IMIDAZO [2, 1-B] [1, 3, 4] THIADIAZOLE DERIVATIVES

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ABSTRACT

Benzoic acid substituted imidazo[2,1-b][1,3,4]thiadiazoles were synthesized using appropriate reaction scheme. The synthesized derivatives were then purified and elucidated for their structure by measuring their melting points, infra-red spectra and proton NMR spectra. The progress of the reaction was determined by using thin layer chromatography technique. The antimicrobial potential of the synthesized derivatives was evaluated against *S. aureus* and *E. coli.* The zone of inhibition (mm) was measured. Reported derivatives showed good to moderate antibacterial activity.

Keywords: Zone of inhibition, thin layer chromatography, 1,3,4-thiadiazole, Imidazo[2,1-b][1,3,4]thiadiazole, anti-bacterial activity, melting points

INTRODUCTION

Infections caused by bacteria can be prevented, managed and treated through the anti-bacterial group of compounds known as antibiotics. Antibiotics are natural, semi-synthetic or synthetic compounds that kill or stop the growth of bacteria. When an antibiotic drug is used against any bacteria, then it will shows two types of responses: firstly bacteria are sensitive and the drugs may cause the inhibition of their growth, division and death. Secondly, the bacteria remains unaffected or resistant[1]. The ability of a microorganism (like bacteria, viruses, and some parasites) to stop the action of an antimicrobial drug is known as antimicrobial resistance (AMR). This may result in, standard treatments become ineffective, infection persisting for long time period and spread to other organs[2]. Antimicrobial resistance of different pathogens has also became widespread[3]. There has been a significant increase in the synthesis of compounds having antimicrobial activities in recent years[4].

The antibiotic resistance in bacteria is mainly due to following reasons: (i) reduction in the efficiency of binding of the drug by modification of active site of the target, (ii) destruction or modification of the antibiotic by enzymes produced by the organism, and (iii) efflux of antibiotic from the cell[5].

Heterocyclic compounds play an important role among organic compounds with biological action and are used for various purposes such as drugs in human and veterinary medicine or as insecticides and pesticides in agriculture[6].1,3,4-Thiadiazole derivatives have established pharmacological activities and have been increasingly investigated due to their broad spectrum of pharmacological properties. 1,3,4-Thiadiazole derivatives possess interesting biological activity probably attributable to them due to strong aromaticity of the ring system which may leads to improve *in vivo* stability and generally, a lack of toxicity for higher vertebrates, including humans when diverse functional groups that interact with biological receptors are attached to aromatic ring[7]. Thiadiazole compounds with good liposolubility of the sulfur atom might also have a positive effect on the biological activity[8]. Consequently, according to literature review, 1,3,4-thiadiazoles have pharmacological

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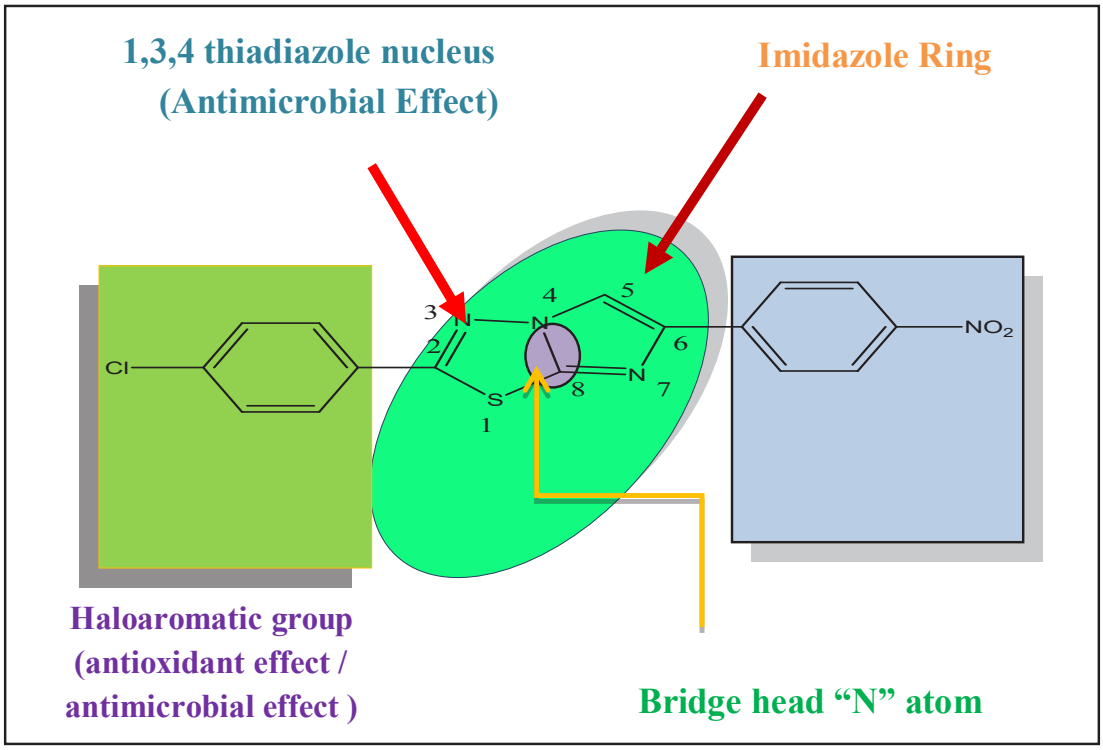


Fig. 1: Structural representation of imidazo[2,1-b][1,3,4]thiadiazoles

properties such as antitubercular[9], antimicrobial[10], antifungal[11], anticonvulsant[12], anti-inflammatory[13] and diuretic[14].

The fusion of an imidazole ring with a [1, 3, 4] thiadiazole nucleus gives rise to a class of heterocyclic systems containing a bridgehead nitrogen atom known as imidazo thiadiazole and is shown in Fig. 1. 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. In this context, it has been reported in the literature that the difference of groups or atoms in the 2- and 6- positions of the imidazo[2,1-b] [1,3,4]thiadiazole derivatives to be synthesized had a significant effect on the variety and effect of their biological activities[15]. In addition, the presence of different groups in the imidazo [2, 1-b] [1, 3, 4] thiadiazole derivatives at C-5 position has been reported in the literature to contribute to the various biological effects[16]. Therefore, in this study, we chose 4-chlorobenzoic acid as a starting material of forming the imidazo [2, 1-b] [1, 3, 4] thiadiazole moiety and the aromatic hydroxy group to possess antioxidant effect.

MATERIALS AND METHODS

Chemical and reagents

The synthesis was carried out using chemicals of LR grade and obtained from Spectrochem Loba Chem. All the solvents used for the reaction were of LR grade and purified before use in different reactions. Thin layer chromatography (TLC) was carried on pre coated plates (Merck 60F254) for monitoring the reaction. Various solvents systems used for developing the chromatograms were: hexane: ethylacetate (3:2 V/V) and methanol: chloroform (9:1 V/V).

Instrumentation

The IR spectra were recorded on FTIR spectrophotometer.^{1}H-NMR spectra were recorded in $CDCl_3$ solution on Bruker Avance II 400 MHz NMR Spectrometer, IIT(Ropar) using tetramethylsilane (TMS) as the internal standard Chemical shifts were recorded as parts per million (ppm).

Determination of antimicrobial activity

The synthesized compounds **(5a – 5b)** were screened against different standard organisms, including *S. aureus and E. coli*. Agar diffusion method at the concentration level of 1000 µg mL^{-1} was applied. Ciprofloxacin was used as reference compound for antibacterial activity. The antimicrobial activity of the newly synthesized compounds was determined by cup plate method in nutrient agar medium. The compounds were tested at a concentration of 1000 µg mL^{-1} and

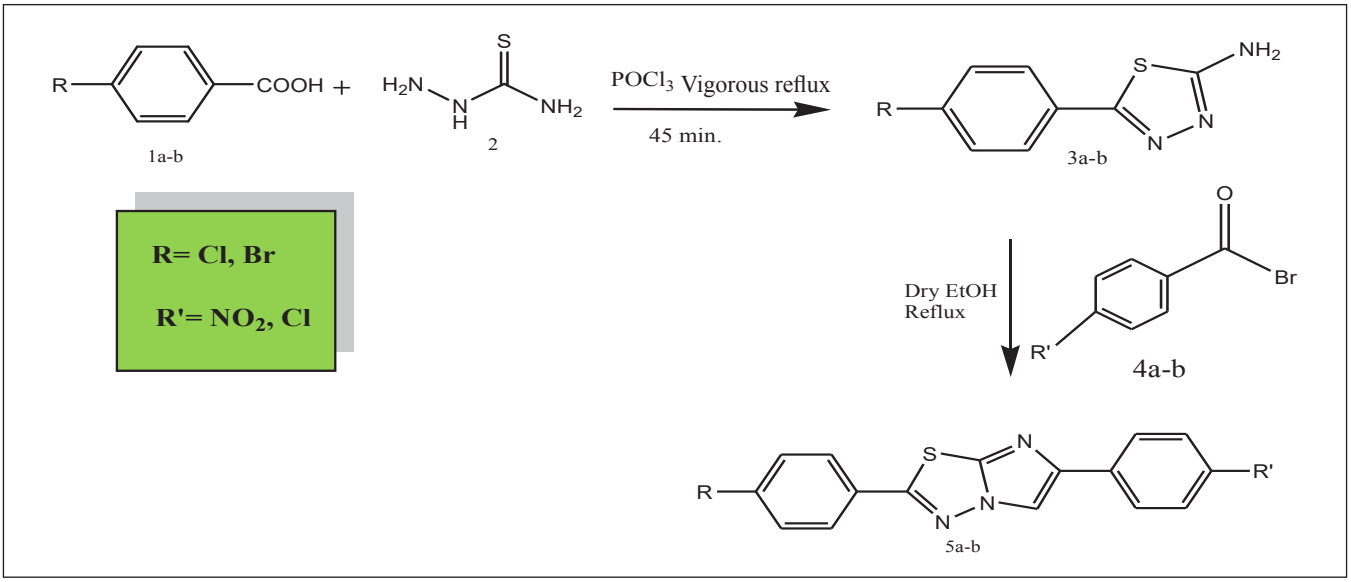


Fig. 2: Reaction scheme for the preparation of substituted imidazo thiadiazoles

were prepared in dimethylsulfoxide (DMSO). The petri dishes used for antibacterial screening were incubated at 37±1 ^{0}C for 24 h; the diameter of zone of inhibition was measured. The results were compared with ciprofloxacin of 1000 µg mL^{-1} concentration.

EXPERIMENTAL

Synthesis of 5-(4-substitutedphenyl)-1,3,4-thiadiazol-2-amine (3a-3b)

The synthesizing compounds were formed in two reaction steps as shown in Fig. 2. 4-Chlorobenzoic acid (1a) or 4-bromobenzoic acid (1b) with thiosemicarbazide (2) were mixed and then $POCl_3$ was added dropwise to the mixture and was refluxed at 75 ^{0}C for 45 min. The mixture was cooled to room temperature and quenched with cold water. Again, the mixture was refluxed for an additional 4 h. Mixture was cooled to room temperature and alkalised to pH 8 using NaOH or KOH solution dropwise under stirring. The precipitates were filtered, washed and recrystallized from ethanol to obtain compounds 3a-b.

Synthesis of 2-(4-substitutedphenyl)-6-(4-substitutedphenyl) imidazo[2,1-b][1,3,4] thiadiazole

Equimolar quantities of 5-(4-chlorophenyl)-1,3,4-thiadaizol-2-amine (3a) or 5-(4-bromophenyl)-1,3,4-thiadiazol-2-amine (3b) and 4-nitro phenacyl bromide (4a) or 4-chlorophenacyl bromide (4b) were separately added to dry ethanol and refluxed for 46 h. The excess of solvent was removed and the solid hydrobromide was filtered, suspended in water and neutralised with aqueous sodium carbonate solution to get free base. The compounds were filtered, washed with distilled water, dried, and recrystallized from ethanol.

RESULTS AND DISCUSSION

Spectral elucidation of the synthesized derivatives are shown in Table I, II and III.

Table I : Physical parameters of the synthesized compounds

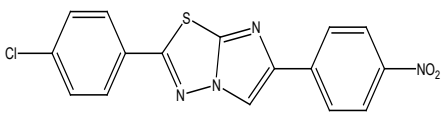
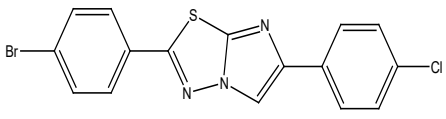
Sr. No.	Compound	Mol. Weight	% Yield	M. P. (°C)	R_f value
1.		356.79	53	288- 290	0.75
2.		256.12	51	201-203	0.6

Table II: Analytical values of compounds (5a-b): IR spectra peaks of synthesized compounds

Compound	5a	5b
(υ $_{-N-H\ stretch}$) cm^{-1}	3449.65	
($\upsilon_{-C-N\ stretch}$) cm^{-1}	1089.30	1275
(υ $_{-C=N\ stretch}$) cm^{-1}	1668.90	1504
(υ $_{-C-H\ stretch}$) cm^{-1}	3053.79	3095
($\upsilon_{-C=C\ stretch}$) cm^{-1}	1668.90	1394
(υ $_{-C-Cl\ stretch}$) cm^{-1}	718.92	727
(υ $_{-C-S\ stretch}$) cm^{-1}	685.51	1066
(υ $_{-C-Br\ stretch}$) cm^{-1}	-	834
(υ $_{-N=O-\ stretch}$) cm^{-1}	1337.99	-

Table III: ^{1}H-NMR of synthesized compounds (5a-b)

Compound ($CDCl_3$, δ ppm)	(m, 8H, Arom-H)	(s, Ar-H, imidazole)
5a	7.5267-8.2901	8.268
5b	7.24-7.77	8.35

Table IV: Zone of inhibition of compounds 5a-5b

Compound	Conc. mg mL^{-1}	Zone of inhibition in cm	
		S. aureus	***E. coli***
Standard	1000	5	4.5
5a	1000	1.6	2.2
	500	1.2	1.7
	250	0.8	1.4
Standard	1000	-	1.8
5b	1000	-	1.8
	500	-	1.3
	250	-	0.9

BIOLOGICAL ACTIVITY

All the imidazo [2,1-b][1,3,4] thiadiazole compounds were evaluated for their *in vitro* antimicrobial activity by agar diffusion method against gram positive bacteria: *S. aureus* and gram negative bacteria: *E. coli.* Ciprofloxacin was used as a reference drug. The culture medium (nutrient agar) was sterilized and poured in 90 mm sterile petri plate under sterile conditions. The lawn of tested bacterial strains *S. aureus* (MTCC 87) and *E. coli* (MTCC 40) was made by spreading 100 µL of log phase bacterial strains on different nutrient agar plates. The imidazo [2,1-b][1,3,4] thiadiazole compound and standard drug (ciprofloxacin) were suspended in DMSO at the concentration of 1000 µg mL^{-1}. Wells in nutrient agar (7 mm diameter) were made and 50 µL of compound suspension was added to the wells. These compounds were allowed to diffuse for at least 2 h and were incubated at 37 °C for 18-24 h. The zones of inhibition for imidazo [2,1-b][1,3,4]thiadiazole compounds were recorded on the next day and are represented in Table IV.

CONCLUSION

The proposed derivatives were synthesized and characterized according to the procedure understood during the literature review. Refluxing method was used for the synthesis. The yield was found to be satisfactory. The IR and proton NMR spectral analysis confirmed the formation of the synthesized derivatives. Compounds 5a and 5b were synthesized and screened for their antimicrobial activity against *S. aureus* and *E. coli*. The inhibition of the compounds was determined by observing the zones of inhibition formed around the cup after 24 h of incubation for antimicrobial activities. Compounds 5a and 5b possess good antimicrobial activity against *E. coli,* whereas both the compounds show minimal activity against *S. aureus* when compared to standard drug ciprofloxacin.

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