Synthesis, Characterization and Antimicrobial Activities of Some Substituted Thiosemicarbazones

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***Abstract*:-The thiosemicarbazone Compounds were synthesized by condensation of thiosemicarbazide with substituted benzaldehydes. The synthesized substituted thiosemicarbazone compounds were characterized by their physical constants, UV, IR and NMR spectra. The antimicrobial activities of these synthesized substituted thiosemicarbazone compounds have been screened by Bauer-Kirby method using human pathogenic bacteria and fungal species. The antimicrobial activities of all synthesized thiosemicarbazone compounds have shown significant activity.**

***Key words*: synthesis, substituted thiosemicarbazone compounds, UV, IR & NMR spectra and Antimicrobial activities**

I. INTRODUCTION

The synthesis, structure and biological activity of some new thiosemicarbazone prepared from aliphatic hydrazides has been the focus of research. Different methods have been employed to synthesize different types of thiosemicarbazone from different starting materials. Hydrazones are characterized by the presence of the triatomic grouping C=N-N-. They can be considered as Schiff bases derived from acid hydrazides. The most important property of hydrazones is their high physiological activity [1-6]. Extensive studies have revealed that the lone pair on trigonally hybridized nitrogen atom of the azomethine group is responsible[7-11] for the chemical and biological activity. It has been reported that metal complexes of hydrazones have diverse applications. They find use as plasticizers, polymerization inhibitors and antioxidants. They are used as fungicides and pesticides in biological and biochemical context.

Moreover, the thiosemicarbazone group plays an important role of the antimicrobial and possesses interesting antibacterial, antifungal [12-14] and anti-tubercular activities [15-20]. In addition, their varied coordinating behaviour makes them interesting candidates for metal-based drugs. Generally, the ligands act synergistically with metals towards their biological activity. These observations have guided the development of new hydrazones with varied biological activities [21].The biological activity of complexes derived from hydrazones have been studied and contrasted with regard to their antibacterial, antitumoral, antiviral, antimalarial and antitubercular properties [22]. It has also been shown that the azomethine N, which has a lone pair of electrons in a sp^2 hybridised orbital, is biologically important [23].

Hydrazones constitute an important class of biologically active drug molecules [24] which has attracted attention of medicinal chemists due to their wide range of pharmacological properties. These compounds are being synthesized as drugs by many researchers in order to combat diseases with minimal toxicity and maximal effects. These predictions has provided therapeutic pathway to develop new effective biologically active hydrazones. A number of hydrazone derivatives have been reported to exert notably antimicrobial, antihypertensive, anticonvulsant, analgesic, anti-inflammatory, antituberculosis, antitumoral, antiproliferative and antimalarial activities, biological activities of various hydrazones are well reported in literature. This review highlights diverse pharmacological activities shown by hydrazones.

Medicinal chemists have also carried out considerable research for novel antimicrobial and anticancer agents bearing hydrazone moiety. Some studies have confirmed that hydrazone derivatives exhibit antifungal and anticancer activities [25-29]. Some researchers have reported anticancer effects of some antifungal agents and carried out considerable research for deciphering the underlying mechanisms of antitumor activity [30-32].In antifungal and anticancer drug design, the lack of selectivity of conventional chemotherapeutic agents and the acquisition of multiple-drug resistance are two major challenging problems. As a consequence of this situation, the search for new effective chemotherapeutic agents has attracted a great deal of interest [33-35]. Several hydrazone derivatives have been reported as insecticides, nematocides, herbicides, rodenticides and antituburculosis in addition to that some of the hydrazone were found to be active against leukemia, sarcoma and illnesses [36,37]. We now carry out another systematic study of their synthesis and biological activity. Herein, the synthesis of the substituted thiosemicarbazones are described and their antimicrobial properties are evaluated

II. EXPERIMENTAL

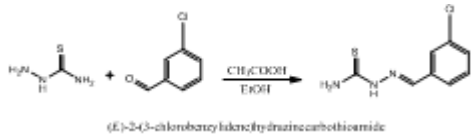
2.1. General

All the chemicals used in the present investigation, have been procured from Sigma–Aldrich Chemical Company. Melting points of all substituted thiosemicarbazones have been determined in open glass capillaries on a Mettler FP51 melting point apparatus and are uncorrected. The UV spectra of all the substituted

thiosemicarbazones have been recorded with ELICO-BL222 spectrophotometer (λ_{max} nm) in spectral grade methanol solvent. Infrared spectra (KBr, 4000–400 cm^{-1}) have been recorded on SHIMADZU Fourier transform spectrophotometer. Bruker AV400 NMR spectrometer operating at 400 MHz has been utilized for recording ^{1}H NMR spectra and 100 MHz for ^{13}C NMR spectra in DMSO solvent using TMS as internal standard.

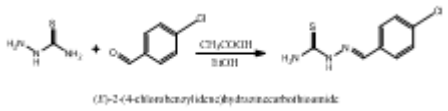
2. 2. Synthesis of substituted (E)-2-(3- chlorobenzylidene) hydrazinecarbothio amide(3CBHC)

A solution of equimolar quantities of thiosemicarbazide (0.01mol) with substituted 3-cholorobenzaldehyde (0.01mol) acetic acid (two drops) and 10 ml of ethanol were shaken occasionally for 1 hour [38]. The completion of the reaction was monitored by TLC continuously. The resultant mixture was cooled at room temperature. Then the precipitate obtained, was filtered at the filter pump and washed several times with cold water then pale yellow solid was obtained as the final product. This crude product was recrystallized from ethanol and glittering colorless solid was obtained. The general scheme for preparation of substituted thiosemicarbazone has shown in **scheme-1.**



2.3. Synthesis of substituted (E)-2- (4-chlorobenzylidene) hydrazinecarbothio amide(4CBHC)

A solution of equimolar quantities of thiosemicarbazide (0.01mol) with substituted 4-cholorobenzaldehyde (0.01mol) acetic acid (two drops) and 10 ml of ethanol were shaken occasionally for 1 hour [38]. The completion of the reaction was monitored by TLC continuously. The resultant mixture was cooled at room temperature. Then the precipitate obtained, was filtered at the filter pump and washed several times with cold water then pale yellow solid was obtained as the final product. This crude product was recrystallized from ethanol and glittering colorless solid was obtained. The general scheme for preparation of substituted Thiosemicarbazone has shown in **scheme-2**



The physical constants of substituted thiosemicarbazone compounds presented in **Table1**. The ultraviolet absorption maxima (λ_{max}, nm), infrared absorptions (ν,cm^{-1}) and NMR chemical shifts (δ, ppm) of substituted thiosemicarbazones are presented in Table 1.

Entry	substituents	M. F.	M. W.	Yield (%)	m.p. (C)
1	3-Cl **(3CBHC)**	$C_8H_8ClN_3S$	213.68	94	198-199
2	4-Cl **(4CBHC)**	$C_8H_8ClN_3S$	213.68	92	210-211

Table 2. The ultraviolet absorption maxima (λ_{max}, nm), infrared absorptions (ν, cm^{-1}) and NMR chemical shifts (δ, ppm) of substituted thiosemicarbazones

ENTRY	X	UV(λ_{max})nm	IR vcm-1	^{1}H CH=N(ppm)	^{13}C C=N(ppm)
1	3-Cl(3CBHC)	315	1685.79	8.041	140.55
2	4-Cl(4CBHC)	316.5	1689.64	8.06	140.9

III. RESULTS AND DISCUSSION

*(E)-2-(3-bromobenzylidene) hydrazinecarbothioamide***:** Yield: 91%, m.p. 209-210 °C. UV λ_{max} (nm): 318.00. IR (KBr, cm^{-1}): ν =1641.42 (CH=N), 997.20 (N-N), 3385.07 (-NH), 3250.05 ($-NH_2$). ^{1}H NMR (DMSO, ppm): δ=8.117 (S, 1H.CH=N), 7.323-7.667 (m, 4H, Ar-H), 8.171 (S, 1H.-NH), 8.567 (S, 2H.-NH_2). ^{13}C NMR (DMSO, ppm): δ (C_1) = 140.75 (CH=N), 136.48 (C_2), 126.84 (C_3), 128.83 (C_4), 132.34 (C_5), 122.25 (C_6), 130.74 (C_7), 178.02 (C=S),

(E)-2-(4-bromobenzylidene) Hydrazinecarbothioamide: Yield: 88%, m.p. 197-198°C. UV λ_{max} (nm): 317.50. IR (KBr, cm^{-1}): ν =1641.42(CH=N), 997.20 (N-N), 3385.00 (-NH), 3251.00(-NH_2).^{1}H NMR (DMSO, ppm): δ=8.015 (S, 1H.CH=N), 7.283-7.864 (m, 4H Ar-H) , 8.239(S, 1H.-NH), 7.946 (S, 2H.-NH_2). ^{13}C NMR (DMSO, ppm): δ (C_1) = 140.23 (CH=N), 132.23 (C_2), 128.50 (C_3, C_7)132.23 (C_4, C_5, C_6), 172.58 (C=S).

3.1 Antimicrobial Activities

In an urge to develop new antimicrobial compound, a number of hydrazones were tested for their antimicrobial activities because of the evolution of drug-resistant microbial pathogens. The fast resistance of bacteria against antibiotics has become a widespread medical problem. Treatment options for these infections are often limited, especially in debilitated and immune compromised patients. The dramatically rising incidence of multi-drug resistant microbial infections in the past few decades has become a serious health care problem. The search for new antimicrobial agents will consequently always remain as an important and challenging task for medicinal chemists. The treatment of bacterial and fungal infectious diseases remains a challenging problem because of the increasing number of multi-drug microbial pathogens [39-41]. Nowadays, the design of new compounds able to deal with resistant bacteria, having new structures and new targets

of action, has become one of the most important areas in the antibacterial research purpose [42].

3.2 Antibacterial sensitivity assay

Antibacterial sensitivity assay was performed using Kirby-Bauer [43] disc diffusion technique. In each Petri plate about 0.5 mL of the test bacterial sample was spread uniformly over the solidified Mueller Hinton agar using sterile glass spreader. Then the discs with 5mm diameter made up of Whatmann No.1 filter paper, impregnated with the solution of the compound was placed on the medium using sterile forceps. The plates were incubated for 24 hours at 37 °C by keeping the plates upside down to prevent the collection of water droplets over the medium. After 24 hours, the plates were visually examined and the diameter values of the zone of inhibition were measured. Triplicate results were recorded by repeating the same procedure. The antibacterial screening effect of the synthesized substituted thiosemicarbazone compounds is shown in **Fig. 1** Plates (1–3).

Fig-1 Antibacterial activity of substituted thiosemicarbazone

The antibacterial activities of all the synthesized substituted thiosemicarbazone compounds have been studied against two gram positive pathogenic strains *Bacillus subtilis, Staphylococcus aureus* and one gram negative strains *Escherichia coli.* The disc diffusion technique was followed using the Kirby–Bauer [43] method, at a concentration of 250 µg/mL with Ciprofloxacin used as the standard drug

Table-3

S. No.	Bacterial species	Standard Antibiotic Disk* *ciprofloxacin	Zone of inhibition (mm)		
			(3CBHC)	(4CBHC)	Control (DMSO)
1	***Bacillus subtilis***	19	18	17	-
2	***Streptococcus pyogenes***	21	19	16	-
3	***Escherichia coli***	22	20	19	-

3.3 Antibacterial activity of substituted thiosemicarbazone compounds

The antibacterial activity of the substituted thiosemicarbazone compounds in **Fig-1** for **Plates (1-3).** The diameter of zone of inhibition (mm) values of antibacterial activity was given in **Table-3**.

3.4 Antibacterial activity against Bacillus subtilis

Analysis of the zone of inhibition(mm) values reveals that substituted thiosemicarbazone compounds (3CBHC and 4CBHC) have shown good antibacterial activity.

3.5 Antibacterial activity against Staphylococcus pyogenes

Analysis of the zone of inhibition(mm) values reveals that substituted thiosemicarbazone compound (3CBHC) only have shown good antibacterial activity.

The substituted thiosemicarbazone compound (4CBHC) have shown moderate antibacterial activity against Staphylococcus pyogenes

3.6 Antibacterial activity against Escherichia coli

Analysis of the zone of inhibition(mm) values reveals that substituted thiosemicarbazone compound (3CBHC) only have shown good antibacterial activity.

The substituted thiosemicarbazone compound (4CBHC) have shown moderate antibacterial activity against *Escherichia coli.*

3.7. Antifungal sensitivity assay

Antifungal sensitivity assay was performed using Kirby-Bauer [43] disc diffusion technique. PDA medium was prepared and sterilized as above. It was poured (ear bearing heating condition) in the Petri-plate which was already filled with 1 ml of the fungal species. The plate was rotated clockwise and counter clock-wise for uniform spreading of the species. The discs were impregnated with the test solution.

The test solution was prepared by dissolving 15 mg of the hydrazone in 1ml of DMSO solvent. The medium was allowed to solidify and kept for 24 hours. Then the plates

were visually examined and the diameter values of zone of inhibition were measured. Triplicate results were recorded by repeating the same procedure.

The antifungal activities of all the synthesized substituted thiosemicarbazone compounds have been studied against pathogenic strains ***Aspergillus niger*** and *Trichoderma viride.* The disc diffusion technique was followed using the Kirby–Bauer [43] method, at a concentration of 250 µg/mL with **Amphotericin-B** used as the standard drug

Antifungal activity of substituted thiosemicarbazon compounds

Table-4

S. No.	Fungal species	Standard Antibiotic Disk* * **Amphotericin-B**	Zone of inhibition (mm)		
			(3CBHC)	**(4CBHC)**	**Control(DMSO)**
2	*Aspergillus niger*	18	17	15	-
3	*Trichoderma viride*	19	18	16	-

4.6 Antifungal activity of substituted thiosemicarbazone compounds

The antifungal effect of the substituted of thiosemicarbazone compounds is shown in **Fig-2** for **Plates (4-5)**. The diameter of zone of inhibition(mm) values of antifungal activity is given in **Table-4**

4.7 Antifungal activity Aspergillus niger

Analysis of the zone of inhibition(mm) values reveals that substituted thiosemicarbazone compound (3CBHC) has shown good antifungal activity.

The substituted thiosemicarbazone compound (4CBHC) has shown moderate antifunga activity against *Aspergillus niger*

4.8 Antifungal activity Trichoderma viride

Analysis of the zone of inhibition(mm) values reveals that substituted thiosemicarbazone compound (3CBHC) has shown good antifungal activity.

The substituted thiosemicarbazone compound (4CBHC) has shown moderate antifunga activity against *Trichoderma viride.*

V. CONCLUSIONS

The authors have been synthesized some substituted some *meta* and *para* substituted Thiosemicarbazones by condensation method to study of the characterization.Their UV, IR, ^{1}H and ^{13}C NMR spectra have been recorded. The antimicrobial activities of all the synthesized Thiosemicarbazones compounds have been evaluated using Bauer-Kirby disc diffusion technique. The substituted thiosemicarbazone compounds (3CBHC and 4CBHC) have shown good antibacterial activity against and antifungal activities

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