FULL LENGTH ARTICLE

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SOUTH ASIAN JOURNAL OF ENGINEERING AND TECHNOLOGY

DOI: 10.26524/sa2

Synthesis and Characterization of (1, 4)-4-benzylidene-1-(1-phenylethylidene) thiosemicarbazide and their Antifungal Activities

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Received : 30-08-2018
Accepted : 19-10-2018

ABSTRACT: The Present research work is aimed to synthesize a serious of various substituted Schiff bases compounds of 1-(1-phenylethylidene)thiosemicarbazide form (1,4)-4-benzylidene-1-(1-phenylethylidene)Thiosemicarbazide condenses with Four different substituted aryl aldehydes under Microwave irradiation and conventional method. The structure for compounds has been determined by UV, FTIR, 1H-NMR and Mass s spectroscopy. All the compounds are evaluated for its anti-fungal activity.

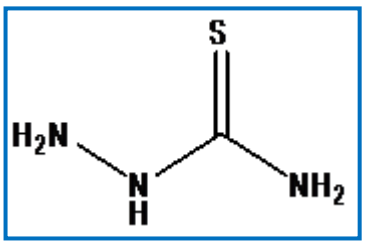
Keywords: 1-(1-phenylethylidene)thiosemicarbazide, Schiff bases, Microwave irradiation and anti-fungal activity.

1 Introduction

Heterocycles have contributed to the development of society from a biological and industrial point of view as well as to improve the quality of life. During the past few decades, interest has been rapidly growing in gaining insight into the properties and transformation of these heterocycles. As a result, variety of new compounds is being added to this field every year [1-6]. Among the sulfur and nitrogen containing heterocyclic compounds, thiosemicarbazides and thiosemicarbazones have potential pharmacological activities. The hydrazides addition to various isothiocyanates is one of the convenient methods of the synthesis of substituted thiosemicarbazides, which are of great interest not only in terms of a possible study of biological activity, but also as the starting compounds for the synthesis. Thiosemicarbazides is a unique class of organic compounds which is not known only for its various biological activities but also as metal-chelating and anticorrosion agents [7-10]. Thiosemicarbazides have occupied an important place in drug industry. Use of these compounds in organic synthesis has become a classical strategy for the synthesis of several heterocycles. Their reactions with compounds containing C=O and C=N groups is an important method for the synthesis of biologically active compounds, viz triazoles and diazoles [11-18]. A better understanding of their biological activity can be derived from their oxidation mechanisms. Thiosemicarbazides derivatives display interesting biological activities, including anticancer, antiHIV, antibacterial, antiviral and antifungal owing to their ability to diffuse through the semi permeable membrane of cell lines. They play an important role in the regulation of plant growth. Due to their abundance in plants and ease of synthesis, this class of compounds has generated great interest for possible therapeutic uses [19-24].

These sulfur and nitrogen donor ligands and their co-ordination complexes have gained special attention due to their activity against protozoa. Some industrially

important activities, such as anticorrosion and antifouling effects have also been observed for these compounds.



Thiosemicarbazides

2. Experimental section

Materials and Methods

All chemicals employed in the present investigation were of analytical grades (AR) which have been in thiosemicarbazide, Acetophenone, 4-methylacetophenone, 4-nitroacetophnone, 4-bromoacetophenone and benzaldehydes were purchased in Sigma Alrich and methanol and acetic acid were purchased in Merck and hydrochloric acid and ethyl acetate were purchased in S.d.Fine and hexane were purchased in Avra. The purity of compounds were checked by TLC (0.5mm thickness) using Silica gel-G coated aluminium plates (Merck) and were visualized by exposing the dry plates into the iodine vapours and exposing UV light.

A variety of physiochemical methods have been employed to characterize the synthesis compounds. A brief account of these methods was given below. The melting points were determined in open capillaries, using Biotas melting apparatus, expressed in ^{o}C (uncorrected). FT-IR spectra were recorded on Alpha-Brukar spectrophotometer (KBr pellets) scanning with the entire region of 4000 - 400 cm^{-1}. UV spectra were recorded on Alpha Bruker UV spectrophotometer. 1H NMR spectra of the substituted thiosemicarbazide, and substituted imine derivatives were recorded on Bruker AV400 spectrometer operating 400 MHz for recording ^{1}H NMR spectra in DMSO solvent using TMS as internal reference (chemical shift in ppm).

Synthesis of substituted Schiff bases

The various substituted Schiff bases have been synthesized through two stages. Each stage has been achieved viz both conventional and micro-wave assisted methods.

Stage 1: Synthesis of 1-(1-phenylethylidene) thiosemicarbazide (A1)

The compound 1-(1-phenylethylidene) thiosemicarbazide (A1) was synthesized two methods.

Method A: Conventional method

An equal molar quantities mixture of compound thiosemicarbazide (0.01mol), acetophenone (0.01mol), concentrated hydrochloric acid (0.01mol) and methanol (10mL). The mixture was stirred under reflux for 5-7 hours. After the completion of reaction mixture (monitored by TLC) was evaporated under reduced pressure, to the residue was added a mixture solution of hexane and diethyl ether and the deposited solid was collected by filtration washed with hexane, diethyl ether and then dried under reduced pressure to give the light brown color solid was obtained was recrystallized using methanol get the needles like brown crystals of 1-(1-phenylethylidene) thiosemicarbazide (A1). The purity of the product was checked by TLC.

Method B: Microwave irradiation method

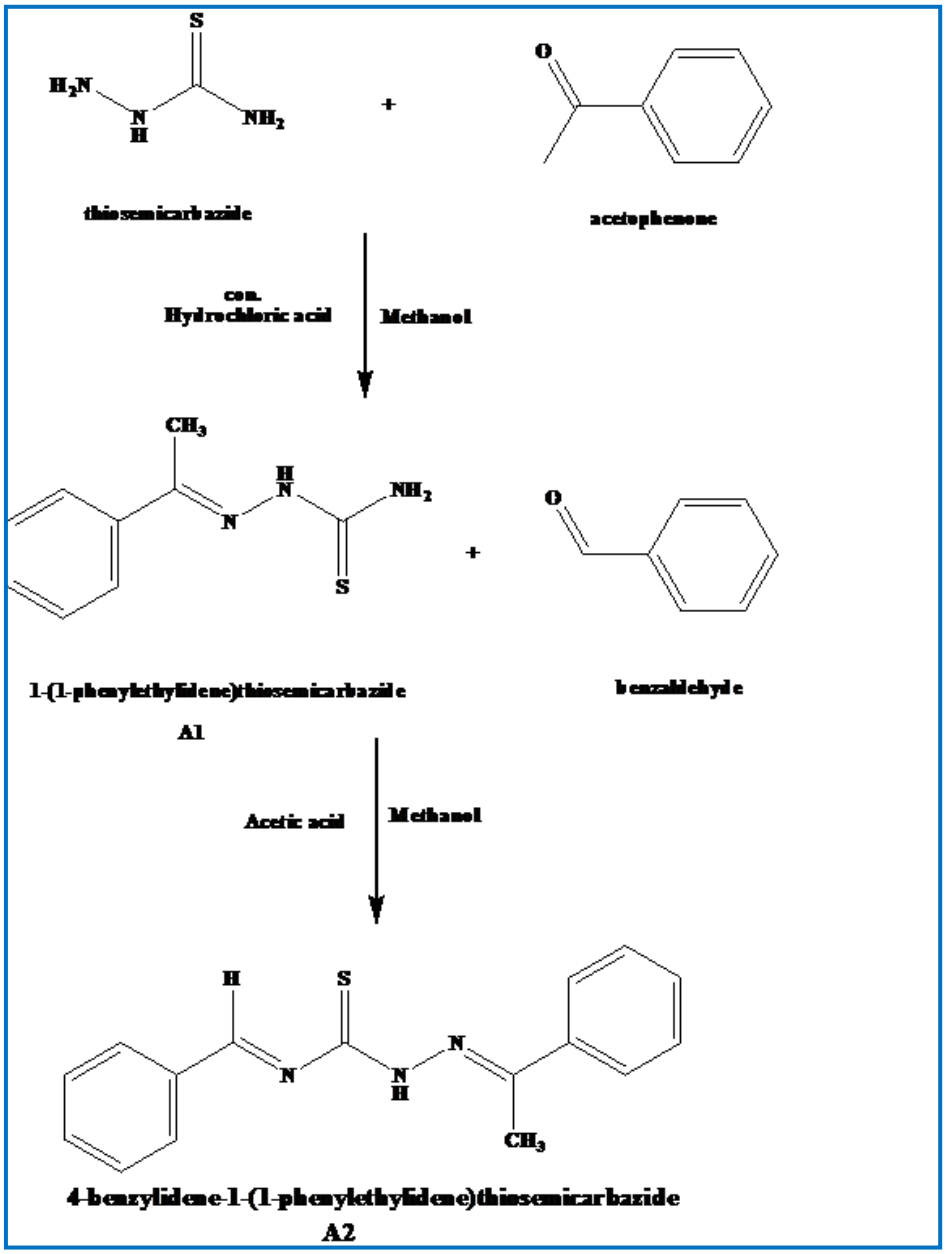
An equal molar quantities mixture of compound thiosemicarbazide (0.01mol), acetophenone (0.01mol), concentrated hydrochloric acid (0.01mol) and methanol (10mL) were grinded in to the mortar. The mixed compounds were taken in a 100mL beaker and it was irradiated in a microwave oven for the 3 minutes at 110W operating at 2450Hz at 30 seconds of intervals. After completion of reaction as followed by TLC examination, then the solvent was evaporated under reduced pressure to the residue was added a mixed solution of hexane and diethyl ether and the deposited solid was resurged by filtration washed with hexane, diethyl ether and then dried under reduced pressure to give the light brown color solid was obtained was recrystallized using methanol get the needles light brown crystals of 1-(1-phenylethylidene) thiosemicarbazide (A1). The purity of the product was checked by TLC.

Stage 2: Synthesis of (1, 4)-4-benzylidene-1-(1-phenylethylidene) Thiosemicarbazide (A2)

The compound (1,4)-4-benzylidene-1-(1-phenylethylidene)thiosemicarbazide (A2) were synthesized two methods.

Method A: Conventional method

An equal molar quantities mixture of compound 1-(1-phenylethylidene) thiosemicarbazide (A1) (0.01mol), benzaldehyde (0.01mol), acetic acid (0.01mol) and methanol (10mL) were added. The mixture was stirred under reflux for 5 hours. After the completion of reaction mixture (monitored by TLC) was evaporated under reduced pressure, to the residue was added a mixture cooled to room temperature and kept overnight the solid was washed with methanol and recrystallized using methanol to get the dark brown color solid was obtained was recrystallized using methanol get the needles dark brown crystals of (1, 4)-4-benzylidene-1-(1-phenylethylidene) thiosemicarbazide (A2). The purity of the product was checked by TLC.



Synthetic Scheme

Method B: Microwave irradiation method

An equal molar quantities mixture of compound 1-(1-phenylethylidene) thiosemicarbazide (A1) (0.01mol), benzaldehyde (0.01mol), acetic acid (0.01mol) and methanol (10mL) were grinded in to the mortar. The mixed compounds were taken in a 100mL beaker and it was irradiated in a microwave oven for the 3 minutes at 110W operating at 2450Hz at 30 seconds of intervals. After completion of reaction as followed by TLC examination. The reaction mixture was cooled to room temperature and kept overnight the solid was washed with methanol and recrystallized using methanol get the needles dark brown crystals of (1,4)-4-benzylidene-1-(1-phenylethylidene) thiosemicarbazide (A2). The purity of the product was checked by TLC.

3. Result and discussion

Based on the careful analysis of literature survey, the target compounds have been synthesized through two stages of chemical reaction. Both stages of reaction carried out viz. conventional and microwave assisted methods. In a first stage, substituted thiosemicarbazide derivatives were synthesized in the presence of hydrochloric acid and the second stage have been achieved condensation between stage 1 compound and benzaldehyde. The yields of the synthesized compounds were found to been significant. The structure of the synthesized compounds was identified by UV, FTIR and confirmed by 1H NMR spectroscopy. All the compounds give the characteristic FTIR and absorption peaks that proved that the presence of particular functional group and 1H NMR spectrum helps to find the structure synthesized compounds.

Interpretation of spectral data's of the Compounds

The (1, 4)-4-benzylidene-1-(1-phenylethylidene) thiosemicarbazide compound were synthesized through two stages.

Interpretation of spectrum to the 1-(1-phenylethylidene) thiosemicarbazide

UV spectrum of compound 1-(1-phenylethylidene) thiosemicarbazide

It is a light brown solid. The formation of product was confirmed by Thin Layer chromatography. UV peak and appear at 204nm (CH=CH) corresponding to the π-π* transition of aromatic double bond and peak at 330nm is due to n-π* transition which clearly indicates the presence of hetero atom. The UV spectrum of compound was shown in the Fig.1.

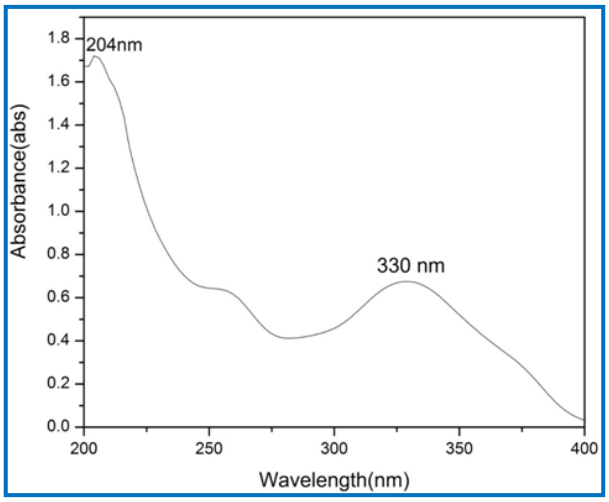


Fig 1 UV spectrum of 1-(1-phenylethylidene) thiosemicarbazide

FT-IR spectrum of compound 1-(1-phenylethylidene) thiosemicarbazide

FT-IR spectrum of the 1-(1-phenylethylidene) thiosemicarbazide was shown in Fig.2. The weak absorption at 3143cm^{-1} is due to the C-H stretching vibration in aromatic system. The weak absorption at 3390 cm^{-1} due to the N-H stretching frequency. The sharp peak at1583cm^{-1}clearly shows C=C bond was attached with conjugation.

The peak was observed in the range of 1434 cm^{-1} which indicates C=N stretching frequency. The peak at 756cm^{-1} was assigned the presences of C-H out-of- plane bending vibration in the ring. The peak at 618cm^{-1} indicates the presences of C=S stretching vibrations. The assignment of the main peak in the FT-IR spectrum of 1-(1-phenylethylidene) thiosemicarbazidephenol was obtained represented by Table 1.

^{1}H NMR spectrum of compound 1-(1-phenylethylidene) thiosemicarbazide

The ^{1}H NMR spectrum of the 1-(1-phenylethylidene) thiosemicarbazide was shown in Fig.3 the DMSO or $CDCl_3$ is used as a solvent and TMS used as internal standard. The proton in the vast majority of

organic compounds resonated at a low field then the proton of TMS is zero; it is possible to device δ scale. The assignment of the peaks in the ^1H NMR spectrum of 1-(1-phenylethylidene) thiosemicarbazide it is represented in the Table 2.

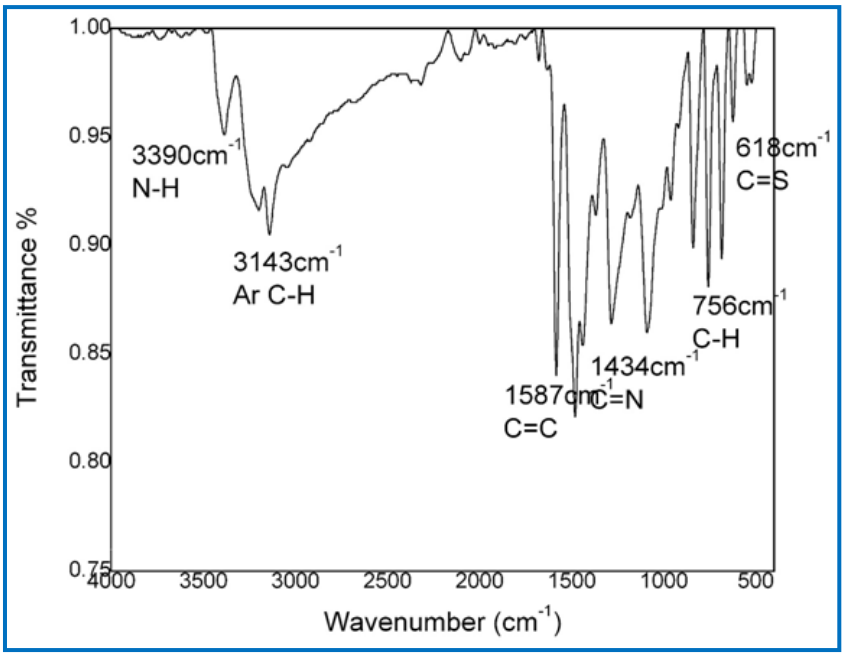


Fig 2 FTIR spectrum of 1-(1-phenylethylidene) thiosemicarbazide

Table 1

Peak Assignments	Wave number(cm^{-1})
C-H stretching vibration in aromatic system	3143
-C=C- bond was connected with conjugation	1583
-C=N stretching frequency	1434
-C-S stretching vibration	616
-C-H out of plane bending vibration	756
NH stretching vibration	3390

Table 2

Proton of different chemical environment	Chemical shift[δ]
Singlet,(1H) NH_2 proton	6.6
Singlet,(1H) N-H proton	8.5
Multiplet,(14H) Aromatic proton	7.1-7.4
Singlet,(3H) CH_3 proton	2.5

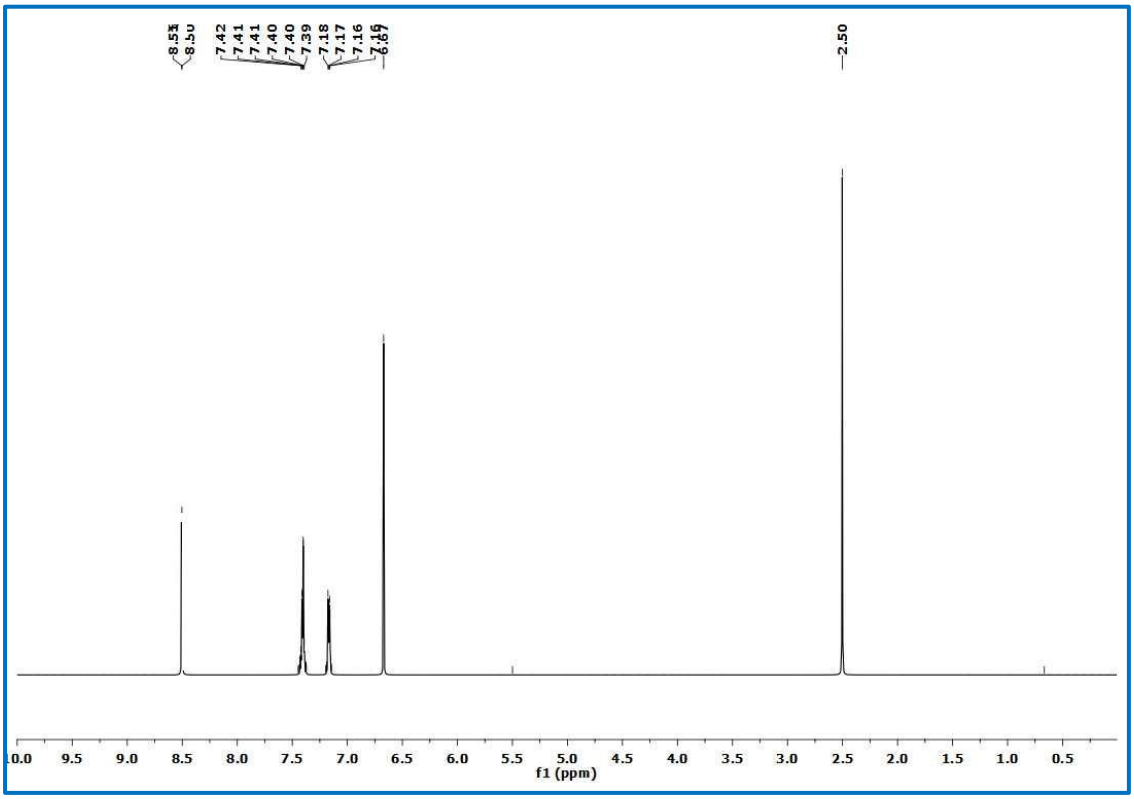


Fig 3 ^{1}H NMR spectrum of 1-(1-phenylethylidene) thiosemicarbazide

UV spectrum of compound (1, 4)-4-benzylidene-1-(1-phenylethylidene) thiosemicarbazide

It is a dark brown solid. The formation of product was confirmed by Thin Layer chromatography. UV peak at 322nm is due to n-π* transition which clearly indicates the presence of hetero atom. The UV spectrum of compound (A1) was shown in the Fig. 4.

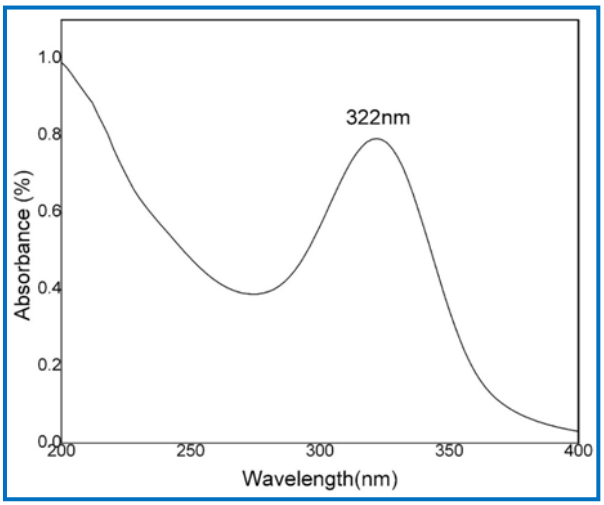


Fig 4 UV spectrum of (1, 4)-4-benzylidene-1-(1-phenylethylidene) Thiosemicarbazide

FT-IR spectrum of compound (1, 4)-4-benzylidene-1-(1-phenylethylidene) thiosemicarbazide

FT-IR spectrum of the (1, 4)-4-benzylidene-1-(1-phenylethylidene) thiosemicarbazide was shown in Fig.5. The weak absorption at 3146cm^{-1} is due to the C-H stretching vibration in aromatic system. The weak absorption at 3403cm^{-1} due to the N-H stretching frequency. The sharp peak at 1580cm^{-1}clearly shows C=C bond was attached with conjugation.

The peak was observed in the range of 1499cm^{-1} which indicates C=N stretching frequency. The peak at 743cm^{-1} was assigned the presences of C-H out-of- plane bending vibration in the ring. The peak at 682cm^{-1} indicates the presences of C=S stretching vibrations. The assignment of the main peak in the FT-IR spectrum of (1, 4)-4-benzylidene-1-(1-phenylethylidene) thiosemicarbazide was obtained represented by Table 3.

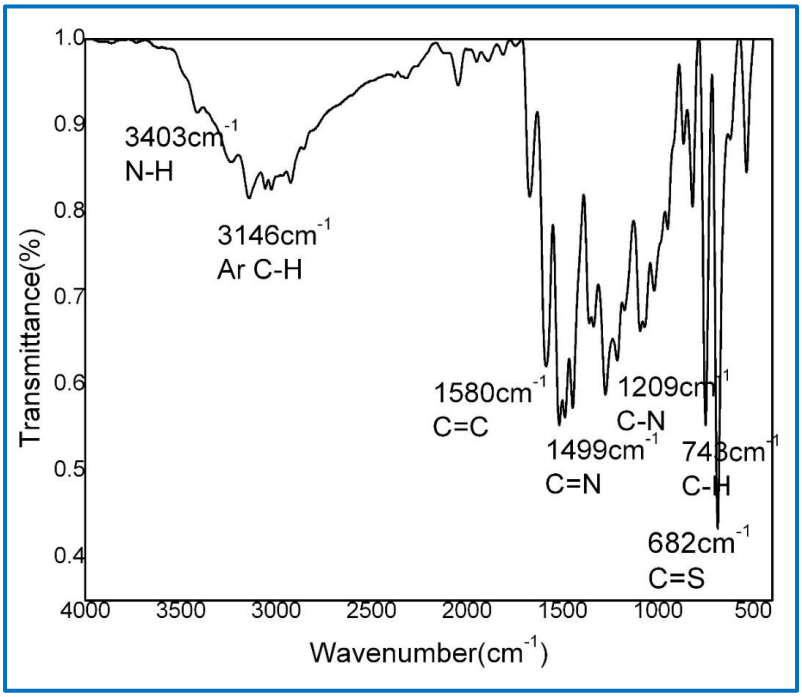


Fig 5 FTIR spectrum of (1, 4)-4-benzylidene-1-(1-phenylethylidene) Thiosemicarbazide

Table 3

Peak Assignments	Wave number(cm^{-1})
C-H stretching vibration in aromatic system	3143
-C=C- bond was connected with conjugation	1580
-C=N stretching frequency	1499
-C=S stretching vibration	682
-C-H out of plane bending vibration	743
N-H stretching vibration	3403

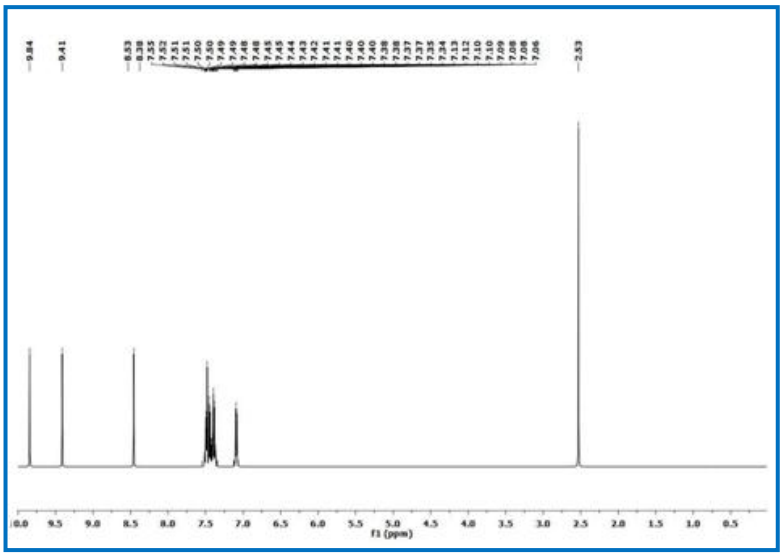


Fig 6 ^{1}H NMR spectrum of (1, 4)-4-benzylidene-1-(1-phenylethylidene) Thiosemicarbazide

Table 4

Proton of different chemical environment	Chemical shift[δ]
Singlet,(1H) imine proton	9.4
Singlet,(1H) N-H proton	8.5
Multiplet,(10H) Aromatic proton	7.0-7.5
Singlet,(3H) CH_3proton	2.5

^1H NMR spectrum of compound (1, 4)-4-benzylidene-1-(1-phenylethylidene) thiosemicarbazide

The ^1H NMR spectrum of the (1, 4)-4-benzylidene-1-(1-phenylethylidene) thiosemicarbazide was shown in Fig.6. The DMSO or $CDCl_3$ is used as a solvent and TMS used as internal standard. The proton in the vast majority of organic compounds resonated at a low field then the proton of TMS is zero it is possible to device δ scale. The assignment of the peaks in the ^1H NMR spectrum of (1,4)-4-benzylidene-1-(1-phenylethylidene) thiosemicarbazide it is represented in the Table 4.

4. Anti-fungicidal activity

All synthesized compounds were screenedfor their antifungal activity by Zone of Inhibition method the following fungicidal and procedures were used.

1. Aspergillus niger
2. Candida albicans

Procedure

The fungicidal activity of all that synthesized compounds was studied at 1000 ppm concentration in vitro. Plant pathogenic organisms used were Aspergillus niger and Candida albicans. The antifungal activity of all the compounds was measured on each of these plant pathogenic strains on a potato dextrose agar (PDA) medium such a PDA medium contained potato 200gm, dextrose 20gm, agar 20gm, and water 1 liter. Five days old cultures were employed. The compounds to be tested were suspended (1000 ppm) in a PDA medium and autoclaved at 120oC for 15 min and 15 atm. Pressure. These media were poured into sterile Petri plates. The percentage inhibition for fungi was calculated after five days using the formula given below,

Percentage of Inhibition $=\frac{\mathbf{100}\ (\boldsymbol{X}-\boldsymbol{Y})}{\boldsymbol{X}}$

Where, X = Area of colony in control plate

Y = Area of colony in test plate Antifungal Activity

The compounds viz, A1, A2, B1, B2, C1, C2, D1 and D2 have been subjected to antifungal studies using little epresentative number of pathogenic organisms like Aspergillus Niger and Candida albicans with respect to Fluconazole used as standard by Zone of inhibition method.

Antifungal activity of all the synthesized compounds by Zone of inhibition methods values of reported in the Table.5. Among all the synthesized compounds C1 and C2 found to possess the excellent fungicidal activity than the other first and second stages of compounds. The compound C1 has the zone of inhibition values of 15 & 16nm and C2 has 21 & 22 nm against the Aspergillus Niger and Candida albican respectively. The increasing activities of these compounds due to the presence of bromine atom.

Similarly electron donating groups like methyl substituted compound (B1 & B2) also found to have very good activity than the compounds of without substituted Compound of A1 & A2 against the Aspergillus niger and Candida albican. In contrast, the nitro substituted compounds have lowest value of antifungal activity which may due to the presence of electron withdrawing nitro group.

From the overall observation, among all the compounds of series substituted Schiff base derivatives have found to have excellent activity than the first stage compounds of substituted thiosemicarbazone. Fig.7.shown that Bar graph of anti-fungicidal activities.

Table 5 Antifungal activities of zone of inhibition (in nm) values of the synthesized Compounds

Compounds	**Zone of inhibition (in nm)**	
	Aspergillus niger	*Candida albicans*
A1	12	14
A2	16	16
B1	16	17
B2	17	19
C1	**15**	**16**
C2	**20**	**21**
D1	10	12
D2	15	16
Fluconazole	30	29

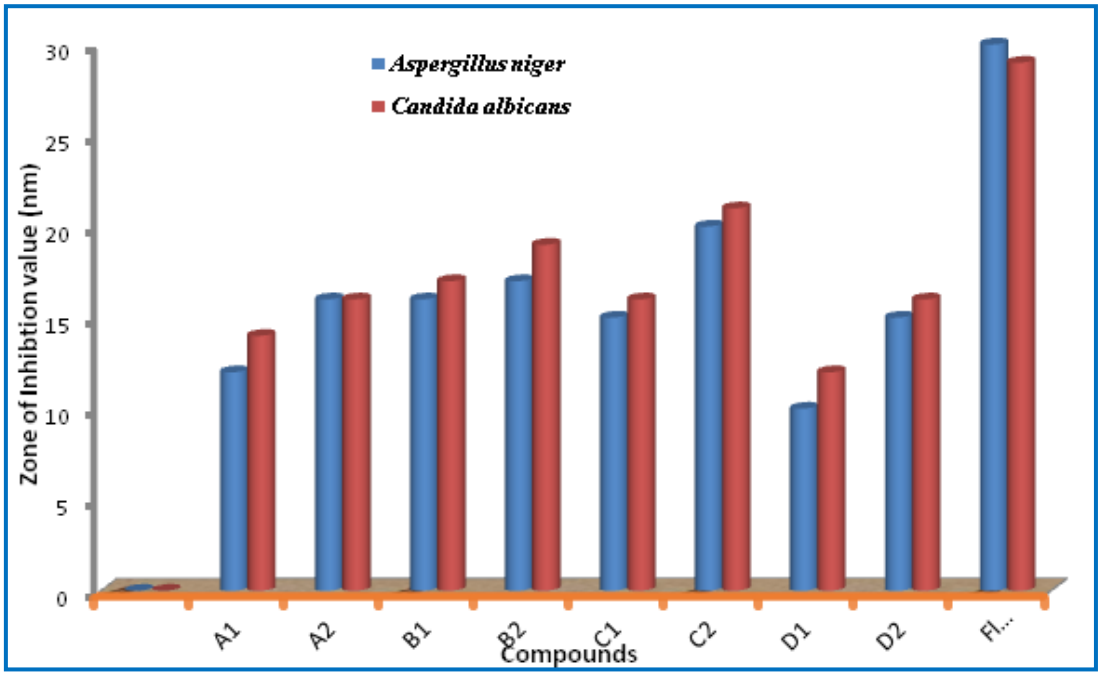


Fig 7 Bar Graph for comparison of antifungal activities of synthesis compounds

Conclusion

It is deals with the general introduction about the substituted thiosemicarbazide and Schiff bases and its applications, synthetic methods. Also, this chapter contains various medicinal applications of substituted thiosemicarbazide and Schiff bases derivatives. Especially in this chapter discussed advantages of microwave irradiation methods rather than the conventional methods.

It is deals with the scope and objectives of the present investigation based on the literature survey. Third chapter dedicated to the conventional and microwave assisted synthesis of target molecule of thiosemicarbazone bearing Schiff bases. The target compound of thiosermicarbazone linked with Schiff bases derivatives have been synthesized via two stages of chemical reaction. In a first stage of chemical reaction have been used to synthesis the various substituted thiosemicarbazide derivatives in the presence of catalytic amount of hydrochloric acid. In a second stage of reaction were carried out by condensation between the thiosemicarbazide molecules with corresponding substituted aldehyde in the presence of acetic acid in which creates the substituted Schiff bases as product. All the stages compounds have been synthesized via conventional and microwave assisted method of reaction. But the microwave assisted synthesis is clean with shorter reaction time, very fast, better homogeneity in temperature with quick transfer of energy into the whole, absence of inertia, mild reaction condition, eco-friendly, excellent yield as compared to conventional methods and reduces the use of volatile organic compounds (VOCs) and finally, it is agreement with the green chemistry protocols.

The fourth chapter dealt the results and discussion of the synthesized compounds of A1, A2, B1, B2, C1, C2, D1 and D2. All synthesized compounds were characterized by UV, FT-IR and 1H NMR spectral techniques and were found to be in agreement with the chemical structures expected. Antifungal activity of all synthesized compounds tested against Aspergillus niger and Candida albicans with respect to fluconazole used as standard.

From the antifungal evaluation of all the synthesized compounds it was concluded that out of eight compounds, C2 showed excellent antifungal activities against both Aspergillus niger and Candida albicans with highest zone of inhibition values 20nm and 21nm respectively.

The activity data obtained during the study will be certainly useful to go for further research for drug designing, drug delivery and synthesizing new thiosemicarbazide based heterocyclic compounds of Schiff bases and its derivatives.

References

1. A. Del Corso, M. Cappiello, U. Mura, Thiol dependent oxidation of enzymes: The last chance against oxidative stress, *International Journal of Biochemistry*, 26 (1994) 745-750.
2. R.B. Freedman, how many distinct enzymes are responsible for the several cellular processes involving thiol: protein-disulphide interchange? *FEBS Letters,* 97 (1979) 201-210.
3. M. Gopalakrishnan, P. Sureshkumar, J. Thanusu, V. Kanagarajan, Unusual formation of N-hydroxy-3,3-dimethyl-2,6-diarylpiperidin-4-one and its thiosemicarbazide derivative-synthesis and antimicrobial activity, *Pharmaceutical Chemistry Journal*, 42 (2008) 271-276.
4. P. Yogeeswari, D. Banerjee, P. Bhat, A. Thomas, M. Srividya, D. Shriram, Novel isatinyl thiosemicarbazones derivatives as potential molecule to combat HIV-TB co-infection, *European Journal of Medicinal Chemistry***,** 46 (2011) 106-121.
5. N. Siddiqui, O. Singh, Antibacterial activity of some 4-N-substituted thiosemicarbazides and thiosemi-carbazones *Indian Journal of Pharmaceutical Sciences*, 65(2003) 423-425.
6. ChristopheBiot, B. Pradines, M.H. Sergeant, J. Gut, P.J. Rosenthal, K. Chibalede, Design, synthesis, and antimalarial activity of structural chimeras of thiosemicarbazone and ferroquine analogues, *Bioorganic and medicinal chemistry letters*, 17 (2007) 6434-6438.
7. Jr. Charles Shipman, S.H. Smith, J.C. Drach, and D.L. Klayman, Antiviral Activity of 2-Acetylpyridine Thiosemicarbazones Against Herpes Simplex Virus, *Antimicrob Agents Chemother,* 19(1981), 682-685.
8. A. Siwek, J, Stefanska, K. Dzitko, A. Ruszezak, Antifungal effect of 4-arylthiosemicarbazides against Candida species. Search for molecular basis of antifungal activity of thiosemicarbazide derivatives, *Journal of Molecular Modeling*, 18 (2012) 4159-4170.
9. A.M. Thomas, A.D. Naik, M. Nethaji, A.R.Chakravarty Synthesis, crystal structure and photo-induced DNA cleavage activity of ternary copper(II)-thiosemicarbazone complexes having heterocyclic bases, *Inorganica chimica acta*, 357 (2004) 2315-2323.
10. H. Beraldo, D. Gambino, The wide pharmacological versatility of semicarbazones, thiosemicarba-zones and their metal complexes, *Mini-Reviews in Medicinal Chemistry*, 4 (2004) 31-39.
11. M.B. Ferrari, F. Bisceglie, G.G. Fava, G. Pelosi, P. Tarasconi, R. Albertini, S. Pelosi, Synthesis, characterization and biological activity of two new polymeric copper (II) complexes with α-ketoglutaric acid thiosemicarbazone, *Journal of inorganic biochemistry*, 89(2002), 36-44.
12. HS Seleem, BA El-Shetary, SME Khalil, M. Mostafa, M. Shebl, Structural diversity in copper(II) complexes of bis(thiosemicarbazone) and bis(semicarbazone) ligands, Journal of Coordination Chemistry, 58 (2005) 479-493.
13. K. Zamani, K. Faghihi, S. Bagheri, M. Kalhor, Microwave assisted rapid synthesis of thiosemicarbazide derlivatices, *Indian Journal Chemistry*, 43 (**2004)** 2716-2718.
14. C.M. Reis, D.S. Pereira, R. Oliveira, L.F. Kneipp, A. Echevarria, Microwave-Assisted Synthesis of New N1,N4-Substituted Thiosemicarbazones, *Molecules,* 16 (**2011**) 10668-10684.
15. M. Sheikhy, A.R. Jalilian, A. Novinrooz, F. J. Motamedi-Sedeh, Synthesis and in vitro antibacterial evaluation of some thiosemicarbazides and thiosemicarbazones, *Scientific research an academic publisher*, 5 (**2012**) 39-42.
16. D.L. Klayman, J.P. Scovill, J.F. Bartosevich, J. Bruce, 2-Acetylpyridine thiosemicarbazones. 5. 1-[1-(2-Pyridyl) ethyl]-3-thiosemicarbazides as potential antimalarial agents, *Journal of Medicinal Chemistry*, 26 (1983) 35-39.
17. M.A. Ashraf, K. Mahmood, A. Wajid, Synthesis, Characterization and Biological Activity of Schiff Bases, *International Conference on Chemistry and Chemical Process*, 10 (2011) 1-7.
18. S. Kalaivani, N.P. Priya, S. Arunachalam, Schiff bases: facile synthesis, spectral characterization and biocidal studies, International *Journal of Appled Biology and Pharmaceutical Technology,* 3 (2012) 219-223.

19. P. Souza, J.A. Garcia-Vazquez, J.R. Masaguer, Synthesis and characterization of copper(II) and nickel(II) complexes of the Schiff base derived from 2-(2-aminophenyl)benzimidazole and salicylaldehyde, *Transition Metal Chemistry*, 10 (1985) 410- 412
20. C. Silva da, D. Silva da, L.V. Modolo, R. B. Alves, M.A. de Resende, C.V.B. Martins, A. de Fatima, Schiff bases: A short review of their antimicrobial activities, Jouranl *of Advanced Research*, 2 (2011) 1-8.
21. H. Naeimi, J. Safari, A. Heidarnezhad, Synthesis of Schiff base ligands derived from condensation of salicylaldehyde derivatives and synthetic diamine, Dyes and Pigments,73 (2007) 251 -253.
22. S.J. Lippard, J.M. Berg, Principles of bioinorganic chemistry, (1994) 23, 26-87, University Science Books, Mill Valley, California, USA.
23. S. Kumar, D.N. Dhar, P.N. Saxena, Synthesis and Antibacterial Studies of Metal Complexes of Cu(II), Ni(II) and Co(II) with Tetradentate Ligand, *Scientific research an academic publisher*, 68 (2009) 181-187.
24. TM. Krygowski, K. Woźniak, R. Anulewicz, D. Pawlak, W. Kołodziejski, E. Grech, A. Szady, Through-Resonance Assisted Ionic Hydrogen Bonding in 5-Nitro-N-salicylideneethylamine, *The Journal of Physical Chemistry A*, 101 (1997) 9399–9404.