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SYNTHESIS, CHARACTERIZATION AND ANTI MICROBIAL SCREENING OF SOME SALICYL HYDRAZIDE SCHIFF BASES

P. V. Hemalatha*[1], T. Sudha[2], V. R. Ravikumar[3] and V. Ganesan[4]

[*1]Department of Pharmaceutical Chemistry, the Erode College of Pharmacy, Tamilnadu.
[2]Department of Pharmaceutical Analysis, Aadhi Parasakthi College of Pharmacy, Tamilnadu.
[3]Department of Pharmacognosy, the Erode College of Pharmacy, Erode Tamilnadu.
[4]Department of Pharmaceutics, the Erode College of Pharmacy, Erode Tamilnadu.

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***Corresponding Author**
P. V. Hemalatha
Department of Pharmaceutical Chemistry, the Erode College of Pharmacy, Tamilnadu.

ABSTRACT

Schiff bases also known as imines are important class of biologically active compounds showing wide variety of pharmacological activity. Salicyl hydrazide was synthesized from methyl salicylate by reaction with hydrazine hydrate. Salicyl hydrazide was then converted to Schiff bases by reacting with various aromatic aldehydes. Salicyl hydrazide reacted with cinnamaldehyde and formed a lime yellow coloured product, 2-hydroxy-N-[3- phenylprop-2-ene-1- ylidene] benzohydrazide (SHC). Similarly salicyl hydrazide reacted with furfural and formed an ash coloured product, N-[(furan-2-yl) methylidene]-2. hydroxy benzohydrazide, (SHF). The synthesized products were characterized by IR and NMR analysis. Salicyl hydrazide derived Schiff bases were evaluated for antimicrobial activity. Both the Schiff bases (SHC and SHF) did show antibacterial and antifungal activity. Salicyl hydrazide derived furfural schiff base (SHF) showed significant antibacterial activity against *Klebsialla pneumoniae*. Both SHC and SHF showed significant antifungal activity against *Candida albicans* and *Aspergillus niger*.

KEYWORDS: Salicyl hydrazide, Schiff base, cinnamaldehyde, furfural, antibacterial, antifungal.

INTRODUCTION

The increasing clinical importance of drug-resistant microbial pathogens has lent additional urgency in microbiological and antifungal research. Microbial infections are a growing problem in contemporary medicine, and the use of antibiotics is inevitable. Antibiotic

resistance is a major problem in hospitals as well as in community settings. In the current situation, where multidrug-resistant bacteria have spread widely, thus the treatment of bacterial infections remains a challenging therapeutic problem. Hydrazones are special group of compounds in the Schiff bases family. They are characterized by the presence of (C=N-N=C), the presence of two inter-linked nitrogen atoms was separated from imines, oximes, etc. as hydrazone Schiff bases of acyl, aroyl and heteroaroyl compounds have additional donor sites like C=O. The additional donor sites make them more flexible and versatile.[1] Schiff bases also known as hydrazones are now well known for their importance in biological fields. They have been explored extensively and found to exhibit a broad range of pharmacological actions like antiproliferative, antimicrobial, anti-inflammatory, antiviral, analgesic antioxidant and antipyretic properties. Schiff bases have also been shown to exhibit a broad range of biological activities, including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory properties.[2-5] Structurally, a Schiff base is a nitrogen analogue of an aldehyde or ketone in which the carbonyl group (C=O) has been replaced by an imine or azomethine (C–NH) group. Schiff are also formed from aldehyde and an acid hydrazide. "Acid hydrazide schiff bases are the compounds obtained by the condensation of an acid hydrazide and a carbonyl compound". Acid hydrazides and their derivatives have gained prominence because of their antibacterial activity and potentiality as versatile coordinating agents. Isonicotonic acid hydrazide is found superior to some other pyridine and nonpyridine hydrazides towards tuberculostatic activity.[6] The remarkable biological activity of acid hydrazides R-CO-NH-NH_2, and its class of Schiff base with aldehydes or ketones forming the corresponding aroyl hydrazones, (R-CO-NH-N=CH-R'), their mode of chelation with transition metal ions present in the living system have been of significant interest in the recent years. Their biological activity may be due to the ability of the ligands to form stable complexes with the metal ions which the fungus needs for its metabolism[7] Salicyl hydrazide is an acid hydrazide used to synthesize Schiff base and was expected to produce wide range of pharmacological activities. In depth review of the literature reveals that salicyl hydrazide has not yet been explored. Therefore, an attempt was made to synthesize novel derivatives of salicyl hydrazide schiff bases and screening of their antimicrobial activity. Synthesized compounds were characterized by IR, ^{1}H NMR spectral analysis.

General procedure for synthesis of Schiff bases

Methyl salicylate was treated with hydrazine hydrate to form salicyl hydrazide. Then the reaction mixture was kept over night for the formation of salicyl hydrazide. It was refluxed

with different aldehydes for 4 hrs using ethanol as the solvent for the formation of Schiff bases.

Synthesis of salicyl hydrazide

Hydrazides have been synthesized from esters by reaction with hydrazine hydrate.[8] So, a similar method was followed for the synthesis of salicyl hydrazide. Equal moles of hydrazine hydrate and methyl salicylate were dissolved separately in ethanol. Hydrazine hydrate was slowly added to the methyl salicylate solution with continuous stirring for 5 minutes. The order of addition is to be strictly followed. The reactants were mixed thoroughly and kept aside overnight. Shining white crystals were formed. The crystals were filtered and washed thoroughly with water to remove any traces of the by-product methanol and also the reagent hydrazine hydrate. The product was then recrystallized from hot ethanol. It was confirmed by IR analysis. The melting point determination confirmed its purity.

Synthesis of Salicyl Hydrazide Derived Cinnamaldehyde Schiff Base (SHC)

Salicyl hydrazide (0.05 mol) was dissolved in 10ml of ethanol in a round bottom flask. To this was added an ethanolic solution of 0.05 moles of cinnamaldehyde. The reaction mixtures was refluxed for 4 hr. Progress and completion of the reaction was analysed by performing TLC. The reaction mixture was cooled and set aside. Shining yellow crystals of the Schiff base was obtained. SHC was filtered using vacuum filter and washed thoroughly with water followed with ethanol. The product was recrystallised using DMF and characterized using IR and NMR analysis.

Synthesis of Salicyl Hydrazide Derived furfuraldehyde Schiff base (SHF)

About 0.05 moles of salicyl hydrazide was dissolved in hot ethanol in a round bottom flask attached to a reflux condenser. 0.05 moles of ethanolic solution of furfuraldehyde was added to the hot salicyl hydrazide solution and continued the reflux condensation for 6 hrs. The reaction mixture was allowed cool by setting aside overnite. The Schiff base formed was filtered over pump and washed thoroughly with water followed by ethanol. The product was recrystallised from DMF and characterized by IR and NMR.

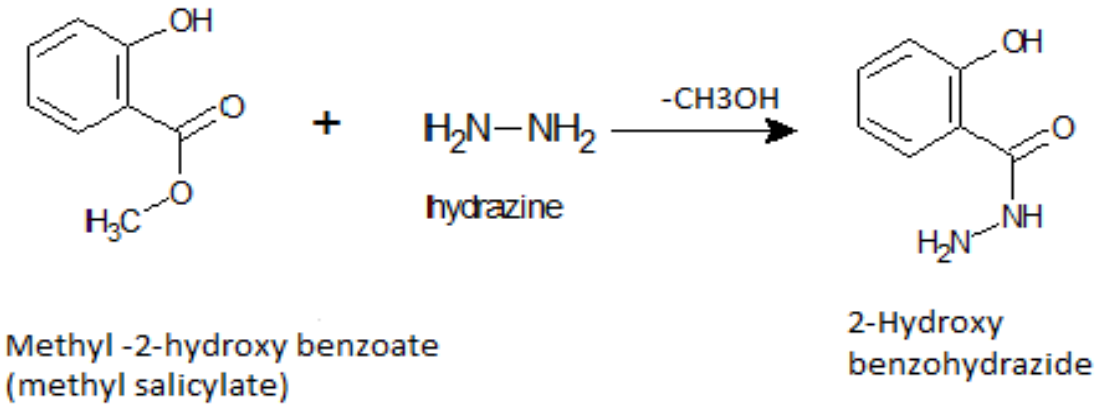

Fig. 1: Scheme for synthesis of salicyl hydrazide.

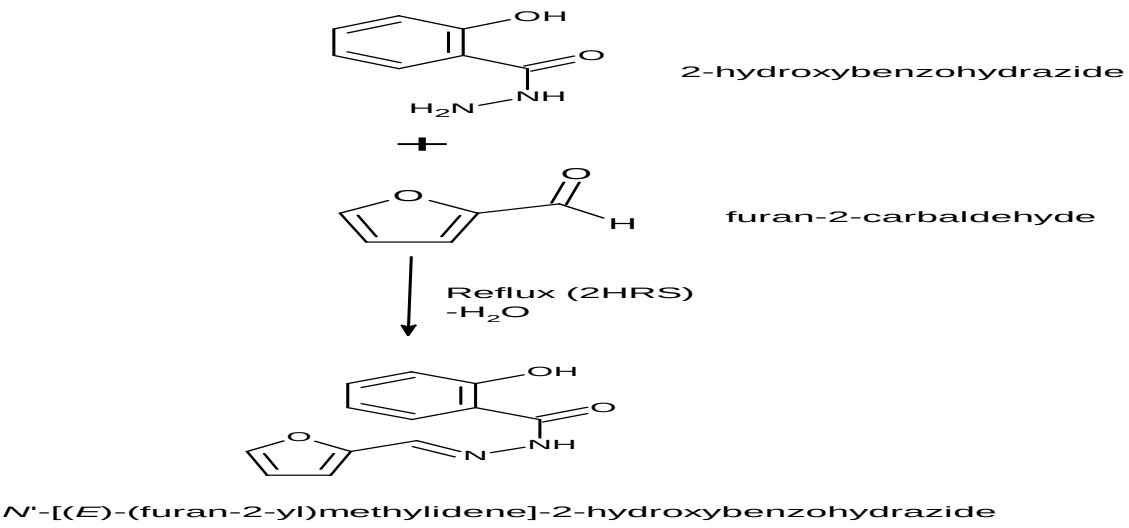

Fig. 2: Scheme for the synthesis of salicyl hydrazide –furfuraldehyde schiff base(SHF).

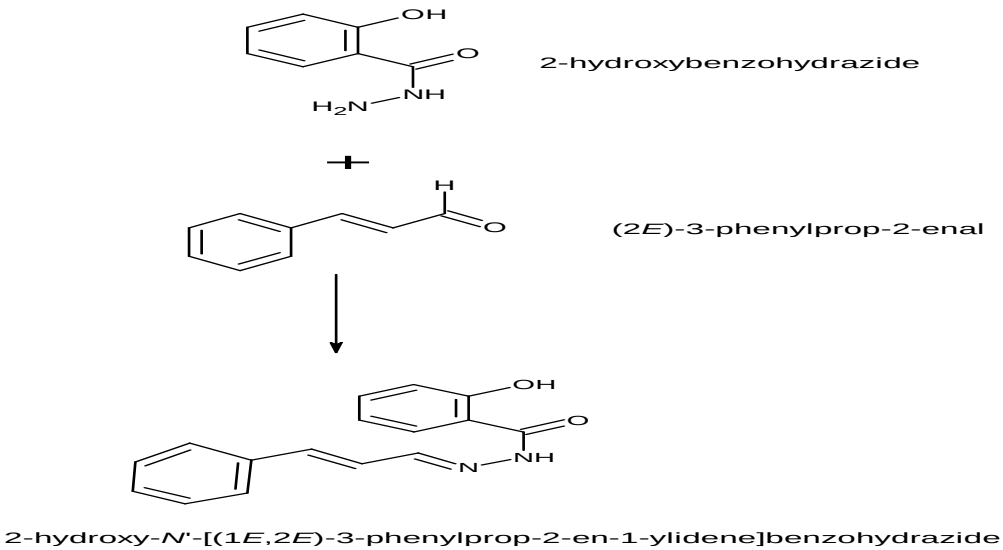

Fig. 3: Scheme for the synthesis of salicyl hydrazide –cinnamaldehyde schiff base(SHC).

Biological activity[9]

The antimicrobial activity against bacteria for the synthesized compounds (SHC and SHF) was determined against four different strains, gram positive and gram negative bacteria bacteria like *S.aureus* and *P. vulgari,s Klebsiella pneumoniae* and *P. aeruginosa* as compared to the standard drug ciprofloxacin. Similarly, the antifungal activity was studied against *Candida.albicans* and *Aspergillus niger.* The results were compared with the standard drug griseofulvin. The antimicrobial activity was assessed by measuring the zone of inhibition,

Antibacterial activity

The newly synthesized compounds were screened for their *in-vitro* antibacterial activity against *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus vulgaris* bacterial strains by cylinder plate method.[10] About 2 % of microbial suspension was added to the quantity of medium per plate i.e., 0.5 ml of suspension per 25 ml of muller hinton nutrient agar medium. The petriplates were allowed for solidification for about 10 min. By using sterile glass (Pyrex) bores, holes are punched out of the inoculated culture medium maintaining approximate distance between well (well diameter: 4 mm). Wells were labelled properly to enable the introduction of the test sample, standard and control precisely. Sample solutions measuring 20 µl, concentration ranging from 250µg/ml to 1000µg/ml were introduced into appropriate wells with the help of micropipette. All the wells were filled with equal volumes of the sample. To minimize the effect of variants the petriplates were stored at room temperature for 1 - 4 hrs, and then the plates were allowed to incubate at 37ºC for a time period of 2-3 days. The zone of inhibition was examined and measured with the help of antibiotic zone reader. A control was also prepared for the plates in the same way using DMSO as a solvent. The petri dishes were prepared in triplicate and antibacterial activity studied in triplicate. Zone of inhibition of each compound was compared with standard drug ciprofloxacin (500 µg/ml). The result tabulated in the Table-1.

Antifungal activity

Newly prepared compounds were screened for their antifungal activity against *Candida albicans and Aspergillus niger* in DMSO by well diffusion technique.[10] Potato dextrose agar media was prepared and adjusted the pH to 5.7. DMSO was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of the inoculated potato dextrose agar media was poured into each petri dish. Excess of suspension was

decanted and plates were dried by placing in an incubator at 37 0C for 1 h. Using a punch, wells were made on these seeded agar plates of 4 mm in diameter. About 20 µl of the test compounds (SHC & SHF) of concentration ranging from 250 µg/ml to 1000 µg/ml in dimethyl sulfoxide (DMSO) were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The petri dishes were prepared in triplicate and maintained at 37^0C for 3 to 4 days. Antifungal activity was determined by measuring the diameter of zone of inhibition.The results were compared with the standard griseofulvin (500 µg/ml) and tabulated in the Table -1.

RESULTS AND DISCUSSION

Salicyl hydrazide was prepared from the salicylic ester. A solution of methyl salicylate in ethanol was mixed with hydrazine hydrate in ethanol. The reaction just requires very simple condition that is keeping the reaction mixture undisturbed overnight, when salicyl hydrazide crystals are formed. The product was characterized by IR and its melting point was determined. The reaction is depicted in figure 1 as scheme I.

Hydrazide derived Schiff bases were prepared using salicyl hydrazide and aldehydes like cinnamaldehyde and furfural. A common preparation method was adopted. The nucleophilic addition reaction between aldehydes and salicyl hydrazide in EtOH followed by the elimination of one water molecule results in the formation of the Schiff base, SHC and SHF as shown in figure 2 and 3. Salicyl hydrazide was refluxed with the aldehyde for 4hrs and kept aside overnite. Cinnamaldehyde produced shinning yellow crystals SHC whereas fufural produced grey coloured crystals SHF. The products were recrystallised from DMSO. The synthesized products were tested for solubility analysis, their melting point was determined and characterized by IR and ^{1}H NMR. The Synthesized compounds SHC &SHF were commonly soluble in DMSO and DMF. The compounds were insoluble in water, alcohol and ether. They were partially soluble in chloroform. The melting point was determined using digital melting point apparatus. The melting point of SHC was determined to be 158º C. The Melting point of SHF was determined to be 136ºC.

pectral Characterization

Spectral Analysis: The Schiff bases were caharacterized by IR and NMR. The analytical data shows satisfactory results confirming the structure of the compounds.

SHC (*Infra Red & ^{1}H NMR)*

The *IR* spectral analysis of the Schiff base SHC shows a band at 3257 cm^{-1}indicating the presence of –OH group. The other bands at 1626 cm^{-1},3174 cm^{-1},1606 cm^{-1} are assignable to C=O(carbonyl), N-H,C=N groups. The presence of phenolic -OH was ascertained by 1535 cm^{-1}. The bending vibrations at 910,783cm^{-1} are assignable to N-H and C-H groups respectively.

The ^{1}H NMR spectrum of the Schiff base analysed using dimethyl-sulphoxide, DMSO. ^{1}H NMR spectrum of the Schiff base SHC showed the signals at 8.2 ppm were assigned to proton of CH=N group. Usually the phenolic proton show signals at δ 7.5 to δ 4.0. But presence of a carbonyl group at ortho position shifts the phenolic proton absorption at δ 11.8 due to intra molecular hydrogen bonding.[11] Aliphatic amino-NH proton show signals at δ 2.5. Signals in the region of δ 6.6 to δ 8.0 were assigned to the aromatic protons.

SHF *(Infra Red & ^{1}H NMR)*

The interpretation of the IR spectrum of SHF shows a band at 3250 cm^{-1} indicating the presence of–OH group. The other bands at 1635 cm^{-1}, 3159 cm^{-1},1608 cm^{-1} are assignable to C=O (carbonyl), N-H, C=N (azomethine) groups respectively. The band at 1159 cm^{-1} represents the presence of –C-O-C- of furfural.[12] The presence of phenolic -OH was ascertained by 1539 cm^{-1}.The bending vibrations at 906 cm^{-1}, 777cm^{-1} are assignable to N-H and C-H groups respectively.

The *^{1}H NMR* spectrum of the Schiff base dissolved in dimethyl-sulphoxide, DMSO showed signals at 8.4 ppm were assigned to proton of CH=N azomethine group. Usually the phenolic proton show signals at δ 7.5 to δ 4.0. But presence of a carbonyl group at ortho position shifts the phenolic proton absorption at δ 11.8 due to intra molecular hydrogen bonding.[11] Aliphatic amino-NH proton show signals at δ 2.5. Signals in the region of δ 6.9 to δ 7.8 were assigned to the aromatic protons of the phenolic and furfural rings.

Antimicrobial Activity

The synthesized hydrazide derived Schiff bases were screened for their *in vitro* anti-bacterial activity against Gram –ve and Gram +ve pathogenic bacteria like *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus vulgaris*. The zone of inhibition was observed. Both SHC and SHF demonstrates inhibitory effect against the bacteria. Especially, SHF showed good activity against good appreciable activity against

Klebsiella pneumoniae. Similarly SHC and SHF were subjected to *in vitro* antifungal activity against *Candida albicans* and *Aspergillus niger.* SHF showed good appreciable antifungal activity against *Candida albicans* and *Aspergillus niger.* The results are tabulated in table 1.

Table. 1: Antimicrobial activity of SHC and SHF.

Drug	Concentration µg/ml	Zone of inhibition in mm for the microorganism*					
		Pseudomonas aeruginosa	***Proteus vulgaris***	***Klebsiella Pneumoniae***	***Staph. aureus***	***Candida albicans***	***Aspergillus niger***
SHC	250	10±0.18	11±0.06	12±0.08	10±0.24	6±0.26	10±0.25
	500	12±0.23	11±0.12	12±0.18	13±0.26	9±0.24	13±0.37
	750	13±0.26	13±0.16	14±0.026	15±0.25	10±0.18	14±0.26
	1000	15±0.18	14±0.33	15±0.26	15±0.26	10±0.08	14±0.18
	1250	15±0.16	15±0.36	15±0.33	16±0.38	14±0.05	13±0.25
SHF	250	10±0.24	11±0.06	12±028	10±0.26	12±0.16	13±0.42
	500	11±0.28	12±0.24	13±0.28	12±0.33	13±0.18	14±0.16
	750	13±0.26	12±0.12	13±0.14	14±0.24	13±0.16	15±0.20
	1000	14±0.16	13±0.14	14±0.16	15±0.18	14±0.14	17±0.36
	1250	15±0.42	13±0.26	16±0.16	15±0.15	16±0.16	18±0.38
Ciprofloxacin	500	15±0.45	16±0.35	13±0.36	18±0.26	-	-
Ketoconazole	500	-	-	-	-	13±0.33	15±0.25

****Each value represent triplicate determination. mean±SEM***

CONCLUSION

Novel salicycl hydrazide derived Schiff bases were synthesized. They were characterized by IR, NMR. The *in vitro* antimicrobial including anti bacterial and antifungal activity were studied. The synthesized compounds did show both anti bacterial and antifungal activity. But salicyl hydrazide derived furfural Schiff base presented appreciable antimicrobial activity.

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