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STRUCTURAL AND BIOLOGICAL ACTIVITIES OF (4Z)-4-(3-PHENYL ALLYLIDENE AMINO)-3-HYDROXY NAPHTHALENE-1-SULFONIC ACID (AC) AND NAPHTHALENE-1-YL-THIOPHENE-2-YLMETHYLENE-AMINE (NT) AS SCHIFF BASES

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ABSTRACT

Schiff bases (4Z)-4-(3-phenyl allylidene amino)-3-hydroxy naphthalene-1-sulfonic acid (AC) and naphthalene-1-yl-thiophene-2-ylmethylene-amine (NT) derived from the condensation reaction of Analar grade 1-amino-2-naphthol 4-sulphonic acid WITH Cinnamaldehyde and naphthalene-1-ylamine and thiophene-2-carboxaldehyde, respectively was prepared. The synthesis was carried out under microwave condition. This Schiff base was analysed by infra-red and UV. The all prepared compounds were assayed for antibacterial (*Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 96, *Echirichia coli* MTCC 1689 and *Proteus vulgaris* MTCC 742) and antifungal (*Aspergillus sp*. and *Candida albicans*) activities by disc diffusion method. The results indicate that all tested compounds show antibacterial activity against *E. coli*, as gram positive and negative bacteria, and antifungal activity against *C. albicans*. But the compounds AC having 1-OH and 1-HSO_3 substituted showed good inhibition against bacteria and fungi as compare to standard drugs.

Key words: (4Z)-4-(3-Phenyl allylidene amino)-3-hydroxy naphthalene-1-sulfonic acid (AC) and naphthalene-1-yl-thiophene-2-ylmethylene-amine (NT) antibacterial, Antifungal.

INTRODUCTION

Schiff bases, named after Hugo Schiff[1], are formed when any primary amine reacts with an aldehyde or a ketone under specific conditions. Structurally, a Schiff base (also known as imine or azomethine) is a nitrogen analogue of an aldehyde or ketone in which the carbonyl group (C=O) has been replaced by an imine or azomethine group. Schiff bases are

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some of the most widely used organic compounds. They are used as pigments and dyes, catalysts, intermediates in organic synthesis, and as polymer stabilisers[2]. Schiff bases also exhibit a broad range of biological activities, including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic properties[2,3]. Imine or azomethine groups are present in various natural, natural-derived, and non-natural compounds. The imine group present in such compounds has been shown to be critical to their biological activities[4-6].

Searching for novel antimicrobial agents and new microbial targets is in demand to intervene to avert the danger caused by these life-threatening infections[7]. The treatment of nosocomial infections such as hospital acquired methicillin resistant *Staphylococcus aureus* (MRSA) and biofilm formers has become an important problem to deal with owing to their multidrug resistance[8,9]. Since the resistance towards the available antibiotics among pathogenic bacteria has grown rapidly, there is a clear need for the development of new and effective antimicrobial agents. Therefore, the success in designing antimicrobial agents, which are distinct from those of the classical antibiotics is the key for treating such infectious diseases known for their chronicity and failure to treat with conventional antibiotics. which will eventually lead to death. Schiff bases have been found to possess pharmacological activities such as antibacterial[10], antifungal[11], antitubercular[12], antimicrobial[13], antiviral[14], antimalarial[15] and anticancer[16]. They are important compound due to their wide range of biological activities and industrial application. They also serve as a back bone for the synthesis of various heterocyclic compounds[17]. The condensing protein, (ketoacyl-acyl carrier protein synthase (KAS), is an essential target for a novel antibacterial drug design against multidrug resistant Gram positive pathogens, impressive efforts have led to the synthesis of peptide and Schiff bases, which can be potential antibiotic agents targeting KAS in Gram positive and Gram negative pathogens.

Several research groups have been involved in the synthesis and biological screening of Schiff bases. The authors[18] showed that the aldehyde Schiff base N-aryl thiosemicarbazones had stronger anti-MRSA potency, being effective at half the concentration of the vancomycin. Furthermore, preliminary results revealed that one of the thiazolidinedione-5-acetic acid amide derivatives exhibits promising antimicrobial activity[19]. Similarly, it is discovered that 2,20-diamino-1-azavinyl aminoamide can be used effectively against a number of both Gram-positive and Gram-negative bacteria[20]. Quite recently, the synthesis and characterization of a number of new Schiff bases derived from metronidazole have been undertaken and their antigiardial and antimicrobial activities were evaluated[21].

In view of the wide interest in the activity and profile of Schiff bases derived from benzaldehydes due to their pharmacological interest, we described herein the synthesis and characterization of two Schiff bases derived from derived from the condensation reaction of analar grade1-amino-2-naphthol 4-sulphonic acid with cinnamaldehyde and naphthalene-1-ylamine and thiophene-2-carboxaldehyde, which some of them are to the best of our knowledge, have not previously been described in the literature. The antimicrobial and antifungal activity of the synthesized compounds was evaluated. Also, published articles concerning antimicrobial activity sometimes lack containing information about the cytotoxicity of such compounds.

EXPERIMENTAL

Materials and methods

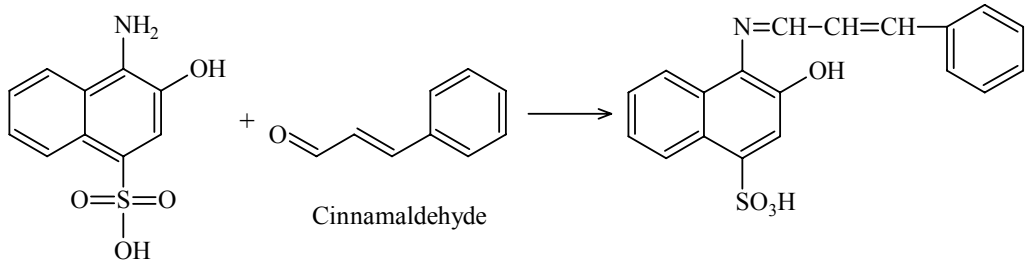
Antimicrobial activities of compounds were tested using well diffusion method. Tested microorganism strains human pathogenic bacteria both in positive and negative bacteria such as *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 96, *Echirichia coli* MTCC 1689 and *Proteus vulgaris* MTCC 742 and two human pathogenic fungi viz. *Aspergillus sp*. and *Candida albicans* were obtained from the Center for Advanced Studies in Botany, University of Madras, Chennai, India under *in vitro* conditions.

In the present study, antimicrobial activities of different compounds were evaluated against four human pathogenic bacteria both positive and negative organisms such as *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 96, *Echirichia coli* MTCC 1689 and *Proteus vulgaris* MTCC 742 and two human pathogenic fungi viz. *Aspergillus sp*. and *Candida albicans* under *in vitro* conditions. The biological screening results of compounds with 10% DMSO as control and with commercial antibiotics viz., tetracyclin and carbendazim are tabulated in the form of minimum inhibitory concentration (MIC).

Antibacterial studies

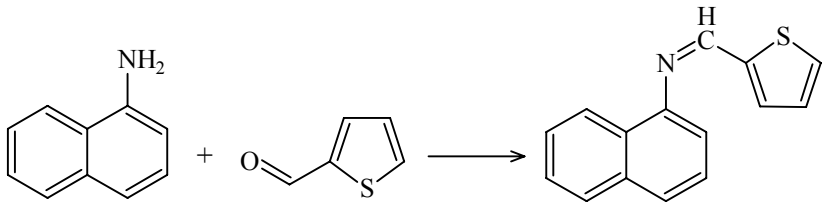
The human pathogenic bacterial cultures positive bacteria viz. *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 96 and negative bacteria viz. *Echirichia coli* MTCC 1689 and *Proteus vulgaris* MTCC 742 were obtained from the Center for Advanced Studies in Botany, University of Madras, Chennai, India and the biological screening of the compounds was performed there itself. The all bacterial strains were maintained on nutrient agar (NA) consisting of the following (g/L): beef extract 10; yeast extract 20; peptone 10; NaCl 5; agar 15; distilled H_2O 1L; pH 7.2 in slants or Petriplates at room temperature

(28-32ºC). Inhibitory concentration (MIC) was determined for compounds AC and NT by well diffusion assay. The compound at the concentration range of 5-100 µg/mL in 10% DMSO was used in this study with tetracyclin as reference control. The minimum inhibitory concentration (MIC) value was taken as the lowest concentration of compound that showed prominent inhibition of bacterial growth after 24 hrs of incubation at 37ºC.



1-Amino-2-naphthol 4-sulphonic acid

Scheme 1a: Chemical structure of the (4Z)-4-(3-phenyl allydene amino)-3-hydroxy naphthalene-1-sulfonic acid



Scheme 1b: Chemical structure of the naphthalene-1-yl-thiophene-2-ylmethylene-amine

Antifungal studies

The human pathogenic fungi viz., *Aspergillus sp*. and *Candida albicans* were obtained from the Center for Advanced Studies in Botany, University of Madras, Chennai, India and the biological screening of compounds was performed there itself. The human pathogens viz., Trichoderma sp. and *Aspergillus sp*. were maintained on potato dextrose agar (PDA) containing the following (g/L): potato 20.0 g; dextrose 20 g; agar 15 g; distilled H_2O; 1L; pH 6.5 in slants or Petriplates at room temperature (28-32ºC). Similar to antibacterial activity the minimum inhibitory concentration (MICs) was determined for the compound 1-8 by well diffusion assay. The compound at the concentration range of 15-90 µg/mL in 10% DMSO was used in this study with carbendazim as reference control. Minimum inhibitory

concentration (MIC) value was taken as the lowest concentration of compound that showed prominent inhibition of fungal growth after 3 days of incubation at 37ºC.

Disc diffusion method

The synthetic compounds were tested *in vitro* for their antimicrobial activity against Gram positive and Gram-negative organisms and Candida at 0.02 g/mL, respectively by Kirby–Baueragar diffusion method. The current NCCLS-LSI document M2-A9, 2006 guidelines recommend using Mueller–Hinton agar medium for bacteria and Sabouraud dextrose agar medium for Candida NCCLS-M27-A 1997.

RESULTS AND DISCUSSION

Synthesis of (4Z)-4-(3-phenyl allylidene amino)-3-hydroxy naphthalene-1-sulfonic acid) and naphthalene-1-yl-thiophene-2-ylmethylene-amine (NT)

All the chemicals used were of Analar grade 1-amino-2-naphthol 4-sulphonic acid and Cinnamaldehyde were mixed together in an Erlenmeyer flask. The mixture was kept under microwave radiation for four minutes on the "M-High" setting and the product obtained was brownish in nature. The resulting solution was evaporated to remove the solvent. The product was washed several times and recrystallized from ethanol. Thin layer chromatography was used to check the purity of the compound. The yield obtained was about 84%.

Elemental analysis

The Schiff base [(4Z)-4-(3-phenyl allydene amino)-3-hydroxy naphthalene-1-sulfonic acid] under study was identified by many techniques. The elemental analysis (CHN) is given in Table 1a. The Schiff base naphthalene-1-yl-thiophene-2-ylmethylene-amine under study was identified by many techniques. The elemental analysis (CHN) is given in Table 1b.

Table 1a: Elemental analysis data for CHN of Schiff base (4Z)-4-(3-phenyl allydene amino)-3-hydroxy naphthalene-1-sulfonic acid

Schiff base	Molecular weight	Percentage in mole fraction					
		C		H		N	
		Calc.	Found	Calc.	Found	Calc.	Found
AC	353	64.58	63.80	4.28	4.05	3.96	3.73

Calc.: Calculated

Table 1b: Elemental analysis data for CHN of Schiff base naphthalene-1-yl-thiophene-2-ylmethylene-amine

Schiff base	Molecular weight	Percentage in mole fraction							
		C		H		N		S	
		Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
NT	237.32	75.91	74.60	4.67	4.52	5.90	5.71	13.51	13.26

Calc.: Calculated

Spectral analysis

Electronic spectra

The electronic spectra of the ligand were recorded on DMSO on Pekin Elmer Lambde E2201 spectro photometer. The electronic absorption spectra of the ligand show λ_{max} at 247 nm and 216 nm for [(4Z)-4-(3-phenyl allydene amino)-3-hydroxy naphthalene-1-sulfonic acid] and naphthalene-1-yl-thiophene-2-ylmethylene-amine.

IR Spectra

IR spectra was recorded on Perkin Elmer 783 spectrophotometer by using KBr pellet. The characterized bands in the IR spectra are shown in the Table 2a and 2b. The band in the region 1614 cm^{-1} is due to (N=C), and bands in the region 1430 cm^{-1} and 3435 cm^{-1} are due to (C=C) and (O-H), (S=O) give 1231 cm^{-1} and (S-O) gives band at 650 cm^{-1} and for (C-N) 1384 cm^{1}, respectively[18]. The infrared spectra are shown in Fig. 1a and 1b.

Table 2a: Characterized band of IR spectra of Schiff base (4Z)-4-(3-phenyl allydene amino)-3-hydroxy naphthalene-1-sulfonic acid

Schiff base	N=C (cm^{-1})	C=C (cm^{-1})	O-H (cm^{-1})	S=O (cm^{-1})	S-O (cm^{-1})	C-N (cm^{-1})
AC	1614	1430	3435	1231	650	1384

Table 2b: Characterized band of FT-IR Spectra of Schiff base naphthalene-1-yl-thiophene-2-ylmethylene-amine

Schiff base	C-H (cm^{-1})	C=C (cm^{-1})	O-H (cm^{-1})	C=O (cm^{-1})	C-N (cm^{-1})
NT	3045	1457	3435	1601	1423

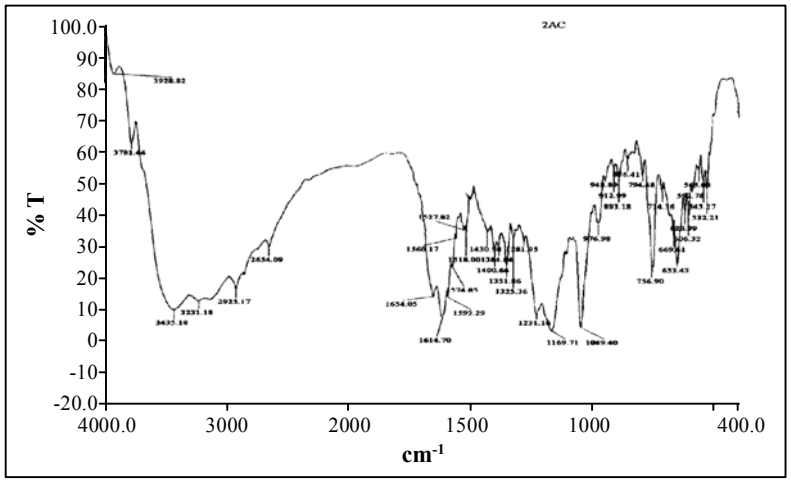


Fig. 1a: IR Spectrum of Schiff base (4Z)-4-(3-phenyl allydene amino)-3-hydroxy naphthalene-1-sulfonic acid

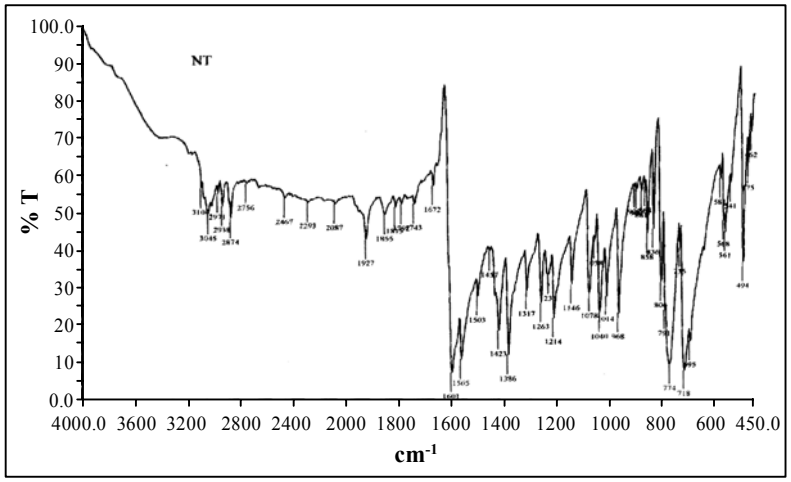


Fig. 1b: FT-IR Spectrum of naphthalene-1-yl-thiophene-2-ylmethylene-amine

Effect of tested compounds on the growth of human bacterial pathogens

All the compounds exhibited different levels of antibacterial activity against the four-tested human pathogenic bacteria are compared to 10% DMSO as control. Further, the antibacterial activity of the test compounds was dose dependent and was remarkable at higher concentrations. The minimum inhibitory concentration (MIC) of compounds AC and NT against bacterial human pathogens as determined by well diffusion method ranged between 5 and 25 µg/mL. Significantly the compound AC shows prominent antibacterial

activity against the both positive and negative tested human pathogenic organisms namely, positive bacteria viz. *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 96 and negative bacteria viz *Echirichia coli* MTCC 1689 and *Proteus vulgaris* MTCC 742; compared to other tested compound AC at lowest concentration ranging from 5 to 10 µg/mL, compared with reference control. Table 3 shows the minimum inhibitory concentration (MIC) values are relatively low for the compound NB as compared with reference control. Overall analysis on the antibacterial activity revealed that the compound AC remarkably inhibited all the pathogenic bacteria in most of the tested concentrations as compared to other compounds and control. The compounds AC having 1-OH and 1-HSO_3 substituted showed good inhibition against bacteria and fungi as compare to standard drugs. Further, the compound AC was also found to be superior then the commercial antibiotics. The minimum inhibitory concentrations (MIC) of compounds AC and NT were determined between 5 and 15 µg/mL as compared to 5 and 50 µg/mL for other compounds and tetracyclin (Table 3). However, the antibacterial activity of compound NT was found to be equal to that of tetracyclin on all the tested pathogens.

Table 3: *In vitro* antibacterial activity against human pathogens (minimum inhibitory concentration in µg/mL) for the compounds

Compounds	Positive bacteria		Negative bacteria	
	B. subtilis **MTCC 441**	***S. aureus*** **MTCC 96**	***E. coli*** **MTCC 1689**	***P. mirabilis*** **MTCC 742**
AC	5	5	5	5
NT	5	10	5	5
Tetracyclin	10	15	10	20
Control	NI	NI	NI	NI

Effect of tested compounds on the growth of human fungal pathogens

Similar to antibacterial activity, all the compounds exhibited different levels of antifungal activity against the two-tested human pathogenic fungi compared to 10% DMSO control. Further, the antifungal activity of the test compounds was dose dependent and was remarkable at higher concentrations. The minimum inhibitory concentrations (MICs) of the compounds AC and NT against human fungal pathogens as determined by well diffusion method ranged between 15 and 90 µg/mL. Among the compounds tested, the compound AC (ranging from 10 to 20 µg/mL) significantly inhibited the two human fungal pathogens namely, *Aspergillus sp.* and *Candida albicans* compared to NT and commercial fungicide,

carbendazim. The compound NT was rated as the second best antifungal activity (ranging from 15 to 25 µg/mL) compared with reference control. Table 4 shows the minimum inhibitory concentration (MIC) values are relatively low for other compound NT as compared with reference control.

Table 4: *In vitro* antibacterial activity against human pathogens (minimum inhibitory concentration in µg/mL) for the compounds

Compounds	Fungus	
	Aspergillus sp.	***Candida albicans***
AC	10	20
NT	15	25
Carbendazim	15	10
Control	NIL	NIL

CONCLUSION

All the synthesized compounds particularly AC has elevated antimicrobial activity against all the selected human pathogenic bacteria and fungi then followed by NT. The compound AC may be developed further as antibiotic drugs as they exhibit better antimicrobial activity against all the test pathogens than the other compounds as well as commercial antibiotics. The compounds AC having 1-OH and 1-HSO_3 substituted showed good inhibition against bacteria and fungi as compare to standard drugs. However, further studies are required to determine their mode of action and their potential application against wide range of human pathogens.

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