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Antimicrobial Studies on Derivatives of Benzocaine

T KOLOCHI Head of the department, PG and Research Department of Chemistry, Arignar Anna Government Arts college, Musiri 621211,Tamil Nadu, India.

A VICTORIA PG and Research Department of Chemistry, Arignar Anna Government, Arts college, Musiri 621211,Tamil Nadu, India.

T POOVENTHIRAN PG and Research Department of Chemistry, Arignar Anna Government Arts college, Musiri 621211,Tamil Nadu, India.

ABSTRACT

The novel Schiff bases from Benzocaine with 5-Formyl salicylic acid and 5-Nitrovaniline, were prepared and characterized by physical and analytical data, FTIR, 1H NMR, UV-Vis, Emission spectra and were screened for antibacterial activity against gram positive bacteria Staphylococcus aureus, Basillus subtilis and gram negative bacteria Escherichia Coli, Klebsiella aerogenes and antifungal activity against Aspergillus niger and Candida albicans by disc diffusion method. Ciprofloxacin and Nystatin were used as standard drug for bacteria and fungus.

KEYWORDS : Benzocaine, 5-Formyl salicylic acid and 5-Nitrovaniline, Schiff bases, Zone of inhibition, Antibacterial activity, Antifungal activity, Ciprofloxacin and Nystatin.

INTRODUCTION

Schiff base organic compounds containing the azomethine group (–CH=N–) are usually prepared by the condensation of a primary amine with an active carbonyl compound[1]. Different types of Benzocaine derivatives posses wide range of biological and pharmacological activities. Antimicrobial agents are the drugs, chemical or other substances that kill or slow the growth of microbes. They are well known for their biological applications as antibacterial, antifungal, anticancer, antiviral[2-4] and antitubercular agents[5-6] which give it attracted remarkable attention[7]. Benzocaine is prepared by direct esterification of p-aminobenzoic acid with absolute ethanol, in the presence of sulfuric acid as dehydrating agent[8]. 5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid and ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene)amino) benzoate are prepared by direct condensation of ethyl-4-amino benzoate with 5-Formyl salicylic acid and 5-Nitrovaniline.The presence of azomethine and benzoate functional group is responsible for antimicrobial activity, which can be altered depending upon the type of substituent present on the aromatic rings. Keeping in view of the pronounced biological activity of the Schiff bases from anesthetic drug, it is thought of worthwhile to study the antimicrobial activity of Schiff bases are derived from Benzocaine with 5-Formyl salicylic acid and 5-Nitrovaniline.

MATERIALS AND METHODS

Ethyl-4-amino benzoate, 5-Formyl salicylic acid, 5-Nitrovaniline, Ethanol, DMSO and $CHCl_3$ were purchased from Alfa Aesar.

Instruments

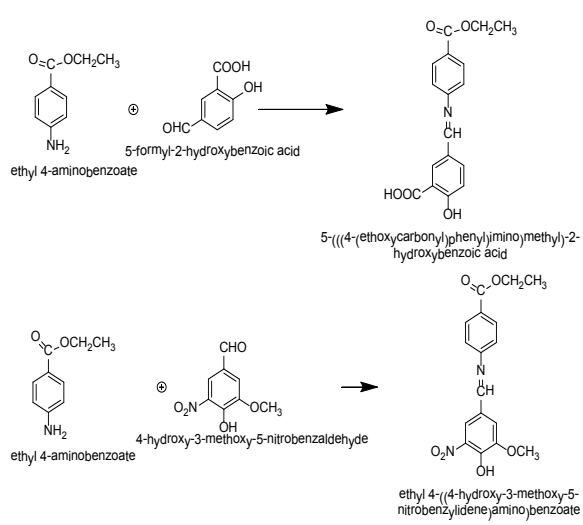
Melting points were determined using Thomas Hoover capillary melting point apparatus. IR spectra were recorded on cary 630 FTIR spectrophotometer. The ^1H NMR spectra were recorded on Bruker AV 300 MHz using DMSO as a solvent. The UV-Visible spectra were recorded on UV-Visible spectrometer. The Fluorescence spectra of 5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid and ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene)amino) benzoate were recorded using Fluorescence spectrophotometer.

Preparation of 5-(((4-(ethoxycarbonyl)phenyl)imino) methyl)-2-hydroxybenzoic acid

3g of Benzocaine was mixed with equivalent amount of 5-Formyl salicylic acid and was grained well in acidic medium at room temperature. The reaction mixture was refluxed for 3 hours. The solid product formed during refluxing was filtered, washed with ethanol and dried over anhydrous $CaCl_2$ in a vaccum desiccator. The melting point was noted. 5-(((4 ethoxycarbonyl) phenyl) imino) methyl)-2-hydroxy benzoic acid was soluble in DMSO and Ethanol.

Preparation of ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene) amino) benzoate

6g of Benzocaine was mixed with equivalent amount of 5-Nitro Vaniline and was grained well in acidic medium at room temperature. The reaction mixture was refluxed for 6 hours. The solid product formed during refluxing was filtered, washed with methanol and dried over anhydrous $CaCl_2$ in a vaccum desiccator. The melting point was noted. Ethyl-4-((4-hydroxy-3-methoxy-5-Nitro Benzylidene)amino)benzoate was soluble in DMSO and Ethanol.



Antimicrobial susceptibility test by Disc diffusion Technique

Principle

Disc impregnated with known concentration of antibiotics are placed on an agar plate that has been inoculated uniformly over the entire plate with a culture of the bacterium to be tested. The plate is incubated for 20 to 28 hours at 38^{0}C. During this period, the antimicrobial agent diffuses through the agar and may prevent the growth of the organism. Effectiveness of susceptibility is proportional to the diameter of the inhibition zone around the disc. Organisms which grow up to the edge of the disc are resistant.

Procedure

The plate was labeled with the name of the culture, sample and standard at the bottom of the plate. Then sterile cotton swab on a wooden applicator stick was dipped into the bacterial suspension. Excess fluid was removed by rotating the swab and was rubbed gently over the plate to obtain uniform distribution of the inoculums. The sterile disc was held on the inoculated plate with the help of micropipette. The sample was leveled in the sterile disc and incubated at 37^{0}C in an incubator. After incubation the diameter of the zone of inhibition of growth was measured.

Antibacterial Activity

The bacterial cultures for *Staphylococcus aureus* (NCIM 2079), *Basillus subtilis* (NCIM 2063), *Klebsiella aerogenes* (NCIM 2098), *Escherichia coli* (NCIM 2065) were obtained from Schiff bases were stored dry at room temperature and dissolved 5µg /ml in DMSO. Ciprofloxacin was used as a standard drug. Zone of Inhibition were measured and compared with the controls.

Antifungal Activity

Pathogenic strains of *Aspergillus niger* (NCIM 105) *and Candida albicans* (NCIM 3102) were obtained from Schiff bases were stored dry at room temperature and dissolved 100 µg /ml in DMSO. Antifungal activities of each compound were evaluated by agar-diffusion method. The effect produced by the sample was compared with the effect produced by the positive control. Reference standard Nystatin 100 µg/disc for fungal.

RESULTS AND DISCUSSION

The physical and analytical data of 5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid and ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene)amino) benzoate are shown in Table 1.

Table 1. The physical and analytical data of the 5-(((4-ethoxycarbonyl) phenyl) imino) methyl)-2-hydroxy benzoic acid and ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene) amino)benzoate

S.No	M.F	M.Wt	Colour	M.Pt	Yield	Elemental analysis(%)			
						C	H	N	O
1	$C_{17}H_{15}NO_5$	313	Bright yellow	138^{o}C	86%	65.17	4.83	4.47	25.5
2	$C_{17}H_{16}N_2O_6$	344	Deep yellow	232 ^{o}C	88%	59.3	4.68	8.14	27.88

FTIR(cm^{-1}) spectra of 5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid.

1602(-HC=N), 2985(-COOH), 1720, (- COO^-)1550(-CO), 1289(-OH).

^{1}HNMR spectra of 5-(((4-ethoxycarbonyl)phenyl)imino) methyl)-2-hydroxy benzoic acid: 6.6(OH), 8.4(-HC=N), 6.55-7.88(Ar-H), 9.87(COOH), 4.3(CH_2)10.

UV-Vis(nm) spectra of 5-(((4-ethoxycarbonyl)phenyl)imino) methyl)-2-hydroxy benzoic acid: 255nm(n→π*), 302nm(π →π*)[11].

FTIR(cm^{-1}) spectra of ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene)amino) benzoate: 1610(-HC=N), 1720(-COO^-), (-C=O), 1543(Ar-NO_2), 1274(-OH).

^{1}H-NMR spectra of **ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene)amino) benzoate:** 6.65(OH), 8.10(-HC=N), 6.60 -7.90(Ar-H), 4.0 (-OCH_3), 4.3(CH_2).

UV-Vis(nm) spectra of ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene)amino) benzoate : 210nm(n →π*), 295nm(π →π*)

Antibacterial activity

Antibacterial activity of 5-(((4-ethoxycarbonyl)phenyl)imino) methyl)-2-hydroxy benzoic acid and ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene)amino) benzoate were screened against bacterial species like gram positive bacteria *Staphylococcus aureus, Basillus subtilis* and gram negative bacteria *Klebsiella aerogenes, Escherichia coli* by disc diffusion method[13] and the results obtained were formulated in Table 3 and Fig 2. The test was carried out in DMSO solution at a concentration of 5µg using Muller Hinton agar media. Ciprofloxacin was used as the standard drug. 5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid and ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene) amino) benzoate were moderately active against all bacterial species.

Table 3. Antibacterial Activity of 5-(((4-ethoxycarbonyl) phenyl)imino)methyl)-2-hydroxybenzoic acid and ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene)amino) benzoate

S.No	Name of the Microorganisms	Zone of inhibition in mm		
		143-B-5-NV	143-B-5-FSA	Standard
1	*Staphylococcus aureus* (NCIM 2079)	16	19	35
2	*Basillus subtilis* (NCIM 2063)	15	15	40
3	*Klebsiella aerogenes* (NCIM 2098)	15	21	30
4	*Escherichia coli* (NCIM 2065)	16	14	38

Fig. 2. ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene)amino) benzoate against $^{+}$ve bacteria and $^{-}$ve bacteria

Antifungal activity

Antifungal screening of 5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid and ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidine)amino) benzoate were carried out against *Aspergillus niger*[12] and *Candida albicans* by disc diffusion method and the results obtained were formulated in Table 2 and Figure 1. The test was carried out in DMSO solution at a concentration of 100 units. Results were compared with standard drug Nystatin at the same concentration. 5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid and ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidine)amino) benzoate were highly active against antifungal activities.

Table 2. Antifungal Activity of 5-(((4-ethoxycarbonyl) phenyl)imino)methyl)-2-hydroxy benzoic acid and ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene)amino) benzoate

S.No	Name of the Microorganisms	Zone of inhibition in mm		
		143-B-5-NV	143-B-5-FSA	Standard
1.	*Aspergillus niger* (NCIM 105)	20	16	35
2.	*Candida albicans* (NCIM 3102)	22	18	32

Fig. 1. 5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid against $^{+}$ve fungal and $^{-}$ve fungal

CONCLUSION

5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid and ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene)amino) benzoate were screened against bacterial and fungal species. 5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid and ethyl-4-((4-hydroxy-3-methoxy-5-nitrobenzylidene)amino) benzoate is moderately active against all bacterial and fungal species.

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