Research Paper Chemistry

Antimicrobial Studies on Derivatives of Benzocaine

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ABSTRACT

To synthesis novel Schiff bases from Benzocaine with 5-Formyl salicylic acid / 5-Nitrovaniline. Schiff bases were characterized by physical characteristice and analytical data,ir spectra,1H NMR,emission spectra UV-Visspectra and" in place of and analytical data,FTIR, 1H NMR,UV-Vis,Emission spectra and werespectra and screened for antibacterial activity against gram positive bacteria Staphylococcus aureus, Basillussubtilis and gram negative bacteria E.Coli, Klebsiellaaerogenes and antifungal activity against Aspergillusniger 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-Nitro vaniline, Schiff bases, zone of inhibition, Antibacterial activity, Antifungal activity, Ciprofloxacin, Nystatin.

INTRODUCTION

Schiff base 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 possess 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[3-4] 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]. (E)-5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid and Ethyl-4-((4-hydroxy-3-methoxy-5-Nitro Benzylidine)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 derived from Benzocaine with 5-Formyl salicylic acid and 5-Nitro vaniline.

MATERIALS AND METHODS

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

Instruments

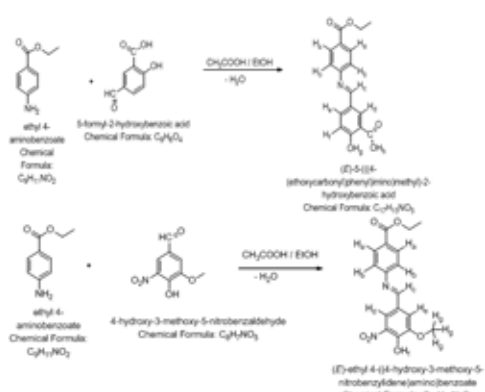
Melting points were determined using Thomas Hoover capillary melting point apparatus. IR spectra were recorded on cary 630 FTIR spectrophotometer The ^{1}H NMR spectra were recorded on Bruker AV 300 MHz using DMSO as a solvent. The UV-Visible spectra were recorded in UV-Visible spectrometer. The Fluorescence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

Procedure of (E)-5-(((4-ethoxycarbonyl)phenyl)imino) methyl)-2-hydroxy benzoic 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 desicator. The melting point was noted. The Schiff base was insoluble in H_2O and soluble in DMSO, Ethanol.

preparation of Ethyl-4-((4-hydroxy-3-methoxy-5-Nitro Benzylidine)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 desiccator. The melting point was noted. 5-(((4-(ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid 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°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 labelled 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 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.

Antifungal Activity

Pathogenic strains of Aspergillusniger *and* Candida albicans were obtained from National Chemical Laboratory (NCL) Pune. 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 fungi.

Antibacterial Activity

The bacterial cultures for Staphylococcus aureus (NCIM2079), Basillussubtilis (NCIM 2063), Klebsiellaaerogenes (NCIM2098), E.coli (NCIM2065) were obtained from National Chemical Laboratory (NCL) Pune. 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.

RESULTS AND DISCUSSION

Physical character: The physical and analytical data of the Schiff bases are shown in Table-1.

CompoundI:(E)-5-(((4-ethoxycarbonyl)phenyl)imino)

methyl)-2-hydroxy benzoic acid.

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FT-IR (γmax cm-1)O : 1602(-HC=N-), 2985(-COOH), 1720, (C O)1550(

C), 1289(-OH)9 .

1H-NMR δ (ppm) : 6.6(OH), 8.4(-HC=N-), 6.55-7.88(Ar-H), 9.87

(COOH), 4.3(CH2)10 .

UV-Vis(nm-1) : 255nm(n –π*), 302nm(π –π*)11 . in place of 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).

1HNMR 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(CH2)10.

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

Compound II :Ethyl-4-((4-hydroxy-3-methoxy-5-nitro Benzylidine)amino) benzoate. FT-IR (γ_{max} cm^{-1}) **:** 1610(-HC=N-), 1720(C-O), (-C=o), 1543(Ar-NO2), 1274(-OH).

^{1}H-NMR δ (ppm) : 6.65(OH), 8.10(-HC=N-), 6.60 -7.90(Ar-H), 4.0 (-O-CH_3), 4.3(CH2).

UV-Vis(nm^{-1}) : 210nm(n →π*), 295nm(π →π*)

Table1: 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-Nitro Benzylidine)amino)benzoate

S.No	M.F	M.Wt	Colour	M. Pt	Yield (in %)	Elemental Analysis (in %)			
						C	H	N	O
1	$C_{17}H_{15}NO_5$	313.3	Bright Yellow	138^{0}C	86	65.17	4.83	4.47	25.5
2	$C_{17}H_{16}N_2O_6$	344.32	Deep Yellow	232^{0}C	88	59.3	4.68	8.14	27.88

Table :2Antifugal Activity of 5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid and Ethyl-4-((4-hydroxy-3-methoxy-5-Nitro Benzylidine)amino)benzoate

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

Table :3 Antibacterial Activity of Schiff bases

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	*Basillussubtilis* (NCIM 2063)	15	15	40
3	*Klebsiellaaerogenes* (NCIM 2098)	15	21	30
4	*E.coli* (NCIM 2065)	16	14	38

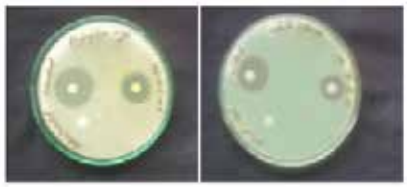

Fig.1:5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acidl against +ve fungi and –ve fungi

Fig.2:Ethyl-4-((4-hydroxy-3-methoxy-5-Nitro Benzylidine)amino)benzoate " against +ve bacteria and –ve bacteria

Antifungal Activity

Antifungal screening 5-(((4-ethoxycarbonyl)phenyl)imino) methyl)-2-hydroxy benzoic acid and Ethyl-4-((4-hydroxy-3-methoxy-5-Nitro Benzylidine)amino)benzoate bases were carried out against Aspergillusniger[12] and Candida albicans by disc diffusion method and the results obtained are 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-Nitro Benzylidine)amino)benzoate were highly active against antifungal activities

"5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid and Ethyl-4-((4-hydroxy-3-methoxy-5-Nitro Benzylidine) amino)benzoate were screened against bacterial species like gram positive bacteria Staphylococcus aureus, Basillussubtilisand gram negative bacteria Klebsiellaaerogenes, E.coli by disc diffusion method[13] and the results obtained are 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. C5-(((4-ethoxycarbonyl)phenyl)imino) methyl)-2-hydroxy benzoic acid and Ethyl-4-((4-hydroxy-3-methoxy-5-Nitro Benzylidine)amino)benzoate were moderately active against all bacterial species.

CONCLUSION

5-(((4-ethoxycarbonyl)phenyl)imino)methyl)-2-hydroxy benzoic acid and Ethyl-4-((4-hydroxy-3-methoxy-5-Nitro Benzylidine)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-Nitro Benzylidine)amino)benzoate were moderately active against all bacterial and fungal species.

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