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Synthesis and pharmacological evaluation of some benzylidene-4-nitroanilines

Ramamurthy SURESH,[1] Saravanan Palanivel SAKTHINATHAN,[1] Dakshnamoorthy KAMALAKKANNAN,[1] Inbasekaran MUTHUVEL,[2,3] and Ganesamoorthy THIRUNARAYANAN[*2]

[1]*PG and Research Department of Chemistry, Government Arts College, C-Mutlur-608 102, Chidambaram, India*
[2]*Department of Chemistry, Annamalai University, Annamalainagar-608002, India*
[3]*Department of Chemistry, MR Government Arts College, Mannargudi-614001, India*

Abstract. A number of nine benzylidene-4-nitroanilines were synthesized by condensation method. The formation of the substituted (*E*)-N-benzylidene-4-nitrobenzenamines has been confirmed from their physical and Ultra-Violet, Infra-Red, NMR spectral data. The evaluation of antimicrobial screening of substituted (*E*)-N-benzylidene-4-nitrobenzenamines was conducted by using standard Bauer-Kirby method. Three gram-positive microbes namely *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus*, and two gram-negative microbes, *Escherichia coli* and *Pseudomonas aeruginosa*, were used for the antibacterial evaluation. The antifungal activities against *Aspergillus niger* and *Penicilium scup* fungal species were also performed. A good antibacterial effect has been possessed by some of the substituted (*E*)-N-benzylidene-4-nitrobenzenamines on the microorganisms utilized in the present study

Keywords: benzylidene-4-nitroanilines; IR and NMR spectra; antimicrobial activities.

1. Introduction

The compounds containing the functional group C=N, known as imines or azomethines, are general called as "Schiff bases" in honor of Schiff [1]. Imines (Schiff's bases) are some of the most extensively used organic compounds exhibiting a widespread range of biological activities, including antibacterial [2], antifungal [3], antiviral [4], anticancer [5], antioxidant [6], anti-inflammatory [7], anti-tubercular [8], antimycobacterial [9], anticonvulsant [10], antiproliferative [4], anti-HIV [11], hypolipienic and antihypertensive [12], analgesic [5], cytotoxic [13], lipoxygenase [14], herbicidal [15], insecticidal [16] and antimalarial [17] activities. Within the above attention, there is no report available about the antimicrobial activities of titled compounds in the literature. Therefore, the authors have been taken efforts to synthesis substituted benzylidene-4-nitroamines by condensation of substituted benzaldehyde and 4-nitroaniline. All the synthesized 4-nitroaniline base aryl imine compounds have been confirmed by using UV, IR and NMR spectral data. By using the standard Bauer–Kirby disc diffusion technique, the antibacterial and antifungal activities of all the substituted benzylidene-4-nitroamines have been studied.

2. Experimental

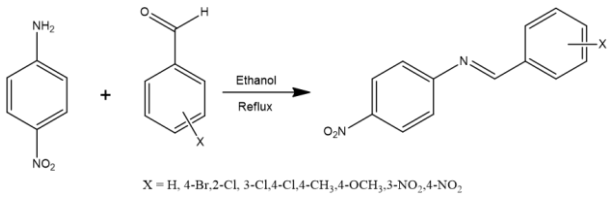
2.1. Materials and methods

From Sigma Aldrich Chemical Company Bengaluru-100, various substituted benzaldehydes were purchased along with substituted 4-nitroaniline. From Himedia, Mumbai, the chemicals *viz.* nutrient broth, Mueller Hinton agar, potato dextrose agar, Tween-80 solution and other relevant materials required for the study in the present work were procured. The microbes used were received and maintained at the Biochemical laboratory PG & Research department of Chemistry Government Arts College, Chidambaram. The melting points of the (*E*)-N-benzylidene-4-nitroaniline compounds were observed in open glass capillaries on SUNTEX melting point apparatus and are uncorrected. The UV spectra of substituted (*E*)-N-benzylidene-4-nitroaniline compounds have been analyzed by using ELICO-BL222 spectrophotometer. The infrared spectra (KBr, 4000-400 cm^{-1}) were recorded in SHIMADZU-2010 FT spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded in Bruker AV400 (400 MHz) spectrometer in $CDCl_3$ solvent, using TMS as internal standard.

2.2. Preparation of (E)-N-benzylidene-4-nitrobenzenamines

By following the already established condensation method of synthesis, (*E*)-N-benzylidene-4-fluorobenzenamine compounds were synthesized and the procedure followed is given below. As shown in Scheme 1, equimolar quantities of 4-nitroaniline (0.01 mol) and benzaldehyde (0.01 mol) were refluxed for 3 h with 20 mL of ethanol [18]. The completion of the reaction was monitored by TLC continuously and the mixture was cooled at room temperature. Then the obtained precipitate was filtered and washed with cold water. A yellow solid was obtained as the final product. This final product was crystallized from ethanol.

* Corresponding author. *E-mail address*: drgtnarayanan@gmail.com (Ganesamoorthy Thirunarayanan)



Scheme 1. Synthesis of substituted (*E*)-N-benzylidene-4-nitroaniline compounds

2.3. Measurement of antibacterial activity

The assay of antibacterial activities was performed by using Kirby-Bauer disc diffusion technique [19]. All the Petri plates were filled by Mueller Hinton agar and they are allowed to solidify. Then the bacteria were spread evenly over the solidified Mueller Hinton agar. The filter paper discs with 5 mm diameter were soaked with the solution of each compound and placed on the medium by using sterile forceps. The plates were incubated for one day at 37 ºC. The diameter values of zone of inhibition [20, 21] were measured after 24 hours by visually examined the plates. The above said methodology was repeated triply.

2.4. Measurement of antifungal activity

The Kirby-Bauer disc diffusion technique [19] was utilized for measuring the antifungal activity of all imines. Exactly 15 mg of the substituted (*E*)-N-benzylidene-4-nitroanilines were dissolved in 1 ml of DMSO. The antifungal sensitivity assay was performed as the antibacterial activity measurement procedure mentioned above. The standard drug is Miconazole.

3. Results and discussion

We have reported our earlier work related to synthesis and evaluation of antimicrobial activities of some imine derivatives from our research laboratory [20]. In continuation of this work, in the present investigation, the authors have synthesized some substituted (*E*)-N-benzylidene-4-nitroaniline compounds by conventional method for evaluation of antimicrobial activities. In the synthesis of these compounds, the electron donating substituents (methoxy and methyl) gave more yield than electron withdrawing substituents (halogens and nitro) in the aldehyde moiety. The obtained yields are more than 76%. The parent compound obtained yield was 82%. The *ortho*- substituted compounds gave less than 3% yield of parent compound. The *meta* and *para* substituted halogens and nitro compounds obtained 76% yields. The methoxy and methyl substituted compounds obtained 84%. These imines are characterized by their physical constants and spectroscopic data as presented in Table 1.

Table 1. Physical constants, infrared, NMR and mass spectral data of substituted benzylidene-4-nitroanilines.

S. No	X	MF	MW	M. p. (ºC)	UV (λ_{max}, nm)	IR (ν, cm^{-1})	NMR (δ, ppm)		Mass (m/z)
							^{1}H	^{13}C	
1	H	$C_{13}H_{10}N_2O_2$	226	104-106 (141-143)[15]	329	1624.04	8.448	163.49	226[M^+]
2	4-Br	$C_{13}H_9BrN_2O_2$	305	178-180 (176-178)[15]	328.5	1614.56	8.405	161.35	305[M^+],303[M^{2+}],
3	2-Cl	$C_{13}H_9ClN_2O_2$	260	108-110	373.5	1618.28	8.945	159.35	260[M^+], 262[M^{2+}],225, 214, 149, 138, 136, 124, 122, 111, 45, 34
4	3-Cl	$C_{13}H_9ClN_2O_2$	260	128-129	372.5	1614.99	8.494	160.99	260[M^+],262[M^{2+}],225,214, 149, 138, 136, 124, 122, 111, 45, 34
5	4-Cl	$C_{13}H_9ClN_2O_2$	260	138-140 (141-142)[15]	326	1616.42	8.426	158.52	260[M+], 262[M^{2+}]
6	4-CH_3	$C_{14}H_{12}N_2O_2$	240	136-137 (128-130)[16]	372	1633.71	8.411	160.56	240[M^+]
7	4-OCH_3	$C_{14}H_{12}N_2O_3$	256	73-74 (69–71) [16]	370	1628.86	8.488	160.05	256[M^+]
8	3-NO_2	$C_{13}H_9N_3O_4$	271	124-126	375	1614.42	8.455	159.87	271[M^+], 149, 225, 136, 135, 122, 45
9	4-NO_2	$C_{13}H_9N_3O_4$	271	109-110 (112-116)[17]	366.5	1599.06	8.436	159.43	271[M+]

3.1. Antibacterial sensitivity assay

The antibacterial effect of substituted benzylidene-4-nitroaniline compounds was displayed in Figure 1 (Plates 1-10). Three gram-positive pathogenic strains, i.e. *Bacillus substilis, Micrococcus luteus, Staphylococcus aureus*, and two gram-negative strains, i.e. *Escherichia coli* and *Pseudomonas aeruginosa*, have been utilized to evaluate the antibacterial activity of substituted benzylidene-4-nitroanilines. The Kirby-Bauer method [19] has been carried out at a concentration of 250 µg/mL with ampicillin taken as the standard drug. The measured values for the zone of inhibition were presented in Table 2.

Table 2. Zone of inhibition (mm) values of antibacterial activity of substituted (*E*)-N-benzylidene-4-nitroanilines.

S. No	X	Zone of inhibition (mm)				
		Gram positive bacteria			Gram negative bacteria	
		B. subtilis	***M. luteus***	***S. aureus***	***E. coli***	***P. aerugi-nosa***
1	H	7	0	9	8	6
2	4-Br	8	6	0	9	0
3	2-Cl	8	0	9	0	0
4	3-Cl	14	0	9	8	0
5	4-Cl	6	6	7	0	7
6	4-CH_3	8	10	7	0	8
7	4-OCH_3	8	0	6	6	0
8	3-NO_2	7	0	8	6	8
9	4-NO_2	8	10	7	7	-
Standard: Ampicillin		11	10	14	8	15
Control: DMSO		-	-	-	-	-

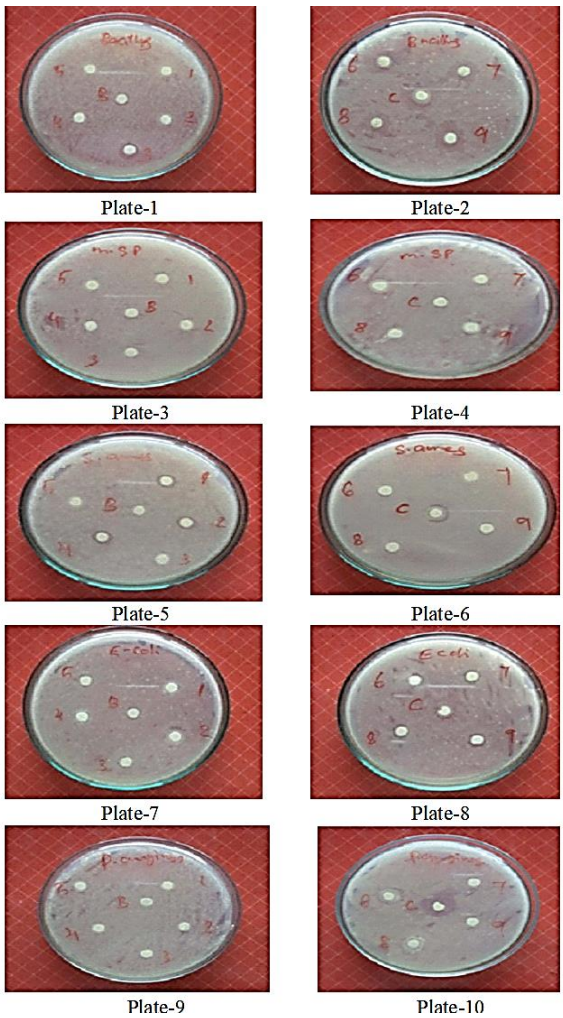


Figure 1. Antibacterial activity of substituted (*E*)-N-benzylidene-4-nitroanilines.

From Table 2, the analysis of the zone of inhibition (mm) values reveals that (*E*)-N-benzylidene-4-nitroaniline compound with 3-Cl substituent has shown excellent antibacterial activity when compared to standard ampicillin against *Bacillus subtilis*. The parent, 4-Br, 2-Cl, 4-OCH_3, 4-CH_3, 3- and 4-NO_2 substituted imines showed good antibacterial activities against *Bacillus subtilis*. The +I, -I and hyper conjugative effects electron donating as well electron withdrawing of the substituents show good antibacterial activity. The imines possessing 4-Cl substituents showed satisfactory antibacterial activity against *Bacillus subtilis*. The 4-CH_3 and 4-NO_2 substituted imines show better antibacterial activity against *Micrococcus luteus* strain. Similarly, the (*E*)-N-benzylidene-4-nitroaniline compound with 4-Br substituent has shown good antibacterial activity against *Micrococcus luteus*. Here the inductive and conjugative effects of the substituents enhance the antibacterial activity against *Micrococcus luteus*. The remaining imines had no antibacterial activity against *Micrococcus luteus*. The parent, 2-Cl, 3-Cl, 4-Cl, 4-CH_3, 3-NO_2 and 4-NO_2 substituted imines show good antibacterial activity against *S. aureus* strains. The 4-OCH_3 substituted imine have least antibacterial activity against *S. aureus*. The 4-Br substituted imines had no antibacterial activity against *S. aureus* strains. Here the electron-donating methoxy substituted imine had a surprising for the lowering the antibacterial activity. The 4-Br substituted imine showed excellent antibacterial activity against *Escherichia coli* strain. The parent and 3-Cl substituted (*E*)-N-benzylidene-4-nitroaniline compounds show good antibacterial activity against *Escherichia coli* strain. The 4-OCH_3 and nitro substituted imines showed good antibacterial activity against *Escherichia coli* strain. The 2-Cl, 4-Cl and 4-CH_3 substituted imines had no antibacterial activity against *E. coli* strain. Here the +I and –I effects of substituents enhances the antibacterial activity and the conjugation lowered the activity. The 4-CH_3 and 3-NO_2 substituted imines showed good antibacterial activity against *P. aeruginosa* strain. The parent and 4-Cl substituents showed satisfactory antibacterial activity against *P. aeruginosa* strain. The 4-Br, 2-Cl, 3-Cl, 4-OCH_3, and 3-NO_2 substituents have no antibacterial activity against *P. aeruginosa*. Here the +I and resonance effects of the substitutes lowered the antibacterial activity against *P. aeruginosa* strain.

3.2. Antifungal sensitivity assay

The antifungal activity of (*E*)-N-benzylidene-4-nitroanilines is shown in Fig. 2 for plates 1-4. All the measured values for the zone of inhibition are given in Table 3.

Table 3. Zone of inhibition (mm) values of antifungal activity of substituted (*E*)-N-benzylidene-4-nitroanilines.

S. No.	X	Zone of inhibition (mm)	
		A. niger	***P. scup***
1	H	6	6
2	4-Br	8	0
3	2-Cl	9	6
4	3-Cl	0	0
5	4-Cl	0	6
6	4-CH_3	6	7
7	4-OCH_3	0	6
8	3-NO_2	7	7
9	4-NO_2	0	6
Standard: Miconazole		11	13
Control: DMSO		-	-

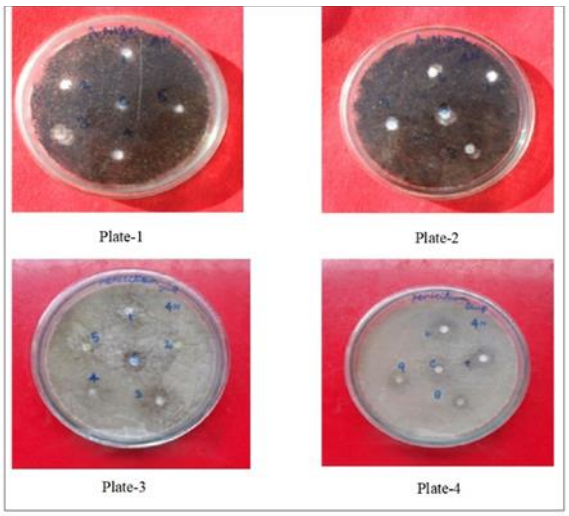


Figure 2. Antifungal activity of substituted (*E*)-N-benzylidene-4-nitroanilines.

From Table 3, the 4-Br and 2-Cl substituted imines show good antifungal activity against *A. niger* fungal strain. The parent, 4-CH_3 and 3-NO_2 substituents showed satisfactory antifungal activity against *A. niger strain.* The 3-Cl, 4-Cl, 4-OCH_3 and 4-NO_2 substituted imines showed no antifungal activity against *A. niger* fungal strains. Here the +I, -I and hyper conjugative effects of substituents showed the antifungal activity and the resonance effect of substituents reduced the activity. The 4-CH_3 and 3-NO_2 substituted imines show good antifungal activity against *P. scup fungal* strain. The parent, 2-Cl, 3-Cl, 4-OCH_3 and 4-NO_2 substituted imines show satisfactory antifungal activity against *P. scup* fungal strain. Here the hyper conjugation and +I effect of the substituents enhanced the antifungal activity. The 4-Br and 3-Cl substituted imines have no antifungal activity against *P. scup* fungal strain.

4. Conclusions

In this present investigation a series of substituted (*E*)-N-benzylidene-4-nitroanilines were synthesized to explore their antimicrobial activity. The (*E*)-N-benzylidene-4-nitroanilines with 3-Cl substituent has shown excellent and parent, 4-Br, 2-Cl, 4-OCH_3, 4-CH_3, 3- and 4-NO_2 substituents showed good antibacterial activity when compared to standard ampicillin against *Bacillus subtilis*. The 4-CH_3 and 4-NO_2 substituted imines showed better and 4-Br substituent showed good antibacterial activity against *Micrococcus luteus* strain. The parent, 2-Cl, 3-Cl, 4-Cl, 4-CH_3, 3-NO_2 and 4-NO_2 substituted imines show good antibacterial activity against *S. aureus strains.* The parent, 4-Br and 3-Cl substituted (*E*)-N-benzylidene-4-nitroaniline compounds show excellent, and 4-OCH_3 and nitro substituted imines show good antibacterial activity against *Escherichia coli* strain. The 4-CH_3 and 3-NO_2 substituted imines showed good antibacterial activity against *P. aeruginosa* strain. The 4-Br and 2-Cl substituted imines show good antifungal activity against *A. niger* fungal strain. The 4-CH_3 and 3-NO_2 substituted imines showed good antifungal activity against *P. scup fungal* strain.

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Conflict of interest

Authors have no conflict of interest to declare.

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