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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 4-NITRO SCHIFF BASES

B.N.B.VAIDEHI*, K.SURYA TEJASWI, N.V.PRABHAKAR AND L.DEVI

Sri Sai Aditya Institute of Pharmaceutical Sciences and Research, Adb road, Surampalem, East Godavari District, Andhra Pradesh, India.

ABSTRACT

The purpose of the research was to synthesize better antibacterial and antioxidant compounds using various substituted aromatic aldehydes for synthesis of 4-Nitro Schiff bases in the presence of glacial acetic acid as catalyst. The synthesized compounds were screened for antioxidant activity by DPPH method and antibacterial activity by Cup Plate method. The antibacterial activity was evaluated against four bacterial strains. IR spectroscopy and elemental analysis were used for characterization of compounds. Some of the synthesized compounds exhibited promising antioxidant and antibacterial activities.

KEYWORDS: **antibacterial activity; antioxidant activity; DPPH; 4-Nitro aniline.**

B.N.B.VAIDEHI
Sri Sai Aditya Institute of Pharmaceutical Sciences and Research, Adb road, Surampalem, East Godavari District, Andhra Pradesh, India.

INTRODUCTION

Compounds with the structure of –C=N – (azomethine group) are known as Schiff bases. Schiff bases form an important class of the most widely used organic compounds and has a wide variety of applications in many fields, e.g., biological, inorganic, analytical and synthetic chemistry[1].The study of Schiff base has been fast developing because they possess excellent characteristics such as structural similarities with natural biological substances, relatively simple preparation procedures and synthetic flexibility that enables the design of suitable structural properties [2,3]. Schiff bases also serve as back bone for synthesis of various heterocyclic compounds. The biological activities reported in the literature on Schiff bases are antibacterial, antifungal, antioxidant, anticonvulsant, CNS depressant, anti-HIV, anti-inflammatory, antitumor, Angiotensin-II receptor antagonist, antioxidant, insecticidal, antimalarial, antiproliferative, antipyretic[4,5,6,7,8,9]. Schiff's base have the potentials to be used in different areas such as color photography, catalysis, metallic deactivators, separation processes and environmental chemistry [10] and they are becomingly important in pharmaceutical, dye, plastic industries[11]. In view of those above biological impotance of Schiff bases we tried to synthesize a series of 4-Nitro Schiff bases by condensation of 4-nitro aniline with substituted aromatic aldehydes and evaluate their antioxidant, antibacterial activities.

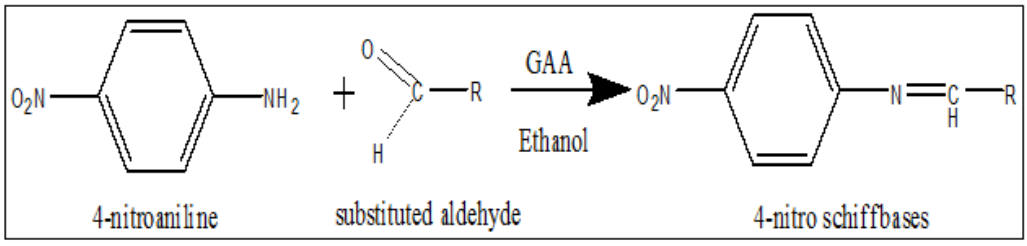
EXPERIMENTAL MATERIALS AND METHODS

All the chemicals and solvents used for this work were obtained from s d fine-chem limited (SDFCL), MUMBAI. Melting point of synthesized compounds was determined in open capillary tube using kshitij melting point apparatus, expressed in ^{0}c and was uncorrected. Silica gel chromatographic plates were used for TLC and solvent systems were chloroform: benzene (1:2) for all compounds. The purity of the compounds was checked by TLC and spots were visualised by iodine vapours[12,13]. IR spectra were recorded in KBr on bruker FT-IR spectrometer. The synthesis of compounds was carried according to Scheme-1.

GENERAL PROCEDURE FOR THE SYNTHESIS OF SCHIFF BASES

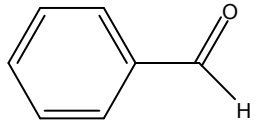
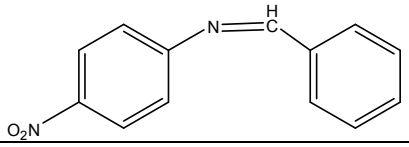
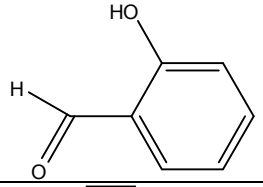
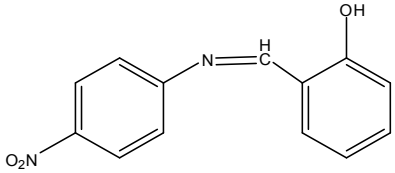
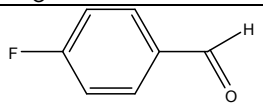
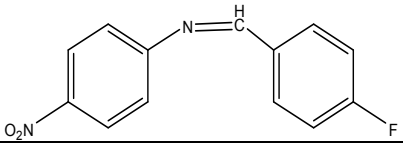
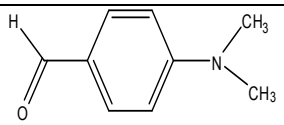
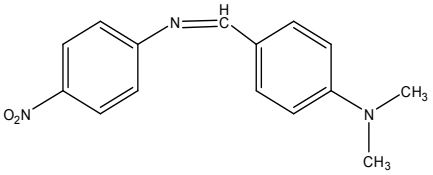
An equimolar quantities of 4-nitroaniline in ethanolic solution was condensed with substituted aldehydes in the presence of few drops of glacial acetic acid. Then the mixture was refluxed for 6-10hr on water bath. The completion of reaction was monitored by TLC (chloroform: benzene 1:2). On completion of reaction, the reaction mixture was cooled and poured in to crushed ice. The separated solid was filtered, washed and recrystallized from methanol. The synthesis of compounds was carried according to Scheme-1

The Physico-chemical data of all the synthesized compounds was represented in table-1.



Scheme-1

Table 1
Physico- chemical data

Entry	Aldehyde	Product	Melting point	Yield (%)	RF Value
1			163-165 ^{o} C	65	0.62
2			180-185^{o}C	76	0.8
3			180-185^{o}C	85	0.54
4			200-202^{o}C	82	0.54

SPECTRAL DATA OF SYNTHESIZED COMPOUNDS

1. N-benzylidene-4- nitroaniline(NBS)

IR (KBr) *v* cm−1: 3219.59 (=C-H stretching), 1628.84 (-C=N), 1589.63 (C=C), 1504.21 (N=O) sym stretching, 1302.30 (N=O) asym stretching, Anal Calcd. For $C_{13}H_{10}N_2O_2$ (181.23): C, 69.02; H, 4.46; N, 12.38; O, 14.14%. Found: C, 68.55; H, 4.36; N, 12.12 O, 14.08%.

2. 2-((4-nitrophenylimino) methyl) phenol (NSS)

IR (KBr) *v* cm−1:3052.43 aromatic (=C-H stretching), 1569.40(-C=C) 1618.89 (-C=N stretching), 1498.37 (N=O) sym stretching, 1228.47 (N=O) asym stretching, 3323.59(O-H stretching),1367.21(C-O-H bending) Anal Calcd. For $C_{13}H_{10}N_2O_3$(242.23): C, 64.66; H, 4.16; N, 11.56; O, 19.82%. Found: C, 64.23; H, 4.09; N, 11.43 O, 19.65%.

3. N-(4-fluorobenzylidene)- 4- nitroaniline (NFS)

IR (KBr) *v* cm−1: 1601.49 (-C=C stretching), 632.08 (-C=N stretching), 3220.73 (=C-H stretching), 1505.91 (N=O) sym stretching, 1444.96 (N=O) asym stretching, 1236.79(Aryl C-F) Anal Calcd. For $C_{13}H_9FN_2O_2$ (244.22) : C, 63.93; H, 3.71;F, 7.78; N, 11.47; O, 13.10%. Found: C, 63.78; H, 3.69; F, 7.65; N, 11.45 O, 13.08%.

4.N,N-dimethyl-4-((4-nitrophenylimino)methyl)aniline (NDS)

IR (KBr) *v* cm−1: 1579.88 (-C=C stretching), 1604.34 (-C=N stretching), 3097.29 (Aromatic =C-H stretching), 2850.34(aliphatic-C-H stretching) 1550.87 (N=O) sym stretching, 1335.70 (N=O) asym stretching Anal Calcd. For C15H15 N_3O_2 (269.30) : C, 66.90; H, 5.61; N, 15.60; O, 11.88%.Found: C, 65.78; H, 5.23; N, 15.42 O, 11%.

ANTIOXIDANT ACTIVITY

MATERIALS AND METHODS

CHEMICALS & EQUIPMENT

All the chemicals used are of analytical grade. DPPH (2, 2-diphenyl-1-picryl hydrazyl) was procured from RESEARCH-LAB FINE CHEM INDUSTRIES, Mumbai. Gallic acid was gifted sample. The different glassware used for the

experimental purpose is of standard quality. UV-Visible double beam (ELICO SL 210), Shimadzu electronic balances were used for analysis.

PREPARATION OF STANDARD DRUG

The standard Gallic acid 50 µg/ml stock solution in ethanol was prepared and further dilution 1 µg/ml, 2.5 µg /ml and 5 µg /ml in ethanol were prepared for the study.

PREPARATION OF TEST SAMPLE

The test sample 1 mg/ml was prepared in ethanol and further dilutions 50µg /ml, 100µg /ml, 300 µg /ml were prepared for the study.

PREPARATION OF 0.1mM DPPH SOLUTION

0.002g of DPPH was weighed on shimadzu electronic balance and dissolved in ethanol and the volume is made to 50ml using ethanol to give 0.1mM DPPH solution.

DPPH (2, 2 DIPHENYL-1-PICRYL-HYDRAZYL) FREE RADICAL SCAVENGING ACTIVITY[14,15,16]:

The stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The absorption maximum of a stable DPPH radical in ethanol was at 517nm.

PRINCIPLE

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as:

Antioxidants react with DPPH, which is a free radical and is reduced to the DPPH-H and the colour turns from purple to yellow as the molar absorptivity (optical density) of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen radical from a free radical scavenging antioxidant to form the reduced DPPH-H. The degree of discoloration indicates the scavenging potential of the antioxidant compounds in terms of hydrogen donating ability.

PROCEDURE

Free radical scavenging activity of 4-Nitro Schiff bases was determined against 0.1mM DPPH in ethanol. 1.0 ml of 0.1mM DPPH solution was added to 3.0ml of test solution in ethanol at the concentrations of 50µg /ml, 100µg /ml, 300 µg /ml. The mixtures were kept in dark for 30min, and then the absorbance was measured at 517nm using UV- visible double beam spectrophotometer. A blank was prepared by taking 0.1mMDPPH and ethanol. Gallic acid was used as standard at the concentration of 1µg /ml, 2.5µg /ml, 5µg/ml. lowering the absorbance of reaction mixture indicates higher free radical scavenging activity. The percentage inhibition can be calculated by the following equation.

$$\% \text{ Inhibition} = \frac{\text{Absorbance(blank)}_{517nm} - \text{Absorbance(sample)}_{517nm}}{\text{Absorbance(blank)}_{517nm}} \times 100$$

ANTIBACTERIAL ACTIVITY

MATERIALS & METHODS

MICRO ORGANISMS

The various organisms like *Staphylococcus aureus* ATCCBAA 1026, *Bacillus subtilis* ATCC 11774, (all Gram positive) and *Pseudomonas aeruginosa* ATCC 10662, *E.coli ATCC 10536* (all Gram negative) were procured from Microbes Speciality Lab, Danavaipeta, Rajahmundry, East Godavari District, Andhra

Pradesh, India. The inhibition zones of synthesized compounds were determined by cup plate method.

ANTIMICROBIAL AGENT

The reference standard Gentamycin was procured from Pradeep Organics and chemicals Pvt. Ltd, Hyderabad.

PREPARATION OF TEST AND STANDARD SOLUTIONS

The test compounds were prepared by dissolving 5mg of compound in 5ml of DMSO resulting in formation of solution at a concentration of 1mg/ml. Then this solution was made up to 10 ml with water to get a concentration of 500 µg/ml .The other concentration of test sample is 800 µg/ml which was prepared in the same manner. The stock solution of reference standard Gentamycin was prepared at a concentration of 50µg/ml in sterile water.

EVALUATION OF ANTIBACTERIAL ACTIVITY BY CUP-PLATE METHOD[17,18,19]

The compounds were tested for their *in vitro* growth inhibitory activity against different bacteria. The sterilized medium (autoclaved at 121°C for 20min) was inoculated using 18hr slant cultures of the test organisms and transferred into sterile petri dishes and allowed to solidify the media. Cups of 8mm diameters were made on solidified media. Solutions of the synthesized compounds at a concentration of 500µg/ml and 800µg/ml were prepared in DMSO. 50µl of each solution was placed in cups by means of sterile pipette. In each plate one cup was used for standard and other two for test solutions. The plates thus prepared were left for 90min in a refrigerator for diffusion. The plates were incubated at 37°C for 24hrs and examined for inhibition zones. The experiment was performed in duplicate and the average diameter of the zones of inhibition was recorded. Gentamycin (50µg/ml) was used as standard.

RESULTS & DISCUSSION

Various 4-Nitro Schiff bases were prepared by condensing 4-nitroaniline with substituted aldehydes[19,20]. The purity of the synthesized compounds were checked by TLC and melting point. The synthesized compounds were analysed by Elemental analysis, and IR Spectroscopy. In vitro antioxidant activities of ethanolic solution of 4-Nitro Schiff bases were evaluated and the results obtained were mentioned. It is observed that the ethanolic solution of 4-Nitro Schiff bases have demonstrated dose dependent increase in the DPPH radical scavenging activity. Where as 5 µg/ml of Gallic acid (STD) has 46.8% inhibition. Of all the synthesized compounds NFS, NSS, NDS was found to exhibit good antioxidant activity.NFS was most efficacious antioxidant among all the compounds. Substitution in the aromatic ring system with halogens like Cl/F, hydroxyl, N-dimethyl amino groups enhanced the antioxidant potency. The IC_{50} values were found to be 35.8 µg /ml and 6.44µg /ml for NFS and Gallic acid respectively. The results of final compounds for antioxidant activity were recorded in table2,3 & figure1,2.

Table 2

Antioxidant activity of 4-nitro schiff bases evaluated by DPPH method

Compounds	%inhibition			IC_{50}
	50µg /ml	100µg /ml	300µg /ml	
NBS	28.9±0.37	32.7±0.55	54.8±0.63	225.6
NSS	53.7±0.68	63.5±0.52	86.6±0.52	39.29
NFS	56.2±0.53	65.8±0.63	94.7±0.75	35.8
NDS	53.4±0.40	67.3±0.55	94.8±0.48	40.2

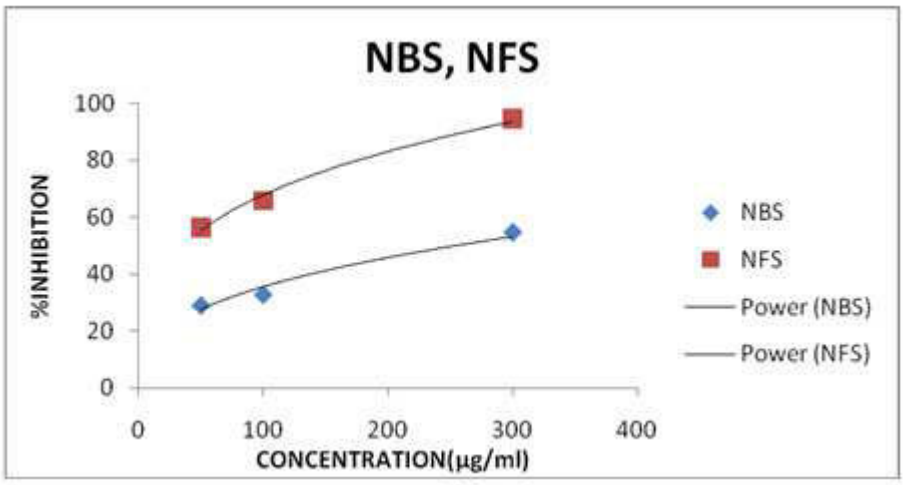
Values are expressed as Mean ± SEM, n=3

TABLE 3
Antioxidant activity of Gallic acid (STD) evaluated by DPPH Method

COMPOUND	%INHIBITION			IC_{50}
	1µg/ml	2.5µg/ml	5µg/ml	
Gallic acid(STD)	38.7±0.47	43.4±0.66	46.8±0.55	6.44

Values are expressed as Mean ± SEM, n=3

Figure 1
Showing the Graphical representation of the % free radical scavenging activity of the 4-Nitro Schiff bases



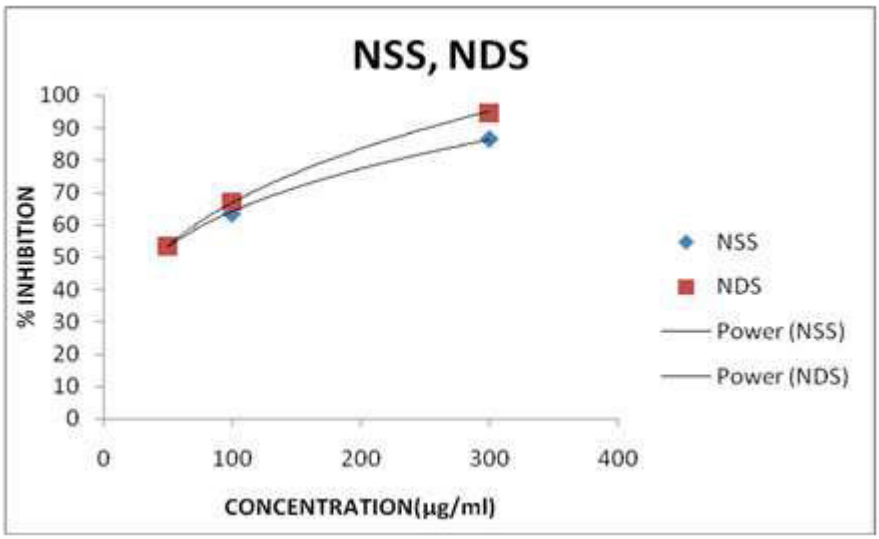
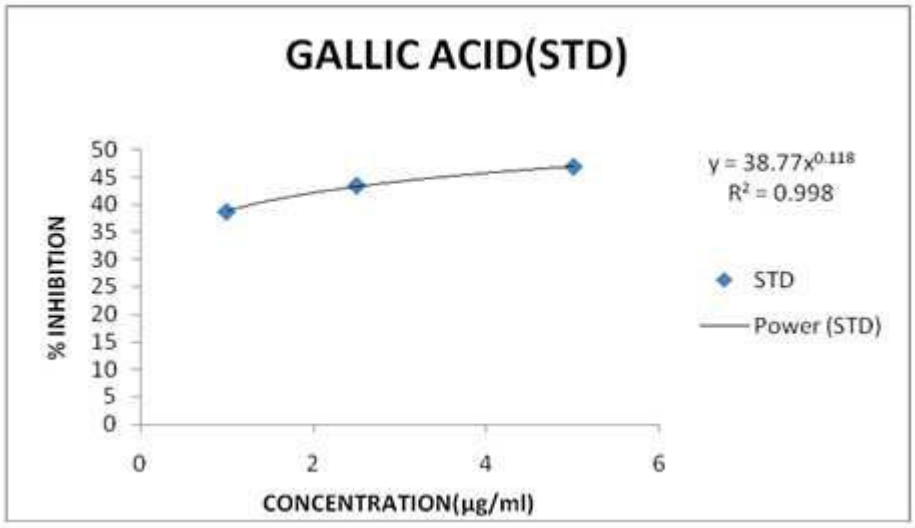


Figure 2
Showing the Graphical representation of the % free radical scavenging activity of the GALLIC ACID (STD)



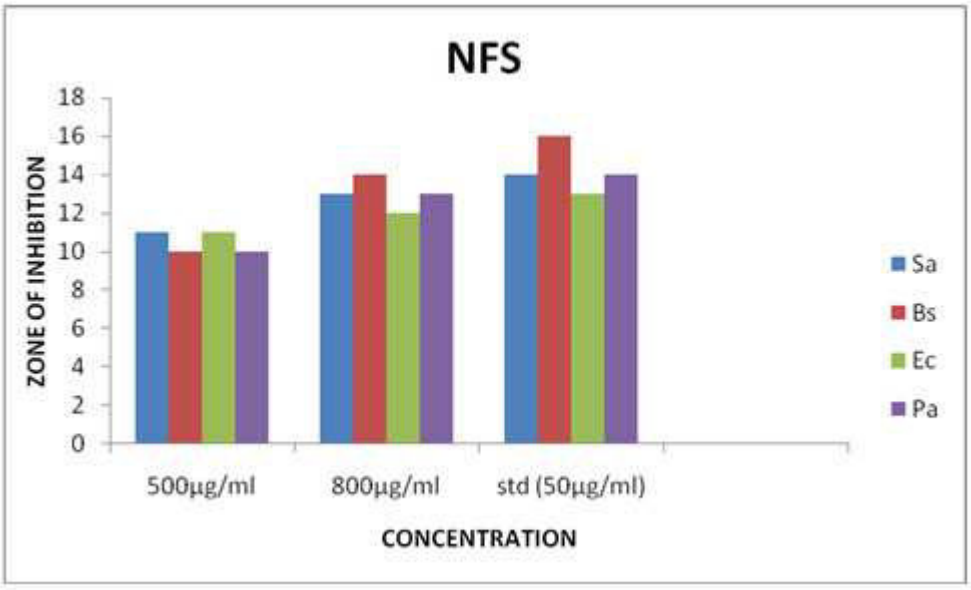
The in vitro antibacterial activity was performed by using cup plate method with different strains of gram positive and gram negative bacteria. The results of final compounds for antibacterial activity were recorded in table 4 & figure 5. The results revealed that synthesized compounds showed varying degree of inhibition against the tested microorganisms. In general, the inhibitory activity against gram positive bacteria was higher than that of gram negative bacteria. Among the synthesized compounds, NFS showed potent antibacterial activity. The activity was due to the presence of an electron withdrawing group like halogen on aromatic ring[21].

TABLE 4
Antibacterial activity of Synthesized Compounds

Entry	Compound	Zone of inhibition(mm)							
		Gram positive bacteria				Gram negative bacteria			
		S.*aureus*		B.*subtilis*		E.*coli*		P.*aeruginosa*	
		500 µg/ml	800 µg/ml	500 µg/ml	800 µg/ml	500 µg/ml	800 µg/ml	500 µg/ml	800 µg/ml
1	NBS	7	9	8	11	9	12	8	10
2	NSS	9	11	7	13	8	11	9	11
3	NFS	11	13	10	1 4	11	12	10	13
4	NDS	10	12	11	12	9	11	9	12
5	Control	-	-	-	-	-	-	-	-
6	STANDARD (Gentamycin 50 µg/ml)	14		16		13		14	

Control – DMSO, -- No activity

Figure 3
Showing the Graphical representation of the Antibacterial activity of NFS



CONCLUSION

In the present study, 4-Nitro Schiff bases from 4-nitro aniline were synthesized and characterized by IR spectral data. Compound NFS showed good anti-bacterial activity against standard drug Gentamycin. Compounds NFS, NSS, NDS were found to exhibit good antioxidant activity when compared to standard drug Gallic acid. NMR & Mass Spectroscopy is required to interpret the synthesized compounds for further analysis.

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