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Synthesis, Characterization of 4-Nitrobenzamide Derivatives and their Antimicrobial Activity

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Abstract

New 4-nitrobenzamide derivatives, 3(a–d) and 3(a_1–d_1) were synthesized and structurally characterized by various spectroscopic techniques such as ^{1}H-NMR, ^{13}C NMR, LCMS and FT-IR spectral studies. All compounds were evaluated for *in vitro* antimicrobial activity. Compounds 3a and 3a_1 were found to be most active and compared to the other synthesized compounds. Compounds 3a and 3a_1 could be a potential antimicrobial agent and these deserve further research.

Keywords
Schiff base, 4-nitrobenzamide, antimicrobial activity.

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INTRODUCTION

A Schiff base is derived from aromatic amines and aromatic aldehydes have a wide range of applications in various fields, example biological, inorganic and analytical chemistry [1-5]. A Schiff base is nitrogen analogue of an aldehyde or ketone wherein the C=O group replaced by a C=N-R cluster. Schiff bases that contain aryl substituents are significantly more stable and more rapidly synthesized, while those which contain alkyl substituents are relatively unstable.

Schiff bases are used in optical and electrochemical sensors, as well as in various chromatographic methods, to enable detection of enhance selectivity [6-8]. Among the organic reagents used, Schiff bases possess excellent characteristics, structural similarities with natural biological substances, relatively simple preparation procedures and the synthetic flexibility that enables design of suitable structural properties [9, 10].

Unfortunately, most Schiff bases are chemically unstable and show a tendency to be involved in various equilibria, like interconversions, hydrolysis,

or formation of ionized species [11, 12]. So, successful application of Schiff bases requires a careful study of their characteristics. These facts open an attractive possibility of application in optical sensors where pH sensitivity is required over limited pH range [13].

The dehydration of the carbinolamine is the rate-determining step of Schiff base formation and this is why the reaction is catalysed by acids. Yet the acid concentration cannot be too high because amines are primary compounds. If the amine is protonated and becomes non-nucleophilic, equilibrium is pulled to the left and carbinolamine formation cannot occur. So, many Schiff base syntheses are best carried out at mildly acidic pH. The dehydration of carbinolamines is also catalysed by base. The compounds containing an azomethine group (-CH=N-) are important in elucidating the mechanism of transamination and racemisation reactions in biological systems [14, 15]. The great flexibility and diverse structural aspects, a wide range of Schiff bases have been synthesized and their complexation behaviors have been studied [16]. They have been synthesized from a variety of compounds, such as amino thiazoles, 2-hydroxy-1-naphthalaniline, amino sugars, aromatic aldehydes, ketones, isatin, triazole ring, thiosemicarbazides, amino acids, pyrazolone, etc [17, 18]. Antimicrobial and anticancer activities of Schiff bases have been reported [19].

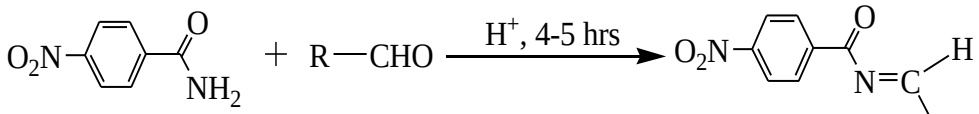
Benzamide is a carbonic acid amide of benzoic acid. Amide is a group of organic chemicals with the general formula RCO-NH_2 in which a carbon atom is attached to oxygen in double bond and also attached to a hydroxyl group, where 'R' groups range from hydrogen to various linear and ring structures or a compound with a metal replacing hydrogen in ammonia such as sodium amide, $NaNH_2$- Amides are divided into subclasses according to the number of substituents on nitrogen. The primary amide is formed by replacement of the carboxylic hydroxyl group by the NH_2, amino group. These observations have encouraged us to synthesize some new Schiff base derivatives containing amine moiety via the -condensation reaction and also under solvent free condition by an efficient and general procedure in the hope to evaluate their potential antimicrobial activity.

MATERIALS AND METHODS

All solvents and reagents were purchased from S. D. Fine Chemicals, India. Melting range was determined by GLNR SELEC apparatus. The UV-visible spectra were recorded on Agilent Technologies Cary 60 UV-visible single beam spectrophotometer with quartz cell of 1.0 cm path length in Methanol. The FT-IR spectra were recorded using FT-IR Agilent Technologies Cary 630 FT-IR infrared spectrophotometer and were quoted in cm^{-1}. The ^1H and ^{13}C NMR spectra were recorded using Bruker DRX 500 spectrometer at 300 MHz with tetramethylsilane as the internal standard. Mass spectral data were obtained by LC/MSD Trap XCT.

General synthetic procedure for 4-nitrobenzamide derivatives (3a-d)

Equimolar concentrations of 4-nitrobenzamide (**1**, 0.5 g) & aryl aldehydes (**2a-d**, 0.25g) and add catalytic amount of glacial acetic acid. Grind well; the solid mass was left over 4-5 hours. The product was dried for several hours and recrystallized from hot alcohol to obtain the pure product and the progress was monitored by TLC. The product was characterized by various spectral studies. Compound was prepared by the method summarized in below scheme 1.



1 **2a-d** **3a-d**

Scheme 1

(E)-N-(2-Nitrobenzylidene)-4-nitrobenzamide (3a)

FT-IR (KBr, cm^{-1}) *v*: 3070 (Ar C-H), 1669 (C=O), 1593 (C=N), 1340 (Ald C-H bending), 1108 (C-N), 706 (Ar C-H bending). ^1H NMR (300 MHz, DMSO-d_6): δ 8.35 (d, 2H, Ar-H), 8.05 (d, 2H, Ar-H), 8.10 (s, 1H, CH), 8.05-7.74 (m, 4H, Ar-H). ^{13}C-NMR (300 MHz, DMSO-d_6): δ 175.9, 152.9, 140.5, 130.3, 121.8. MS: m/z, M^+ 300.

(E)-N-(4-(Dimethylamino) benzylidene)-4-nitrobenzamide (3b)

FT-IR (KBr, cm^{-1}) *v*: 3186 (Ar C-H), 1658 (C=O), 1589 (C=N), 1230 (Ald C-H bending), 1064 (C-N), 700 (Ar C-H bending). ^1H NMR (300 MHz, DMSO-d_6): δ 8.38 (d, 2H, Ar-H), 8.10 (d, 2H, Ar-H), 8.10 (s, 1H, CH), 7.74 (d, 2H, Ar-H), 6.75 (d, 2H, Ar-H), 2.80 (s, 6H, CH_3). ^{13}C-NMR (300 MHz, DMSO-d_6): δ 172.4, 163.0, 152.5, 140.5, 130.2, 120.8, 111.0, 40.5. MS: m/z, M^+ 298.

(E)-N-(3-Methoxybenzylidene)-4-nitrobenzamide (3c)

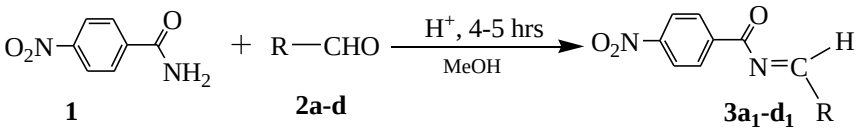
FT-IR (KBr, cm^{-1}) *v*: 3185 (Ar C-H), 1656 (C=O), 1589 (C=N), 1340 (Ald C-H bending), 1118 (C-N), 1090 (C-O-C), 862 (Ar C-H bending). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.34 (d, 2H, Ar-H), 8.00 (d, 2H, Ar-H), 8.15 (s, 1H, CH), 7.25-6.84 (m, 3H, Ar-H), 6.80 (s, 1H, Ar-H), 3.70 (s, 3H, CH_3). ^{13}C-NMR (300 MHz, DMSO-d_6): δ 172.5, 162.4, 153.5, 142.7, 130.2, 120.5, 110.0, 55.0. MS: m/z, M^+ 285.

(E)-N-(3,4,5-Trimethoxybenzylidene)-4-nitrobenzamide (3d)

FT-IR (KBr, cm^{-1}) *v*: 2942 (Ar C-H), 1664 (C=O), 1586 (C=N), 1321 (Ald C-H bending), 1120 (C-N), 991 (C-O-C), 844 (Ar C-H bending). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.38 (d, 2H, Ar-H), 8.00 (d, 2H, Ar-H), 8.10 (s, 1H, CH), 6.54 (s, 2H, Ar-H), 3.75 (s, 9H, CH_3). ^{13}C-NMR (300 MHz, DMSO-d_6): δ 171.3, 163.4, 152.5, 142.5, 130.5, 121.5, 112.0, 56.0. MS: m/z, M^+ 345.

General synthetic procedure for 4-nitrobenzamide derivatives (3a$_1$-d$_1$)

Equimolar concentrations of 4-nitrobenzamide (**1**, 0.5 g) & aryl aldehydes (**2a-d**, 0.25g) were refluxed at 60 °C for 4-5 hr using methanol (25 ml) and add 2-3 drops of conc. H_2SO_4. The solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. The organic layer was washed with brine solution and finally water wash was given to organic layer and dried with anhydrous sodium sulphate. The ethyl acetate was used as a solvent for recrystallization. The product was characterized by different spectral studies. Compounds were prepared by the method summarized in below Scheme 2.



1 **2a-d** **3a$_1$-d$_1$**

Scheme 2

(E)-N-(2-Nitrobenzylidene)-4-nitrobenzamide (3a$_1$)

FT-IR (KBr, cm^{-1}) *v*: 3071 (Ar C-H), 1669 (C=O), 1592 (C=N), 1340 (Ald C-H bending), 1108 (C-N), 705 (Ar C-H bending). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.34 (d, 2H, Ar-H), 8.04 (d, 2H, Ar-H), 8.20 (s, 1H, CH), 8.05-7.70 (m, 4H, Ar-H). ^{13}C-NMR (300 MHz, DMSO-d_6): δ 175.8, 152.5, 140.4, 130.3, 121.0. MS: m/z, M^+ 300.

(E)-N-(4-(Dimethylamino) benzylidene) – 4 - nitrobenzamide (3b$_1$)

FT-IR (KBr, cm^{-1}) *v*: 3185 (Ar C-H), 1654 (C=O), 1580 (C=N), 1235 (Ald C-H bending), 1065 (C-N), 710 (Ar C-H bending). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.37 (d, 2H, Ar-H), 8.13 (d, 2H, Ar-H), 8.12 (s, 1H, CH), 7.75 (d, 2H, Ar-H), 6.74 (d, 2H, Ar-H), 2.82 (s, 6H, CH_3). ^{13}C-NMR (300 MHz, DMSO-d_6): δ 171.4, 163.2, 152.5, 140.5, 130.2, 120.8, 111.0, 40.5. MS: m/z, M^+ 298.

(E)-N-(3-Methoxybenzylidene)-4-nitrobenzamide (3c$_1$)

FT-IR (KBr, cm^{-1}) *v*: 3185 (Ar C-H), 1654 (C=O), 1586 (C=N), 1342 (Ald C-H bending), 1115 (C-N), 1090 (C-O-C), 863 (Ar C-H bending). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.34 (d, 2H, Ar-H), 8.07 (d, 2H, Ar-H), 8.14 (s, 1H, CH), 7.25-6.83 (m, 3H, Ar-H), 6.81 (s, 1H, Ar-H), 3.71 (s, 3H, CH_3). ^{13}C-NMR (300 MHz, DMSO-d_6): δ 172.3, 162.2, 153.1, 142.6, 130.1, 120.4, 110.2, 55.1. MS: m/z, M^+ 285.

(E)-N-(3,4,5-Trimethoxybenzylidene)-4-nitrobenzamide (3d$_1$)

FT-IR (KBr, cm^{-1}) *v*: 2941 (Ar C-H), 1662 (C=O), 1585 (C=N), 1320 (Ald C-H bending), 1120 (C-N), 991 (C-O-C), 845 (Ar C-H bending). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.36 (d, 2H, Ar-H), 8.05 (d, 2H, Ar-H), 8.20 (s, 1H, CH), 6.52 (s, 2H, Ar-H), 3.74 (s, 9H, CH_3). ^{13}C-NMR (300 MHz, DMSO-d_6): δ 171.2, 163.2, 152.1, 142.4, 130.4, 121.1, 112.0, 56.3. MS: m/z, M^+ 345.

Antibacterial activity

The strains of bacteria were subjected to disc diffusion method according procedure [20] with negligible modification. The suspension of bacteria was prepared from the overnight culture and 1x10^6 CFU/mL cells and inoculated on to nutrient agar, the sterile disc of 6 mm size was loaded with 5 µL of different serial dilutions of synthesized compounds (200 µg/disc) were added. The sterile distilled water and Streptomycin served as negative and positive control, respectively. The plates were sealed and incubated at 37°C for 24h to examine zone of inhibition [21].

Antifungal activity

The antifungal assay is executed with an aid of food poisoning technique [22]. Potato dextrose agar (PDA) medium is used in antifungal study. The media containing plates with different concentrations of synthesized compounds are inoculated at the centre with 5 mm inoculum disc of pathogenic fungus and incubated at 25°C for 7 days. The sterile distilled water and nystin served as negative and positive control, respectively.

RESULTS AND DISCUSSION

Chemistry

Schiff bases were synthesized by the method summarized in the above Scheme.[1]H-NMR, [13]C NMR, LC-MS and FT-IR spectral studies were used for characterization. Structure and physical data of the synthesized compound was depicted in Tables 1 & 2, respectively.

Table-1: Structure of synthesized compounds 3a-d and 3a[1]-d[1]

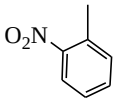
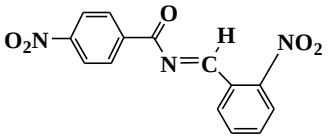
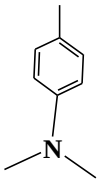
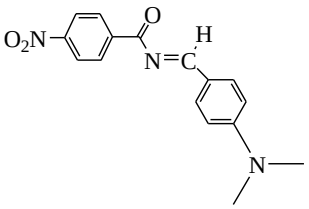
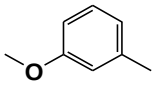
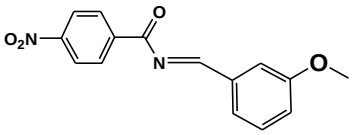
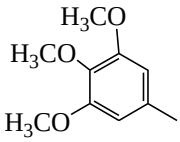
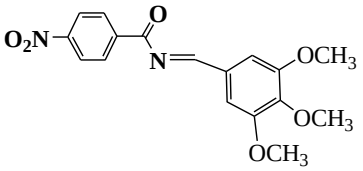
Compound	Aryl aldehydes	R	Structure
3a & 3a$_1$	2-Nitrobenzaldehyde		
3b & 3b$_1$	4-(Dimethylamino)benzaldehyde		
3c & 3c$_1$	3-Methoxybenzaldehyde		
3d & 3d$_1$	3,4,5-Trimethoxybenzaldehyde		

Table-2: Physical data of synthesized compounds 3a-d and 3a[1]-d[1]

Compound	Mol. Formula	Mol. Wt.	Yield (%)	UV-Visible (λ_{max})	Melting Point	Colour	Solubility
3a	$C_{14}H_9N_3O_5$	299.2	75.45	425	130 ˚C	Brown	Ethanol
3b	$C_{16}H_{15}N_3O_3$	297.3	73.20	435	140˚C	Brown	Ethanol
3c	$C_{15}H_{12}N_2O_4$	284.3	78.12	410	132 ˚C	Brown	Ethyl acetate
3d	$C_{17}H_{16}N_2O_6$	344.3	76.50	450	145˚C	Black	DMSO
3a[1]	$C_{14}H_9N_3O_5$	299.2	74.00	425	130 ˚C	Brown	Ethanol
3b[1]	$C_{16}H_{15}N_3O_3$	297.3	72.10	435	140˚C	Brown	Ethanol
3c[1]	$C_{15}H_{12}N_2O_4$	284.3	78.00	410	130 ˚C	Brown	Ethyl acetate
3d[1]	$C_{17}H_{16}N_2O_6$	344.3	75.50	450	145˚C	Black	DMSO

UV-Visible spectra of synthesized compounds were illustrated in the **Figure 1** displaying the peak between 410 to 450 nm which confirms the formation of products. The peaks were broad with high intensity and the synthesis of nanoparticles was achieved within 24 hours of incubation time. The change in colour was observed for the synthesized compounds which were confirmed by UV-Visible spectra as part of primary confirmation. The UV-visible absorption spectra of **3a** showed a peak at λ_{max} = 425 nm. The UV-visible absorption spectra of **3b** showed a peak at λ_{max} = 435 nm. The UV-visible absorption spectra of **3c** showed a peak at λ_{max} = 410 nm. The UV-visible absorption spectra of **3d** showed a peak at λ_{max} = 450 nm.

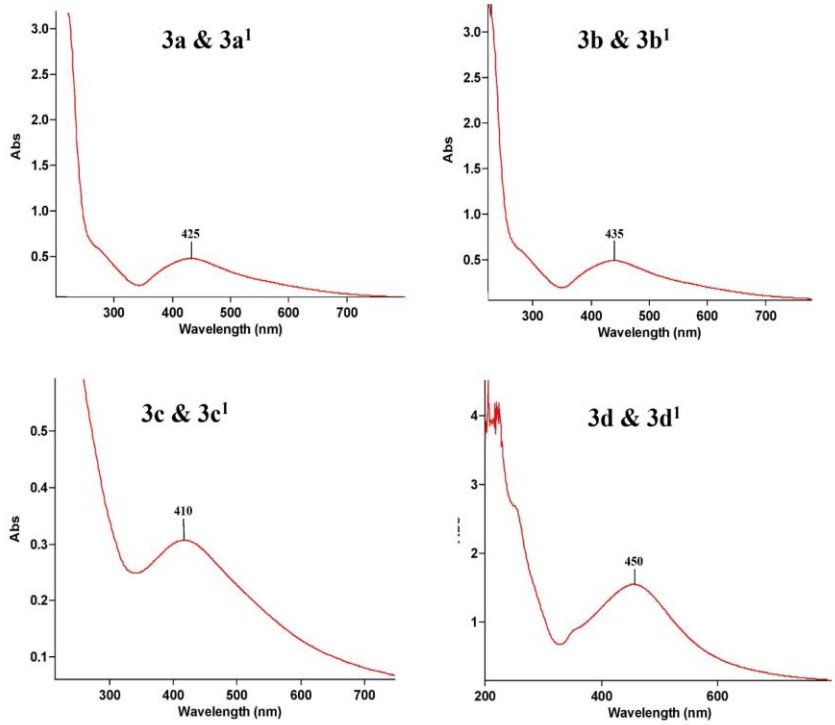


Fig-1: UV-Visible spectra of 3a-d and 3a^1-d^1

The IR spectrum of the compound was run using single beam FT-IR. In the present study, the compounds, **3a** and **3b** give an absorption band in the region of 1118 and 1142 cm^{-1} due to C-N, respectively. The FT-IR spectrum of **3a** is shown in **Figure 2**. The absence of NH_2 and C=O absorption bands in the IR spectra confirmed that the synthesized compounds were obtained via condensation. However, the changes in integral intensities and bandwidths, especially of the bands originating from NH_2 stretching vibrations didn't show in products. An absorption band in the region of 1230 cm^{-1} due to C-N of compounds **3c** and **3d**.

The infrared spectrum in the 1593 cm^{-1}-1589 cm^{-1} region has been determined for C=N in synthesized compounds **3a-d** and **3a^1-d^1**. The proton spectral data of **1** shows resonance at δ 5.45 ppm (s, 2H, NH_2). In all the synthesized compounds, the above resonance disappeared and additional resonances assigned to the -CH=N- (δ 8.20 – 8.10 ppm) was observed, which confirmed the condensation between the amino group and carbonyl group. ^{13}C NMR spectra present the correct number of carbon atoms at the appropriate chemical shift values. The mass spectra showed molecular ion peak, which is in agreement with the molecular formula.

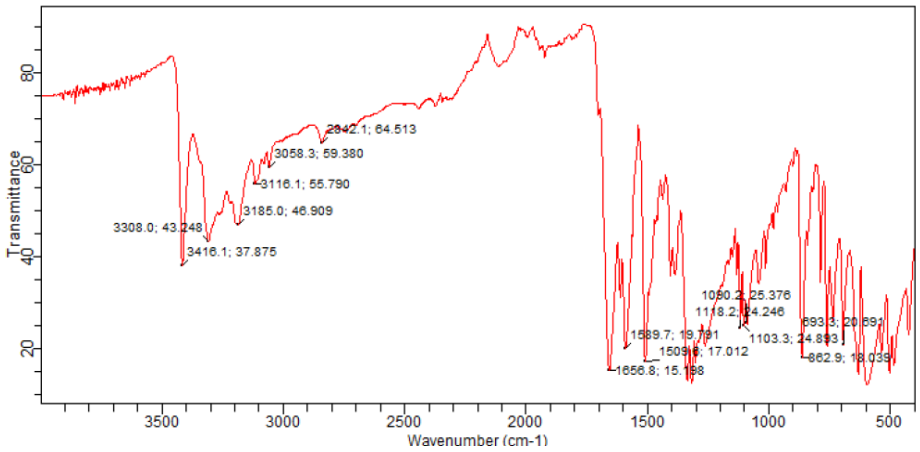


Fig-2: FT-IR analysis of compound 3a

Antibacterial activity

The synthesized compounds were evaluated for the biocidal potency against both Gram-positive and Gram-negative bacteria in disc diffusion method. The compounds **3a-d** and **3a^1-d^1** assessed the antibacterial and antifungal activity showed compounds **3a** and **3a^1** is the highly potent compared to the other synthesized analogs carryout in the present investigations. These results were depicted in the **Table 3**, and the promising lead molecule was further used to confirm the dose depended action in the further study. In antimicrobial activity, **3a** and **3a$_1$** found to be a relatively better compound in inhibiting the growth of the microorganisms. All the synthesized compounds were found to be a relatively good in inhibiting the growth of the microorganisms at 200 µg/ml (**Figure 3**).

Table-3: *In vitro* antibacterial and antifungal activity of new compounds

Compound	*S.typhi*	*S.paratyphi*	*Klebsiella*	*Pseudomonas*	*S.aureus*	*S.mitis*	*F.oxysporum*
	Zone of inhibition (mm)						% Inhibition
3a & 3a$_1$	16.5	14.1	12.4	14.5	18.5	13.1	74.1
3b & 3b$_1$	15.4	13.3	12.2	14.2	17.1	13.2	72.3
3c & 3c$_1$	11.3	10.4	10.2	12.3	14.5	10.3	59.4
3d & 3d$_1$	13.2	12.2	11.3	13.5	16.4	12.6	68.5
Streptomycin	18.2	15.3	13.4	14.8	19.2	14.6	-
Nystatin	-	-	-	-	-	-	80.0

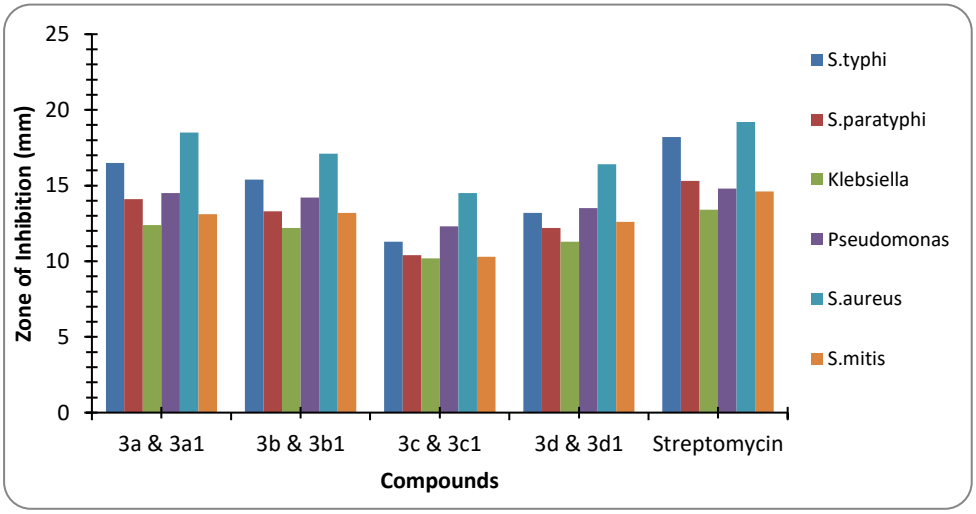


Fig-3: Antibacterial activity of 3a-d and 3a^1-d^1

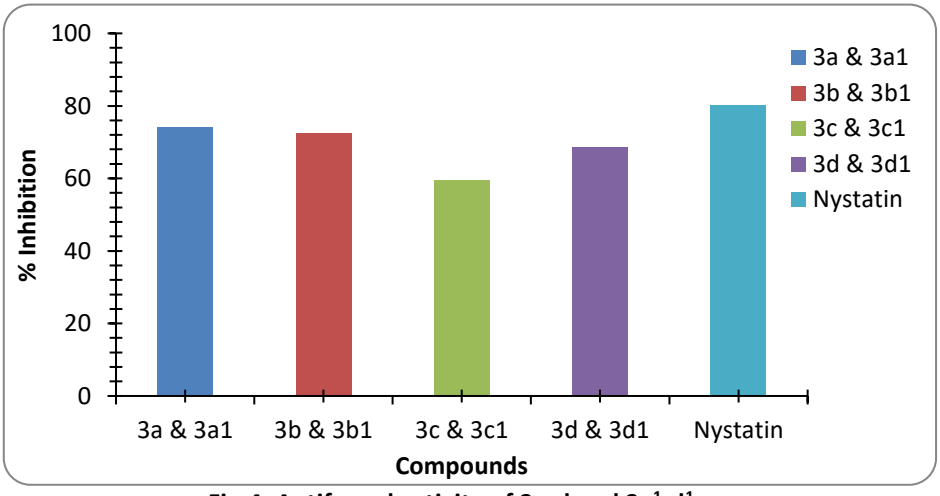


Fig-4: Antifungal activity of 3a-d and 3a^1-d^1

CONCLUSION

In conclusion, new Schiff bases compounds were synthesized from 4-nitrobenzamide with different aldehydes. These compounds were characterized by different spectral studies. This work presented a simple method to prepare new compounds and form in good yield. The compounds assessed the antimicrobial and compounds 3a and $3a_1$ showed highly potent compared to the other synthesized analogues carryout in the present investigations. On the basis of their activity, these derivatives were identified as viable leads for further studies.

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