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ARTICLE

Design, synthesis, and antimicrobial evaluation of some nifuroxazide analogs against nosocomial infection

Deepak K. Dwivedi[1,2] | **Adarsh Sahu**[1] | **Sachin J. Dighade**[2] | **Ram Kishore Agrawal**[1]

[1]Department of Pharmaceutical Sciences, Dr. Harisingh Gour Central University, Sagar, India

[2]Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Education and Research, Wardha, India

Correspondence
Deepak K. Dwivedi, Department of Pharmaceutical Sciences, Dr. Harisingh Gour Central University, Sagar, India.
Email: dk.dwivedi95@gmail.com

Abstract

A series of 10 *p*-substitutedbenzoylmethylene hydrazide derivatives **4a-j** were synthesized by protecting carboxylic group of 4-hydroxybenzoic acid using methanol and sulfuric acid than reacting it with hydrazide to form 4-hydroxybenzohydrazide followed by reacting with a variety of aldehydes and evaluated for their activity against nosocomial infection. All the synthesized compounds were characterized by Fourier-transform infrared (FT-IR), ^{1}H nuclear magnetic resonance (NMR), and mass spectral data. The in vitro antimicrobial potential of synthesized compounds was estimated against prominent strains of nosocomial pathogens (*Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger*). The antimicrobial evaluation revealed compounds **4b**, **4c**, **4d**, **4e**, **4f**, and **4j** to be the most active compounds of the series with IC_{50} value for antibacterial in the range 0.39 to 0.75 μM/mL. Furthermore, the in vitro cytotoxic potential of the compounds was appraised by hemolytic assay. The results showed that some of the synthesized compounds exhibited marked activity.

1 | INTRODUCTION

Nosocomial infections (NIs) are hospital-acquired infections (HAIs).[1] As per World Health Organization (WHO), the classical definition of the term encompasses any infection occurring in a patient in a hospital or any other healthcare facility in which the infection was not present or incubating at the time of admission. Consequences of NIs range from mild nausea, vomiting, to fatal meningitis.[2] Currently, NIs has become a common occurrence in both developed and developing countries, accounting for approximately 1.4 million incidences worldwide.[3] The pathogens predominantly implicated include resistant bacterial strains of *Staphylococcus aureus* and *Escherichia coli*; fungal strains of Candida and Aspergillus have also been incriminated in the certain manifestations of NIs.[4] The strains being resistant to the first line antimicrobial chemotherapy considerably limit the therapeutic intervention of NIs.[5]

Nifuroxazide is an oral nitro-furan antibiotic drug. It has excellent characteristics such as wide spectrum of activity and better chances for structure manipulation for better activity. Consequently, NF is advocated as an outstanding lead compound (Figure 1). Scientific literature reports modification of the NF moieties to yield structures, which were successfully investigated as monoamine oxidase inhibitors,[6] antifungal,[7] anticonvulsant,[8] anti-inflammatory,[9] bactericidal,[10] and trypanocidal agents.[11] The findings have emphasized the potential of these molecular structures for the development of novel drugs. Herein, we report the design, synthesis, and evaluation of the antimicrobial activity of *p*-substitutedbenzoylmethylene hydrazide derivatives against NIs. The nifuroxazide hydrazone was substituted with 10 different aldehydes elected according to physiochemical properties such as electron releasing, withdrawing, and electron distribution. Antimicrobial activity was evaluated against microorganisms

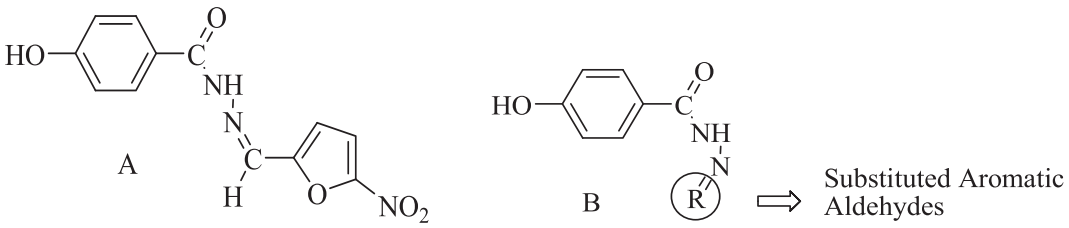
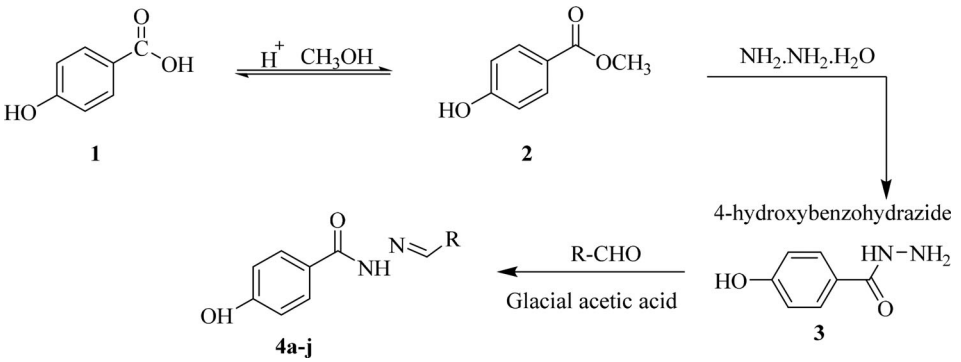


FIGURE 1 (A) Chemical structure of nifuroxazide (NF) (B) General structure of substituted analogs



SCHEME 1 General scheme for the synthesis of *p*-substitutedbenzoylmethylene hydrazide derivatives **4a-j**

reported as popular pathogens (*S. aureus*, *E. coli*, and *Aspergillus niger*). The molecular characterization was investigated by infrared (IR), nuclear magnetic resonance (NMR), and mass spectroscopic analysis.

The nifuroxazide hydrazones constitute an important class of biologically active agents such as antimicrobial, antiprotozoal, antichagasic, and anti-inflammatory agents. An extensive literature search revealed that nifuroxazide are demonstrated to be the noble bioactive molecules. Hence, it is worth to synthesize some nifuroxazide derivatives for better antimicrobial activity. The essential of new antibacterial and antifungal agents is justified since microorganisms are being resistant to the existing drugs available in the market.[12,13]

2 | RESULT AND DISCUSSION

The benzhydrazide derivatives could also be synthesized from *p*-substituted methyl benzoates. Thus, based on literature reports, the methyl esters were synthesized from the corresponding *p*-hydroxy-benzoic acid using dried methanol and sulfuric acid and refluxing it for 3 to 4 hours. Then *p*-hydroxy methyl benzoate **2** was treated with hydrazine hydrate under reflux condition for 5 to 6 hours to furnish *p*-hydroxy benzhydrazide **3**, a mixture of equimolar proportion of *p*-hydroxybenzhydrazides with substituted aromatic aldehydes *p*-substitutedbenzoylmethylene hydrazide derivatives (Scheme 1).[14]

The structures **4a-j** (Figure 2) of the synthesized compounds was elucidated using Fourier-transform IR (FT-IR), ^{1}H NMR, and mass spectroscopic methods. The IR spectra of synthesized compounds **4a-j** showed hydrazine N—H bands in 3400 to 3200 cm^{-1}, C═O bands in 1620 to 1750 cm^{-1} and C═N bands in 1600 to 1620 region. Cl and NO_2 bands were seen in IR (7231560-1523 to 1367-1350).^{1}H NMR spectra of compounds (**a-j**) showed two signals in the δ 5.435 ppm and δ 8.7 to 7.7 ppm, which were attributed to the OH and N═CH proton, respectively.

The synthesized nifuroxazide analogs were evaluated against prominent strains of nosocomial pathogens. Activity demonstrated by compounds **4b** (*m*-OH), **4c** (*p*-Cl), **4d** (*m*-NO2), **4e** (*p*-dimethylamine), **4f** (*p*-diethylamine), and **4j** (*p*-Br) were comparatively better than that exhibited by the reference standard. The anti-nosocomial activity of the synthesized nifuroxazide analogs was evaluated against three prominent strains (*S. aureus*, *E. coli*, and *A. niger*) of nosocomial pathogens by the cup-plate method.

The IC_{50} value obtained for each compound and that obtained for the reference standards (nifuroxazide, ciprofloxacin, and miconazole) are listed in Figure 3. It was observed that in comparison with the reference standards the compounds **4b**, **4c**, **4d**, **4e**, **4f**, and **4j** demonstrated superior efficacy with respect to compounds **4a**, **4g**, **4h**, and **4i**. The most potent compound of the series is compound **4d** (*m*-NO_2) (IC_{50} value 0.39, 0.42, and 0.68 μg/mL).

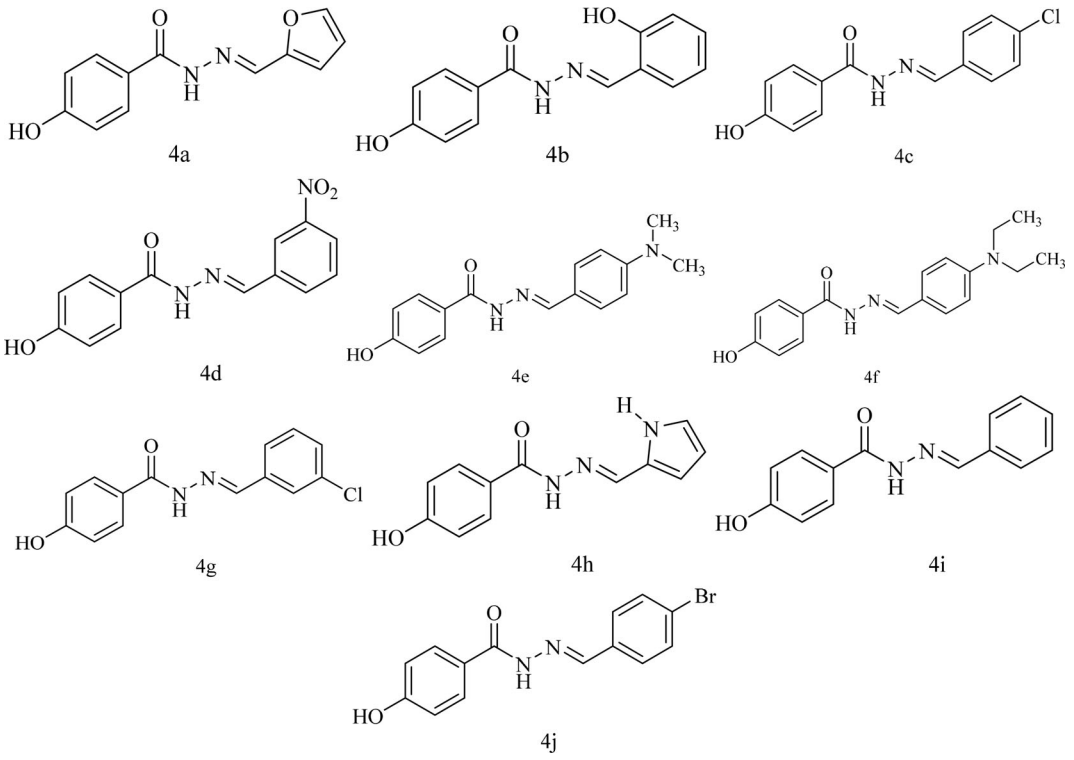
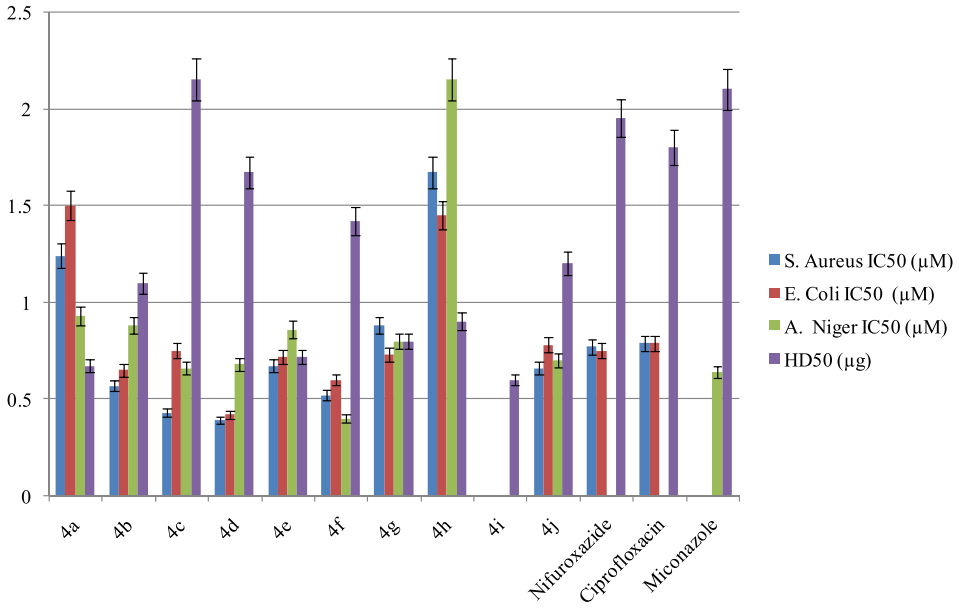


FIGURE 2 Structure of *p*-substitutedbenzoylmethylene hydrazide derivatives **4a-j**

FIGURE 3 In vitro antibacterial, antifungal, and hemolytic activity (human erythrocytes) of the **4a-j** antibacterial strain, *Staphylococcus aureus* (SA); *Escherichia coli* (EC); Antifungal strain, *Aspergillus niger*



In the context of cytotoxic, HD_{50} was used. HD_{50} stands for median hemolytic dose. It is the dose at which 50% hemolysis of human erythrocytes takes place. Nifuroxazide, ciprofloxacin, and miconazole were used as the reference drugs for the study. HD_{50} value of the compounds ranged between 0.6 and 2.5 μg/mL. All the synthesized compounds exhibited mild to moderate cytotoxic propensity. The least cytotoxic compound of the series was found to be compound **4i** while maximum cytotoxicity was observed for compound **4c** with HD_{50} value 2.15 μg/mL. It can be stated that the presence of non-substituted pyrrole may be responsible for the increased cytotoxicity of the compound.

2.1 | IC_{50}—the half maximal inhibitory concentration, HD_{50}—median hemolytic dose

From the results obtained, it can be deduced that substitution at the *p*- position of aromatic ring with an electron releasing group **4e** *p*-((dimethyl amino) benzylidene), **4f** *p*-(diethyl amino) benzylidene), and *m*- position in the aromatic ring with electron withdrawing group **4d** (*m*-nitrobenzylidene), **4g** (*m*-chloro-benzylidene), and **4b** (*m*-hydroxy-benzylidene) may be responsible for the increase in activity of parent nucleus. The substitution at *m*-position in the aromatic ring with NO_2 group yielded the superior compound of the series. Furthermore, the presence of non-substituted pyrrole may have resulted in a decreased bioactivity as observed in the case of compound **4h**. This was recognized as the least active compound of the series.

3 | EXPERIMENTAL SECTION

Reagents and solvents were procured from LOBA Chemie and Himedia Lab. Reaction progress was monitored by thin-layer chromatography (TLC) on silica gel precoated F254 and melting point. The spectra of compounds were recorded using FT-IR - 8400S (Shimadzu), ^{1}H NMR (Bruker, 400 MHz FT NMR), and mass spectra (Q-Tof Micro mass spectrometer).

3.1 | General procedure for the synthesis of *p*-substitutedbenzoylmethylene hydrazide derivatives 4a-j

A mixture of equimolar proportion of *p*-hydroxybenzhydrazides (2 g, 0.0025 mol) and substituted aromatic aldehydes (2 mL, 0.0025 mol), in a solution of ethanol/acetic acid/sulfuric acid/water (20:8:7:8), and three drops of glacial acetic acid was added; mixture was refluxed. The insoluble product was filtered off and purified by recrystallization in *N*,*N*-dimethyl formamide.

3.2 | Spectral data of the final compounds 4a-j

3.2.1 | 4a. *N′*-((4H-furan-2-yl) methylene)-4-hydroxybenzhydrazide

Yield 71%; black color; m.p. 270-273°C, λmax = 371.40; IR (KBr) 3500-3200 cm^{-1} (OH) (Broad), 3400-3200 cm^{-1} (N—H), 1745.24 cm^{-1} (C═O), 1606.59 cm^{-1} (N═C), ^{1}H NMR (DMSO), δ ppm: 10.86 (1H, s, N*H*); 10.11 (1H, s, OH), 7.86 (1H, s, NH), 7.41-7.39 (2H, Ar—H), 7.23-7.22 (2H, d, *J* 8 Hz); 5.59 (1H, t, *J* 8 Hz), 2.86-2.82 (2H, m). LC/MS- m/z calculated for $C_{12}H_{10}N_2O_3$: 230.06, found 230.16 (M^+).

3.2.2 | 4b. *N′*-(2-hydroxybenzylidene)-4-hydroxybenzhydrazide

Yield 60%; light brown color; m.p. 130-135°C, λmax = 343.60; IR (KBr) 3500-3200 cm^{-1} (OH) (Broad), (N═C), 3400-3200 cm^{-1} (N—H), 1637.24 cm^{-1} (C═O), 1610.59 cm^{-1},^{1}H NMR (MeOD), 11.68 (1H, s, N*H*); 11.01 (1H, s, OH); 9.69 (1H, s, OH); 8.79 (1H, s, *H*C═N), 8.11 (2H, s, Ar—H); 7.62-7.58 (2H, m, Ar—H), 6.97-6.93 (4H, m, Ar—*H*); LC/MS- m/z calculated for $C_{14}H_{12}N_2O_3$: 256.08, found 256.12 (M^+).

3.2.3 | 4c. *N′*-(4-chlorobenzylidene)-4-hydroxybenzhydrazide

Yield 79%; yellow color; m.p. 200-202°C, λmax = 382.40; IR (KBr) 3500-3200 cm^{-1} (OH) (Broad), 3400-3200 cm^{-1} (N—H), 1637.24 cm^{-1} (C═O), 1610.59 cm^{-1} (N═C), 723 cm^{-1} (Cl), ^{1}H NMR (DMSO), δ ppm: 10.86 (1H, s, N*H*); 10.11 (1H, s, O*H*); 7.86 (1H, s, —N═C*H*); 7.41-7.30 (4H, m, Ar—H); 7.03-6.99 (2H, m, Ar—H); 5.60 (2H, d, *J* 8 Hz). LC/MS- m/z calculated for $C_{14}H_{11}N_2O_2Cl$: 273, found 273.21(M^+).

3.2.4 | 4d. *N′*-(3-nitrobenzylidene)-4-hydroxybenzhydrazide

Yield 78%; faint yellow crystal; m.p. 250-255°C, λmax = 313.20; IR (KBr) 3500-3200 cm^{-1} (OH) (Broad), 1637.24 cm^{-1} (C═O), 1610.59 cm^{-1} (N═C), 1560-1523, 1367-1350 cm^{-1} (NO_2), 3400-3200 cm^{-1} (N—H), ^{1}H NMR (DMSO), δ ppm: 11.59 (1H, s, N*H*); 9.73 (1H, s, O*H*); 8.44 (1H, s); 8.12 (1H, d, *J* 4 Hz); 7.89-7.86 (2H, d, *J* 12 Hz), 7.75 (3H, t, *J* 8 Hz); 7.08 (1H, d, *J* 8 Hz, Ar—H); 6.88 (2H, d, *J* 8 Hz, Ar—H); LC/MS- m/z calculated for $C_{14}H_{11}N_3O_4$: 285, found 285.31(M^+).

3.2.5 | 4e. *N′*-(4-(dimethylamino) benzylidene)-4 hydroxybenzhydrazide

Yield 65%; red crystal; m.p. 170-175°C, λmax = 391.50.; IR (KBr) 3500-3200 cm^{-1} (OH) (Broad), 1637.24 cm^{-1} (C═O), 1610.59 cm^{-1} (N═C), 1068 cm^{-1} (N—C); ^{1}H NMR

(DMSO), δ ppm: 11.66 (1H, s, N*H*); 9.76 (1H, s, O*H*); 8.31 (2H, s, —N═C*H*); 7.64-7.62 (2H, m, Ar—H); 6.98-6.96 (2H, m, Ar—H), 6.54-6.48 (4H,m, Ar—H); LC/MS- m/z calculated for $C_{16}H_{17}N_3O_2$: 283, found 283.41(M^+).

3.2.6 | 4f. (*N′*-(4-(diethylamino) benzylidene)-4-hydroxybenzhydrazide

Yield 71%; Yellow crystal; m.p. 180-185°C, λmax = 491.50; IR (KBr) 3500-3200 cm^{-1} (OH) (Broad), 1637.24 cm^{-1} (C═O), 1610.59 cm^{-1} (N═C), 1068 cm^{-1} (N—C); ^{1}H NMR (DMSO), δ ppm: 11.58 (1H, s, N*H*); 10.11 (1H, s, O*H*); 8.39 (1H, s, —N═C*H*); 7.86 (2H, s, Ar—H); 7.71-7.69 (2H, d, *J* 8 Hz); 7.30 (2H, d, *J* 4 Hz); 6.62 (2H, d, *J* 8 Hz, Ar—H), 3.62 (4H,d, (CH_2)$_2$); 1.52 (6H, d, *J* 8 Hz, (CH_2CH_3)$_2$). LC/MS- m/z calculated for $C_{18}H_{21}N_3O_2$: 311.1634, found 311.41(M^+).

3.2.7 | 4g. (*N′*-(3-chlorobenzylidene)-4-hydroxybenzhydrazide)

Yield 59%; Orange crystal; m.p. 150-155°C, λmax = 483.00; IR (KBr) 3500-3200 cm^{-1} (OH) (Broad), 1647.24 cm^{-1} (C═O), 1620.59 cm^{-1} (N═C), 1066 cm^{-1} (N—C); ^{1}H NMR (DMSO), δ ppm: 11.69 (1H, s, N*H*); 10.02 (1H, s, O*H*); 8.61 (1H, s, —N═C*H*); 7.39-7.35 (4H, m, Ar—H); 7.30-7.29 (2H, m, *ArH*); 6.97 (2H, d, *J* 8 Hz); 6.86 (1H, t, *J* 8 Hz, Ar—H). LC/MS- m/z calculated for $C_{14}H_{11}ClN_2O_2$: 274.05, found 275.05(M^++ 1).

3.2.8 | 4h. (*N′*-((1H-pyrrole-2-yl)] methylene)-4-hydroxybenzohydrazide)

Yield 85%; Orange crystal; m.p. 150-155°C, λmax = 440.00.; IR (KBr) 3400-3200 cm^{-1} (OH) (Broad), 1648.74 cm^{-1} (C═O), 1640.59 cm^{-1} (N═C), 1068 cm^{-1} (N—C);^{1}H NMR (DMSO), δ ppm: 11.35 (1H, s, N*H*); 10.90 (1H, s, N*H*); 9.89 (1H, s, OH); 7.97 (1H, s, —N═C*H*); 7.78-7.77 (4H, m, Ar—H); 7.35-7.29 (2H, m, *ArH*); 6.96 (2H, d, *J* 8 Hz); 6.87 (1H, t, *J* 4 Hz, Ar—H), 6.20-6.17 (1H,m, *Pyrolle H*). LC/MS- m/z calculated for $C_{12}H_{11}N_3O_2$: 229.08, found 230.05 (M^++ 1).

3.2.9 | 4i. (*N′*-benzylidene-4-hydroxybenzhydrazide)

Yield 72%; Orange crystal; m.p. 165-169°C, λmax = 420.00.; IR (KBr) 3400-3200 cm^{-1} (OH) (Broad), 1648.74 cm^{-1} (C═O), 1640.59 cm^{-1} (N═C), 1068 cm^{-1} (N—C);^{1}H NMR (DMSO), δ ppm: 11.69 (1H, s, N*H*); 9.72 (1H, s, OH); 8.82 (1H, s, —N═C*H*); 7.97-7.93 (2H, m, Ar—H); 7.78-7.75 (2H, m, *ArH*); 7.58 (2H, d, *J* 8 Hz); 6.87 (1H, t, *J* 4 Hz, Ar—H). LC/MS- m/z calculated for $C14H12N_2O_2$: 240.08, found 240.05 (M^++ 1).

3.2.10 | 4j. (N′-(4-bromobenzylidene)-4-hydroxybenzhydrazide)

Yield 82%; Buff color crystal; m.p. 175-179°C, λmax = 450.00.; IR (KBr) 3400-3200 cm^{-1} (OH) (Broad), 1648.74 cm^{-1} (C═O), 1640.59 cm^{-1} (N═C), 1068 cm^{-1} (N—C); ^{1}H NMR (DMSO), δ ppm: 11.65 (1H, s, N*H*); 9.72 (1H, s, OH); 8.37 (1H, s, —N═C*H*); 7.83 (2H, d, *J* 8 Hz, Ar—H); 7.64-7.57 (4H, m, *ArH*); 6.88-6.86 (1H, m, Ar—H). LC/MS- m/z calculated for $C_{14}H_{11}BrN_2O_2$:318.00, found 318.56 (M^++ 1), 319.76 (M + 2).

3.3 | Procedure of antimicrobial assay

The synthesized targeted compounds **4a**-**j** were screened by the cup-plate method. For antibacterial activity, G (+) bacteria *S. aureus* (ATCC 25923), G (−) *E. coli* (ATCC 8739), and for antifungal activity, G (+) *A. niger* (ATCC 16404) have been used. The strains were collects from a microbiological laboratory, IPER College, Wardha, Nagpur. The culture was grown in nutrient agar medium (antibacterial) and Sabouraud's agar medium (antifungal), and nifuroxazide, ciprofloxacin, and miconazole were used as reference standards.[15] IC_{50} was calculated by graph pad prism.

3.4 | Procedure of cytotoxic study (hemolytic assay)

Erythrocyte suspension was prepared by centrifuging 10 mL of whole human blood with isotonic buffer for 10 minutes at 3000 rpm. The supernatant was separated, and packed cells were suspended with an equal volume of normal saline and re-centrifuged. The process was repeated until a clear supernatant was obtained. From the resultant, 10% erythrocyte suspension was prepared. To 2 mL of saline solution, 1 mL of PBS and 1 mL sample solution, and 0.5 mL were added and incubated at 37°C for 30 minutes. After incubation, the reaction vessel was allowed to cool, and absorbance was measured spectrophotometrically at 560 nm. Percentage hemolysis and HD_{50} of the compound under study were calculated.[16]

4 | CONCLUSION

The present work led to the synthesis and development of 10 antimicrobial molecules of *p*-substituted benzoylmethylene hydrazide derivatives that have shown significant activity with good yield. The derivatives were synthesized by reacting *p*-substituted benzhydrazide with a variety of aldehydes. The compounds were characterized by using ultraviolet (UV), IR, ^{1}H NMR, and mass spectroscopy. The synthesized targeted compounds **4a**-**j** were screened against nosocomial infection using cup-plate method. Thus, preliminary in vitro antimicrobial activity of compounds **4b**, **4c**, **4d**, **4e**, **4f**, and **4j** demonstrated superior efficacy with respect to compounds **4a**, **4b**, **4c**, and **4d** against tested organism. The substitution at *m*-position in the aromatic ring with NO_2 group yielded the superior compound of the series. The compounds **4a**-**j** was also evaluated for in vitro cytotoxicity assay in human erythrocytes, which confirms the low cytotoxicity thereby helping in the design of novel potent compounds.

ORCID

Deepak K. Dwivedi https://orcid.org/0000-0003-1164-5181

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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