Synthesis, Spectral Characterization, and Biological Activity Studies of Isoxazolines

K. Mayuri, M. Ajitha
Department of Pharmaceutical Chemistry, Ucpsc, Sultanpur, Jntuh, Hyderabad, Telangana.

ABSTRACT :
The emergence of antimicrobial resistance has necessitated the continuous search for novel bioactive molecules with broad-spectrum efficacy. In the present study, a series of novel heterocyclic derivatives (4a–4d) were synthesized and evaluated for their antibacterial and antifungal activities using the agar well diffusion method. The antimicrobial potency was determined by measuring the zone of inhibition (mm) and minimum inhibitory concentration (MIC, µg mL⁻¹) values against selected Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis), Gram-negative bacteria (Escherichia coli, Salmonella typhi), and fungal strains (Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, and Fusarium moniliforme).
The results revealed that compound 4c exhibited the most significant antibacterial and antifungal activities, with low MIC values (6.25 µg mL⁻¹) across most tested strains and inhibition zones nearly comparable to the reference drugs Ofloxacin and Fluconazole. Compound 4b showed moderate activity, particularly against P. chrysogenum and Gram-positive bacteria, whereas compounds 4a and 4d demonstrated comparatively weaker antimicrobial effects with higher MIC values against Gram-negative bacteria and certain fungi. The superior performance of 4c suggests the presence of favorable structural features that enhance its ability to interact with microbial targets, indicating a strong structure–activity relationship.
In conclusion, compound 4c emerged as the most promising lead molecule with potent broad-spectrum antimicrobial activity, warranting further pharmacological evaluation and optimization. These findings highlight the potential of heterocyclic derivatives as effective candidates in the development of new antimicrobial agents to combat resistant pathogens.
Keywords: Antibacterial, Antifungal, MIC, Heterocyclic derivatives, Structure–activity relationship.

I. INTRODUCTION

Heterocyclic chemistry

The majority of known organic molecules are heterocyclic and heterocycles dominate the fields of biochemistry, medicinal chemistry, dye stuffs, photographic science and are of increasing importance in many others, including polymers, adhesive and molecular engineering. Most pharmaceuticals are based on heterocycles. Heterocycles, containing nitrogen are most abundant in nature than those containing oxygen or sulfur. Most of the alkaloids, which are nitrogenous bases occurring in plants and many antibiotics including penicillin and streptomycin, also contain heterocyclic ring system. Many natural pigments such as indigo, heamoglobin and anthocyanin are heterocycles. Most of the sugars, their derivatives including vitamin C for instance, exist largely in the form of five membered or six membered ring containing nitrogen. Lifesaving drugs, poisons and medicines (both natural and synthetic) such as sulfathiazole, pyrethrin, ratennone, strychnine, reserpine, the ergot alkaloids caffeine, cocaine, barbiturates etc. are heterocyclic.

Research in the field of medicinal chemistry is mainly focussed on the development of new and better drugs and their successful introduction into clinical practice. The basis of understanding the drug design lies in the awareness of the relationship between the chemistry of a particular compound or group of compounds and their interactions with the body, which is known as structure activity relationship and the mechanism by which the compound influences the biological system, which is known as its mode of action.[**1-7]**

With ever increasing number of drug resistant microbes, there is indeed an urgent need to develop new drugs which exhibit clear advantages over the already existing respective drugs.

Such advantages may be:

1) a qualitative or quantitative improvement in activity,
2) a partial or total absence of undesirable side effects,
3) a lower toxicity,
4) more nutritive value,
5) improved stability and

6) a decrease in production cost. Any drug must ideally have a broad spectrum of activity, with a rapid bactericidal action.[**4, 8-12]**

II. EXPERIMENTAL WORK

Present Work

Isoxazolines possesses broad spectrum of biological activities which are largely employed in the area of pharmaceuticals and therapeutics such as insecticidal, antibiotic, antitumour, anticancer, antibacterial, antifungal, antiviral, anti-microbial, anti-TB, anti-inflammatory and ulcerogenic. etc. Some fluorinated methyliminobenzoxazolines and their derivatives have been patented as plant protecting acaricides, fungicides and insecticides. The different isoxazole and isoxazolines derivatives exist as lead component in bioactive natural products (e.gmuscimol and Ibotenic acid) and in many drugs available in market such as valdecoxib, cloxacillin and flucloxacillin. Besides the therapeutic applications, the substituted isoxazoles are also served as an efficient substrates in synthesis useful building blocks such as γ-amino alcohols, β-hydroxy ketones, β-hydroxy nitriles.
In the biomedical research, always there is a scope for the development of novel, safe and economical new chemical compounds which possessing diversified applications. The literature survey also reveals that, if two pharmacophores are connected together may generate novel molecular templates that are able to exhibit interesting, effective biological properties.

During the course of present research work, this effective biological and medicinal importance of substituted isoxazoline derivatives promoted us to continue the present work on isoxazolines containing halogen, benzyloxy, methoxy, hydroxyl groups. Hence, we describe the synthesis and characterization of isoxazoline derivatives with potential biological activities.

The present work describes synthesis of isoxazolines from hydroxylamine hydrochloride and 1-(2-hydroxynaphthalen-1-yl)-3-phenyl prop-2-en-1-one by conventional method

Experimental procedure

Melting points were determined on an electro thermal apparatus using open capillaries and are uncorrected. Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365nm) or with an iodine vapor. IR spectra were recorded on a Shimadzu-fourier transform infra red **(FTIR)-8400 Spectrophotometer** using **KBr** disc. 1H NMR spectra were recorded on a **Bruker DPX-400 MHz** spectrometer. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. The mass spectral data were obtained with a **SHIMADZU-GCMS-QC-2010.**

General Method for the Synthesis of Hydroxy Chalcones

A mixture of 1-(2-hydroxynaphthalen-1-yl)ethanone(1mmol) with different substituted 2,3,4,5-tetramethylbenzaldehyde(1mmol) were dissolved in minimum quantity 90% ethyl alcohol in warm condition to this solution 40% of aq.KOH were added drop wise with constant shaking at room temperature. The reaction mixture was freely corked and kept in bulb oven at about 55-60°C for about 12-13 hrs. The resulting reaction mixture were poured into cold water and neutralized by dilute HCl. The separated solid was filtered (with suction pump), washed with ice cold water, dried and recrystalized from ethanol to get corresponding hydroxy chalcones.

(Scheme -1)

Synthesis of 3-(3-ethoxy-4-hydroxyphenyl)-1-(2-hydroxy naphthalene-1-yl) prop-2-en-1-one (3a)

1-(2-hydroxynaphthalen-1-yl)ethanone (0.01 mol) and 3-(benzyloxy)-4-methoxybenzaldehyde(0.01 mol) were dissolved in minimum quantity of 90% ethyl alcohol in warm condition to this solution 40% of aq.KOH were added drop wise with constant shaking at room temperature. The flask of reaction mixture was freely corked & kept in bulb oven at about 55-60°C for about 12 hrs. The resulting reaction mixture were neutralized by dilute HCl & poured into cold water. The obtained solid was filtered (with suction pump), washed with ice cold water, dried &recrystalized from ethanol to get 3-(3-(benzyloxy)-4-methoxy phenyl)-1-(2-hydroxy naphthalen- 1-yl)prop-2-en-1-one.

Synthesis of 3-(3-bromo-4-hydroxy-5-methoxyphenyl)-1-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one. (3b)

1-(2-hydroxynaphthalen-1-yl)ethanone (0.01 mol) and 3-bromo-4-hydroxy-5-methoxybenzaldehyde(0.01 mol) were dissolved in minimum quantity of 90% ethyl alcohol in warm condition to this solution 40% of aq.KOH were added drop wise with constant shaking at room temperature. The flask of reaction mixture was freely corked & kept in bulb oven at about 55-60°C for about 12 hrs. The resulting reaction mixture were neutralized by dilute

HCl & poured into cold water. The obtained solid was filtered (with suction pump), washed with ice cold water, dried &recrystalized from ethanol to get 3-(3-bromo-4-hydroxy-5-methoxyphenyl)-1-(2-hydroxynaphthalene- 1-yl) prop-2-en-1-one.

Synthesis of 3-(3,4-dimethoxyphenyl)-1-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one. (3c)

1-(2-hydroxynaphthalen-1-yl)ethanone (0.01 mol) and 3,4-dimethoxybenzaldehyde(0.01 mol) were dissolved in minimum quantity of 90% ethyl alcohol in warm condition to this solution 40% of aq.KOH were added drop wise with constant shaking at room temperature. The flask of reaction mixture was freely corked & kept in bulb oven at about 55-60°C for about 12 hrs. The resulting reaction mixture were neutralized by dilute HCl & poured into cold water. The obtained solid was filtered (with suction pump), washed with ice cold water, dried &recrystalized from ethanol to get 3-(3-bromo-4-hydroxy-5-methoxyphenyl)-1-(2-hydroxynaphthalene- 1-yl) prop-2-en-1-one.

Synthesis of 3-(3-bromo-4,5-dimethoxyphenyl)-1-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one. (3d)

1-(2-hydroxynaphthalen-1-yl)ethanone (0.01 mol) and 3-bromo-4,5-dimethoxybenzaldehyde(0.01 mol) were dissolved in minimum quantity of 90% ethyl alcohol in warm condition to this solution 40% of aq.KOH were added drop wise with constant shaking at room temperature. The flask of reaction mixture was freely corked & kept in bulb oven at about 55-60°C for about 12 hrs. The resulting reaction mixture were neutralized by dilute HCl & poured into cold water. The obtained solid was filtered (with suction pump), washed with ice cold water, dried &recrystalized from ethanol to get 3-(3-bromo-4,5-dimethoxyphenyl)-1-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one.

General Method for the Synthesis of Isoxazolines

A mixture of simple and substituted hydroxyl chalcones and hydroxylamine hydrochloride in 2-ethoxy ethanol containing traces of acetic acid was refluxed for 5- 6 hrs. After completion of reaction, it was allowed to cool at room temperature and poured into ice cold water. The obtained solid was filtered and washed with cold water, dried and recrystallized form ethyl alcohol to obtain corresponding substituted isoxazoline derivatives.**(Scheme)**

The purity of synthesized isoxazolines was checked by TLC using petroleum ether/ethyl acetate (7:3) as the mobile phase on precoated sheets of silica gel-G using iodine vapour and UV lamp. Their structures were assigned by elemental and spectral analysis (IR, 1H NMR and MS).

Synthesis of 1-(5-(3-benzyloxy)-4-methoxyphenyl)-4, 5-dihydroisoxazol-3-yl) naphthalene-2-ol (4a).

A mixture of *3-(3-ethoxy-4-hydroxyphenyl)-1-(2-hydroxy naphthalene-1-yl) prop-2-en-1-one*(0.01mol) and hydroxylamine hydrochloride (0.02mol) was dissolved in 2-ethoxy ethanol (15ml), 2-3 drops of acetic acid was added and the reaction mixture was refluxed for 5-6 hrs. The progress of the reaction was monitored by TLC using petroleum ether/ethyl acetate (7:3) as the mobile phase. Reaction mixture then poured into cold water. The separated solid was filtered, washed with water, dried and then recrystallized from ethanol to 1-(5-(3-benzyloxy)-4-methoxyphenyl)-4, 5-dihydroisoxazol-3-yl) naphthalene-2-ol.

Synthesis of 1-(5-(3-bromo-4-hydroxy-5-methoxyphenyl)-4,5-dihydro-1,2-oxazol-3-yl) naphthalen-2-ol.(4b)

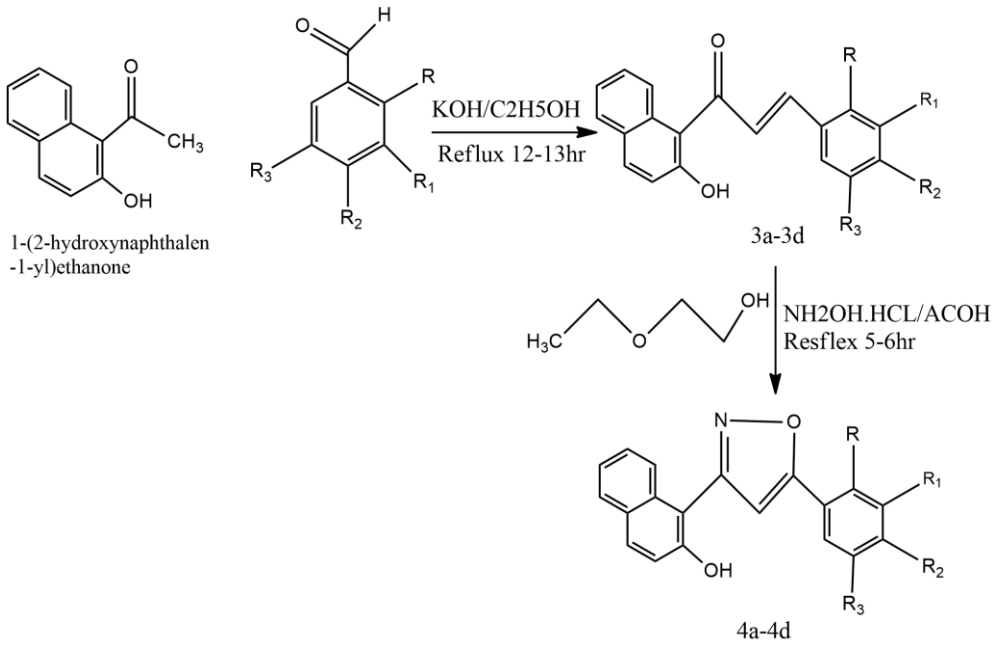
A mixture of *3-(3-bromo-4-hydroxy-5-methoxyphenyl)-1-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one*(0.01mol) and hydroxylamine hydrochloride (0.02mol) was dissolved in 2-ethoxy ethanol (15ml), 2-3 drops of acetic acid was added and the reaction mixture was refluxed for 5-6 hrs. The progress of the reaction was monitored by TLC using petroleum ether/ethyl acetate (7:3) as the mobile phase. Reaction mixture then poured into cold water. The separated solid was filtered, washed with water, dried and then recrystallized from ethanol to 1-(5-(3-bromo-4-hydroxy-5-methoxyphenyl)-4,5-dihydro-1,2-oxazol-3-yl) naphthalen-2-ol.

Synthesis of 1-(5-(3,4-dimethoxyphenyl)-4,5-dihydroisoxazol-3-yl)naphthalene-2-ol.(4c)

A mixture of *3-(3,4-dimethoxyphenyl)-1-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one*(0.01mol) and hydroxylamine hydrochloride (0.02mol) was dissolved in 2-ethoxy ethanol (15ml), 2-3 drops of acetic acid was added and the reaction mixture was refluxed for 5-6 hrs. The progress of the reaction was monitored by TLC using petroleum ether/ethyl acetate (7:3) as the mobile phase. Reaction mixture then poured into cold water. The separated solid was filtered, washed with water, dried and then recrystallized from ethanol to 1-(5-(3,4-dimethoxyphenyl)-4,5-dihydroisoxazol-3-yl)naphthalene-2-ol.

Synthesis of 1-(5-(3-bromo-4,5-dimethoxyphenyl)-4,5-dihydro-1,2-oxazol-3-yl)naphthalene-2-ol.(4d)

A mixture of *3-(3-bromo-4,5-dimethoxyphenyl)-1-(2-hydroxynaphthalen-1-yl)prop-2-en-1-one* (0.02mol) was dissolved in 2-ethoxy ethanol (15ml), 2-3 drops of acetic acid was added and the reaction mixture was refluxed for 5-6 hrs. The progress of the reaction was monitored by TLC using petroleum ether/ethyl acetate (7:3) as the mobile phase. Reaction mixture then poured into cold water. The separated solid was filtered, washed with water, dried and then recrystallized from ethanol to 1-(5-(3-bromo-4,5-dimethoxyphenyl)-4,5-dihydroisoxazol-3-yl)naphthalene-2-ol.



1-(2-hydroxynaphthalen-1-yl)ethanone

3a-3d

4a-4d

4a. R= H, R1= OCH2C6H5, R2 = OCH3, R3 = H
4b. R= H, R1= OCH3, R2 =OH, R3 = Br
4c. R= H, R1= OCH3, R2 = OCH3, R3 = H
4d. R= H, R1= OCH3, R2 = OCH3, R3 = Br

SCHEME

Antimicrobial activities

Antibacterial Activity

All the synthesised compounds were used for antibacterial tests. The pure culture of pathogenic bacteria used for antibacterial activity was sub cultured and characterised by standard method of identification.

The above mentioned quantities of peptone, beef extract and agar were mixed with two litres of double distilled water. The pH of this medium was adjusted at 6.8 with the help of 0.1 N hydrochloric acid and 0.1N sodium hydroxide. This medium was then transferred into conical flask, plugged and autoclaved at 121°C for 15 minutes. For further experimentations they were cooled and placed under aseptic conditions.

Pathogenicity Test - *Cup or Well Method* [13]

For evaluation of antimicrobial activity, several methods can be used such as turbidometric method, agar streak dilution method, paper disc method, serial dilution method, test tube method, cup or well method *etc*. For the present investigation cup or well method was used.

Nutrient agar medium was sterilized by autoclaved at 15 psi and 121°C for twenty minutes. Sterilized petri dishes were placed in laminar flow bench. One end of the lid of each petri dish was lifted and approximately 15- 20 ml. of molten agar medium was poured into it and left for solidification. These were then inoculated with 0.2 ml suspension of organism by spread plate method. Three or four wells of 12 mm diameter were made in the medium with the help of a sterile borer and filled with 50 ppm solution of testing compound in DMF.

Similarly other wells were made for standard drug and filled with standard concentration. These petri dishes were sealed with para film and incubated at 37°C in an incubator. The petri dishes were examined for zone of inhibition after 48 hr.

Antifungal Activity

Corning and borosil glasswares were used for the experimentation. All the glassware used during experiment were cleaned by dilute chromic acid followed by teepol. These were thoroughly washed with distilled water and were dried before being autoclaved at 180°C in hot air oven for almost 2 hours.

Preparation of a growth medium for fungi

Potato dextrose agar (PDA) medium was used as a growth medium. The medium consist of following ingredients and preparative method has been described in preceding paragraph

Peeled potato = 400 g
Dextrose = 40 g
Agar = 30 g
Distilled water = 2000 mL

Potato (400gm) was cut into small pieces and boiled in 2000mL distilled water till they can easily be penetrated by glass rod. After sieving through two fold muslin cloth the volume of extract was again made to 2000 mL. It was boiled further after mixing 30 g of agar and 40 g of dextrose and was again filtered.

Pathogenicity test [13]

All the fungal studies were performed using agar well assay method. The method was applied exactly in the same way as has been done in antibacterial study. Antifungal activities of all the synthesized compounds were studied by this method. For the sake of comparison process was repeated with the standard drug Gresiofulvin.

III. RESULTS AND DISCUSSION

Physical characteristics of the synthesized compound

Table-5.1. Physical and Analytical Data of Isoxazolines (4a-d)

Compound	Colour	Mol. Formulae	M.P. (0C)	% Yield	Elemental Analysis Found (in %)		
					C	H	X
4a	Greenish	C27H23O4N	175	83	76.21	5.44	---
4b	Yellow	C20H16O4NBr	180	84	57.97	3.86	19.32
4c	Yellow	C21H19O4N	189	82	72.20	5.44	---
4d	Faint Yellow	C21H18O4NBr	187	77	58.87	4.20	18.69

Spectral characteristics of synthesized compounds

Compound 4a:

IUPAC Name: 1-(5-(3-benzyloxy)-4-methoxyphenyl)-4, 5-dihydroisoxazol-3-yl) naphthalene-2-ol.

IR: ν max cm^{-1}:3406 (OH), 3026 (-CH2-), 1612, 1599, 1568 (Ar),1510(C=N), 1467 (C-O).

1H NMR (CDCl3) : δ 3.20 (dd, 1H, HA), 3.60 (dd, 1H, HB), δ 3.90 (s, 3H, OCH3), δ 4.50 (dd, 1H, HX),δ 5.10(s, 2H, OCH2), δ 6.80-8.00 (m, 9H Ar-H),δ 11.40 (s, 1H, OH).

MS (m/z) : 425(M+).

Compound 4b:

IUPAC Name: 1-(5-(3-bromo-4-hydroxy-5-methoxyphenyl)-4,5-dihydroisoxazol-3-yl)naphthalen-2-ol.

IR: ν max cm^{-1}:3452 (OH), 2824 (-CH3), 1641, 1631 (Ar),1564 (C=N), 1460(C-O), 723 (Ar-Br).

^{1}H NMR (CDCl3) : δ 3.25 (dd, 1H, HA), δ 3.55 (dd, 1H, HB),δ 3.95 (s, 3H, OCH3), δ 4.55 (dd, 1H, HX),δ 6.90-8.10 (m, 8H Ar-H), δ 11.70 (s, 1H, OH),δ 12.10 (s, 1H, OH).

MS (m/z):414(M+).

Compound 4c:

IUPAC Name: 1-(5-(3,4-dimethoxyphenyl)-4,5-dihydroisoxazol-3-yl)naphthalene-2-ol.

IR: ν max cm^{-1}:3373 (OH), 2929 (-CH3), 1612, 1599 (Ar),1512 (C=N), 1375 (C-O).

^{1}H NMR (CDCl3) : δ 3.10 (dd, 1H, HA), δ 3.30 (s, 3H, OCH3),δ 3.58 (dd, 1H, HB), δ 3.80 (s, 3H, OCH3),δ 4.40 (dd, 1H, HX), δ 6.85-8.00 (m, 9H Ar-H),δ 11.50 (s, 1H, OH).
MS (m/z):349(M+).
Compound 4d:

IUPAC Name: 1-(5-(3-bromo-4,5-dimethoxyphenyl)-4,5-dihydroisoxazol-3-yl)naphthalene-2-ol.
IR: ν max cm^{-1}:3383 (OH), 3047, 2854(-CH3), 1629, 1560, 1491(Ar),1467 (C=N), 1371 (C-O), 748 (Ar-Br).
^{1}H NMR (CDCl3) : δ 3.20 (dd, 1H, HA), δ 3.40 (s, 3H, OCH3),δ 3.60 (dd, 1H, HB), δ 3.90 (s, 3H, OCH3),δ 4.59 (dd, 1H, HX), δ 6.90-8.15 (m, 8H Ar-H),δ 11.50 (s, 1H, OH).
MS (m/z):428(M+).

Antimicrobial activity

Antibacterial Activity

In the present investigations, the antibacterial activity of newly synthesized substituted chalcones and their heterocyclic analogs was evaluated against some gram-positive bacterial strains like *Staphylococcus aureus, Bacillus subtilis,*and some gram-negative bacterial strains like *Escherichia coli,* and *Salmonella typhi* by using the cup well method.

Antifungal Activity

The antifungal activity of newly synthesized substituted chalcones and their heterocyclic analogues were evaluated against *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum* and *Fusarium moniliforme.*

Table 5.2: Antibacterial Activity of Isoxazolines

Sl. No.	Compounds	zone of inhibition in mm (MIC values µg mL-1)			
		Gram +ve bacteria		Gram –ve bacteria	
		S.aureus	*B.subtilis*	*E.coli*	*S.typhi*
1	4a	15 ± 0.9 (6.25)	16 ± 1.0 (6.25)	17 ± 1.0 (25)	16 ± 0.9 (25)
2	4b	20 ± 1.1 (6.25)	21 ± 1.0 (6.25)	22 ± 0.9 (12.5)	20 ± 0.8 (12.5)
3	4c	23 ± 1.0 (6.25)	24 ± 0.8 (6.25)	25 ± 1.0 (6.25)	22 ± 0.9 (6.25)
4	4d	18 ± 0.8 (6.25)	19 ± 0.9 (6.25)	20 ± 1.0 (12.5)	18 ± 1.0 (12.5)
5	Ofloxacin	25 (6.25)	27 (6.25)	29 (6.25)	26 (6.25)

Results were expressed Mean±SD, n=3

Table 5.3: Antifungal Activity of Isoxazolines

Sl. No.	Compounds	zone of inhibition in mm (MIC values µg mL-1)			
		A. niger	*A. flavus*	*P.chrysogenum*	*F.moniliforme*
1	4a	16 ± 0.7 (12.5)	18 ± 0.9 (12.5)	17 ± 1.0 (25)	19 ± 0.8 (25)
2	4b	20 ± 1.0 (6.25)	19 ± 0.8 (12.5)	21 ± 0.7 (6.25)	20 ± 0.9 (12.5)
3	4c	22 ± 0.9 (6.25)	21 ± 0.8 (6.25)	23 ± 1.0 (6.25)	21 ± 0.9 (6.25)
4	4d	15 ± 0.8 (12.5)	16 ± 1.0 (12.5)	18 ± 0.8 (12.5)	15 ± 0.9 (25)
5	Fluconazole	22 (6.25)	23 (6.25)	24 (6.25)	23 (6.25)

Results were expressed Mean±SD, n=3

From the screening studies, it is evident that the synthesized isoxazoline derivatives 4bshowed good antibacterial activity against all the tested organisms. It was further observed that the halogen compounds were better over other compound this could be due to effective binding in to the active site of the target where as electron rich compounds **4c** with –OMe substituent, showed best activity near to that of standard drug while compound **4a** shows less activity, it might be due to the benzyloxy group adjacent to –OMe substituent.
The synthesized isoxazolines were evaluated for antifungal activity using four fungal species and we found that the compounds **4c**was shown good antifungal activity same as that of standard drugs used whereas the compounds **4b**shows moderate antifungal activity. The compound **4d** were inactive in whole test.

CONCLUSION

The synthesized compounds (4a–4d) were evaluated for their antimicrobial potential against selected bacterial and fungal strains. The results indicate that all compounds exhibited noticeable activity, though with varying potency. Among the tested derivatives, **compound 4c consistently showed the most pronounced broad-spectrum antimicrobial activity**, displaying low MIC values (6.25 μg mL^{-1}) and larger zones of inhibition against both Gram-positive (*S. aureus*, *B. subtilis*) and Gram-negative (*E. coli*, *S. typhi*) bacteria. Its antifungal efficacy was also remarkable, with strong inhibition against *A. niger*, *A. flavus*, *P. chrysogenum*, and *F. moniliforme*, nearly comparable to the standard drug Fluconazole.

In contrast, **compounds 4a and 4d showed relatively weaker activity,** particularly against Gram-negative bacteria and certain fungal strains, suggesting limited spectrum coverage. **Compound 4b demonstrated moderate to good antimicrobial activity**, with improved inhibition against *P. chrysogenum* and Gram-positive bacteria.

Overall, these findings suggest that **compound 4c emerges as the lead molecule with potent and broad-spectrum antibacterial and antifungal activity**, making it a promising candidate for further optimization and development.

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