Synthesis and *in vitro* Antibacterial and Antifungal Activities of Benzoxazole Derivatives

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Summary: The *in vitro* antibacterial and antifungal activities of twenty-nine (29) benzoxazole derivatives were tested against fifteen *Gram*-positive and sixteen *Gram*-negative strains. Out of the twenty-nine compounds, eighteen compounds **3-5, 7-9, 11-13, 15-25** showed a broad range of activity against tested *Gram*-positive microorganisms, whereas rest of the compounds **6, 10, 14** and **26-31** were found to be completely inactive against all the tested strains of *Gram*-positive bacteria. Five compounds **8**, **11-13**, and **15** showed activities against *Gram*-negative strains whereas the rest were devoid of any activity. Twenty-one (21) out of twenty-nine (29) compounds possessed antifungal activity. The structures of the synthetic compounds were confirmed by IR, EIMS and ^{1}H-NMR spectral data.

Keywords: Benzoxazole, Antibacterial, Antifungal Activities.

Introduction

The number of acute infections caused by multidrug-resistant *Gram*-positive pathogens has reached an alarming level in hospitals and the neighborhood. Infections caused by these organisms pretense a serious challenge to the scientific community and the need for an effective therapy has led to a search for novel antibacterial agents [1-3].

Heterocycles considerably are the largest classical divisions of organic chemistry and are of enormous biological importance. Benzoxazole is a heterocyclic aromatic organic compound; it is an important pharmacophore and a privileged structure in medicinal chemistry. This compound is bicyclic in nature which consists of the fusion of benzene and oxazole.

The analogues of benzoxazole are known for their interesting biological activity long back. In the recent era, numbers of derivatives have been found to posses marked biological activities such as cyclooxygenase inhibiting [4], antiulcer [5], anticonvulsant [6], hypoglycaemic [7], antituberculor [8], antifungal [9], anti-inflammatory [10], antitumor [11] as well as anticancer activities [12].

In view of broad spectrum of resistant strains of microorganism there is an imperative need for the development of new antimicrobial agents to care for the patients infected with multidrug-resistant bacteria and fungi. In view of wide spectrum of resistant strains of microorganism there is an urgent need for the development of new antimicrobial agents to treat the patients infected with multidrug-resistant bacteria and fungi. In continuation of our earlier work on biologically active heterocyclic derivatives coumarin, chromones, imidazoles, oxadiazoles, thiadiazoles, triazoles, thiazolidine carboxylic acid and other closely related molecules [13-16], we synthesized twenty-nine derivatives of benzoxazoles and screened for their antibacterial activity against fifteen *Gram*-positive and sixteen *Gram*-negative bacterial strains. Eighteen compounds **3-5, 7-9, 11-13, 15-25** showed a broad range of activity against tested *Gram*-positive microorganisms whereas five compounds **8**, **11-13**, and **15** showed activities against *Gram*-negative strains. Twenty-one (21) out of twenty-nine (29) compounds found to possess antifungal activity.

Results and Discussion

Chemistry

To a solution of substituted benzaldehyde and 2-aminophenol, diethyl ether, silica gel were added and thoroughly mixed. Solvent was removed under reduced pressure on a rotary evaporator to

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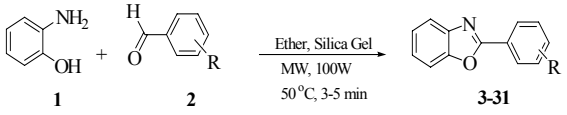
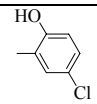
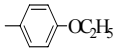
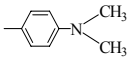
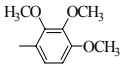
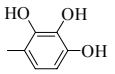
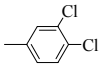
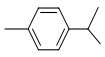
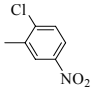
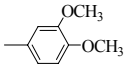
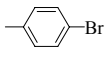
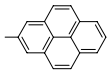
afford slurry. The solid supported reaction mixture was irradiated by microwaves which were operated at 100 W for 3-5 min at 50 °C. After cooling, the product was extracted with ethyl acetate. The extract was then filtered and the filtrate was evaporated under reduced pressure on a rotary evaporator to afford the crude product. The product was purified by recrystallization from MeOH (Scheme-1)



1 **2** **3-31**

S. No.	R	S. No.	R	S. No.	R
3		13		23	
4		14		24	
5		15		25	
6		16		26	
7		17		27	
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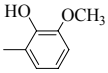
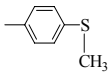
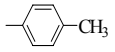
9		19		29	
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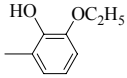
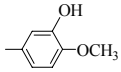
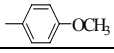
11		21		31	
12		22			

Scheme-1: Synthesis of Benzoxazole Derivatives.

Antibacterial Activity

Primary screening

Twenty-nine synthetic derivatives were screened for their antibacterial activity against fifteen *Gram*-positive *Staphylococcus aureus, Staphylococcus aureus Staphylococcus saprophyticus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus fecalis, Streptococcus pneumoniae, Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Micrococcus luteus, Micrococcus luteus, Corynebacterium xerosis, Corynebacterium hoffmanii* and *Corynebacterium diptherae* (Table-1) and sixteen Gram negative *Salmonella typhi, Salmonella typhi, Salmonella typhi para A, Salmonella typhi para B, Pseudomonas aeruginosa, Pseudomonas aeruginosa, Escherichia coli, Escherichia coli, Escherichia coli (MDR), Klebsiella pneumoniae, Shigella dysenteriae, Shigella flexeneri, Salmonella boydii, Proteus mirabilis, Proteus vulgaris* and *Enterobacter aerogenes* bacterial strains. The activity was determined *via* the growth inhibition of microorganisms *i.e.* the zone of inhibition was measured in millimetres. Ampiciline was used as standard and compounds with zone of inhibitions less than 10 millimetres were not considered as antibacterial agents.

Structure-Activity Relationship of Antibacterial

Eighteen compounds **3-5**, **7-9**, **11-13**, **12-25** showed a broad range of activity against tested *Gram*-positive microorganisms, whereas rest of the compounds **6**, **10**, **14**, and **26-31** were found to be completely inactive against all the tested strains of *Gram*-positive bacteria.

Compound **3** showed activity against five *Gram*-positive *S. aureus, Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Micrococcus luteus*. Compound **4** is active against six Gram positive *S. aureus, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Micrococcus luteus*. Compound **5** was active against five *Gram*-positive *S. aureus, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, and Bacillus thuringiensis*. Compound **7** was active against *Staphylococcus aureus, Micrococcus luteus, Micrococcus luteus, Corynebacterium xerosis, and Corynebacterium hoffmanii.* Compounds **8, 11** and **12** were active against *Staphylococcus aureus, Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Micrococcus luteus, Micrococcus luteus, Corynebacterium xerosis, and Corynebacterium hoffmanii*) Compound **9** was active against (*Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Micrococcus luteus, Corynebacterium xerosis, and Corynebacterium hoffmanii*. Compound **13** was active against *Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Micrococcus luteus, Micrococcus luteus,*

Corynebacterium xerosis, and Corynebacterium hoffmanii. Compound **15** was found to be active against nine strains of *Gram*-positive bacteria *Staphylococcus aureus, Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Corynebacterium xerosis, and Corynebacterium hoffmanii*. Compound **16** was active against *Staphylococcus aureus, Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis, Corynebacterium xerosis, Corynebacterium hoffmanii, and Corynebacterium diptherae*. Compound **17** was active against *Staphylococcus aureus, Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis, Bacillus subtilis, Corynebacterium xerosis, Corynebacterium hoffmanii, and Corynebacterium diptherae*. Compound **18** was active against only three strains of *Gram*-positive bacteria *Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis*. Compounds **19** and **20** showed activity against ten tested bacterial strains *Staphylococcus aureus, Staphylococcus aureus Staphylococcus saprophyticus, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Corynebacterium xerosis, Corynebacterium hoffmanii* and, *Corynebacterium diptherae*. Compound **21** was active against four *Gram*-positive bacterial strains *Staphylococcus aureus, Staphylococcus aureus, Staphylococcus saprophyticus,* and *Staphylococcus epidermidis*. Compounds **22** and **23** were effective against *Staphylococcus aureus, Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis,* and *Bacillus thuringiensis*. Compound **24** exhibited against only two strains *Staphylococcus saprophyticus,* and *Staphylococcus epidermidis*. Compound **25** showed against six tested strains *Staphylococcus aureus, Staphylococcus aureus, Bacillus cereus Bacillus thuringiensis, Corynebacterium xerosis,* and *Corynebacterium hoffmanii*.

Table-1: *In vitro* antibacterial activity of pure compounds 3-31 against *Gram*-positive bacteria (Zone of Inhibition in mm)

C.No	*S. aureus*	*S. aureus*	*S. saprophy-ticus*	*S. epidermidis*	*S. pyogenes*	*S. fecalis*	*S. pneumoniae*	*B. cereus*	*B. subtilis*	*B. thuringiensis*	*M. luteus*	*M. luteus*	*C. xerosis*	*C. hoff-manii*	*C. hoffmani-idiptherae*
3	11	00	00	00	00	00	00	15	16	12	00	10	00	00	00
4	11	15	00	00	00	00	00	15	14	13	00	10	00	00	00
5	19	15	00	00	00	00	00	16	14	12	00	00	00	00	00
6	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
7	00	10	00	00	00	00	00	00	00	00	12	18	14	10	00
8	18	16	20	15	00	00	00	16	17	14	11	30	19	11	00
9	00	18	17	15	00	00	00	14	15	14	00	15	12	10	00
10	00	00	00	00	00	00	00	00	00	00	00	10	00	00	00
11	09	10	13	10	00	00	00	15	15	13	12	19	18	12	00
12	20	16	20	17	00	00	00	16	14	13	13	18	17	10	00
13	00	00	00	00	00	00	00	10	12	12	11	11	17	11	00
14	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
15	19	10	21	14	00	00	00	10	10	10	00	00	13	12	00
16	15	16	18	12	00	00	00	00	00	00	00	00	12	12	13
17	22	18	22	16	00	00	00	00	10	00	00	00	14	13	14
18	10	00	17	13	00	00	00	00	00	00	00	00	00	00	00
19	21	14	28	18	00	00	00	10	20	11	00	00	14	12	14
20	18	10	22	16	00	00	00	11	10	12	00	00	13	15	15
21	16	13	17	14	00	00	00	00	00	00	00	00	00	00	00
22	24	17	30	18	00	00	00	12	11	10	00	00	00	00	00
23	14	10	18	15	00	00	00	00	12	11	00	00	00	00	00
24	00	00	22	16	00	00	00	00	00	00	00	00	00	00	00
25	15	10	00	00	00	00	00	11	00	10	00	00	13	12	00
26	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
27	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
28	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
29	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
30	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
31	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00

Key: 00 = No zone of inhibition

Structure-Activity Relationship of Antibacterial

Eighteen compounds **3-5**, **7-9**, **11-13**, **12-25** showed a broad range of activity against tested *Gram*-positive microorganisms, whereas rest of the compounds **6**, **10**, **14**, and **26-31** were found to be completely inactive against all the tested strains of *Gram*-positive bacteria.

Five compounds **8**, **11-13** and **15** showed activities against *Gram*-negative strains. Compounds **8, 11-13** all four compounds showed activity against *Salmonella typhi para A,* whereas compound **15** exhibited the activity against three tested *Gram*-negative strains *E. coli, E. coli* and *E. coli* (MDR). The results are shown in Table-2.

Minimum Inhibitory Concentration (MIC) Against Bacteria

The minimum inhibitory concentration (MIC) of seventeen derivatives **5, 7-9, 11-13** and **15-24** was determined using the disc diffusion method. The results are shown in Table-3 and it can be seen that compound **5** was active against three *Gram*-positive bacteria (*S. aureus, S. aureus,* and *B. cereus*. Compound **7** inhibited only *M. luteus* and compound **8** was effective against five *Gram*-positive strains (*S. aureus, S. aureus, S. saprophyticus, M. luteus,* and *Corynebacterium xerosis*). Compound **9** displayed activity against *S. aureus,* and *S. saprophyticus* and compound **11** was active against *M. luteus,* and *Corynebacterium xerosis*. Compound **12** was active against five strains *S. aureus, S. aureus, S. saprophyticus, S. epidermidis* and *Corynebacterium xerosis.* Compound **13** was found to be active against only *Corynebacterium xerosis*. Compound **15** exhibited against S. *aureus,* and *S. saprophyticus.* Compound **16** demonstrated activity against only one *Gram*-positive strain *S. saprophyticus*. Compound **17** exhibited antibacterial activity against *S. aureus, S. aureus,* and *S. saprophyticus*. Compound **18** showed activity only against *S. saprophyticus*. Compound **19** showed activity against *S. aureus, S. saprophyticus, S. epidermidis*. Compound **20** inhibits *S. aureus, S. saprophyticus.* Compound **21** displayed activity against only *S. saprophyticus.* Compound **22** was found to be active against four tested strains S. *aureus, S. aureus, S. saprophyticus,* and *S. epidermidis.* Compounds **23** and **24** both inhibited *S. saprophyticus.*

The minimum inhibitory concentration (MIC) of three derivatives **8, 11** and **12** was determined against sixteen *Gram*-negative bacteria and results are shown in Table-4. The results showed that compounds **8** and **12** exhibited good activity against *S. typhi para A,* however, compound **11** showed excellent activities against *Gram*-negative bacterial strains *S. typhi para A, E. coli* and *Klebsiella pneumoniae*.

Table-2: *In vitro* antibacterial activity of pure compounds 3-31 against *Gram*-negative bacteria (Zone of Inhibition in mm)

C. No.	*S. typhi*	*S. typhi*	*S. typhi para A*	*S. typhi para B*	*P. aeruginosa*	*P. aeruginosa*	*E. coli*	*E. coli*	*E. coli(MDR)*	*K. pneumoniae*	*S. dysenteriae*	*S. flexeneri*	*S. boydii*	*P. mirabilis*	*P. vulgaris*	*Entero-bacter*
3	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
4	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
5	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
6	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
7	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
8	00	00	20	00	00	00	00	00	00	00	00	00	00	00	00	00
9	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
10	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
11	00	00	19	00	00	00	00	00	00	00	00	00	00	00	00	00
12	00	00	21	00	00	00	00	00	00	00	00	00	00	00	00	00
13	00	00	14	00	00	00	00	00	00	00	00	00	00	00	00	00
14	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
15	00	00	00	00	00	00	10	10	11	00	00	00	00	00	00	00
16	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
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18	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
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21	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
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25	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
26	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
27	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
28	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
29	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
30	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
31	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00

Key: 00 = No zone of inhibition

Table-3: The MICs (μg/disc or mg/ml) of pure compounds against *Gram*-positive bacteria.

C.No.	*S. aureus*	*S. aureus*	*S. saprophyticus*	*S. epidermidis*	*S. pyogenes*	*S. fecalis*	*S. pneumoniae*	*B. cereus*	*B. subtilis*	*B. thuringiensis*	*M. luteus*	*M. luteus*	*C. xerosis*	*C. hoffmanii*	*C. diptherae*
5	**00**	**100**	**00**	**00**	**00**	**00**	**00**	**50**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
7	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**50**	**50**	**00**	**00**
8	**50**	**100**	**50**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**12.5**	**00**	**00**	**00**
9	**00**	**50**	**50**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**50**	**00**	**00**
11	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**50**	**50**	**00**	**00**
12	**50**	**100**	**50**	**50**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**50**	**00**	**00**
13	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
15	**25**	**00**	**50**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
16	**00**	**00**	**50**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
17	**25**	**50**	**25**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
18	**50**	**00**	**50**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
19	**00**	**00**	**12.5**	**50**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
20	**25**	**00**	**25**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
21	**00**	**00**	**50**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
22	**25**	**50**	**12.5**	**50**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
23	**00**	**00**	**50**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
24	**00**	**00**	**25**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
Ampicilin	**>10**	**>10**	**>10**	**>10**	**ND**	**ND**	**ND**	**50**	**>10**	**>10**	**>10**	**>10**	**>10**	**>10**	**>10**

Key: 00 = No zone of inhibition

Table-4: The MICs (μg/disc or mg/ml) of pure compounds against *Gram*-negative bacteria.

C.No.	*S. typhi*	*S. typhi*	*S. typhi para A*	*S. typhi para B*	*P. aeruginosa*	*P. aeruginosa*	*E. coli*	*E. coli*	*E. coli(MDR)*	*K. pneumoniae*	*S. dysenteriae*	*S. flexeneri*	*S. boydii*	*P. mirabilis*	*P. vulgaris*	*Entero-bacter*
8	**00**	**00**	**50**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
11	**00**	**00**	**50**	**00**	**00**	**00**	**00**	**50**	**00**	**50**	**00**	**00**	**00**	**00**	**00**	**00**
12	**00**	**00**	**25**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
Ampicilin	**>10**	**>10**	**00**	**>10**	**ND**	**00**	**00**	**00**	**00**	**ND**	**>10**	**00**	**>10**	**>10**	**>10**	**00**

Key: 00 = No zone of inhibition

In Vitro Antifungal Activity

Primary Screening

Twenty-nine compounds were screened for antifungal activity against fourteen fungal strains Table-5. The results showed that the compounds **3-5**, **8-9**, **16-20**, **23-26**, and **31** possessed broad spectrum of antifungal activity. Compounds **6, 7**, **10, 13-15**, **21-22, 27** and **30** not showed the zone of inhibition greater than 10 and considered to be inactive. Compounds **3-5, 8-9, 23-25** exhibited activities against two fungi *Candida albicans* and *Candida albicans*. Compound **3** was found to be active against six fungal strains *Saccharomyces cervisiae, Trichophyton rubrum*, *Trichophyton tonsurans, Trichophyton mentagrophyte*, *Microsporum canis* and *Microsporum gypseum*. Compound **4** was found to be active against *Candida albicans, Saccharomyces cervisiae, Trichophyton rubrum*, *Trichophyton tonsurans, Trichophyton mentagrophyte*, *Microsporum canis* and *Microsporum gypseum.* Compound **5** was active against *Candida albicans.* Compound **8** showed activity against five fungal strains *Candida albicans, Trichophyton rubrum*, *Trichophyton tonsurans, Trichophyton mentagrophyte*, *Microsporum canis.* Compounds **9** and **11** were active against *Candida albicans.* Compound **12** was active against four fungal strains *Trichophyton rubrum*, *Trichophyton tonsurans, Trichophyton mentagrophyte*, *Microsporum canis.* Compounds **16-18** were found to have potential against *Trichophyton rubrum*, *Trichophyton tonsurans,* and *Trichophyton mentagrophyte.* Compounds **19-20** both were found to be active against four strains of fungi *Trichophyton rubrum*, *Trichophyton tonsurans, Trichophyton mentagrophyte*, *Microsporum canis.* Compounds **23-24** were found to be active against *Candida albicans* and *Candida albicans*. Compound **25** have potential against *Candida albicans*, *Candida albicans, Trichophyton rubrum*, *Trichophyton tonsurans, Trichophyton mentagrophyte* and *Microsporum canis*. Compounds **26, 28-29** were found to be active against *Trichophyton mentagrophyte* and *Microsporum canis*. Compound **31** was found be active against *Microsporum canis.*

Minimum Inhibitory Concentration (MIC) Against Fungi

The minimum inhibitory concentration (MIC) of eleven derivatives **12, 16-20, 23-24 and 26-28** was determined by the drug incorporation method and the results are tabulated in Table-6. Compounds **23-24** were found to be active against *C. Albicans*. Compounds **12** and **20** showed inhibition against *T. rubrum, T. mentagrophyte* and *Microsporum canis.* Four compounds **16, 19, 27-28** were active against *T. tonsurans* and *T. mentagrophyte*. Compounds **17** and **26** were active against *T. mentagrophyte* and *Microsporum canis*, respectively. Compound **18** was active against *T. tonsurans* and *T. mentagrophyte.*

Table-5: *In vitro* antifungal activity of pure compounds (Zone of Inhibition in mm).

S. No.	*A. niger*	*A. flavus*	*Penicillium sp*	*H. hosporum*	*Fusarium sp*	*Rhizopus sp*	*C. albicans*	*C. albican*	*S. cervisiae*	*T. rubrum*	*T. tonsurans*	*T. mentagrophyte*	*M. canis*	*M gypseum*
3	00	00	00	00	00	00	00	00	10	17	13	15	13	12
4	00	00	00	00	00	00	11	00	10	17	13	15	13	12
5	00	00	00	00	00	00	12	00	00	00	00	00	00	00
6	00	00	00	00	00	00	00	00	00	00	00	00	00	00
7	00	00	00	00	00	00	00	00	00	00	00	00	00	00
8	00	00	00	00	00	00	13	00	00	17	17	13	16	00
9	10	00	00	00	00	00	15	00	00	00	00	00	00	00
10	00	00	00	00	00	00	00	00	00	00	00	00	00	00
11	00	00	00	00	00	00	14	00	00	00	00	00	00	00
12	00	00	00	00	00	00	00	00	00	13	17	18	18	00
13	00	00	00	00	00	00	00	00	00	00	00	00	00	00
14	00	00	00	00	00	00	00	00	00	00	00	00	00	00
15	00	00	00	00	00	00	00	00	00	00	00	00	00	00
16	00	00	00	00	00	00	00	00	00	15	16	17	00	00
17	00	00	00	00	00	00	00	00	00	14	15	19	00	00
18	00	00	00	00	00	00	00	00	00	13	17	20	00	00
19	00	00	00	00	00	00	00	00	10	14	15	19	20	00
20	00	00	00	00	00	00	00	00	00	12	17	17	19	00
21	00	00	00	00	00	00	00	00	00	00	00	00	00	00
22	00	00	00	00	00	00	00	00	00	00	00	00	00	00
23	00	00	00	00	00	00	18	12	00	00	00	00	00	00
24	00	00	00	00	00	00	17	12	00	00	00	00	00	00
25	00	00	00	00	00	00	16	14	00	14	15	13	12	00
26	00	00	00	00	00	00	00	00	00	00	00	16	19	00
27	00	00	00	00	00	00	00	00	00	00	00	00	00	00
28	00	00	00	00	00	00	00	00	00	00	00	18	24	00
29	00	00	00	00	00	00	00	00	00	00	00	20	23	00
30	00	00	00	00	00	00	00	00	00	00	00	00	00	00
31	00	00	00	00	00	00	00	00	00	00	00	00	30	00

Key: 00 = No zone of inhibition

Table-6: The MICs (μg/disc or mg/ml) of pure compounds against Fungi.

S. No.	*A. niger*	*A. flavus*	*Penicillium sp*	*H. hosporum*	*Fusarium sp*	*Rhizopus sp.*	*C. albicans*	*C. albicans*	*S. cervisiae*	*T. rubrum*	*T tonsurans*	*T. mentagrophyte*	*M. canis*	*M. gypseum*
12	00	00	00	00	00	00	00	00	00	00	50	50	50	00
16	00	00	00	00	00	00	00	00	00	00	00	50	50	00
17	00	00	00	00	00	00	00	00	00	00	00	25	00	00
18	00	00	00	00	00	00	00	00	00	00	50	25	00	00
19	00	00	00	00	00	00	00	00	00	00	00	50	25	00
20	00	00	00	00	00	00	00	00	00	00	50	50	50	00
23	00	00	00	00	00	00	50	00	00	00	00	00	00	00
24	00	00	00	00	00	00	50	00	00	00	00	00	00	00
26	00	00	00	00	00	00	00	00	00	00	00	00	50	00
27	00	00	00	00	00	00	00	00	00	00	00	50	25	00
28	00	00	00	00	00	00	00	00	00	00	00	25	25	00
Griseofulvin	**>200**	**>200**	**>200**	**>200**	**>200**	**>200**	**>200**	**>200**	**>200**	**>200**	**>200**	**>200**	**>200**	**>200**

Key: 00 = No zone of inhibition

Experimental

Sensitivity Test Procedure

The disc diffusion technique was adopted to determine the antibacterial activity of the test compounds [17, 18]. Sterile disc containing 200 μg of compound/disc were prepared. The sensi test agar (Oxoid, Hampshire, England) plates were seeded with 24 h old culture gown in trypsicase soya broth (TSB; Oxoid) containing 10^7 cfu mL^{-1} (cfu: colony forming units) using sterile cotton swabs to obtain a confluent lawn. The prepared discs were placed on to the surface at different positions and the plate were incubated at 37 $^{\circ}$C for 24 h the results were recorded by measuring the zones of inhibition in mm against each compound. Ampiciline was used as standard drug.

Minimum Inhibitory Concentration (MIC)

Compounds showing promising antibacterial activity (Zone of inhibition 10 to 30 mm) were selected for minimum inhibitory concentration studies. The minimum inhibitory concentration (MIC) was determined using the disc diffusion technique by preparing discs containing 10, 25, 50 and 100 μg/mL of the compounds and applying the protocol [17,18]. The tube dilution technique for determination of the MIC was not used as the compounds were incompatible with the ingredients of the medium.

Antifungal Activity

Antifungal activity of all compounds was studied against fourteen fungal cultures. Sabouraud dextrose agar (Oxoid) was seeded with 10^{-5} cfu mL^{-1} (cfu: colony forming units) fungal spore suspension

and transferred to Petri plates. Discs soaked with 20 μL (10 mg/mL in DMSO) of all compounds, were placed at different positions on the agar surface. The plates were incubated at 32 $^{\circ}$C for seven days. The results were recorded as zone of inhibition in mm [19].

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined against dermatophytes and other filamentous fungi by drug incorporation method [19] on Sabouraud dextrose agar (Oxoid). The agar slants containing varying concentrations (25, 50, 100, 200 and 300 μg/mL) of test compounds along with the parent compound were prepared and the slants were inoculated with the standard amount of test fungus and incubated at 30 $^{\circ}$C for 7 days and results were recorded.

Conclusion

Conclusively, present study reveals that benzoxazole derivatives may lead to good antimicrobial agent.

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