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Antibacterial and Antifungal Activities of Benzimidazole and Benzoxazole Derivatives

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The in vitro antibacterial and antifungal activities of six benzimidazole and benzoxazole derivatives were tested against standard strains and 59 clinical isolates. Of the six compounds, only compounds II and III (both benzoxazoles) were active, whereas the rest were devoid of any activity. Considerable growth inhibition of all of the standard strains, including fungi and gram-positive and gram-negative bacteria, resulted when they were treated with these compounds. Fifty-nine clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were tested for susceptibility to the two compounds. The most susceptible were the *S. aureus* isolates. The two compounds were of comparable activity against all of the isolates, with compound III showing a slightly higher activity than compound II. Their respective minimal inhibitory concentrations for 90% inhibition of *S. aureus* were 25 and 50 μg/ml. The gram-negative bacteria were resistant to the two compounds and required minimal inhibitory concentrations of 200 μg/ml for a similar degree of inhibition.

The antimicrobial activities of imidazoles and benzimidazoles have long been established. Derivatives of these compounds are known for their antibacterial (7, 14), trichomonacidal (1, 2), anthelmintic (3), fungicidal (5, 6, 13, 15), and antiviral (4, 8) activities. The success with these compounds stimulated the search for new biologically active derivatives. Several thousands of analogs of imidazole and benzimidazole have been synthesized and screened for pharmacological activity. Some of these compounds exhibited anticancer activity (10). In a recent review on the chemistry of imidazoles and benzimidazoles (9), a list of commercially available compounds and their therapeutic uses was included.

The objective of the present work was to investigate the antibacterial and antifungal activities of a benzimidazole derivative and five benzoxazole analogs of benzimidazole.

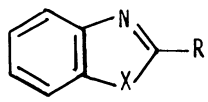
MATERIALS AND METHODS

Organisms. The sources of the standard strains used in this study, and their strain numbers, are as follows. *Escherichia coli* 10418, *Proteus vulgaris* 4635, *Proteus mirabilis* 3177, *Proteus rettgeri* 7475, *Pseudomonas aeruginosa* 10662, *Streptococcus faecalis*

TABLE 1. *Combined antibacterial and antifungal activities of benzimidazole derivatives and dimethylformamide*[a]

Organism	Diameter of inhibition zone (mm) for compound[a]:													
	I		II		III		IV		V		VI		DMF	
	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2
Staphylococcus aureus	11	15	40	51	47	57	15	10	17	12	18	11	10	17
Escherichia coli	–	–	15	20	19	25	–	–	–	–	–	–	–	–
Proteus vulgaris	10	21	30	45	35	42	11	19	12	24	12	19	15	24
P. mirabilis	–	16	18	36	19	35	–	17	–	17	–	17	–	16
P. rettgeri	10	20	31	40	34	40	13	20	13	21	13	21	15	20
Pseudomonas aeruginosa	–	–	12	16	18	20	–	–	–	–	–	–	–	–
Streptococcus faecalis	–	–	16	20	20	23	–	–	–	–	–	–	–	–
Mycobacterium fortuitum	–	–	24	30	26	29	–	–	–	–	–	–	–	–
Candida albicans	–	19	22	43	15	41	–	19	–	18	–	17	–	18
Saccharomyces cerevisiae	–	17	25	45	18	41	–	17	–	19	–	17	–	18
Aspergillus niger	–	11	19	37	15	37	–	10	–	11	–	12	–	12

[a] C_1, 5 mg/ml in 50% dimethylformamide (DMF); C_2, 10 mg/ml in 100% DMF; –, no inhibition.



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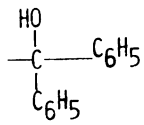
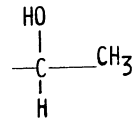
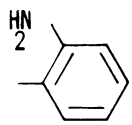
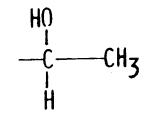
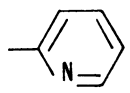
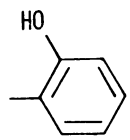
I	O	
II	O	
III	O	
IV	NH	
V	O	
VI	O	

FIG. 1. *Chemical structures of benzimidazole and benzoxazole derivatives.*

775 (enterococcus group D), *Staphylococcus aureus* 6571 (Oxford Hospital), *Mycobacterium fortuitum* 8573, and *Saccharomyces cerevisiae* 10716 were from the National Collection of Type Cultures Public Health Laboratory (London, United Kingdom). *Candida albicans* 3153 and *Aspergillus niger* 2022 were from the Mycological Reference Laboratory, London School of Hygiene (London). The 59 clinical isolates were obtained from King Abdulaziz University Hospital, Riyadh.

Media. For all bacterial strains brain heart infusion broth was used for subculture, and Mueller-Hinton agar was used for both growth inhibition studies and determination of minimum inhibitory concentrations (MICs). The media used for yeasts and fungi were Sabouraud glucose broth and agar, respectively.

Chemicals. The benzimidazole derivative and the five benzoxazoles were synthesized in the Chemistry Laboratory, College of Pharmacy, University of Riyadh (11; Zubair and Al-Badr, unpublished data). The structures of all the compounds are given in Fig. 1. Stock solutions (10 mg/ml) were prepared in dimethylformamide. Dilutions were made in sterile distilled water to give the working solutions.

Measurement of growth inhibition and of MICs. The diffusion technique generally used for antibiotic screening was adopted for growth inhibition studies; the diameter of the cup was 6 mm. The MICs were determined by the twofold agar dilution method (12).

RESULTS AND DISCUSSION

Preliminary values for growth inhibition of different microorganisms treated with benzimidazole and benzoxazole derivatives are shown in Tables 1 and 2. The values in Table 1 represent the combined activities of the compounds and solvent. The inhibitory activity of the solvent was subtracted from the joint activities, and the result is shown in Table 2. Only compounds II and III showed activity against all of the standard test organisms, including fungi and gram-positive and gram-negative bacteria. The two active compounds were benzoxazoles. The three other benzoxazoles and the benzimidazole derivative were devoid of activity against all of the organisms tested.

The majority of imidazoles, benzimidazoles, and their derivatives and analogs are used mainly as fungicides and anthelmintics (9). However, compounds I and II were active against both bacteria and fungi. The two compounds appeared to be more active against gram-positive bacteria than against gram-negative bacteria, with the exception of *Streptococcus faecalis*, which exhibited a susceptibility similar to that of the gram-negative bacteria. In a few instances, the diameters of the inhibition zones did not differ markedly with the two concentrations of the test compounds, e.g., for compound II against *P. vulgaris*, *P. mirabilis*, *P. rettgeri*, and *P. aeruginosa*. The inhibition zone increased with decrease in drug concentration on only one occasion, i.e., compound III against *P. vulgaris*.

Table 3 shows the MICs of compounds II and III against the standard organisms, and Table 4 shows the MICs of compounds II and III needed to inhibit 50, 75, and 90% of the clinical isolates. The standard organisms varied in their susceptibilities to the two compounds. *S. aureus* showed the lowest MIC, followed by *M. fortuitum*, the fungi, *S. faecalis*, and the gram-nega-

TABLE 2. *Antibacterial and antifungal activities of benzimidazole derivatives*[a]

Organism	Diameter of inhibition zone (mm) for compound:											
	I		II		III		IV		V		VI	
	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2
Staphylococcus aureus	–	–	30	34	37	40	–	–	–	–	–	–
Escherichia coli	–	–	15	20	19	25	–	–	–	–	–	–
Proteus vulgaris	–	–	15	21	20	18	–	–	–	–	–	–
P. mirabilis	–	–	18	20	19	19	–	–	–	–	–	–
P. rettgeri	–	–	16	20	19	20	–	–	–	–	–	–
Pseudomonas aeruginosa	–	–	12	16	18	20	–	–	–	–	–	–
Streptococcus faecalis	–	–	16	20	20	23	–	–	–	–	–	–
Mycobacterium fortuitum	–	–	24	30	26	29	–	–	–	–	–	–
Candida albicans	–	–	22	25	15	23	–	–	–	–	–	–
Saccharomyces cerevisiae	–	–	25	27	18	23	–	–	–	–	–	–
Aspergillus niger	–	–	19	25	15	25	–	–	–	–	–	–

[a] C_1, 5 mg/ml; C_2, 10 mg/ml; –, no inhibition. Values represent the activities of the drugs after subtracting the activity of the solvent.

TABLE 3. *MICs of compounds II and III against standard organisms*

Organism	Compound	MIC (μg/ml)
Escherichia coli NCTC 10418	II	100
	III	100
Proteus vulgaris NCTC 4635	II	100
	III	100
P. mirabilis NCTC 3177	II	100
	III	100
P. rettgeri NCTC 7475	II	100
	III	100
Pseudomonas aeruginosa NCTC 10662	II	200
	III	100
Streptococcus faecalis NCTC 775	II	100
	III	100
Staphylococcus aureus NCTC 6571	II	6.2
	III	6.2
Mycobacterium fortuitum NCTC 8573	II	25
	III	25
Saccharomyces cerevisiae NCTC 10716	II	25
Candida albicans 3153[b]	II	50
	III	50
Aspergillus niger 2022[b]	II	50
	III	50

[a] NCTC—National Collection of Type Cultures, London.

[b] From the Myocological Reference Laboratory, London School of Hygiene, London.

TABLE 4. *Activity of compounds II and III against clinical isolates*

Organism (no. of isolates)	Compound	MIC (μg/ml) to inhibit:			
		Range	50%	70%	90%
S. aureus (21)	II	6.2–200	25	25	50
	III	6.2–200	12.5	25	25
E. coli (26)	II	100–200	100	200	200
	III	100–200	100	200	200
P. aeruginosa (12)	II	100–200	200	200	>200
	III	100–200	200	200	>200

tive organisms. *P. aeruginosa* was the most resistant to compound II. The most susceptible of the isolates were those of *S. aureus*. The two compounds showed similar activities against all isolates, compound III being slightly more active than compound II against *S. aureus* isolates. Their MICs for 90% inhibition were 25 and 50 μg/ml, respectively. On the other hand, the gram-negative bacterial isolates were resistant to the two compounds, the MICs of which for 90% inhibition were equal to or greater than 200 μg/ml.

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