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Synthesis of Some Benzimidazole Derivatives and Their Antibacterial and Antifungal Activities

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A number of new benzimidazole derivatives were synthesized by the reaction of benzimidazole with appropriate alkyl halides. The compounds synthesized were identified by ^{1}H NMR, ^{13}C NMR, FT-IR spectroscopic techniques and elemental analysis. All compounds studied in this work were screened for their *in vitro* antimicrobial activities against the standard strains *i.e.*, *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and the yeasts *Candida albicans* and *Candida tropicalis*. Some of the compounds were found effective to inhibit the growth of Gram positive (*E. faecalis* and *S. aureus*) at MIC values between 50-400 µg/mL. None of the compounds exhibit antimicrobial activity against Gram negative bactreria (*E. coli* and *P. aeruginosa*) at the concentrations studied (6.25-800 µg/mL). Nine of the tested compounds showed antifungal activity with a range of the MICs between 50 and 400 µg/mL.

Key Words: Benzimidazole derivatives, Antibacterial activities, Antifungal activities, *in vitro* Studies.

INTRODUCTION

Benzimidazole derivatives constitute an important class of heterocyclic compounds for their versatile phamalogical activities such as antibacterial, antifungal, antihelmintic, antiallergic, antineoplastic, local analgesic, antihistaminic, antiulcer, vasodilative, hypotensive and spasmoliytic activities[1,2]. For example, omeprazole, which contains benzimidazole and pyridine, is the best selling antiulcer drug nowadays[3]. Like benzimidazole derivative, morpholine and piperidines derivatives also important heterocyclic compounds for their pharmacological activities[4-8]. Many pharmaceutical drugs contains a piperidine and morpholine rings. Because these groups tends to impart drug-like physical properties such as water solubility and bioavailability. Examples of drugs that contain piperidines include mesoridazine, thioridazine, haloperidol, droperidol, benperidol and risperidone. Piperidine is also a structural element of many pharmaceutical drugs such as raloxifene, minoxidil, thioridazine and mesoridazine. Piperidine is also commonly used as a strong base for the deprotection of amino acids in solid-

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phase peptide synthesis. Morpholine derivatives used as agricultural fungicides in cereals are known as Ergosterol Biosynthesis Inhibitors. Morpholine is also widely used in organic synthesis.

It is also observed that many benzimidazole derivatives and related heterocyclic compounds have shown considerable antimicrobial activities against standard strains; *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and the yeasts *Candida albicans* and *Candida tropicalis*[9-14].

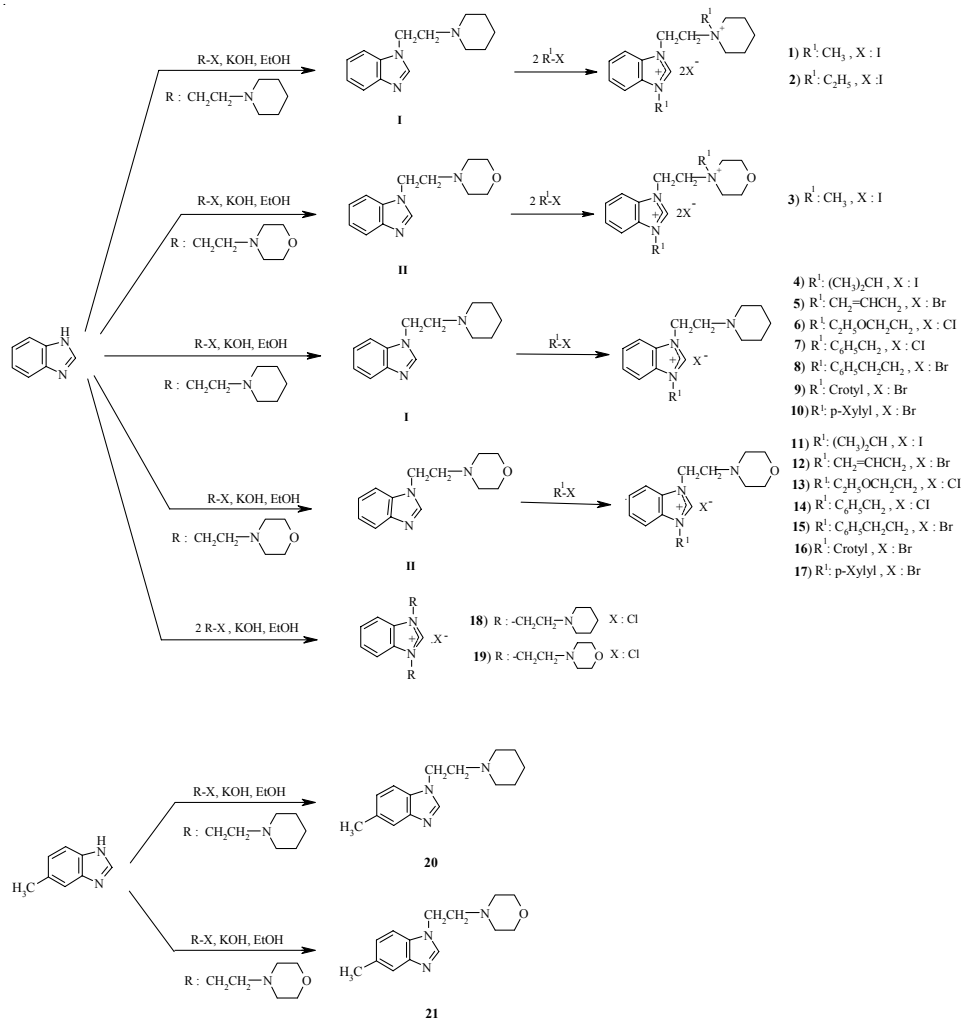
The aim of this study is to synthesize new benzimidazole derivatives contain morpholine and piperidine moiety with combination of the two active heterocyclic part in one compound and investigate their antibacterial and antifungal activities.

EXPERIMENTAL

All chemicals using in this study (except benzimidazole) were purchased from Aldrich Chemical Co. (Dorest, UK) or Merck (Darmstadt, Germany). ^{1}H NMR and ^{13}C NMR spectra (chemical shift in δ, ppm) were recorded using Bruker DPX-300 high performance digital FT-NMR (Bruker WM360, Bruker Instruments, Inc., Billercia, USA) spectrometers. Infrared spectra were recorded as KBr pellets in the range 4000-400 cm^{-1} on an ATI Unicam, Mattson 1000 spectrophotometer (Unicam Ltd., Cambridge, UK). Elemantal analysis was performed by the elemental analysis laboratory of The Scientific and Techonological Research Centre of Inonu University at Malatya (Turkey). Melting points were recorded using an electrothermal melting point apparatus, Electrothermal 9200 (Electrothermal Engineering Ltd., Essex, UK) and uncorrected.

General outline of synthesis: The reactions involve nucleophilic substitution between benzimidazole and appropriate alkyl halides according to the procedure indicated in **Scheme-I** and described in experimental section. Benzimidazole was synthesized from the reaction of 1,2-diaminobenzene and formic acid as reported in literature[15]. The compounds **1-4** were prepared by treating benzimidazole and appropriate alkyl halides similar to the literature procedure[16,17]. The compounds **5-21** were also synthesized from appropriate 1-alkylbenzimidazole and alkyl halides. The compounds **22** and **23** were synthesized from benzimidazole and two equivalent appropriate alkyl halides.

Preparation of 1-(2-piperidinoethyl)benzimidazole (1): To a mixture of benzimidazole (2.00 g; 16.9 mmol) and KOH (2.38 g, 42.5 mmol) in EtOH (20 mL) was added 2-chloroethylpiperidine hydrochloride (3.12 g, 16.9 mmol) and the mixture was heated under reflux for 8 h. The mixture was then cooled, after which potassium chloride was filtered off and washed with a little EtOH. The solvent was then removed from the filtrate *in vacuo*. The residue was treated with chloroform (10 mL) and the chloroform exract was washed with NaOH solution, then water. The volatiles were driven off *in vacuo* to give an oily residue. The residue was crystallized from Et_2O yield: 2.3 g, 59 %; m.p. 59 °C.



Scheme-I: Synthesis pathways of the new benzimidazole derivatives

Similar to compound **1**, compounds **2-4**, were synthesized treating benzimidazole or 5-metilbenzimidazole with appropriate alkyl halides.

Preparation of 1-(2-piperidinoethyl)-3-ethylbenzimidazolium iodide (6): A solution of 1-(2-piperidinoethyl)benzimidazole (1.00 g; 4.37 mmol) and ethyl iodide (0.70 mL, 8.74 mmol) in DMF (3 mL) was heated under reflux for 4 h. The mixture was then cooled to room temperature, after which the solvent was removed from the filtrate *in vacuo* and Et_2O (5 mL) was added. The precipitate was then crystallized from EtOH/Et_2O (2:1). Yield: 1.66 g, 70 %; m.p. 206-207 °C.

Similar to compound **6**, compounds **5**, **7** and **8** were synthesized from benzimidazole derivative with appropriate alkyl halides.

Preparation of 1-(2-piperidinoethyl)-3-isopropylbenzimidazolium iodide (9): A solution of 1-(2-piperidinoethyl)benzimidazole (1.00 g; 4.37 mmol) and isopropyl iodide (0.45 mL, 4.50 mmol) in DMF (2 mL) was heated under reflux for 4 h. The mixture was then cooled to room temperature, after which the solvent was then removed from the filtrate *in vacuo* and Et_2O (5 mL) was added. The precipitate was then crystallized from EtOH/Et_2O (2:1). Yield: 1.45 g, 83 %; m.p. 201-202 °C.

Similar to compound **9**, compounds **10-21** were synthesized from benzimidazole derivative with appropriate alkyl halides.

Preparation of 1,3-di-(2-morpholinoethyl)benzimidazolium chloride (23): A mixture of benzimidazole (2.00 g; 16.9 mmol) and KOH (2.85 g, 51 mmol) in EtOH (30 mL) was added 2-chloroethylmorpholino hydrochloride (6.30 g, 33.8 mmol) and the mixture was heated under reflux for 8 h. The mixture was then cooled, after which potassium chloride was filtered off and washed with a little EtOH. The solvent was then removed from the filtrate *in vacuo* and Et_2O (5 mL) was added. The precipitate was then crystallized from EtOH/Et_2O (2:1). Yield: 4.3 g, 66 %; m.p. 242-243 °C.

Similar to compound **23**, compound **22** was synthesized by treating benzimidazole with 2-chloroethylpiperidine hydrochloride.

Biological activity

Methods of antimicrobial testing: Antimicrobial activities of the compounds were determined by using agar dilution procedure outlined by the National Committee for Clinical Laboratory standards[16,17]. Minimal inhibitory concentrations for each compound were investigated against standard bacterial strains *i.e.*, *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and the yeasts *Candida albicans* and *Candida tropicalis* obtained from the Department of Microbiology, Faculty of Medicine Inönü Universty, Turkey. The stock solutions of the compounds were prepared in DMSO which had no effect on the microorganisms in the concentrations studied. All of the dilutions were done with distilled water. The concentrations of the tested compounds were 800, 400, 200, 100 µg/mL. Ampicilin and fluconazole from FAKO (Istanbul, Turkey) were used as a reference compound for the experimental conditions. A loopful (0.01 mL) of the standardized inoculum of the bacteria and yeasts (10^6 CFUs/mL) was spread over the surface of agar plates. All the inoculated plates were incubated at 35 °C and results were evaluated after 16-20 h of incubation for bacteria and 48 h for yeasts. The lowest concentration of the compounds that prevented visible growth was considered to be the minimal inhibitory concentration (MIC).

RESULTS AND DISCUSSION

The structure of all compounds synthesized were identified by ^{1}H NMR, ^{13}C NMR, FT-IR and micro analysis. Colour, yields, melting points or boiling points and analytical data of the newly synthesized compounds are given in Table-1. ^{1}H NMR spectral data are given in Table-2 and ^{13}C NMR spectral data in Table-3. The

antimicrobial and antifungal activity results (MIC) are given in Tables 4 and 5, respectively. Tables 4 and 5 also contain results for ampicillin and fluconazole as reference compounds.

TABLE-1
COLOUR, YIELDS, MELTING POINTS AND ANALYTICAL DATA OF SYNTHESIZED COMPOUNDS

Compd. No.	Colour	m.p. (ºC)	Yield (%)	Found (required) (%)		
				C	H	N
1	Yellow	209-210	86	37.12 (37.43)	4.77 (4.87)	7.99 (8.18)
2	White	206-207	70	39.34 (39.93)	5.08 (5.36)	7.53 (7.76)
3	Yellow	211-212	90	34.40 (34.95)	4.43 (4.46)	7.77 (8.15)
4	White	201-202	83	51.05 (51.12)	6.42 (6.51)	10.45 (10.52)
5	Cream	161-162	81	58.02 (58.28)	6.62 (6.85)	11.62 (12.00)
6	White	135-136	82	63.46 (63.98)	8.11 (8.29)	12.14 (12.44)
7	Cream	159-160	86	69.32 (70.86)	7.13 (7.31)	11.22 (11.81)
8	White	127-128	74	63.47 (63.75)	6.50 (6.76)	9.81 (10.14)
9	Cream	161-162	85	59.12 (59.33)	7.05 (7.14)	11.22 (11.53)
10	White	155-56	87	63.11 (63.75)	6.51 (6.76)	10.02 (10.14)
11	White	250-251	73	47.32 (47.88)	5.53 (5.98)	10.22 (10.47)
12	White	131-132	75	54.07 (54.54)	6.20 (6.25)	11.58 (11.93)
13	White	109-110	63	59.66 (60.07)	7.60 (7.65)	12.31 (12.36)
14	White	130-131	86	67.02 (67.11)	6.52 (6.71)	11.28 (11.74)
15	White	135-136	85	59.88 (60.56)	6.21 (6.24)	9.80 (10.09)
16	White	133-134	89	55.34 (55.73)	6.41 (6.55)	11.29 (11.47)
17	Cream	143-144	82	60.18 (60.56)	6.16 (6.24)	9.87 (10.09)
18	White	185-186	64	66.63 (66.93)	8.39 (8.76)	14.43 (14.87)
19	White	242-243	66	59.79 (59.92)	7.60 (7.62)	14.48 (14.71)
20	Cream	181/1.2mm Hg	63	74.02 (74.07)	8.58 (8.64)	17.02 (17.28)
21	Cream	172/1.2mm Hg	60	68.16 (68.57)	7.52 (7.75)	17.02 (17.14)

In this work, 21 benzimidazole derivatives were synthesized and tested against standard strains of Gram-positive and Gram-negative bacteria and yeasts. As can be seen in Table-4, the compounds **6**, **7**, **9**, **10**, **11**, **12**, **18**, **19** and **20** were found effective in inhibiting the growth of Gram-positive bacteria with MICs values between 50-400 µg/mL. None of the compounds studied here showed considerable antimicrobial activity against Gram-negative bacteria *E. coli* and *P. aeruginosa*. On the other hand, the compounds **1**, **2**, **4**, **7**, **9**, **10**, **11**, **12**, **17**, **18**, **19** and **21** were found effective against *C. tropicalis* with MIC values of 50-400 µg/mL. These compounds also showed activity *C. albicans* with a range of MICs between 100-400 µg/mL. Among the tested compounds **9**, **10**, **11**, **12**, **18**, **19** and **21** were the most effective compounds with MICs 50 µg/mL against *C. tropicalis*. These compounds also showed considerable antimicrobial activity against Gram-positive bacteria and *C. albicans*. From the data obtained in this work, it is suggested that the 2-ethoxyethyl, benzyl, 2-phenylethyl, *p*-xylyl and crotyl moiety may play a crucial role in the antimicrobial activity when attached to the nitrogen atom of the benzimidazole moiety.

TABLE-2
^{1}H NMR SPECTROSCOPIC DATA, WITH ASSIGNMENTS,
FOR THE NEW COMPOUNDS

Compd. No.	R^1	5-CH_3-benzim.	CH_2CH_2-ring(R)	Ring	2-CH	Ar-H
1	3.2(s), 4.1(s)	-	4.0(t), 5.1(t)	1.8(t), 3.5(t)	9.4(s)	7.7-7.9(m)
2	1.3(t), 1.5(t)	-	3.9(t), 5.0(t)	1.9(t), 3.5(t)	9.5(s)	7.7-7.9(m)
3	3.4(s), 4.0(s)	-	4.0(t), 5.1(t)	3.6(s), 4.1(s)	9.5(s)	7.7-7.9(m)
4	1.6(t), 5.0(t)	-	3.7(t), 4.9(t)	1.8(t), 3.3(t)	9.5(s)	7.6-7.9(m)
5	3.95(s), 5.2(q), 6.1(m)	-	3.5(t), 4.6(t)	1.6(t), 3.1(t)	9.4(s)	7.6-7.9(m)
6	1.0(t), 3.0(t), 3.5(t)	-	3.9(t), 4.6(t)	1.4(t), 2.6(t)	9.3(s)	8.6-8.9(m)
7	5.8(s), 7.4(m)	-	2.8(t), 4.7(t)	1.4(t), 2.4(t)	10.1(s)	7.6-8.1(m)
8	3.4(s), 7.2(s)	-	3.2(t), 4.9(t)	1.4(t), 2.5(t)	9.9(s)	7.6-8.0(m)
9	1.6(t), 5.0(t),5.7(t),6.0(q)	-	3.6(t), 4.9(t)	1.7(t), 3.2(t)	9.4(s)	8.6-8.9(m)
10	2.1(s), 5.4(s), 7.1(m)	-	3.1(t), 4.5(t)	1.8(t), 3.1(t)	9.3(s)	7.6-7.9(m)
11	1.6(t), 5.0(s)	-	3.8(t), 4.9(t)	3.4(s), 3.9(s)	9.5(s)	7.6-7.9(m)
12	4.1(s), 5.1(q), 6.0(m)	-	3.6(t), 4.9(t)	3.2(s), 3.8(s)	9.5(s)	7.7-7.9(m)
13	1.0(t), 3.4(t), 5.1(s)	-	3.8(t), 4.7(t)	3.5(s), 3.9(s)	10.1(s)	7.7-8.2(m)
14	5.6(s), 7.3(m)	-	3.0(t), 4.6(t)	2.6(s), 3.6(s)	9.3(s)	7.5-7.7(m)
15	3.2(t), 5.1(t),7.3(m)	-	3.8(t), 4.8(t)	3.6(s), 3.9(s)	10.3(s)	7.7-8.1(m)
16	1.7(d), 5.1(d), 5.8(t), 6.1(q)	-	3.7(t), 5.0(t)	3.4(s), 3.9(s)	10.0(s)	7.7-7.9(m)
17	2.2(s), 5.5(s), 7.2(m)	-	3.3(t), 4.7(t)	2.9(s), 3.7(s)	9.3(s)	7.6-7.8(m)
18	–	-	2.7(t), 4.7(t)	1.4(t), 2.4(t)	9.9(s)	7.7-8.1(m)
19	–	-	3.8(t), 5.1(t)	3.4(s), 3.9(s)	10.6(s)	8.0-8.2(m)
20	–	2.5(s)	2.6(t), 4.2(t)	1.4(t), 2.4(t)	8.1(s)	7.2-7.9(m)
21	–	2.4(s)	2.5(t), 4.1(t)	2.3(s), 3.5(s)	8.1(s)	7.1-7.9(m)

Chemical shifts (δ) relative to $Si(CH_3)_4 = 0$; abbreviations, s = singulet, d = doublet, t = triplet, q = quarted, m = multiplet, spectra for compounds **1-19** recorded in DMSO-d_6, for compounds **20-21** recorded in $CDCl_3$.

TABLE-3
^{13}C NMR SPECTROSCOPIC DATA, WITH
ASSIGNMENTS, FOR THE NEW COMPOUNDS

Compd. No.	^{13}C-NMR spectroscopic data
1	19.25, 20.31, 33.94, 48.24, 58.15, 60.85, 113.76, 118.97, 122.59, 126.68, 130.54, 131.65, 143.49
2	7.45, 13.93, 18.99, 20.45, 22.40, 42.52, 52.50, 53.65, 54.24, 58.39, 113.76, 126.75, 130.77, 130.88, 142.64
3	33.89, 34.24, 47.89, 52.01, 54.01, 60.20, 63.92, 113.91, 114.25, 127.19, 131.04, 131.23, 132.17, 143.85, 144.00
4	21.23, 21.58, 22.68, 50.74, 52.64, 53.16, 113.93, 114.13, 126.61, 130.41, 130.94, 131.16, 141.41
5	19.06, 22.59, 24.96, 43.55, 46.42, 53.46, 55.71, 58.13, 69.15, 110.49, 113.85, 119.33, 126.44, 130.88, 143.10
6	20.48, 23.06, 43.54, 46.42, 53.46, 55.71, 58.13, 69.07, 110.48, 113.75, 119.32, 126.34, 130.88, 143.09, 144.15
7	23.56, 25.33, 49.53, 49.90, 53.49, 113.84, 126.51, 128.20, 128.86, 130.57, 131.18, 134.07, 142.94
8	23.34, 24.89, 34.73, 47.61, 53.46, 113.69, 126.37, 126.83, 128.47, 128.79, 130.91, 136.87, 142.51

9	13.28, 17.57, 21.36, 22.51, 43.73, 48.46, 52.50, 53.48, 113.89, 121.99, 123.26, 126.56, 130.99, 132.94, 142.83
10	21.32, 47.38, 49.18, 54.37, 54.78, 63.18, 114.62, 128.36, 130.12, 131.48, 131.94, 132.11, 139.14, 142.71
11	21.56, 50.83, 51.48, 53.17, 63.27, 114.03, 114.16, 126.66, 130.46, 131.14, 141.52
12	17.15, 41.19, 46.56, 52.30, 53.34, 62.92, 113.98, 121.93, 123.16, 126.57, 131.00, 131.75, 133.07, 142.98
13	14.79, 23.36, 24.91, 43.48, 46.73, 53.46, 55.79, 65.56, 67.10, 113.76, 113.93, 126.30, 131.01, 143.00
14	43.03, 49.56, 52.33, 55.09, 65.47, 113.96, 125.55, 126.49, 126.85, 128.52, 128.90, 130.14, 131.11, 134.28, 143.30
15	34.31, 47.97, 51.37, 53.43, 63.01, 113.86, 126.33, 128.46, 128.81, 130.62, 131.13, 136.94, 142.13, 143.10
16	13.32, 17.60, 43.81, 48.56, 51.30, 53.34, 62.92, 113.98, 121.93, 123.16, 126.57, 131.00, 131.75, 133.07, 142.98
17	20.67, 49.49, 49.76, 52.37, 54.99, 65.23, 113.92, 126.56, 128.38, 129.42, 130.58, 130.94, 131.14, 138.14, 142.80
18	19.20, 23.76, 25.45, 43.97, 53.81, 56.56, 59.36, 113.74, 126.23, 131.02, 142.71
19	51.27, 53.47, 63.08, 111.87, 113.89, 117.41, 123.97, 126.60, 131.07, 143.18, 144.43
20	21.51, 24.15, 25.94, 42.61, 54.72, 57.99, 109.15, 114.87, 115.52, 119.57, 123.70, 124.33, 137.91, 140.99
21	21.47, 42.15, 53.37, 57.43, 66.63, 109.18, 114.73, 115.78, 119.41, 123.81, 131.75, 137.21, 140.71, 142.92

Chemical shifts (δ, ppm) relative to $Si(CH_3)_4 = 0$; spectra for compounds **1-19** recorded in DMSO-d_6, for compounds **20-21** recorded in $CDCl_3$.

TABLE-4
MINIMUM ANTIBACTERIAL INHIBITORY CONCENTRATIONS (μg/cm^3)
OF THE TESTED COMPOUNDS

Compd. No.	Tested microorganisms*			
	E. faecalis	*S. aureus*	*E. coli*	*P. aeruginosa*
Ampicillin	0.78	0.39	3.12	>75
1	>800	>800	>800	>800
2	>800	400	800	800
3	>800	>800	>800	>800
4	>800	400	>800	800
5	>800	800	800	800
6	800	100	800	>800
7	100	50	800	>800
8	200	50	800	>800
9	800	400	>800	>800
10	>800	>800	>800	>800
11	>800	>800	>800	>800
12	>800	800	>800	800
13	>800	>800	>800	>800
14	>800	200	>800	>800

15	400	50	>800	>800
16	>800	400	>800	>800
17	>800	800	>800	800
18	>800	>800	>800	>800
19	>800	>800	>800	>800
20	>800	>800	>800	800
21	800	800	>800	>800

TABLE-5
MINIMUM ANTIFUNGAL INHIBITORY CONCENTRATIONS (μg/cm^3)
OF THE TESTED COMPOUNDS

Compd. No.	Tested organism		Compd. No.	Tested organism	
	C. albicans	*C. tropicals*		*C. albicans*	*C. tropicals*
Fluconzalo	1.25	1.25	**11**	800	800
1	800	800	**12**	800	800
2	800	800	**13**	800	800
3	800	800	**14**	400	50
4	400	400	**15**	200	50
5	800	800	**16**	800	400
6	400	50	**17**	400	50
7	100	50	**18**	800	800
8	400	50	**19**	800	800
9	200	50	**20**	800	800
10	400	100	**21**	200	200

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