Research Article

Evaluation of Benzophenone-N-ethyl Morpholine Ethers as Antibacterial and Antifungal Activities

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Microorganisms are closely associated with the health and welfare of human beings. Whereas some microorganisms are beneficial, others are detrimental. Bacterial infections often produce inflammation and pains and in some instances, infections result in high mortality. Any subtle change in the drug molecule, which may not be detected by chemical methods, can be revealed by a change in the antimicrobial activity and hence microbiological assays are very useful. A series of substituted hydroxy benzophenones and benzophenone-N-ethyl morpholine ethers were screened for their antibacterial and antifungal activities. Antibacterial activity against *S. aureus*, *E. aerogenes*, *M. luteus*, *K. pneumonia*, and *S. typhimurium*, *S. paratyphi-B* and *P. vulgaris* bacterial strains and antifungal activity against *C. albicans*, *B. cinerea*, *M. pachydermatis*, *C. krusei* fungal strains were carried out. The bioassays indicated that most of the synthesized compounds showed potential antibacterial and antifungal agents.

1. Introduction

Bacteria are extremely pathogenic causing infection and widespread use of commercially available antibiotics led to the developed resistance or ability to produce substance which block the action of antibiotics or change their target and produce undesirable side effects [1]. Although, a number of different classes of antibacterial and antifungal agents have been discovered during the last two decades, the use is limited due to development of microbial resistance [2]. In recent decades, problems of multidrug-resistant microorganisms have reached an alarming level in many countries around the world. Resistance to a number of antimicrobial agents (b-lactam antibiotics, macrolides, quinolones, and vancomycin) among variety of clinically significant species of bacteria is becoming increasingly important global problem. Also a number of recent clinical reports describe the increasing occurrence of methicillin the resistant *Staphylococcus aureus* (MRSA) which is the most disturbing cause of nosocomial infections in developed countries [3, 4]. Infections caused by these microorganisms pose a serious challenge to the medicinal community and the need for an effective therapy has led to search for novel antimicrobial agents.

Phenolic compounds are commonly known for their antioxidant, anti-inflammatory, and antimicrobial activities [5]. During the past years extensive evidences have been accumulated to establish the efficiency of benzophenone analogues as antimicrobial agent [6–10]. Benzophenone analogue (garcinol) has been isolated from the stem bark of Garcinia *huillensis* grown in Zaire and used in central-African traditional medicine and also extracts obtained from Rheedia brasiliensis fruit (bacupari) containing bioactive compound of benzophenone analogous which showed activity against Streptococcus mutans at low concentrations (1.25–2.5 μg/mL). Also prenylated benzophenone isolated from roots of Cudrania cochinchinensis (Moraceae) was tested for its antimicrobial activities against vancomycin-resistant enterococci. This has exhibited chemotherapeutical activity against Gram-positive and Gram-negative *cocci*, mycobacteria, and fungi [11–13]. Recently Selvi et al. have

shown antifungal activity of benzophenone analogues, at its lower concentration [14]. Besides chloro substituted benzophenones have exhibited more antifungal activity [15].

The pharmacological effect of morpholine compounds in different biological fields is of great importance for researchers and investigators. Morpholine derivative plays an important role in the treatment of several diseases. Heterocyclic ring systems having morpholine nucleus have aroused great interest in recent years due to their variety of biological activities [16]. Morpholine derivatives were reported to possess antimicrobial activity [17, 18] and inflammatory activities in albino rats [19]. Morpholine derivatives find their wide spectrum of antimicrobial activity and exhibit anthelmintic, bactericidal, and insecticidal activity [20, 21].

Another important pharmacophore group of morpholine nucleus incorporated in a wide variety of therapeutically important drugs, one of which is linezolid (PNU-10766, commercially available antimicrobial drug), possess 4-(2-fluorophenyl) morpholine moiety [22, 23]. In addition, series of benzophenone derivatives, some of which contained a morpholine group possess good antifungal and antibacterial activities [24].

In continuation of our studies for the synthesized compound [19], we report herein antibacterial and antifungal activities of benzophenone-N-ethyl morpholine ethers.

2. Experimental Section

2.1. Chemistry: Materials and Methods. IR spectra were recorded in Nujol on FT-IR Shimadz 8300 spectrophotometer, ^{1}H NMR spectra were recorded on a Bruker 300 MHz NMR spectrophotometer in CDCl$_3$, and chemical shift was recorded in parts per million down field from tetramethylsilane. Mass spectra were obtained with a VG70-70H spectrophotometer and important fragments are given with the relative intensities in the brackets. Elemental analysis results are within 0.4% of the calculated value.

2.2. General Procedure for the Synthesis of Substituted Benzophenone-N-ethyl Morpholine Ethers (5a–j). Substituted 2-methylphenyl benzoates (**3a–j**) were synthesized by stirring 2-methyl phenol (1, 1 equivalent) with corresponding benzoyl chlorides (**2a–j**, 1 equivalent) for 30 minutes in alkaline medium using 10% sodium hydroxide solution with excellent yield. Substituted hydroxyl benzophenones (**4a–j**) were synthesized by refluxing a mixture of **3a–j** (1 equivalent) and anhydrous aluminum chloride (1.5 equivalents) for 20 minutes. Condensation of **4a–j** (1 equivalent) with 4-(2-chloroethyl) morpholine hydrochloride (1 equivalent) for 3 hours in presence of anhydrous potassium carbonate (1.5 equivalents) and dimethyl sulphoxide furnished substituted benzophenone-N-ethyl morpholine ethers (**5a–j**) with good yield. The compounds **5a-j** were characterized by IR, ^{1}H NMR, and mass spectrophotometer [19].

2.3. Biology: Materials and Methods for the Antimicrobial Activity. Streptomycin and ciprofloxacin (Sigma) were used as positive controls against bacteria. Fluconazole and ketoconazole (Himedia, Mumbai) were used as positive controls against fungi.

2.3.1. Tested Microbes. The following gram-positive bacteria were used for the experiments; *S. aureus*, *S. aureus* (MRSA), *E. aerogenes*, and *M. luteus*. The gram-negative bacteria included *K. pneumoniae*, *S. typhimurium*, *S. paratyphi-B*, and *P. vulgaris*. In addition, fungi *C. albicans*, *B. cinerea*, *M. pachydermatis*, and *C. krusei* were also used for the experiments. All cultures were obtained from the Department of Microbiology, Manasagangotri, Mysore.

2.3.2. Preparation of Inoculums. Bacterial inoculums were prepared by growing cells in Mueller Hinton Broth (MHA) (Himedia) for 24 h at 37°C. These cell suspensions were diluted with sterile MHB to provide initial cell counts of about 104 CFU/mL. The filamentous fungi were grown on sabouraud dextrose agar (SDA) slants at 28°C for 10 days and the spores were collected using sterile doubled distilled water and homogenized.

2.3.3. Disc Diffusion Assay. Antibacterial activity was carried out using a disc diffusion method [25]. Petri plates were prepared with 20 mL of sterile Mueller Hinton Agar (MHA) (Himedia, Mumbai). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 mins. The tests were conducted at 1000 μg/disc. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Streptomycin (10 μg/disc) was used as positive control. The plates were incubated for 24 h at 37°C for bacteria and 48 h at 27°C for fungi. Zone of inhibition was recorded in millimeters and the experiment was repeated twice.

2.3.4. Minimum Inhibitory Concentration (MIC). Minimum inhibitory concentration studies of synthesized compounds were performed according to the standard reference method for bacteria [26] and filamentous fungi [27]. Required concentrations (1000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL, 62.5 μg/mL, 31.25 μg/mL, and 15.62 μg/mL) of the compound were dissolved in DMSO (2%) and diluted to give serial two-fold dilutions that were added to each medium in 96 well plates. An inoculum of 100 mL from each well was inoculated. The antifungal agent's ketoconazole, fluconazole for fungi and streptomycin, and ciprofloxacin for bacteria were included in the assays as positive controls. For fungi, the plates were incubated for 48–72 h at 28°C and for bacteria the plates were incubated for 24 h at 37°C. The MIC for fungi was defined as the lowest extract concentration, showing no visible fungal growth after incubation time. 5 ml of tested broth was placed on the sterile MHA plates for bacteria and incubated at respective temperatures. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate.

TABLE 1: In vitro antibacterial activity of compounds **5a–j**.

| Compounds | Zone of inhibition in mm | | | | | | | |
| | Gram-positive bacteria | | | | Gram-negative bacteria | | | |
	S. aureus	*S. aureus* (MRSA)	*E. aerogenls*	*M. luteus*	*K. pneumonia*	*S. typhimurium*	*S. Paratyphi-B*	*P. vulgaris*
5a	10	9	9	11	9	11	8	12
5b	13	11	10	11	9	10	8	9
5c	15	12	14	13	12	15	13	11
5d	16	12	14	12	10	17	8	13
5e	20	18	22	24	25	26	29	28
5f	24	14	19	17	15	24	9	18
5g	25	15	15	19	14	21	13	17
5h	23	12	18	19	12	21	9	14
5i	19	9	12	15	14	17	11	12
5j	11	8	9	12	11	10	8	9
Streptomycin	16	20	21	24	21	22	17	23

TABLE 2: In vitro antifungal activity of compounds **5a–j**.

| Compounds | Zone of inhibition in mm | | | |
	C. albicans	*B. cinerea*	*M. pachydermatis*	*C. krusei*
5a	8	9	10	11
5b	9	8	10	7
5c	14	10	13	13
5d	10	9	15	14
5e	18	16	21	23
5f	14	10	11	16
5g	9	12	13	19
5h	13	15	11	14
5i	12	10	13	20
5j	10	9	12	15
Ketoconazole	21	11	25	17

3. Result and Discussion

The reaction sequence for the title compounds is outlined in Scheme 1. Compounds **5a–j** have been prepared as previously reported by our group [19]. The antimicrobial activities of synthesized compounds were screened against eight bacteria and four fungi using in vitro disc diffusion method. The results revealed that most of the synthesized compounds exhibited antimicrobial activities against *Staphylococcus aureus*, *Staphylococcus aureus* (MRSA), *Enterobacter aerogenes*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Salmonella paratyphi*-B, *Proteus vulgaris*, *Candida albicans*, *Botyritis cinerea*, *Malassesia pachydermatis*, and *Candida krusei* organisms. The results are summarized in Tables 1 and 2. Compounds **5e, 5f, 5g, 5h**, and **5i** showed good activity more than standard drug against *S. aureus*. Compound **5e** with chlorogroup at meta position in phenyl ring showed good activity against both Gram-positive and

Gram-negative bacteria among all synthesized compounds compared with the standard. Among compounds **5b–d** in which methyl group is substituted at para, ortho, and meta position, respectively, compound **5d** showed good activity against *S. aureus* and *S. typhimurium* Compound **5e** showed significant antifungal activity against *B. cinerea* and *C. krusei* and compound **5h** with fluoro group at para position showed more activity against *B. cinerea*. Similarly compound **5g** with bromo group at para position and **5i** with two chloro groups at ortho and para position showed more activity against *C. krusei* compared to standard drug. In contrast, compounds **5a** with methoxy group, **5b–d** with methyl group, and **5j** without any substituent exhibited lowest activity and this can be attributed to the electron releasing effect. The MIC values of active compounds **5c–i** against bacteria and fungi are given in Tables 3 and 4. Significant MIC values were observed against Gram-positive and Gram-negative bacteria. Compounds **5d–h** showed good activity against *S. aureus*. In comparison to compound **5i**, in compounds **5d** and **5e,** the potency against *S. aureus* has been increased by onefold. Interestingly the presence of halo group in phenyl ring in compounds **5g** and **5h** increased the potency against *S. aureus* by twofold. In comparison to compound **5i** in compound **5e** the potency is increased by twofold against bacteria *S. paratyphi*-B, threefold by *S. aureus* (MRSA) and *S. typhimurium*, fourfold by *M. luteus*, *K. pneumonia*, and fivefold by *E. aerogens*. Besides, the potency of compound **5e** is increased by twofold against fungi *B. cinerea*, three fold by *C. krusei* and fourfold by *C. albicans* compared to compound **5i**. In general, compound **5e** showed better activity than standard drugs for most of the tested bacteria and fungi.

4. Conclusion

In conclusion, this study is considered with respect to synthesis of morpholine analogues **5a–j** as new budding antimicrobials. These novel compounds were evaluated for their
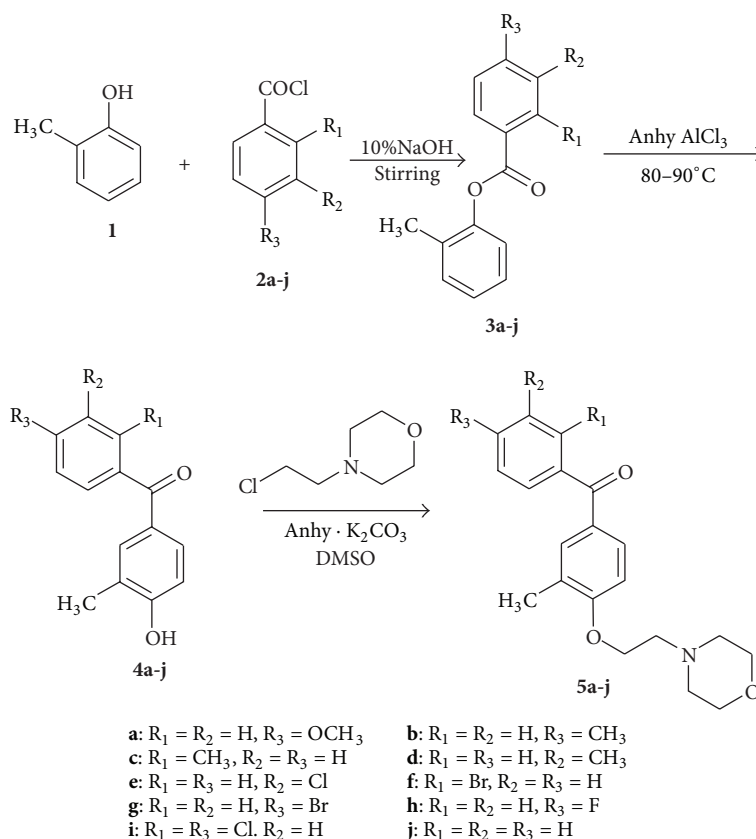
SCHEME 1

TABLE 3: MIC (μg/mL) of compounds **5a–j** against tested bacteria.

| Compounds | Minimum inhibitory concentration (μg/ mL) | | | | | | | |
| | Gram-positive bacteria | | | | Gram-negative bacteria | | | |
	S. aureus	S. aureus (MRSA)	E. aerogenes	M. luteus	K. pneumoniae	S. typhimurium	S. Paratyphi-B	P. vulgaris
5c	250	500	250	250	500	62.5	500	500
5d	31.25	125	125	63.5	63.5	250	125	250
5e	31.25	62.5	15.62	15.62	15.62	15.62	125	<15.62
5f	15.62	12.5	62.5	12.5	250	15.62	500	125
5g	15.62	250	250	32.5	250	31.25	500	62.5
5h	15.62	250	62.5	62.5	550	31.25	500	125
5i	62.5	500	500	250	250	125	500	500
Streptomycin	6.25	>100	25	6.25	6.25	30	ni	6.25
Ciprofloxacin	<0.78	>100	<0.78	>100	<0.78	>100	6.25	<0.78

ni: no inhibition.

activities against eight bacteria and four fungi. Compound **5e** with chloro group at meta position in phenyl ring was found to be more than 1.6 times active against *S. aureus* (MRSA) bacteria than streptomycin and ciprofloxacin and moreover more than 6.4 times active against *M. luteus* and *S. typhimurium* bacteria than ciprofloxacin. Compound **5e** was also found to be more than 3.2 times active against *C. albicans* fungi than fluconazole. In contrast, compounds **5a** with methoxy group and **5b–d** with methyl group exhibited lowest

activity and this can be attributed to the electron releasing effect. These results showed that synthesized compound **5e** might be a potential antibacterial and antifungal agent.

Conflict of Interests

The authors declare that they have no conflict of interests with respect to the content of the paper.

TABLE 4: MIC (μg/mL) of compounds **5a–j** against tested fungi.

Compounds	Minimum inhibitory concentration (μg/mL)			
	C. albicans	*B. cinerea*	*M. pachydermatis*	*C. krusei*
5c	125	500	250	125
5d	250	250	125	125
5e	31.5	125	250	31.5
5f	250	250	500	250
5g	500	500	250	250
5h	500	250	500	250
5i	500	500	250	250
Fluconazole	>100	ni	12.5	12.5
Ketoconazole	25	25	15	15

ni: no inhibition.

Acknowledgments

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