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Efficacy of 5-(2-aroyl)aryloxy methyl-2-phenyl-1,3,4-oxadiazoles as antibacterial and antifungal agents

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ABSTRACT

Research and development of potent and effective antimicrobial agents represent one of the most important advances in therapeutics; the main aim of these efforts is not only control the serious infections, but also prevention and treatment of some infectious complications of other therapeutic modalities. A series of 5-(2-aroyl)aryloxy methyl-2-phenyl-1,3,4-oxadiazoles were screened for their antibacterial and antifungal activities. Anti-bacterial activity against B. cereus, *S. aureus, B. subtilis, S. aureus (MRSA), E. aerogenes, M. luteus, K. pneumonia, P. aeruginosa, S. typhimurium, E. coli, paratyphi-B, P. vulgaris* bacterial strains and anti-fungal activity against *C. albicans, A.niger, F.solani, A.flavus, B.cinerea, C.krusei, M. pachydermatis, C.parapsilosis, F.moniliforme, C.gloeosporioides* fungal strains were carried out. The bioassays indicated that most of the synthesized compounds showed potential antibacterial and anti-fungal activity.

INTRODUCTION

Infectious diseases are still a major threat to public health despite the tremendous progress in human medicine and their control remains a huge challenge since vaccines are only available against a limited number of pathogens. Most of the current anti-infective suffers from considerable limitations in terms of antimicrobial spectrum and side-effects, and their widespread overuse has led to drug resistance (Fauci, 2001). Over the past few decades, the problems posed by multi-drug resistant microorganisms have reached an alarming level in many countries around the world. The use of most antimicrobial agents is limited, not only by the rapidly developing drug resistance, but also by the unsatisfactory status of the present treatment of bacterial and fungal infections (Fidler, 1998; Hong, 2001; Oren *et al.,* 1998). Infections caused by those microorganisms represent a serious challenge to the medical community; hence, the development of new antimicrobial agents is an important goal.

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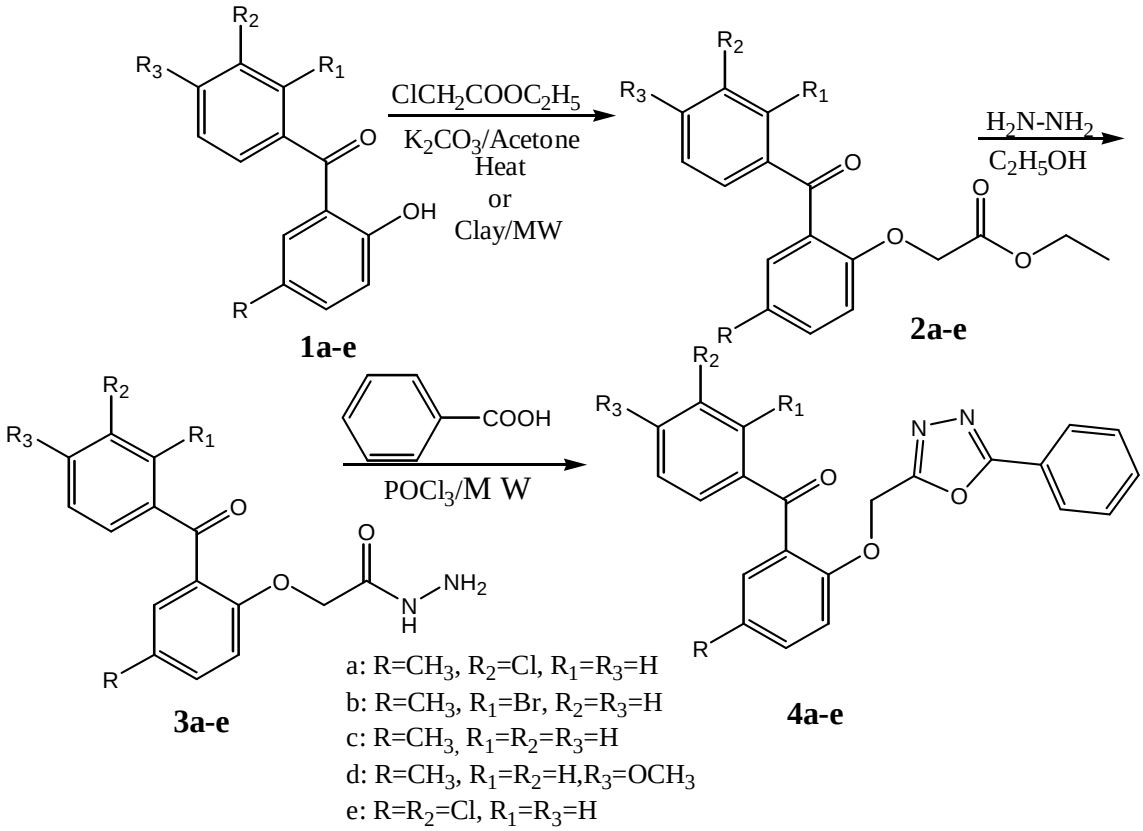
In particular, increasing drug resistance among gram-positive bacteria such as *staphylococci, enterococci* and *streptococci* is a significant health matter (Ramya, 2009). There is a real perceived need for the discovery of new compounds endowed with antimicrobial activities. The newly prepared compounds should be more effective and possibly act through a distinct mechanism from those of well-known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant. During the past years extensive evidences have been accumulated to establish the efficiency of benzophenone analogues as antimicrobial agent (Khanum *et al.,* 2005; Trusheva *et al.,* 2004; Lokvam *et al.,* 2000; Curtze *et al.,* 1998). Benzophenone analogue used in central-African traditional medicine and this has been shown to exhibit chemotherapeutical activity against gram-positive and gram-negative cocci, mycobacteria and fungi (Bakana *et al.,* 1997). Recently Selvi et al have shown antifungal activity of benzophenone analogues, at its lower concentration (Tamil Selvi *et al.,* 2003). Besides chloro substituted benzophenones have exhibited more antifungal activity (Grote *et al.,* 2002). 1,3,4-Oxadiazole is associated with potent pharmacological activity due to the presence of toxophoric N C O linkage (Rigo1985). 1,3,4-oxadiazoles are biologically active,



Scheme 1

synthetically useful and important heterocyclic compounds. Various biological activities are reported to be associated with 1, 3, 4-oxadiazoles like antibacterial (Revanasiddappa and Subrahmanyam 2010) , antifungal (Hansong *et al.,* 2002; Shetgiri and Nayak 2005) , anti inflammatory(Mohd Amir and Shikha Kumar 2003) etc., Similarly 2,5-disubstituted-1,3,4- oxadiazole derivatives possess broad spectrum of activities like antifungal (Adams *et al.,* 1986), anticonvulsant (Omar *et al.,* 1984) , anticancer (Bhat *et al.,* 1984)etc., Moreover, a large number of oxadiazoles (Bhat *et al.,* 2005; Sahin *et al.,* 2002; Priya and Balakrishna Kalluraya 2005; Xia-Juan Zou *et al.,* 2002) have been shown to exhibit significant antimicrobial activity against *S. aureus, C. albicans, C. krusei, C. parapsilosis, T. paradoxa, E. Coli, B. subtilis* and *P. aeruginosa.* Encouraged by these reports the present study for antibacterial and antifungal activities has been undertaken for the synthesized compound (Khanum *et al.,* 2004).

EXPERIMENTAL SECTION

Chemistry

Materials and Methods

Chemicals were purchased from Aldrich Chemical Co. TLC was performed on preactivated (110°C) silica gel plates using hexane chloroform and acetone as eluent. Melting points were determined with Thomas Hoover capillary melting point apparatus and are uncorrected. A simple household microwave oven operating at 2450 MHz (power 900W), equipped with a turntable was used. IR spectra were recorded in Nujol on FT-IR Shimadzu 8300 spectrophotometer, ^{1}H NMR spectra were recorded on a Bruker 300 MHz NMR spectrophotometer in $CDCl_3$ and chemical shifts were recorded in parts per million down field from tetramethylsilane. Mass spectra were obtained with a VG70-70H spectrophotometer. Elemental analysis results are within 0.4% of the calculated value.

SYNTHESIS

5-(2-aroyl) aryloxy-methyl -2-phenyl-1, 3,4oxadiazoles (4a-e)

Thermal method

A mixture of 3a (0.5 g, 1.6 mmol) and benzoic acid (0.19 g, 1.6 mmol) in phosphorus oxy chloride (2 ml) was refluxed for 8 h at 120^{o}C. The mixture was cooled and poured onto crushed ice, made basic by sodium-bi-carbonate solution and the resulting solid was filtered. The crude material was purified by chromatography on silica gel column using hexane/chloroform/ acetone (7:2:1, v/v) as an eluent. The solvent was removed under reduced pressure to afford 5-[2(3-chloro) benzoyl] phenoxy-

Table. 1: Antibacterial activity of the compounds: **4a-e:** MIC in µg /mL.

Compounds	Name of microorganism (MIC in µg /mL)											
	Gram positive bacteria						Gram negative bacteria					
	B. cereus	S. aureus	B. subtilis	S. aureus (MRSA)	E. aerogens	M. luteus	K. pneumonia	P. aeruginosa	S. typhimurium	E. coli	S. Paratyphi-B	P. vulgaris
4a	4.68	9.37	9.37	4.68	18.75	9.37	9.37	18.75	18.75	4.68	9.37	9.37
4b	9.37	4.68	9.37	9.37	9.37	18.75	9.37	9.37	4.68	9.37	9.37	18.75
4c	18.75	18.75	18.75	9.73	18.75	9.37	18.75	18.75	18.75	9.73	18.75	9.37
4d	18.75	9.37	18.75	9.37	18.75	9.37	18.75	9.37	9.37	9.37	18.75	18.75
4e	4.68	18.75	9.37	18.75	9.37	9.37	9.37	9.37	9.37	4.68	9.37	18.75
Streptomycin	2.34	2.34	4.68	1.17	2.34	2.34	4.68	4.68	2.34	2.34	4.68	4.68

Table. 2: Antifungal activity of the compounds: **4a-e** MIC in µg /mL.

Compounds	Name of the microorganism MIC in µg /mL									
	C. albicans	A. niger	F. solani	A. flavus	B. cinerea	C. krusei	M. pachydermatis	C. parapsilosis	F. moniliforme	C. gloeosporioides
4a	9.37	4.68	9. 37	18.75	18.75	9.37	9.37	18.75	9.37	4.68
4b	9.37	4.68	9.37	9.37	9.37	9.37	9.37	4.68	18.75	9.37
4c	18.75	18.75	18.75	9. 37	18.75	18.75	9. 37	18.75	18.75	18.75
4d	18.75	9.37	18.75	9.37	18.75	18.75	18.75	9.37	9.37	9.37
4e	18.75	4.68	9.37	18.75	9.37	9.37	9.37	18.75	18.75	18.75
Ketoconazole	4.68	2.34	2.34	1.17	4.68	2.34	4.68	4.68	2.34	2.34

methyl-2-phenyl-1, 3, 4-oxadiazoles 5-[2(3-chlorobenzoyl)-4-methylphenoxy] methyl-2-phenyl-1, 3, 4-oxadiazoles (4a).

Microwave irradiation method

In a typical synthetic procedure, a mixture of 3a (0.5 g, 1.6 mmol), benzoic acid (0.19 g, 1.6 mmol) and clay (1:3 w/w) was thoroughly mixed in the solid state using a vortex mixer and irradiated in an unmodified household microwave oven at its 50% power for 10 min. Upon completion of the reaction followed by TLC examination, the product was extracted into dichloromethane (3×20 ml), the combined organic extract dried with anhydrous sodium sulphate and solvent was removed under reduced pressure to afford pure 4a. The compounds 4a-e was characterized by IR, ^{1}H NMR and mass spectrophotometer (Khanum *et al.*, 2004).

BIOLOGY

Materials and methods for the antimicrobial activity

Streptomycin was used as positive controls against bacteria. ketoconazole (Himedia, Mumbai) were used as positive controls against fungi.

Tested microbes

The following gram positive bacteria were used for the experiments; *B. cereus, staphylococcus aureus (MTCC 7443), B. subtilis, Staphylococcus aureus* (MRSA) (MTCC 84), *Enterobacter aerogenes* (MTCC 111), *Micrococcus luteus* (MTCC 1538). The gram negative bacteria included *Klebsiella pneumoniae* (MTCC 109), *P. aeruginosa, Salmonella typhimurium* (MTCC 2488), *Escherichia coli, Salmonella paratyphi-B* (MTCC 733), *Proteus vulgaris* (MTCC 321). In addition, fungi *Candida albicans* (MTCC 227), *A.niger, F. solani, A.flavus, Botyritis cinerea* (MTCC 2880), *Candida krusei* (MTCC 231), *Malassesia pachydermatis, C.parapsilosis, F. moniliforme C. gloeosporioides* were also used for the experiments. All cultures were obtained from the Department of Microbiology, Manasagangotri, Mysore.

Preparation of inoculums

Bacterial inoculums were prepared by growing cells in Mueller Hinton Broth (MHA) (Himedia) for 24 h at 37^{o}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^{o}C for 10 days and the spores were collected using sterile doubled distilled water and homogenized.

Disc diffusion assay

Antibacterial activity was carried out using a disc diffusion method (Murray 1995) 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^{o}C for bacteria and 48 h at 27^{o}C for fungi. Zone of inhibition was recorded in millimeters and the experiment was repeated twice.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration studies of synthesized compounds were performed according to the standard reference method for bacteria (Duraipandiyan and Ignacimuthu 2009) and filamentous fungi (Clinical and Laboratory Standards Institute 2008). Required concentrations (1000 µg/ml, 500µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml and 15.62 µg/ml) of the compound was 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 anti-fungal agent's ketoconazole, fluconazole for fungi and streptomycin, ciprofloxacin for bacteria were included in the assays as positive controls. For fungi, the plates were incubated

for 48-72 h at 28ºC and for bacteria the plates were incubated for 24 h at 37ºC. 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.

RESULT AND DISCUSSION

The reaction sequence for the title compounds is outlined in Scheme 1. Compounds **4a-e** has been prepared as previously reported by our group (Khanum *et al.,* 2004). The antimicrobial screenings of the synthesized compounds were undertaken using disc diffusion method. The screening results of the tested compounds against the gram negative bacteria *(Klebsiella pneumoniae, P. aeruginosa, Salmonella typhimurium, Escherichia coli, Salmonella paratyphi-B, Proteus vulgaris),* gram positive bacteria *(B. cereus, Staphylococcus aureus, B. subtilis, Staphylococcus aureus (MRSA), Enterobacter aerogenes, Micrococcus luteus)* in addition to the pathogenic fungi *Candida albicans, A.niger, F. solani, A. flavus Botyritis cinerea, Candida krusei, Malassesia pachydermatis, C.parapsilosis, F. moniliforme C. gloeosporioides* microorganisms are summarized in Table **1** and **2**. The obtained data revealed that most of the compounds showed moderate to excellent activities against the tested microorganisms. Compounds **4a, 4b, 4e** showed good activity among all the synthesized compounds compared with the standard drug. Compound **4a** showed good bacterial activity against *B. cereus, S. aureus (MRSA)* and *E. coli.* Compound **4b** showed moderate activity against *S. aureus* and *S. typhimurium* and compound **4e** against *B. cereus* and *E. coli*. Compound **4a** with chloro group at the meta position in benzoyl ring and methyl group at para position in phenyl ring of benzophenone, **4b** with bromo group at ortho position in benzoyl ring and methyl group at para position in phenyl ring of benzophenone and **4e** with chloro group at meta position in benzoyl ring and para position in phenyl ring of benzophenone showed good activity against both gram-positive and gram-negative bacteria.

Compound **4a** showed significant antifungal activity against *A. niger* and *C. gloeosporioides*, Compound **4b** showed activity against *A.niger* and *C. parapsilosis* and **4e** showed activity against *A.niger*. Compound **4c** and **4d** without any halogen substituent exhibited lowest activity and this can be attributed to the electron releasing effect. Significant MIC values were observed against gram positive, gram negative bacteria and antifungal activity. In comparison, compound **4a** is more potent than **4b** and **4b** is more potent than **4e**. In general, compound **4a** showed better activity for most of the tested bacteria and fungi.

CONCLUSION

In conclusion, this study is with respect to synthesis of 5-(2-aroyl)aryloxy methyl-2-phenyl-1,3,4-oxadiazoles analogues 4a-**e** as new budding antimicrobials. These novel compounds were evaluated for their activities against twelve bacteria and ten fungi. Compound **4a** with chloro group showed better activity for most of the tested bacteria and fungi.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with respect to the content of the manuscript.

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