ANTIFUNGAL ACTIVITY OF A BIFUNCTIONALIZED ALLENE ETHANOL EXTRACTS

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INTRODUCTION

The present treatments of bacterial and fungal infections are a bit unsatisfactory, owing to rapidly developing drug resistance and side effects. This effect has a negative impact on the usage of most antimicrobial agents[12]. Antibiotic resistance continues to rise and the dawn of the much forewarned post-antibiotic era has arguably broken [9]. Antimicrobial resistance (AMR) is not a recent phenomenon, but it is a critical health issue today. Over several decades, to varying degrees, bacteria causing common infections have developed resistance to each new antibiotic, and AMR has evolved to become a worldwide health threat. The evolving public health threat of antimicrobial resistance (AMR) is driven by both appropriate and inappropriate use of anti-infective medicines for human and animal health and food production, together with inadequate measures to control the spread of infections. On World Health Day (WHD) 2011[13], in a six-point policy package, countries were called upon to (1) commit to a comprehensive, financed national plan with accountability and civil society engagement, (2) strengthen surveillance and laboratory capacity, (3) ensure uninterrupted access to essential medicines of assured quality, (4) regulate and promote rational use of medicines in animal husbandry and to ensure proper patient care, (5) enhance infection prevention and control, and (6) foster innovations and research and development of new tools [13]. Small-molecule drugs, for the time being, remain an essential component of infection treatment and prevention. Two proven strategies within this paradigm are the development of new drugs with direct antibacterial activity (e.g. daptomycin) and adjuncts with antibiotic-enhancing activity (e.g. tazobactam) [9]. Efforts to develop synthetic antibacterial drugs have not been abandoned but are now more focused on derivatization of natural molecules and synthesis of natural-product-like compounds using well-known natural product scaffolds [4]. The current global pandemic of antibiotic resistance shows no signs of abating, although it may be changing direction. An important milestone has been the 3rd World Health Organization (WHO) Patient Safety Challenge on antibiotic resistance, subsequently upgraded to an entire WHO Concern, with last year’s World Health Day on this topic. With a dearth of new antibiotics
coming to market, the need for action to avert a developing global crisis in health care is increasingly urgent.  

In this paper, the antifungal activity of a Bifunctionalized Allene with unprotected hydroxy group (Dimethyl (1-(1-hydroxyethyl)-3-methylpenta-1,2-dienephosphonate) (BA-2)) has been studied as part of the exploration for new and novel bio-active compounds.

**MATERIALS AND METHODS**

**Test organisms**

*Aspergillus niger, Penicillium claviforme, Saccharomyces cerevisiae, Candida albicans 8673 and Candida glabrata 72* were obtained from the National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria. All the isolates were checked for purity and maintained in slants of Nutrient agar.

**Media used**

They were maintained on Potato Dextrose Agar (PDA, Oxoid, Hampshire, UK) plate sat 29°C and subcultured on a monthly basis until sporulation. The spores were harvested after establishing a good growth rate of each of the fungal cultures and were filtered with sterile cotton filter, to avoid the presence of conidia and mycelia. The spore's suspension in PBS (pH 7.0) were adjusted to the final concentrations in the range of 10^7-10^8 spores/mL.

**Compound tested**

Bifunctionalized Allene with unprotected hydroxy group (Dimethyl (1-(1-hydroxyethyl)-3-methylpenta-1,2-dienephosphonate) (BA-2)) was synthesised in the Laboratory of Toxicochemical Chemistry, Department of Organic Chemistry & Technology of the Konstantin Preslavsky University of Shumen, Bulgaria (figure 1) [7].

![Figure 1](image)

**Preparing the solution of BA-2**

The solutions of BA-2 (50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml) were freshly prepared in ethanol.

**Assay for Antifungal Activity**

Antifungal assay was performed by the well diffusion method using soft 0.8% agar. Agar medium was added to sterile Petri dishes seeded with 100 µl of each test bacterial strains. Wells of equal distance were dug on the seeded plates. Each well was filled up with 100 µl of the BA-2 and antibiotics tested. After adjusting the pH at 6.5 by NaOH, the activity of the BA-2 was checked. The plates were incubated at 37°C for 48 hours. The antifungal activity was assayed by measuring the diameter of the inhibition zone formed around the well with digital caliper[3]. All experiments were performed in triplicate.

**Determination of Minimum inhibitory concentrations (MICs)**

The minimum inhibitory concentrations of BA-2, that shows antimicrobial activity, were determined by 2-fold dilution methods as described by [11] and MICs were read in g/ml after overnight incubation at 37°C. All experiments were made in replicate.

**Determination of Minimum fungal concentration (MFC)**

The MFC were carried out to check whether the test microbes were killed or only their growth was inhibited. Potato Dextrose Agar was prepared and sterilized at 121°C for 15 minutes, the medium was poured into sterile petridishes and were allowed to cool and solidify. The contents of the MFC in the serial dilution were then subcultured onto the prepared medium, incubation was made at 37°C for 24 h, after which each plate was observed for colony growth. The lowest concentration of the BA-2 without a colony growth was recorded as the MFC.

**RESULTS**

In the present study the effects of BA-2 on five pathogenic fungi were evaluated. The effects were compared with widely used antibiotic Fluconazole. According to NCCLS, the antibiotic Fluconazole used is known to have broad spectrum antifungal activity [10]. The effects of BA-2 on the microorganisms were summarized in Table 1.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone of inhibition (mm)</th>
</tr>
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<tbody>
<tr>
<td>A. niger</td>
<td>15.53±0.19</td>
</tr>
<tr>
<td>P. claviforme</td>
<td></td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>0</td>
</tr>
<tr>
<td>C. albicans 8673</td>
<td>13.84±0.18</td>
</tr>
<tr>
<td>C. glabrata 72</td>
<td>16.25±0.02</td>
</tr>
<tr>
<td>Ethanol (96%)</td>
<td>13.22±0.01</td>
</tr>
<tr>
<td>Fluconazole 150µg/ml</td>
<td>13.49±0.02</td>
</tr>
</tbody>
</table>

*Data are presented as average values ± standard deviation in mm.*

BA-2 at concentration 50 mg/ml for 24 hours notably inhibited growth of A. niger (15.53 mm mean zone of inhibition) and C. glabrata72 (16.25 mm mean zone of inhibition). On the contrary, BA-2 had no activity against C. albicans (13.84 mm...
mean zone of inhibition), which are comparable to the inhibitory effect of standard drug. BA-2 did not inhibited *P. claviforme* and *S. cerevisae*.

Our assay for antifungal activity of BA-2 was conducted by testing different concentrations of the compound on various pathogens to determine the MICs. We used five concentrations – 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. The results are shown in Table 2.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC (mg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>50mg/ml</td>
</tr>
<tr>
<td></td>
<td>25mg/ml</td>
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<tr>
<td></td>
<td>12.5mg/ml</td>
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<td></td>
<td>6.25mg/ml</td>
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<tr>
<td></td>
<td>3.125mg/ml</td>
</tr>
<tr>
<td>A. niger</td>
<td>+</td>
</tr>
<tr>
<td>P. claviforme</td>
<td>-</td>
</tr>
<tr>
<td>S. cerevisae</td>
<td>-</td>
</tr>
<tr>
<td>C. albicans 8673</td>
<td>-</td>
</tr>
<tr>
<td>C. glabrata 72</td>
<td>+</td>
</tr>
</tbody>
</table>

The MFC of BA-2 was determined to be one of the major pathogenic yeast and fungi. The results revealed variability in the inhibitory concentrations of BA-2 for given fungi. MIC of BA-2 at concentration 50 mg/ml for 24 hours notably inhibited growth only of *A. niger*. In contrast, MIC of BA-2 at concentration 25 mg/ml for 24 hours notably inhibited growth of yeast *C. glabrata* 72 and *C. albicans*. The probable reason for the higher MIC reported for *A. niger* is considered to be one of the major global public health threats and the magnitude of the problem recently prompted a number of international and national bodies to take actions to protect the public.

The occurrence of drug resistant strains with less susceptibility to antibiotics due to mutation challenges the researchers to invent newer drugs. At this scenario, evaluation of antimicrobial substances from various sources is considered to be a pivotal role. Nevertheless, further studies are required to explore the mechanism of biochemical active principle in the Bifunctionalized Allenes for the inhibitory action on various pathogens selected in the study.

Based on the results obtained we can conclude that the examined BA-2 has bactericidal activity towards both pathogenic yeast and Fungi Imperfecta from *P. claviforme* and years *S. cerevisae*. MFC was not reported.

The BA-2 possesses biological activity, which is not well studied. We know only from literary data that they are used for inhibiting the biosynthesis of sterol from the pathogen responsible for *Pneumocystis-carinii* pneumonia (PCP) – a disease similar to AIDS[12]. In our previous studies was shown that the Bifunctionalized Allenes with protected hydroxy group (*Dimethyl 3-methyl-1-[1-(tetrahydro-2H-pyran-2-ylxy)-ethyl]-hepta-1,2-dienephosphonate*) (BA-1) exhibited antibacterial [5] and antifungal activity [6]. The results obtained show for the first time the existence of antifungal activity of BA-2 towards various pathogenic yeast and fungi.
References


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