https://doi.org/10.5272/jimab.2020263.3328

Journal of IMAB - *Annual Proceeding (Scientific Papers)*. 2020 Jul-Sep;26(3)

Journal of IMAB
ISSN: 1312-773X
https://www.journal-imab-bg.org

Original article

ANTIFUNGAL ACTIVITY OF A PHOSPHORYLATED 3-(α-HYDROXYALKYL) ALLENES ETHANOL EXTRACTS

Sevginar Ibryamova[1], Hasan Hasanov[2], Tsveteslava Ignatova-Ivanova[1]
1) Department of Biology, Shumen University, Shumen, Bulgaria.
2) Department of Chemistry, Shumen University, Shumen, Bulgaria.

ABSTRACT:

Antifungal effects of a *4-(Diphenylphosphinoyl)-2-methyl-4-phenylbuta-2,3-dien-1-ol* (PA-2) on pathogenic yeast and fungi had been established. PA-2 (50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml) exerted different inhibitory effect on different yeast and fungi cells in vitro. The effects of PA-2 on eukaryotic cells have not been studied yet. The present study was aimed to assess the antifungal activity of PA-2on pathogenic yeast and fungi. Experimental approach: In vitro antifungal test: *Aspergillus niger, Penicillium claviforme, Saccharomyces cerevisae, Candida albicans 8673 and Candida glabrata 72* were treated for 24 hours with PA-2(50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml), Fluconazole (150 mg/ml). The antifungal activity was assayed by the goot diffusion method with a digital caliper. Determination of minimum inhibitory concentrations (MICs): The MIC of PA-2, that shows antifungal activity was determined by **2-fold dilution methods** and MICs were read in ìg/ml after overnight incubation at 37ºC. Determination of Minimum fungal concentration (MFC): The MFC was carried out to check whether the test microbes were killed or only their growth was inhibited. Potato Dextrose Agar (PDA, Oxoid, Hampshire, UK) was prepared and sterilized at 121ºC for 15 minutes, the medium was poured into sterile petri dishes and was allowed to cool and solidify. The contents of the MIC in the serial dilution were then subcultured onto the prepared medium, incubation was made at 37ºC for 24 h, after which each plate was observed for colony growth. **All experiments were performed in triplicate.**

The lowest concentration of the PA-2 without a colony growth was recorded as the MFC. PA-2 had higher antifungal activity than the tested antibiotic (Fluconazole).

Keywords: *4-(Diphenylphosphinoyl)-2-methyl-4-phenylbuta-2,3-dien-1-ol*, Antibacterial activity, Antibiotic,

INTRODUCTION

Since the penicillin era, antibiotics have been viewed as wonder drugs that could be prescribed without fear of harm, despite early warnings of consequences such as antibiotic resistance and side-effects. In 1945, Sir Alexander Fleming famously warned of the danger of over-reliance on antibiotics and the threat of bacteria developing resistance. 68 years later, his prediction has been realised [1]. The use has spread into many nonmedical areas and has been unregulated, both legally and illegally. Antibiotic resistance is perceived as a complex medical problem. Antibiotics are different from all other drug groups in that the effects of their use extend far beyond individual patients. Even more worrying is the accumulating evidence that antibiotic use in seriously ill but uninfected patients can actually increase mortality [2]. Use of antibiotics, which is unnecessary (eg, for growth promotion) or where alternatives exist (eg, routine prevention) should be phased out. The international organisations WHO, OIE and FAO should provide a clear definition of "unnecessary routine prevention". Governments across the globe should then revise existing legislation or draft new legislation accordingly. Additionally, all key stakeholders should commit to the prudent and rational use of antimicrobials. The environmental release of antibiotics from all sectors needs to be monitored and controlled. Strategies need to identify and focus control on hot-spots for horizontal resistance gene transfer such as wastewater treatment facilities. The rapid pandemic spread of multiresistant bacteria and the paucity of new effective antibiotics is placing patients' safety at risk worldwide. The infrastructure of antibiotic discovery both in academia and in the industry is at a dangerously low level and needs to be rebuilt. A new sustainable global model for the discovery, development, and distribution of antibiotics needs tî be developed in which the private and public sectors work in partnership and the large scientific bottlenecks for the discovery of antibiotics with new mechanisms of action have to be solved in collaboration between academia, SMEs and major pharmaceutical companies [3]. Pharmaceutical companies are increasingly turning away from participating in the development of new antibiotics, due to the regulatory environment and the financial risks. There is an urgent need for innovations in antibiotic research, as classical discovery platforms (e.g., mining soil *Streptomycetes*) are no longer viable options. In addition to discovery platforms, a concept of an ideal antibiotic should be postulated, act as a blueprint for future drugs, and aid researchers, pharmaceutical companies, and relevant stakeholders in selecting lead compounds [4].

In this paper, the antifungal activity of a *4-*

(Diphenylphosphinoyl)-2-methyl-4-phenylbuta-2,3-dien-1-ol ethanol extracts (PA-2) has been studied as part of the exploration for new and novel bioactive compounds.

MATERIALS AND METHODS

Test organisms

Aspergillus niger, Penicillium claviforme, Saccharomyces cerevisae, 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.

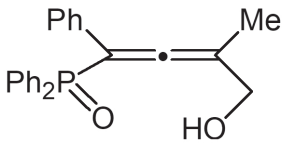
Media used

They were maintained on Potato Dextrose Agar (PDA, Oxoid, Hampshire, UK) plate sat 29ºC 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 a sterile cotton filter, to avoid the presence of conidia and mycelia. The spore's suspensions in PBS (pH 7.0) were adjusted to the final concentrations in the range of 105-106 spores/mL.

Compound tested

4-(Diphenylphosphinoyl)-2-methyl-4-phenylbuta-2,3-dien-1-ol (PA-2) was synthesised in the laboratory of organic chemistry, department of organic chemistry & technology of the "Konstantin Preslavsky" University of Shumen, Bulgaria (figure 1) [5].

Fig. 1. Structural formula of PA-2



4-(Diphenylphosphinoyl)-2-methyl-4-phenylbuta-2,3-dien-1-ol. Yellow oil, yield: 84%. Eluent for TLC: ethyl acetate:hexane = 1:1, Rf 0.62; IR (neat, cm^{-1}): 1180 (P=O), 1439, 1493 (Ph), 1948 (C=C=C), 3381 (OH). ^{1}H-NMR (600.1 MHz): δ_H 2.07 (d, *J*=7.5 Hz, 3H, Me), 2.71 (s, 1H, OH), 4.71- 4.94 (m, 2H, CH_2), 7.32-7.89 (m, 15H, 3Ph). ^{13}C-NMR (150.9 MHz) δ_C 14.7 (*J*=5.0 Hz, CH_3), 62.7 (*J*=4.6 Hz, CH_2), 97.4 (*J*=184.3 Hz, C), 110.5 (*J*=8.0 Hz, C), 124.0-139.7 (m, 3Ph), 211.0 (*J*=2.0 Hz, C). ^{31}P-NMR (242.9 MHz): δ_P 32.8. Anal. Calcd. for $C_{23}H_{21}O_2P$: C 76.65, H 5.87; found: C 76.69, H 6.92.

Preparing the solution of PA-2

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

Assay for antifungal activity

The antifungal assay was performed by the good 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 ìl of the ba-2 and antibiotics tested. After adjusting the ph at 6.5 by NaOH, the activity of the PA-2 was checked. The plates were incubated at 37ºC for 48 hours. The antifungal activity was assayed by measuring the diameter of the inhibition zone formed around the well with digital caliper [6,7]. All experiments were performed in triplicate.

Determination of minimum inhibitory concentrations (mics)

The minimum inhibitory concentrations of PA-2, that shows antimicrobial activity, were determined by 2-fold dilution methods as described by [8] and mics were read in ìg/ml after overnight incubation at 37ºC. All experiments were made to replicate.

Determination of minimum fungal concentration (MFC)

The MFC was carried out to check whether the test microbes were killed or only their growth was inhibited. Potato dextrose agar was prepared and sterilized at 121ºC for 15 minutes, the medium was poured into sterile Petri dishes and was 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 37ºC for 24 h, after which each plate was observed for colony growth. The lowest concentration of the PA-2 without a colony growth was recorded as the MFC.

RESULTS AND DISCUSSION

In the present study, the effects of PA-2on five pathogenic fungi and were evaluated. The effects were compared with widely used antibiotic Fluconazole. According to NCCLS, the used antibiotic Fluconazole is known to have broad-spectrum antifungal activity [9,10]. The effects of BA-2 on the microorganisms were summarized in Table 1.

Table 1. Effect of PA-2 on test organisms.

Microorganisms	Zone of inhibition (mm)*
A. niger	20,47±0,01
P. claviforme	22,38±0,3
S. cerevisae	11,48±0,2
C. albicans 8673	18,20±0,1
C. glabrata 72	23,22±0,05
Ethanol (96%) (Negative control)	12.65±0.05
Fluconazole150µg/ml	13.52±0.02
Chloronitromycin (250 µg/ml)	14.06±0.19

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

PA-2 at concentration 50 mg/ml for 24 hours notably inhibited the growth of *A. niger* (20.47 mm mean zone of inhibition), *P. claviforme niger* (22.38 mm mean zone of inhibition), *C. albicans 8673*(18.20 mm mean zone of inhibition), and *C. glabrata72* (23.22 mm mean zone of

inhibition). On the contrary, PA-2 had no activity against *S. cerevisae* (11.48 mm mean zone of inhibition), which are comparable to the inhibitory effect of standard drug.

Our assay for antifungal activity of PA-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 2. The MIC of PA-2

Microorganisms	MIC (mg/ml)				
	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml
A. niger	+	+	+	+	
P. claviforme	+	+			
S. cerevisae	+		+		
C. albicans 8673	+				
C. glabrata 72	+		+		

Results are mean ± SEM of three separate trails.

The results revealed variability in the inhibitory concentrations of PA-2 for given fungi. MIC of PA-2 at concentration 50 mg/ml for 24 hours notably inhibited growth of yeast *S. cerevisae, C. glabrata* 72 and *C. albicans* and fungi *A. niger* and *P. claviforme*. In contrast, MIC of PA-2 at concentration 25 mg/ml for 24 hours notably inhibited growth only of fungi *A. niger* and *P. claviforme.* MIC of PA-2 at concentration 12,5 mg/ml for 24 hours notably inhibited growth of yeast *S. cerevisae* and *C. glabrata* 72 and fungi *A. niger*. MIC of PA-2 at concentration 6,5 mg/ml for 24 hours notably inhibited growth only fungi *A. niger*. The probable reason for the higher MIC reported for eukaryotic microorganisms is the complex structure of their cell.

Our next task was to determine the Minimum fungal concentration (MFC) in regards with determining the bactericidal or bacteriostatic activity of the examined PA-2. We used five concentrations – 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml. The results are shown in Table 3.

Table 3. The MFC of PA-2

Microorganisms	MFC (mg/ml)*				
	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml
A. niger			+		
P. claviforme	-	-	-	-	-
S. cerevisae	-	-	-	-	-
C. albicans 8673		+			
C. glabrata 72		+			

*Results are mean ± SEM of three separate trails.

MFC of PA-2 at concentration 25 mg/ml for 24 hours notably inhibited the growth of *C. glabrata* and *C. albicans 8673*. MFC of PA-2 at concentration 12.5 mg/ml for 24 hours notably inhibited growth only of fungi *A. niger.* For Fungi Imperfecta from *P. claviforme* and year *S. cerevisae* MFC it was not reported.

Based on the results obtained we can conclude that the examined PA-2 has bactericidal activity towards both pathogenic yeast and Fungi Imperfecta but in different concentrations.

The PA-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 [11,12]. Similar chemical compounds were synthesized at the laboratory for organic chemistry at Shumen University. [13-21]. For most of them the already have data that possess antimicrobial and antifungal [7]. The obtained results show for the first time the existence of antifungal activity of BA-3 towards various pathogenic yeasts and fungi.

In Europe and the United States of America, *Candida glabrata* has emerged as the second most common cause of invasive candidiasis and an increasing number of reports show its importance in mucosal or bloodstream infections [22]. Systemic infections due to *C. glabrata* are characterized by a high mortality rate and they are difficult to treat due to their intrinsically low susceptibility to azoles, particularly fluconazole [23-24]. Numerous *C. glabrata* isolates have shown primary resistance to Fluconazole, while others easily develop Fluconazole resistance after exposure to the treatment [25;26]. Chassot et al. [27] reported a case of IC due to *C. glabrata* in which the infecting strain acquired resistance to Flucytosine, Fluconazole, Voriconazole,

and Caspofungin through successive independent events following prolonged exposure to each class of antifungal agent.

Consequently, the continued application of antifungal susceptibility testing for the conventional and new antifungal agents is critical to detect the emergence of resistance in this important opportunistic fungal pathogen. Future studies should include testing Bifunctionalized allenes and as a component of combined antifungal therapy for invasive and refractory mould infections.

The occurrence of drug-resistant strains with less susceptibility to antibiotics due to mutation challenges the researchers to invent newer drugs. At this scenario, the 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.

CONCLUSIONS

The Phosphorylated 3-(α-Hydroxyalkyl) allenes PA-2 at 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml concentrations showed significant antifungial activity on selected pathogens inclinical isolates.

Acknowledgements

The contributors express their gratitude for the funding by the project by Shumen University project RD-08-104/30. 01. 2020 Department of Biology and National Program Young Scientists and Postdoctoral Students.

REFERENCES:

1. Howard SJ, Catchpole M, Watson J, Davies SC. Antibiotic resistance: global response needed. *Lancet Infect Dis.* 2013 Dec;13(12): 1001-3. [PubMed] [Crossref]

2. Klompas M. Overuse of Broad-Spectrum Antibiotics for Pneumonia. *JAMA Intern Med.* 2020 Feb 17. [PubMed] [Crossref]

3. Laxminarayan R, Duse A, Wattal C, Zaidi AKM, Wertheim HFL, Sumpradit N, et al. Antibiotic resistance-the need for global solutions. *Lancet Infect Dis.* 2013 Dec;13(12): 1057-98. [PubMed]

4. Gajdács M. The Concept of an Ideal Antibiotic: Implications for Drug Design. *Molecules.* 2019 Mar 3;24(5): 892. [PubMed]

5. Hasanov HH, Ivanov IK, Christov VC. Bifunctionalized allenes. Part XX. A convenient and efficient regioselective synthesis of phosphorylated 3-(α-hydroxyalkyl) allenes. *Bulgarian Chemical Communications.* 2017; 49(Special Edition B):25-32. [Internet]

6. Akl MA, Mostafa MM, Abdel Hamid AI, Hassanin EE, Abdel-Raouf M. Assessment of The Antimicrobial Activities of Trioctylphosphine Oxide Modified Silica Nanoparticles. *Egypt J Chem.* 2020 Apr;63(4):1325-1339. [Crossref]

7. Ignatova-Ivanova Ts, Stefanova I, Ismailov I E, Ivanov IK, Christov VCh. *In Vitro* Studies of antifungal activity of a bifunctionalized allene ethanol extracts. *Int J Recent Sci Res.* 2015; 6(5):4352-4355.

8. Al-Abdalall AHA. Effect of plants extracts on the growth of *Candida albicans and Staphylococcus aureus. Afr J Pharm Pharmacol.* 2016 Apr;10(16): 337-345. [Crossref]

9. Gajdács M. The Concept of an Ideal Antibiotic: Implications for Drug Design. *Molecules.* 2019 Mar;24(5), 892. [PubMed]

10. Lin J, Nishino K, Roberts MC, Tolmasky M, Aminov RI, Zhang L. Mechanisms of antibiotic resistance. *Front Microbiol.* 2015 Feb 5;6:34. [PubMed]

11. Konda S, Raparthi S, Bhaskar K, Munaganti RK, Guguloth V, Nagarapu L, et al. Synthesis and antimicrobial activity of novel benzoxazine sulfonamide derivatives. *Bioorg Med Chem Lett.* 2015 Apr 1; 25(7):1643-6. [PubMed]

12. Zhao C, Rakesh KP, Ravidar L, Fang W-Y, Qin H-L. Pharmaceutical and medicinal significance of sulfur (S VI)-Containing motifs for drug discovery: A critical review. *Eur J Med Chem.* 2019 Jan 15;162:679-734. [PubMed]

13. Ismailov IE, Ivanov IK, Christov VCh. Bifunctionalized allenes. Part XIII. A convenient and efficient method for regioselective synthesis of phosphorylated α-hydroxyallenes with protected and unprotected hydroxy group. *Molecules.* 2014 May 16;19(5):6309-29. [PubMed]

14. Ismailov IE, Ivanov IK, Christov VCh. Bifunctionalized allenes. Part XV. Synthesis of 2,5-dihydro-1,2-oxaphospholes by electrophilic cyclization reaction of phosphorylated α-hydroxyallenes. *Molecules.* 2014 Jul 29;19(8):11056-76. [PubMed]

15. Ismailov IE, Ivanov IK, Christov VC. Bifunctionalized allenes. Part XIV. A convenient and efficient regioselective synthesis of phosphorylated β-hydroxyallenes with protected and unprotected hydroxy group. *Bulgarian Chemical Communications.* 2014; 46(Special Issue A):39-46. [Internet]

16. Christov VC, Ismailov IE, Ivanov IK. Bifunctionalized Allenes. Part XVI. Synthesis of 3-Phosphoryl-2,5-dihydrofurans by Coinage Metal-Catalyzed Cyclo-isomerization of Phosphorylated α-Hydroxyallenes. *Molecules.* 2015 Apr 21;20(4):7263-75. [PubMed]

17. Christov VC, Ismailov IE, Ivanov IK. Bifunctionalized allenes. Part XVII. Synthesis of 2,5-Dihydro-1,2-Oxaphospholes and 4-Phosphoryl-3,6-Dihydro-2H-Pyrans by Electrophilic Cyclization and Coinage Metal-Catalyzed Cycloisomerization of Phosphorylated β- Hydroxyallenes. *Int J Rec Sci Res.* 2015 Jun;6(6): 4526-4537.

18. Ismailov IE, Ivanov IK, Christov VC. Trifunctionalized Allenes. Part VI. Synthesis of 2,5-Dihydro-1,2-oxaphospholes, Furan-2(5H)-ones and 5,6-Dihydro-2H-pyrans by Electrophilic Cyclization and Cycloisomerization of 4-Phosphorylated 6-Hydroxyhepta-2,3-

dienoates. *Heterocycles*. 2019 Sep; 98(9):1236-1243. [Crossref]

19. Ismailov IE, Ivanov IK, Christov VCh. Trifunctionalized allenes. Part III. Electrophilic cyclization and cycloisomerization of 4-phosphorylated 5-hydroxypenta-2,3-dienoates: An expedient synthetic method to construct 2,5-dihydro-1,2-oxaphospholes, furan-2(5H)-ones and 2,5-dihydrofurans. *Phosphorus Sulfur Silicon Relat Elem.* 2020; 195(4): 314-323. [Crossref]

20. Ismailov IE, Ivanov IK, Christov VC. Trifunctionalized Allenes. Part IV. Cyclization Reactions of 4-Phosphorylated 5-Hydroxyhexa-2,3-dienoates. *Lett Org Chem.* 2020; 17(9):726-733. [Crossref]

21. Ismailov IE, Ivanov IK, Christov VCh. Trifunctionalized allenes. Part V. Competitive electrophilic cyclization and cycloisomerization of 4-phosphorylated 5-hydroxy-5-methylhexa-2,3-dienoates. *Bulgarian Chemical Communications.* 2020; 51(1):121-128. [Internet]

22. Pfaller MA, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. Twenty Years of the SENTRY Antifungal Surveillance Program: Results for *Candida* Species From 1997–2016. *Open Forum Infect Dis.* 2019 Mar 15;6(Suppl 1):S79-S94. [PubMed]

23. Pfaller MA, Diekema DJ. Rare and emerging opportunistic fungal pathogens: concern for resistance beyond Candida albicans and Aspergillus fumigatus. *J Clin Microbiol.* 2004 Oct;42(10):4419-31. [PubMed]

24. Kumar A, Nair R, Kumar M, Banerjee A, Chakrabarti A, Rudramurthy SM, et al. Assessment of antifungal resistance and associated molecular mechanism in Candida albicans isolates from different cohorts of patients in North Indian state of Haryana. *Folia Microbiol (Praha).* 2020 Aug;65(4):747-754. [PubMed]

25. Gao Y, Li H, Liu S, Zhang X, Sun S. Synergistic effect of fluconazole and doxycycline against Candida albicans biofilms resulting from calcium fluctuation and down regulation of fluconazole-inducible efflux pump gene overexpression. *J Med Microbiol.* 2014 Jul; 63(Pt 7):956-961. [PubMed]

26. Esfahani AN, Golestannejad Z, Khozeimeh F, Dehghan P, Maheronnaghsh M, Zarei Z. Antifungal effect of Atorvastatin against Candida species in comparison to Fluconazole and Nystatin. *Med Pharm Rep.* 2019 Oct;92(4):368-373. [PubMed]

27. Chassot F, Venturini TP, Piasentin FB, Santurio JM, Svidzinski TIE, Alves SH. Activity of antifungal agents alone and in combination against echinocandin-susceptible and -resistant Candida parapsilosis strains. *Rev Iberoam Micol.* 2019 Jan-Mar; 36(1):44-47. [PubMed]

Please cite this article as: Ibryamova S, Hasanov H, Ignatova-Ivanova T.Antifungal Activity of a Phosphorylated 3-(a-Hydroxyalkyl) allenes ethanol extracts. *J of IMAB.* 2020 Jul-Sep;26(3):3328-3332.
DOI: https://doi.org/10.5272/jimab.2020263.3328

Received: 24/08/2020; Published online: 21/09/2020

Address for correspondence:
Tsveteslava Ignatova-Ivanova
Department of Biology, Shumen University "Konstantin Preslavski"
115, Universitetska Str., Shumen, Bulgaria
E-mail: ts.ignatovaivanova@shu.bg