PHARMACOLOGICAL IMPORTANCE OF NOVEL SPIRO DERIVATIVES AGAINST HUMAN PATHOGENIC FUNGI

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Human mycoses have become a threat to health world-wide. Unfortunately there are only a limited number of antimycotic drugs in use. In the present study, antifungal activity of earlier synthesized spiro-1,4-dihydropyridines (1,4-DHPs) was investigated. The antifungal activity of spiro-1,4-DHPs compounds were screened against *Aspergillus flavus*, *A. fumigatus*, and *Candida albicans* by using Disc Diffusion and Modified Microdilution method. Among six spiro-1,4-DHPs compounds tested all of them showed stronger antifungal activity possibly through inhibiting the synthesis of chitin in cell wall against *A. flavus*, *A. fumigatus*, and *C. albicans* as compared to fluconazole, a standard antifungal drug. The combination of compounds showed that the synthesized compounds had synergistic, additive effects as compared to currently used drugs as an antifungal agent. These results indicated that these designed compounds were potential chitin synthase inhibitors and had excellent antimycotic activity for the treatment of fungal infections.

Key words: spiro-1,4-DHPs; drug resistant; fungi; combination; drugs; antimycotic

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INTRODUCTION

Fungal infection is a leading cause of mortality in the immunocompromised population, such as patients exposed to anticancer chemotherapy, radiation therapy, parenteral nutrition, or organ transplantation [1]. This population also includes patients infected with HIV, which disrupts the immune system [2]. In contrast to the increased incidence of fungal infections, the number of efficient and less toxic antifungal antibiotics is limited. Also, the increased use of antifungal agents leads to the emergence of drug-resistant fungal strains [3, 4]. Therefore, it is urgent to develop new and safe antifungal agents. Unlike human cells, fungi have a cell wall structure, which is an ideal target for antifungal drugs. The fungal cell wall is highly dynamic and essential for cell viability, morphogenesis, and pathogenesis. Although the components of the fungal cell wall vary among species, most fungal cell walls consist of a core of β-1,3-glucan polysaccharide covalently cross-linked to chitin, forming the primary scaffold structure. As a key component of the fungal cell wall, chitin contributes to about 2% (w/w) of fungal dry weight [5]. The 1,4-dihydropyridines (DHPs), a class of drugs possess a wide variety of biological and pharmacological actions. It represents one of the most important groups of calcium-channel modulating agents and has experienced widespread use in the cardiovascular disease treatment, which includes antihypertensive, antianginal, vasodilator and cardiac depressants activities. It also shows antibacterial, anticancer, antileishmanial, anticoagulant, anticonvulsant, antitubercular, antioxidant, antiulcer, CFTR, antimalarials, neuroprotection properties, HIV-1 protease inhibitors, antifertility activities and many more. There are many drugs available in the market, which contain 1,4-dihydropyridines ring as a basic scaffold. Dihydropyridines are a group of pyridine-based molecules. Chemically, it is a family of hydrogenated N-hetero-aromatic members. Various derivatives with different substituents are known to exist with them positioned at 2,6,3,5, and 1,4, which can be synthesized via cyclic condensation type reaction, Hantzsch pyridine synthesis [6]. The implications of 1,4-dihydropyridines (DHPs) are known to be evident and satisfactory for more than four decades now, and newer studies and theories still persist [7]. DHPs' analogy with 1,4-dihydronicotinamide has theorized its possible use in studying the molecular mechanisms as model compounds. They are postulated for providing the base for newer cardiovascular drugs development [8, 9]. A number of drugs commonly used in present times bear the 1,4-DHP ring-like calcium channel blockers [10-12]. Spiro oxindole derivatives are also becoming key building blocks for drug discovery as these templates have been shown to exhibit a variety of interesting biological activities, such as antioneoplastic [13], antibiotic [14], cytostatic [15], monoamine transporter inhibiting [16], bradykinin antagonist [17] and cell cycle inhibitor activity [18]. Due to their structure, they interact with a wide range of receptors; this activity has resulted in significant interest in developing efficient methods to prepare spiro compounds. A DHP skeleton is identified as a novel

scaffold and its capability to exhibit pleiotropy [19]. Certain DHP derivatives and DHP encompassing formulations are observed to depict antioxidant action [20-22], contributing to their notable pleiotropic effects precipitating into antiaging activity, neuroprotective effect, anticancer movement, antibacterial action [23] and among others. When exposed to certain chemical, biological and electrochemical processes, these derivatives tend to release free radicals. It is notable that hydrogen donors like phenol, amine, etc, tend to have an anticancer capacity basically via restricting oxidation and free radical processes. Being a significant H-donor, 1,4-dihydropyridines initiate similar actions. It further supports their use in cancer conditions. Additionally, synergistic effects might be witnessed on their concomitant use with antioxidants [24]. DHP model compounds have been used in clinical trials in recent decades due to their impressive range of pharmacological activities. Hypotensive, antimicrobial, anticoagulant, antioxidant, antitubercular, anticonvulsant, antiulcer, neuroprotective and antimalarial effects have been reported, among others as well [25]. Increased risk of fungal diseases in immunocompromised patients, emerging fungal pathogens, limited repertoire of antifungal drugs and resistance development against the drugs demands for development of new and effective antifungal agents. Some antifungal agents target cell wall structure. Nikkomycin and polyoxin are specific chitin synthase inhibitors, and nikkomycin Z is under clinical trial for the treatment of fungal infections, such as coccidioidomycosis [26]. Echinocandins inhibit β-1,3-glucan synthesis in the cell wall, and this group of agents is wildly used for the treatment of fungal infections caused by yeast, such as *Candida* species, or molds, such as *Aspergillus* [27]. A recent study indicated a synergistic effect for the combination of echinocandins and nikkomycin Z against infections caused by *C. albicans* using a mouse model [28]. Therefore, chitin inhibitors could be used in combination with enchinocandins for the treatment of fungal infections.

In the present study, the antifungal activity of spiro-1,4-dihydropyridines (1,4-DHPs) compounds were screened against *Aspergillus flavus*, *Aspergillus fumigatus*, and *Candida albicans*.

METHODS

Spiro-1,4-DHPs derivatives (**4a-4f**), tested in this study, were synthesized as described earlier by Sharma et al. [29].

In Vitro Screening of the Synthesized Compounds Using the Disc Diffusion Method

Compounds were screened for their antifungal activity against *A. fumigatus*, *A. flavus*, and *C. albicans* by disc diffusion method [30]. Standard size Whatman No.1 filter paper discs, 6.0 mm in diameter ("Sigma-Aldrich", USA), sterilized by dry heat at 140°C in an oven for one hour were used to determine the antifungal activity. SDA medium for disc diffusion test was prepared. After sterilization, it was poured into sterilized Petri plates and allowed to solidify. A suspension that was just turbid by visual inspection was prepared by suspending in 0.9% NaCl solution and the homogeneous suspension was used for inoculation and test inoculum was maintained at $(1\text{-}5)\times10^6$ CFU/ml. The spore suspension of each of the fungi was prepared from 8 to 10-day-old cultures separately. The suspension was vortexed and 0.1 µl aliquots were spread over the respective agar medium plates Sterilized filter paper discs were soaked in 50 µg/ml concentration of pure compounds and their mixture of compounds. Similarly, a solution of Fluconazole of 50 µg/disc used as the reference agent for comparison of the antifungal activity was prepared. These discs were then placed over the plates preceded with respective microorganisms. The plates were incubated at 30°C for 48-72 h. Three replicates were used in each case and average values were calculated. The diameter of the inhibition zones was measured in mm and the activity index was calculated on the basis of the size of the inhibition zone. The activity of compounds was measured by the following formula:

$$\text{Activity Index (AI)} = \frac{\text{Inhibition zone of Compound}}{\text{Inhibition zone of standard drugs}}.$$

Determination of Minimum Inhibitory Concentration by a Modified Microdilution Method [31]

The Minimum Inhibitory Concentration (MIC) of a mixture of compounds against *A. fumigatus*, *A. flavus*, and *C. albicans* were determined by a microdilution method with slight modification [31]. Sterilized brain heart infusion agar semisolid agar media were poured into the sterilized culture tubes and allowed to solidify. Test inoculum was prepared in 0.9% NaCl solution, the suspension was vortexed properly. Different concentrations of mixture of compounds were added in media containing culture tubes, afterwards a standard platinum loopful (~0.005 ml, Flexiloop, "Himedia", USA) of the inoculum suspension was inserted deep into each tube of medium containing a different concentration of compounds as well as compound free control. The culture tubes were then incubated at 28°C for 48-72 h to determine the MIC. MIC was defined as the lowest concentration that did not yield visual growth after the incubation period. All experiments were performed in triplicates.

RESULTS AND DISCUSSION

Due to emerging fungal infections and increased risk of fungal diseases in immunocompromised patients, there is a clear need in new antifungal drugs, firstly due to limited repertoire of antifungal drugs and

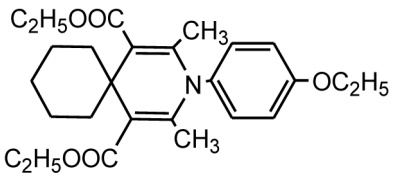
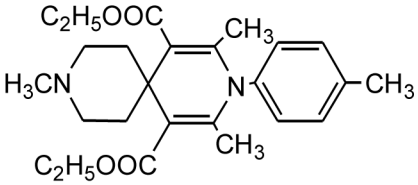
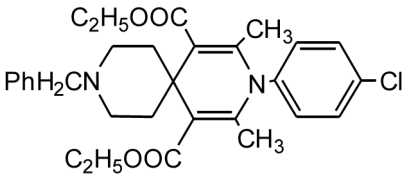
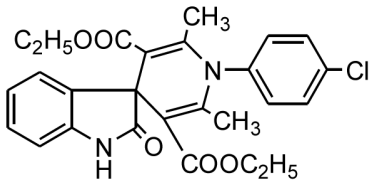
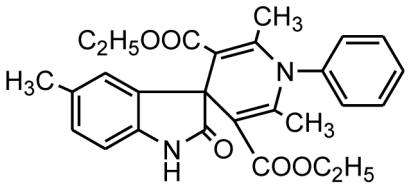
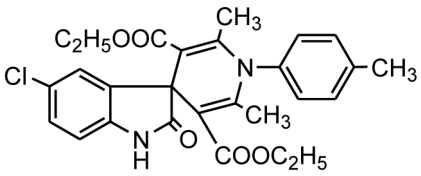
secondly, due to developing drug resistance against the available antifungal drugs. With greater knowledge of fungal metabolism efforts are being made to inhibit specific enzymes involved in different biochemical pathways for the development of antifungal drugs.

Chitin synthase is one such promising target as it is absent in plants and mammals. In the context of antifungal agent development, different chitin synthase inhibitors have been synthesized by a green chemical approach. Previous researchers interest on the green chemical synthesis of biodynamic spiro and annulated derivatives [32, 33] using nontraditional approach, an attempt has been made to synthesize various spiro-1,4-DHPs derivatives. A series of novel N-substituted spiro-1,4-DHPs derivatives (**4a-4f**) (Table 1) has been synthesizes in one pot under microwave irradiation using solvent free conditions and tested for an antidermatophytic activity [29]. In the course of search for potent chitin synthase inhibitors, we report here an antifungal activity of these six compounds of spiro-1,4-DHPs derivatives (**4a-4f**) against common fungal pathogens.

Screening of compounds (spiro-1,4-DHPs derivatives, **4a-4f**) for antimycotic activity against *A. fumigatus*, *A. flavus*, and *C. albicans*, the dominant etiological agents in immunocompromised patients, was performed using the disc diffusion and microdilution method. The results obtained showed the significant antifungal activity of compounds alone and in combinations (Table 1, Fig. 1). In the present study, compound **4c** showed excellent antifungal activity against *A. fumigatus* followed by **4a**, **4e**, **4b**, **4f**, and **4d**. Similarly, compound **4e** exhibited inhibitory action against *A. flavus* followed by **4a**, **4f**, **4c**, **4b**, and **4d**. In the current study, **4b** compound also showed significant antifungal activity against *C. albicans* followed by **4a**, **4d**, **4e**, **4f**, and **4c**. A mixture of compounds showed excellent and synergistic antimycotic activity as compared to single compounds and reference antibiotics. The mixture of compounds was prepared by adding 8.3 µg/ml from each six compounds (**4a** to **4f**) so total concentration of mixture of compounds was 50 µg/ml. Synergistic activity of spiro-1,4-DHPs derivatives was determined in the present study, by preparing the mixture of all six compounds in equal proportion so additive effect of all six compounds in one mixture can be studied against fungal pathogens. The diameter of inhibition zone obtained from the mixture of compounds (**4a-4f**) at total concentration 50 µg/ml were 87 mm, 86 mm, and 84 mm against *C. albicans*, *A. fumigatus*, and *A. flavus* (Table 2). In the further study, MIC was also determined by the modified Provine & Hadley method [31]. The results showed that the mixture of compounds (**4a-4f**) exhibited inhibitory action at 0.08 µl/ml to 0.9 µl/ml against *A. fumigatus* (Table 3). At 0.08 µl/ml concentration, no growth of *A. fumigatus* was observed after 6 days of incubation at 28°C. This minimum inhibitory concentration of mixture of spiro derivatives (**4a-4f**) showed no growth up to 0.9 µl/ml concentration. Present results indicate that these compounds can be used as active antimycotic agents in pharmaceutical industries for drug formulation. MIC of the mixture of compounds against *A. flavus* and *C. albicans* was found to be 0.1 µl/ml and 0.09 µl/ml respectively. These results were observed after 6 days, 12 days, 18 days, and 24 days, no growth was observed up to 30 days at that low concentration. Control was taken without addition of mixture of compounds showed 100% growth of *A. fumigatus*, *A. flavus*, and *C. albicans*. Our present findings suggested that these mixtures of compounds were found to be more additive, syngeristic and effective in inhibiting the growth of *A. fumigatus*, *A. flavus*, and *C. albicans* as compared to standard drugs used. These results concluded that mixture of compounds and single compounds can be used as an antifungal agent against *A. fumigatus*, *A. flavus*, and *C. albicans*, as a pharmaceutical drug active ingredient to combat the fungal infections in immunocompromised patients. Similarly, other researchers also reported that DHPs exhibited effective anticoagulant action by restricting calcium currents with high potency via calcium channels. They were explored in this area owing to their therapeutic use as hypotensive, anti-inflammatory, and anti-ischemic agents [34]. Sirisha et al., synthesized compound 4-substituted-2,6-dimethyl-3,5-bis-N-(heteroaryl)-carbamoyl-1,4-dihydropyridine was also found to have anticancer, antitubercular, and antibacterial activities *in vitro* and this derivative was discovered to be incredibly successful in the fight against cancer [35]. Tempone et al. reported the antileishmanial action of the drug nimodipine, and the structural damage to parasites caused by these medications [36]. This medication was found to be particularly selective against *Leishmania chagasi* promastigotes and intracellular amastigotes, with 50% inhibitory concentrations of 81.2 µM and 21.5 µM, respectively.

Calcium channel blockers have shown to be an effective antileishmanial compound *in vitro* [36]. Echinocandins are considered as the first-line therapy for invasive *Candida* infections [28]. However, treatment failures associated with resistant isolates harboring fks1 hot-spot mutations have been reported [37]. In recent years, the synergistic effects of nikkomycin and echinocandin have been studied, which suggests that the combination of two antifungal agents can be used in the treatment of infections caused by echinocandin-resistant strains [38]. These studies highlight the potential of combination therapies that target the synthesis of the two major structural polysaccharides found in most fungi, in achieving fungicidal regimens that would prevent the emergence of resistance mechanisms. In addition, cell wall synthase inhibitors, applied in combination with antagonists of the signalling pathways that regulate synthase expression and activity, may have potential as potent antifungal combination therapies [39-41].

Table 1. Antifungal activity of synthesized spiro-1,4-DHPs derivatives against *A. fumigatus*, *A. flavus*, and *C. albicans*

Compounds	Product	IZ of compounds (50 μg/ml)		
		A. fumigatus	*A. flavus*	*C. albicans*
4a		78	65	72
4b		75	73	75
4c		80	76	66
4d		72	79	70
4e		76	80	68
4f		75	77	65

In this and other tables IZ means Inhibition Zone measured in mm; IZ of Fluconazole is 42 mm against *A. fumigatus*; IZ of Fluconazole is 41 mm against *A. flavus*; IZ of Fluconazole is 38 mm against *C. albicans*.

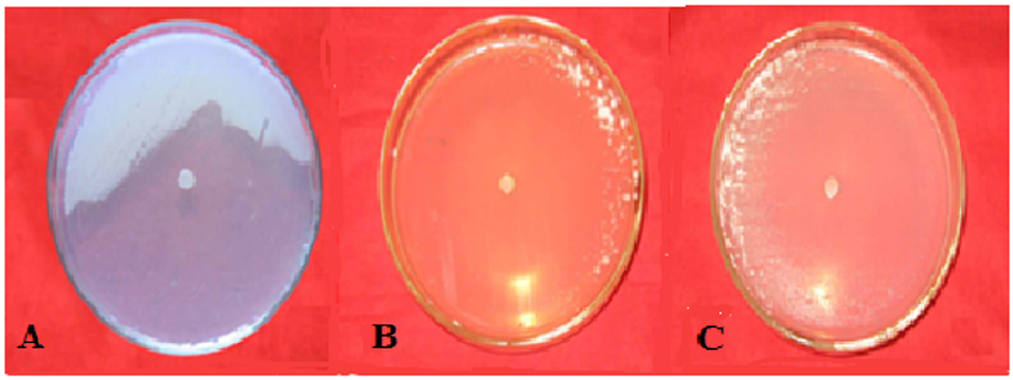


Figure 1. Antifungal activity of mixture of compounds against: (**A**) *C. albicans*, (**B**) *A. fumigatus*, (**C**) *A. flavus*.

Table 2. Antifungal activity of mixture of all compounds (novel synthesized spiro-1,4-DHPs derivatives, **4a-4f**) against *A. fumigatus*, *A. flavus*, and *C. albicans*

Compounds	Test strain	IZ of mixture of compounds (50 μg/ml)	IZ of Fluconazole (50 μg/ml)	AI
Mixture of compounds (**4a-4f**)	*A. flavus*	84 mm	41 mm	2.0
	A. fumigatus	86 mm	42 mm	2.0
	C. albicans	87 mm	38 mm	2.2

IZ is Inhibition Zone (in mm) including the diameter of disc (6 mm), AI (Activity index).

Table 3. MIC of Mixture of compounds (novel synthesized spiro-1,4-DHPs derivatives, **4a-4f**) against *A. flavus*, *A. fumigatus*, *C. albicans*

Different concentrations of compound used in μm/ml	Growth visually inspected in different concentrations of compounds against *A. flavus*	Growth visually inspected in different concentrations of compounds against *A. fumigatus*	Growth visually inspected in different concentrations of compounds against *C. albicans*
0.07	+4	+1	+2
0.08	+3	0	+1
0.09	+2	0	0
0.1	+1	0	0
0.2	0	0	0
0.3	0	0	0
0.4	0	0	0
0.5	0	0	0
0.6	0	0	0
0.7	0	0	0
0.8	0	0	0
0.9	0	0	0
Control without mixture of compounds	100% growth	100% growth	100% growth

The growth was scored in the following manner: **4+**, growth comparable to that of the oil free control; **3+**, growth approximately 75% that of the control; **2+**, growth approximately 50% that of the control; **1+**, growth 25% or less that of the control; and **0**, no visible growth.

CONCLUSIONS

The results of this study indicate that spiro-1,4-dihydropyridines are a genus of pharmacologically active molecules that serve as multifunctional potent leads and can be substituted in a variety of positions. On the basis of biological experiments, we have proved that these compounds have excellent antifungal activity *in vitro* to control the fungal infections and it can be used as a potent antifungal drug for immunocompromised patients as a medicinally important pharmaceutical application.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or using animals as subjects.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

REFERENCES

1. *Köhler J.R., Hube B., Puccia R., Casadevall A., Perfect J.R.* (2017) Fungi that infect humans. Microbiology Spectrum, **5**(3), 5-3. DOI: 10.1128/microbiolspec.FUNK-0014-2016
2. *Limper A.H., Adenis A., Le T., Harrison T.S.* (2017) Fungal infections in HIV/AIDS. Lancet Infectious Diseases, **17**(11), e334-e343. DOI: 10.1016/S1473-3099(17)30303-1
3. *Perlin D.S., Rautemaa-Richardson R., Alastruey-Izquierdo A.* (2017) The global problem of antifungal resistance: Prevalence, mechanisms, and management. Lancet Infectious Diseases, **17**(12), e383-e392. DOI: 10.1016/S1473-3099(17)30316-X
4. *Sanglard D.* (2016) Emerging threats in antifungal-resistant fungal pathogens. Front. Med., **3**, 11. DOI: 10.3389/fmed.2016.00011
5. *Holan Z., Pokorný V., Beran K., Gemperle A., Tuzar Z., Baldrián J.* (1981) The glucan-chitin complex in *Saccharomyces cerevisiae*. Arch. Microbiol., **130**(4), 312-318. DOI: 10.1007/BF00425946

6. *Kreutzer D., Ritter C.A., Hilgeroth A.* (2020) Novel nonsymmetrical 1,4-dihydropyridines as inhibitors of nonsymmetrical MRP-efflux pumps for anticancer therapy. Pharmaceuticals, **13**(7), 146. DOI :10.3390/ph13070146
7. *Cataldi M., Bruno F.* (2012) 1,4-Dihydropyridines: The multiple personalities of a blockbuster drug family. Transl. Med. UniSa, **4**, 12-26.
8. *Pajuste K., Rucins M., Domracheva I., Sobolev A., Pikun N., Plotniece M., Duburs G., Pajuste K., Plotniece A.* (2020) Data for the cytotoxicity, self-assembling properties and synthesis of 4-pyridinium-1,4-dihydropyridines. Data Brief, **33**, 1-16. DOI: 10.1016/j.dib.2020.106545
9. *Rucins M., Plotniece A., Bernotiene E., Tsai W.B., Sobolev A.* (2020) Recent approaches to chiral 1,4-dihydropyridines and their fused analogues. Catalysts, **10**(9), 1019. DOI: 10.3390/catal10091019
10. *van der Vossen A.C., Cransberg K., de Winter B., Schreuder M.F., van Rooij-Kouwenhoven R.W., Vulto A.G., Hanff L.M.* (2020) Use of amlodipine oral solution for the treatment of hypertension in children. Int. J. Clin. Pharmacy, **42**(3), 848-852. DOI:10.1007/s11096-020-01000-9
11. *Palatnick W., Jelic T.* (2020) Calcium channel blocker and beta blocker overdose, and digoxin toxicity management. Emergency Medicine Practice, **22**(9), 1-42.
12. *Velena A., Zarkovic N., Gall Troselj K., Bisenieks E., Krauze A., Poikans J., Duburs G.* (2016) 1,4-dihydropyridine derivatives: Dihydronicotinamide analogues – model compounds targeting oxidative stress. Oxid. Med. Cell. Longev., **2016**, 1892412. DOI: 10.1155/2016/1892412
13. *Cui C.B., Kakeya H., Okada G., Onose R., Osada H.* (1996) Novel mammalian cell cycle inhibitors, tryprostatins A, B and other diketopiperazines produced by *Aspergillus fumigatus*. I. Taxonomy, fermentation, isolation and biological properties. J. Antibiotics, **49**(6), 527-533.
14. *Tsuda M., Mugishima T., Komatsu K., Sone T., Tanaka M., Mikami Y., Shiro M., Hirai M., Ohizumii Y., Kobayashi J.I.* (2003) Speradine A, a new pentacyclic oxindole alkaloid from a marine-derived fungus *Aspergillus tamarii*. Tetrahedron, **59**(18), 3227-3230. DOI: 10.1016/S0040-4020(03)00413-7
15. *Zhang X., Smith C.D.* (1996) Microtubule effects of welwistatin, a cyanobacterial indolinone that circumvents multiple drug resistance. Mol. Pharmacol., **49**(2), 288-294.
16. *Efange S.M., Kamath A.P., Khare A.B., Kung M.P., Mach R.H., Parsons S.M.* (1997) N-hydroxyalkyl derivatives of 3β-phenyltropane and 1-methylspiro [1H indoline-3,4'-piperidine]: Vesamicol analogues with affinity for monoamine transporters. J. Med. Chem., **40**(24), 3905-3914. DOI: 10.1021/jm970326r
17. *Goehring R.R.* (1995) Synthesis of a spirocyclic oxendole analogue as a putative replacement for Pr02-Pro3-Gly4-Phe5 in bradykinin antagonists. Organic Preparations Procedures International, **27**(6), 691-694. DOI: 10.1080/00304949509458534
18. *Edmondson S., Danishefsky S.J., Sepp-Lorenzino L., Rosen N.* (1999) Total synthesis of spirotryprostatin A, leading to the discovery of some biologically promising analogues. J. Am. Chem. Soc., **121**(10), 2147-2155. DOI: 10.1021/ja983788i
19. *Zhou L.M., Qu R.Y., Yang G.F.* (2020) An overview of spirooxindole as a promising scaffold for novel drug discovery. Exp. Opin. Drug Discov., **15**(5), 603-625. DOI: 10.1080/17460441.2020.1733526
20. *Vijesh A.M., Isloor A.M., Peethambar S.K., Shivananda K.N., Arulmoli T., Isloor N.A.* (2011) Hantzsch reaction: Synthesis and characterization of some new 1,4-dihydropyridine derivatives as potent antimicrobial and antioxidant agents. Eur. J. Med. Chem., **46**(11), 5591-5597. DOI: 10.1016/j.ejmech.2011.09.026
21. *Mehta P., Verma P.* (2013) Antimicrobial activity of some derivatives of 1,4-dihydropyridines. J. Chem., **2013**, 1-4. DOI: 10.1155/2013/865128
22. *Olejníková P., Švorc Ľ., Olšovská D., Panáková A., Vihonská Z., Kovaryová K., Marchalín Š.* (2014) Antimicrobial activity of novel C2-substituted 1,4-dihydropyridine analogues. Scientia Pharmaceutica, **82**(2), 221-232. DOI: 10.3797/scipharm.1311-04.
23. *Sepehri S., Sanchez H.P., Fassihi A.* (2015) Hantzsch-type dihydropyridines and biginelli-type tetra-hydropyrimidines: A review of their chemotherapeutic activities. J. Pharm. Pharm. Sci., **18**(1), 1-52. DOI: 10.18433/J3Q01V
24. *Tirzit G.D., Dubur G.Y.* (1972) 1,4-dihydropyridines as inhibitors of free-radical reactions. Chem. Heterocycl. Comp., **8**(1), 126-127. DOI: 10.1007/BF00478512
25. *Khedkar S., Auti P.B.* (2014) 1,4-Dihydropyridines: A class of pharmacologically important molecules. Mini Rev. Med. Chem., **14**(3), 282-290.
26. *Nix D.E., Swezey R.R., Hector R., Galgiani J.N.* (2009) Pharmacokinetics of nikkomycin Z after single rising oral doses. Antimicrob. Agents Chemother., **53**(6), 2517-2521. DOI: 10.1128/AAC.01609-08
27. *Sucher A.J., Chahine E.B., Balcer H.E.* (2009) Echinocandins: The newest class of antifungals. Ann. Pharmacother., **43**(10), 1647-1657. DOI: 10.1345/aph.1M237.
28. *Cheung Y.Y., Hui M.* (2017) Effects of echinocandins in combination with nikkomycin Z against invasive *Candida albicans* bloodstream isolates and the fks mutants. Antimicrob. Agents Chemother., **61**(11), e00619-17. DOI: 10.1128/AAC.00619-17
29. *Sharma G., Sharma R., Sharma M., Dandia A., Bansal P.* (2013) Synthesis and synergistic, additive inhibitory effects of novel spiro derivatives against ringworm infections. Russ. J. Bioorg. Chem., **39**(3), 318-328. DOI: 10.1134/S106816201303014X
30. *Gould J.C., Bowie J.H.* (1952) The determination of bacterial sensitivity to antibiotics. Edinb. Med. J., **59**(4), 178-199.
31. *Provine H., Hadley S.* (2000) Preliminary evaluation of a semisolid agar antifungal susceptibility test for yeasts and molds. J. Clin. Microbiol., **38**(2), 537-541. DOI: 10.1128/JCM.38.2.537-541.2000
32. *Dandia A., Arya K.* (2007) Synthesis and cytotoxic activity of trisubstituted-1,3,5-triazines. Bioorg. Med. Chem. Lett., **17**(12), 3298-3304.
33. *Dandia A., Sharma G., Singh R., Laxkar A.* (2009) A new strategy for the synthesis of novel spiro [indoline-3, 2'-thiazolo [5, 4-e] pyrimido [1, 2-a] pyrimidine] derivatives. Arkivoc, **14**, 100-108.
34. *Kumar R.S., Idhayadhulla A., Nasser A.J.A., Selvin J.* (2011) Synthesis and anticoagulant activity of a new series of 1,4-dihydropyridine derivatives. Eur. J. Med. Chem., **46**(2), 804-810. DOI: 10.1016/j.ejmech.2010.12.006
35. *Sirisha K., Achaiah G., Reddy V.M.* (2010) Facile synthesis and antibacterial, antitubercular, and anticancer activities of novel 1,4-dihydropyridines. Archiv Der Pharmazie, **343**(6), 342-352. DOI: 10.1002/ardp.200900243
36. *Tempone A.G., Taniwaki N.N., Reimão J.Q.* (2009) Antileishmanial activity and ultrastructural alterations of *Leishmania chagasi* (L.) treated with the calcium channel blocker nimodipine. Parasitol. Res., **105**(2), 499-505. DOI: 10.1007/s00436-009-1427-8

37. *Baixench M.T., Aoun N., Desnos-Ollivier M., Garcia-Hermoso D., Bretagne S., Ramires S., Dannaoui E.* (2007) Acquired resistance to echinocandins in *Candida albicans*: Case report and review. J. Antimicrob. Chemother., **59**(6), 1076-1083. DOI: 10.1093/jac/dkm095
38. *Niimi K., Monk B.C., Hirai A., Hatakenaka K., Umeyama T., Lamping E., Niimi M.* (2010) Clinically significant micafungin resistance in *Candida albicans* involves modification of a glucan synthase catalytic subunit GSC1 (FKS1) allele followed by loss of heterozygosity. J. Antimicrob. Chemother., **65**(5), 842-852. DOI: 10.1093/jac/dkq073
39. *Munro C.A., Selvaggini S., de Bruijn I., Walker L., Lenardon M.D., Gerssen B., Gow N.A.* (2007) The PKC, HOG and Ca^{2+} signaling pathways co-ordinately regulate chitin synthesis in *Candida albicans*. Mol. Microbiol., **63**(5), 1399-1413.
40. *Fortwendel J.R., Juvvadi P.R., Pinchai N., Perfect B.Z., Alspaugh J.A., Perfect J.R., Steinbach W.J.* (2009) Differential effects of inhibiting chitin and 1,3-β-D-glucan synthesis in ras and calcineurin mutants of *Aspergillus fumigatus*. Antimicrob. Agents Chemother., **53**(2), 476-482. DOI: 10.1128/AAC.01154-08
41. *Steinbach W.J., Reedy J.L., Cramer R.A., Perfect J.R., Heitman J.* (2007) Harnessing calcineurin as a novel anti-infective agent against invasive fungal infections. Nature Reviews Microbiology, **5**(6), 418-430. DOI: 10.1038/nrmicro1680

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ФАРМАКОЛОГИЧЕСКОЕ ЗНАЧЕНИЕ НОВЫХ СПИРО-1,4-ДИГИДРОПИРИДИНОВ ПРОТИВ ПАТОГЕННЫХ ДЛЯ ЧЕЛОВЕКА ГРИБОВ

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Микозы человека представляют серьезную угрозу для здоровья населения во всем мире. Для лечения этих заболеваний применяется ограниченное число противогрибковых препаратов. В данной работе исследовали противогрибковую активность ранее синтезированных спиро-1,4-дигидропиридинов (1,4-DHPs). Противогрибковую активность соединений спиро-1,4-DHPs проверяли в отношении *Aspergillus flavus*, *A. fumigatus* и *Candida albicans* с использованием метода дисковой диффузии и модифицированного микроразведения. Оценка противогрибковой активности против лекарственно-устойчивых вариантов грибов показала, что исследованные соединения обладают значительной противогрибковой активностью. Все шесть исследованных соединений спиро-1,4-DHPs проявляли более сильную противогрибковую активность в отношении *A. flavus, A. fumigatus* и *C. albicans* по сравнению с флуконазолом — стандартным противогрибковым препаратом, — по-видимому, за счёт ингибирования синтеза хитина в клеточной стенке. Три из шести соединений (**4c**, **4e** и **4b**) были наиболее эффективны в отношении *A. fumigatus*, *A. flavus*, *C. albicans* соответственно. Комбинация соединений показала, что синтезированные вещества обладают синергетическим, аддитивным действием по сравнению с применяемыми в настоящее время препаратами в качестве противогрибкового средства. Полученные результаты свидетельствуют о том, что синтезированные соединения являются потенциальными ингибиторами хитинсинтазы и обладают превосходной антимикотической активностью для лечения грибковых инфекций.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: спиро-1,4-дигидропиридины; лекарственная устойчивость; грибы; комбинация; препараты; антимикотик

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