International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 8, 2016

Short Communication

ANTIFUNGAL ACTIVITY OF BIPHENYL-2,6-DIETHANONE DERIVATIVES

MEGHA RIKHI[1], SHANU HODA[2], SAHIL NAGPAL[2], POOJA VIJAYARAGHAVAN[2], SEEMA BHATNAGAR[1]*

[1]Novel Molecule Synthesis Laboratory, Amity Institute of Biotechnology, Amity University, Noida 201303, Uttar Pradesh, India, [2]Antimycotic and drug susceptibility laboratory, Amity Institute of Biotechnology, Amity University, Noida-201303, Uttar Pradesh, India
Email: sbhatnagar1@amity.edu

Received: 19 Dec 2015 Revised and Accepted: 20 Jun 2016

ABSTRACT

Objective: The objective of the study was to evaluate the antifungal activity of biphenyl-2,6-diethanone derivatives against *Cryptococcus neoformans*.

Methods: Antifungal activity of biphenyl derivatives were evaluated against *C. neoformans.* Zone of inhibition by disc diffusion method and minimum inhibitory concentration (MIC) using micro-broth dilution method was performed as per clinical and laboratory standard institute (CLSI). Melanin was extracted using 1M KOH, purified using 6M HCL and its reduction was assayed spectrophotometrically at 530 nm. Laccase activity was measured using L-DOPA as substrate and was assayed spectrophotometrically at 480 nm. Time kill assay was also performed to compare the antifungal potency of the test compound against azole drug.

Results: Zone of inhibition of 12 mm diameter was estimated against *C. neoformans*. MIC_{80} of compound **1e** was calculated as 50µg/ml. 63.67% decrease in melanization and 57.44% laccase activity reduction was determined. The Time-kill assay illustrated that the compound **1e** inhibited the growth of *C. neoformans* cells in almost the same duration as observed in fluconazole.

Conclusion: The outcome of *in vitro* antifungal studies indicated that compound **1e** demonstrated maximum reduction of melanin and laccase activity in *C. neoformans.* In conclusion, biphenyl-2,6-diethanone derivatives possess significant antifungal property which can be explored further for lead generation.

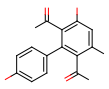
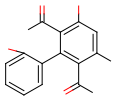
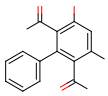
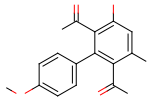
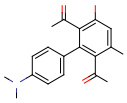
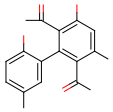
Keywords: *Cryptococcus neoformans*, Antifungal, Biphenyl-2,6-diethanone, Laccase, Melanin

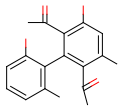
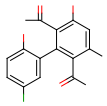
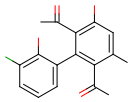
C. neoformans has emerged as a serious human pathogen in the past few years. It causes lung infections and may spread in the brain; causing meningoencephalitis. It has become the leading mycological cause of morbidity and mortality among immunocompromised patients [1]. It forms a creamy colored colony on potato dextrose agar (PDA) but produces melanized cells in the presence of exogenous precursor like L-DOPA which is catalysed by laccase enzyme of *C. neoformans*. Melanin has been reported to increase virulence and antifungal drug resistance by growing in a biofilm. Currently, azole antifungal drugs are the mainstay for the treatment of cryptococcosis infection. However, increased antifungal drug resistance caused through drug efflux by the fungal cellular machinery raises an urgent need to discover novel antifungal agents targeting cryptococcosis infection [2, 3].

Biphenyl scaffolds have received considerable attention due to their wide range of biological activities. Substituted biphenyls have been reported as an important pharmacologically important molecules exhibiting significant antifungal activity against *Candida albicans* and *Aspergillus niger* [4,5]. This literature prompted us to investigate antifungal activity of biphenyl-2,6-diethanone derivatives. The synthesis of derivatives of biphenyl-2,6-diethanones has been previously done by our group (unpublished results). The present study focusses on evaluating antifungal activity of synthesized biphenyl-2,6-diethanone derivatives against *C. neoformans*. The biphenyl-2,6-diethanone derivatives evaluated for antifungal activity are depicted in table 1. These derivatives were synthesized using procedures previously reported [6].

C. neoformans, ATCC 66031 (KwikStik, Himedia) was maintained on PDA plates at 30ºC for 36 h* [7]. Efficacy of the compound was tested by measuring the zone of inhibition using disc diffusion method. Fluconazole (1 mg/ml) and methanol were used as positive and negative controls respectively. *In vitro* susceptibility assay was performed using broth microdilution method in a 96 well plate as per CLSI protocol, formerly known as NCCLS [8]. MIC_{80} was calculated to find the concentration at which the test compound was potent enough to kill 80% of *C. neoformans* cells. This concentration was taken up for further experiments.

Table 1: Substituted Biphenyl-2,6-diethanone derivatives

Compound name	Compound structure
1a	
1b	
1c	
1d	
1e	
1f	

1g	
1h	
1i	

In *C. neoformans*, melanin has a complex architecture with multiple layers and a granular surface which enhances their virulence [9]. Literature reports have indicated that cryptococcosis infection is associated with the presence of melanized colonies in the brain tissue. Hence, the effect of the test compounds on melanin formation was determined according to the method described by Rosas *et al.*, 2000 [10]. Melanin was extracted using glycolytic and proteolytic enzymes, denaturant, organic extractions, and finally boiling in 6M HCl. The absorbance was measured spectrophotometrically at 530 nm.

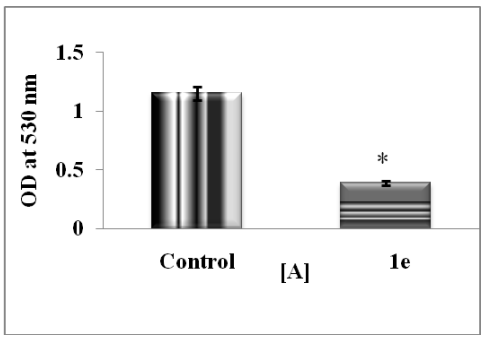
C. neoformans utilizes the laccase enzyme that catalyses the synthesis of melanin in the presence of the precursor molecule like L-DOPA. The laccase activity was determined in suspensions of permeabilized cells as performed by Ikeda *et al.*, 2002 [11]. *C. albicans* was used as a negative control since it is reported to exhibit a lack of laccase enzyme production. The optical density of the supernatant obtained after each time interval was measured spectrophotometrically at 480 nm.

Time kill assay was done to check the survival rate of *C. neoformans* with and without the test compound. The test compound at MIC_{80} concentration was added to the potato dextrose broth (PDB) and 10^6cells/ml of *C. neoformans* was added. The suspension of PDB with test compound served as blank. Fluconazole served as positive control for which PDB and fluconazole suspension was taken as blank. The time course study was spectrophotometrically measured in terms of absorbance at 480 nm.

The experimental results were expressed as mean±standard error of mean of triplicates. Where applicable, the data were subjected to one-way and two-way analysis of variance (ANOVA) followed by bonferroni post test. A p-value of ≤ 0.05 was regarded as significant.

The present study showed that compound 1e exhibited retardation in growth with zone of inhibition of 12 mm and MIC_{80} of compound 1e was calculated as 50µg/ml against *C. neoformans*.

It was found that compound 1e exhibited 63.67% reduction of melanization in *C. neoformans* at MIC_{80} concentration (fig. 1). Earlier studies have reported that unpigmented conidia are unable to quench ROS and were effectively eradicated by the host defence system [12]. Hence, compound 1e may be a potent lead for upcoming antifungal medication.



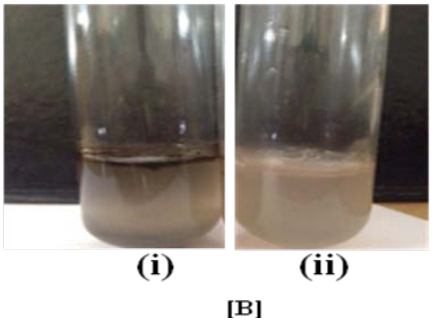


Fig. 1: Reduction in melanin production. [A] Illustrates reduction in melanization in *C. neoformans* in the presence of compound 1e. The data are mean±SEM from 3 samples for each group and analyzed by one-way ANOVA where * P<0.05 significant from control; [B] (i) melanization produced in *C. neoformans*; (ii) reduction in melanin content of *C. neoformans* in presence of compound 1e

Laccase enzyme is responsible for the oxidization of polyphenolic substrate and iron into reactive intermediates which causes cell membrane damage in the host [13]. The compound 1e exhibited a significant reduction in laccase activity of *C. neoformans*. The laccase activity was reduced to 57.44% after 4 h* of treatment with compound 1e as calculated from the slope obtained from the straight line equation. The activity exhibited by the *C. albicans* was used as a negative control since it has been reported to exhibit a lack of laccase enzyme production as depicted from fig. 2.

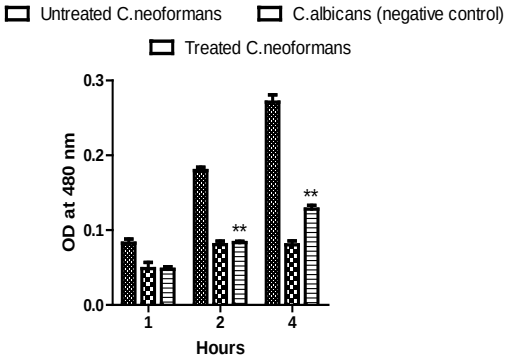


Fig. 2: Illustrates the reduction of laccase activity in *C. neoformans* in the presence of compound 1e. The data are mean±SEM from 3 samples for each group and analyzed by two-way ANOVA followed by Bonferroni Post-test where ** P<0.01 significant from control

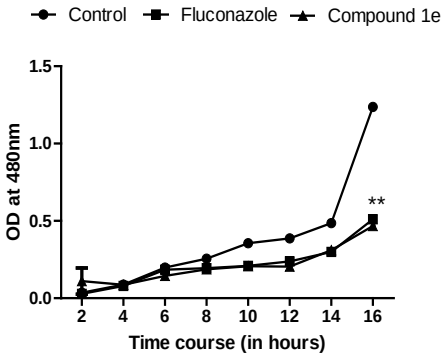


Fig. 3: Illustrates absorbance of *C. neoformans* liquid culture at 600 nm in the presence of fluconazole and compound 1e. The data are mean±SEM from 3 samples for each group and analyzed by two-way ANOVA followed by Bonferroni Post-test where ** P<0.01 significant from control

The Time-kill assay results have illustrated that the compound 1e inhibited the growth of *C. neoformans* cells in almost the same duration of time as reported for the commercially available drug, fluconazole (fig. 3).

Amongst the synthesized biphenyl-2,6-diethanone derivatives, compound 1e exhibited maximum antifungal activity against *C. neoformans* which may be attributed to the presence of nitrogen in the biphenyl scaffold. Thus, biphenyl-2,6-diethanone derivatives can behave as potentially promising antifungal agent. Present study concludes that compound 1e had a potential role in inhibition of melanin synthesis and laccase activity hence, can be used as therapeutic agents to kill *C. neoformans* or in the treatment of infected persons.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Amity University for infrastructure and facilities. Author, MR thanks financial assistance through Amity Science, Technology and Innovation Foundation (ASTIF) fellowship provided by Amity University.

CONFLICT OF INTERESTS

All authors declare no conflicts of interest

REFERENCES

1. Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS-100 y after the discovery of *Cryptococcus neoformans*. Clin Microbiol Rev 1995;8:515-48.
2. Chuck SL, Sande MA. Infections with Cryptococcus neoformans in the acquired immunodeficiency syndrome. N Engl J Med 1989;321:794–9.
3. Kovacs JA, Kovacs AA, Polis M, Wright WC, Gill VJ, Tuazon CU, *et al.* Cryptococcosis in the acquired immunodeficiency syndrome. Ann Intern Med 1985;103:533–8.
4. Shashikumar ND, Krishnamurthy G, Bhojyanaik HS, Lokesh MR, Jithendrakumara KS. Synthesis of new biphenyl substituted quinoline derivatives, preliminary screening and docking studies. J Chem Sci 2014;126:205–12.
5. Kaur A, Kumar R, Kalidhar U. Synthesis, spectral studies and biological activities of some novel biphenyl imidazole[2,1-b][1,3,4]thiadiazole derivatives. Res J Pharm Biol Chem Sci 2012;3:1084-96.
6. Sharma A, Pandey J, Tripathi RP. An efficient regioselective synthesis of functionalized biphenyls via sequential reactions of aromatic aldehydes and β-keto esters or ketones. Tetrahedron Lett 2009;50:1812-6.
7. Van Duin D, Cleare W, Zaragoza O, Casadevall A, Nosanchuk JD. Effects of voriconazole on *Cryptococcus neoformans.* Antimicrob Agents Chemother 2004;48:2014–20.
8. Pfaller MA, Chaturvedi V, Espinel-Ingroff A, Ghannoum MA, Gosey LL, Odds FC, *et al.* Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard. 2nd Edition. NCCLS document M27-A2 Pennsylvania, USA; 2002;22:1-29.
9. Mayer AM, Staples RC. Laccase: new functions for an old enzyme. Phytochemistry 2002;60:551-65.
10. Rosas AL, Nosanchuk JD, Feldmesser M, Cox GM, McDade HC, Casadevall A. Synthesis of polymerized melanin by *Cryptococcus neoformans* in infected rodents. Infect Immun 2000;68:2845–53.
11. Ikeda R, Sugita T, Jacobson ES, Shinoda T. Laccase and melanization in clinically important *Cryptococcus* species other than *Cryptococcus neoformans.* J Clin Microbiol 2002;40:1214-8.
12. Brakhage AA, Liebmann B. *Aspergillus fumigatus* conidial pigment and cAMP signal transduction: significance for virulence. Med Mycol 2005;43 Suppl 1:S75-S82.
13. Zhu V, Williamson PR. Role of laccase in the biology and virulence of *Cryptococcus neoformans*. FEMS Yeast Res 2004;5:1-10.

How to cite this article

- Megha Rikhi, Shanu Hoda, Sahil Nagpal, Pooja Vijayaraghavan, Seema Bhatnagar. Antifungal activity of biphenyl-2,6-diethanone derivatives. Int J Pharm Pharm Sci 2016;8(8):378-380.