# Synthesis and Antifungal Activity of Perylene Bisimide Derivatives

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**Abstract**

In this study, we have synthesized several perylene bisimide derivatives and evaluated the antifungal activity against human pathogenic yeasts (*Candida* spp.) and filamentous fungi (*Aspergillus* spp., *Fusarium* spp., and *Trichophyton* spp.). Three compounds (**2-4**) from the nine compounds tested presented relevant activity against *Candida albicans*, *C. parapsilosis* and *C. tropicalis* with minimum inhibitory concentrations ($MIC_{90}$) values of 3.1-25.0 µg/mL. These three compounds also showed activity against filamentous fungi, *F. oxysporum*, *T. rubrum* and *T. mentagrophytes* at a concentration of 50 µg/mL. The resultant data indicates that perylene bisimides (**2-4**) display wide spectrum and relevant antifungal activity. Compound **4** represents a new scaffold for the development of antifungal agents and warrant further structure activity relationship studies.

**Keywords**

Antifungal, *Candida*, *Fusarium*, *Trichophyton*, perylene, synthesis

## Introduction

The frequency of opportunistic fungal infection has increased drastically, mainly in patients who are immunocompromised due to organ transplant, leukaemia or HIV infection.[1] *Candida albicans* is responsible for most infections caused by fungi; however, the incidence of non-albicans species that are resistant, less susceptible or potentially resistant to currently antifungal drugs, such as *Candida parapsilosis, Candida krusei*, *Candida tropicalis*, appears to be increasing.[2] Filamentous fungi infections are less frequent than Candida species infection, but are associated with high mortality rates.[3]

Despite the addition of new classes of antifungals, the number of currently available drugs for the treatment of fungal infections remains limited. Many of them are associated with a substantial toxicity and display very complex structures. Therefore, there is a continuing need to develop novel and simpler antifungal agents being more effective and less toxic.

Perylene derivatives are compounds used in a variety of industrial applications for decades, especially in the dye-sensitized solar cells, organic light-emitting diodes, and organic thin film transistors.[4] Moreover, these compounds have shown some anticancer, antiviral, antidepressant, and antibacterial activities.[5]

In the course of one investigation of dye-sensitized solar cells and exfoliation of single-walled carbon nanotubes and graphite, a synthetic study resulted in the preparation of several perylene bisimides, compounds **1-9** (Figure 1). Taking into account that perylene bisimides have shown interesting biological properties, we decided to study the antifungal properties of the synthesized materials.

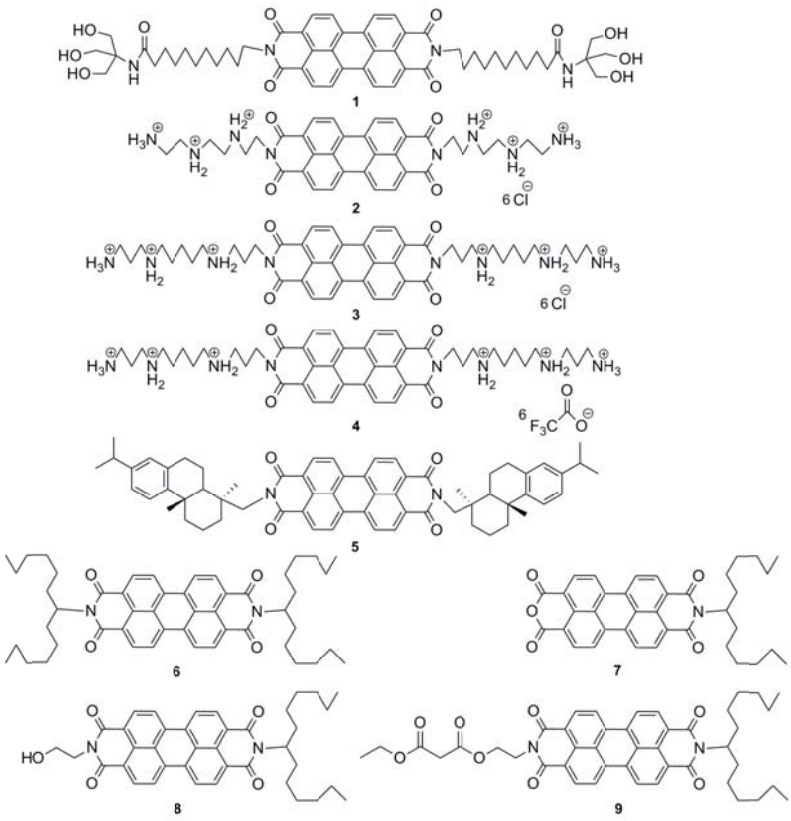

**Figure 1**. Tested perylene bisimides

## Results and discussion

The tested compounds **1-6** were synthesized starting from perylene-3,4,9,10-tetracarboxylic dianhydride by condensation with the corresponding amine following reported procedures.[6,7,8,9] Compounds **7-9** were prepared following the literature for similar compounds.[10]

With compounds **1-9** in hand, the antifungal activity against human pathogenic yeasts (*Candida* spp.) and filamentous fungi (*Aspergillus* spp., *Fusarium* spp., and *Trichophyton* spp.) was evaluated. Three compounds (**2-4**) from the nine compounds tested presented relevant activity (Table 1). Compound **2** was active against *Candida albicans*, *C. parapsilosis* and *C. tropicalis* with minimum inhibitory concentrations ($MIC_{90}$) values of 25.0, 22.0 and 14.0 μg/mL, respectively. Similarly, compound **3** was active against these three yeasts with $MIC_{90}$ values of 7.0, 5.0 and 6.0 μg/mL,

respectively, while compound **4** showed a $MIC_{90}$ value below 3,1 µg/mL, being this compound the most potent. These three compounds also showed activity against filamentous fungi, *F. oxysporum*, *T. rubrum* and *T. mentagrophytes* at a concentration of 50 µg/mL. However, we didn't find any activity of perylene derivatives against *Aspergillus* spp at the tested concentrations.
The most active compounds, **3** and **4**, are the result of condensation of perylene dianhydride and the polyamine spermine. Both compounds are ammonium derivatives, compound **3** results from acidification with HCl and compound **4** from acidification with trifluroacetic acid. From the observed results, it seems that the trifluoroacetate anion in the ammonium derivatives is important for enhanced biological activity.

**Table 1.** Geometric Means of Minimal Inhibitory Concentration (GM – MIC, µg/mL) of perylene bisimide derivatives **1-9.**

| **GM – MIC (µg/mL)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Compound** | ***Ca4*** | ***Cp2*** | ***INM13*** | ***Fo7*** | ***Tr1*** | ***Tm1*** | ***Afu8*** | ***Afl6*** | ***INM7*** |
| **1** | * | * | * | * | * | * | * | * | * |
| **2** | 25 | 22 | 14 | 50 | 50 | 50 | * | * | * |
| **3** | 7 | 5 | 6 | 50 | 50 | 50 | * | * | * |
| **4** | <3,125 | <3,125 | <3,125 | 50 | 50 | 50 | * | * | * |
| **5** | * | * | * | * | * | * | * | * | * |
| **6** | * | * | * | * | * | * | * | * | * |
| **7** | * | * | * | * | * | * | * | * | * |
| **8** | * | * | * | * | * | * | * | * | * |
| **9** | * | * | * | * | * | * | * | * | * |
| **ITZ** | - | 0.5 | - | - | - | - | - | - | - |
| **AMB** | - | - | - | - | - | - | 1 | 0.5 | - |
| **TERB** | - | - | - | - | <0.0078 | <0.0078 | - | - | - |

***** MIC > 50 µg/mL; ***Ca4:*** *Candida albicans* ATCC 10231***; Cp2:*** *Candida parapsilosis* ATCC 22019***; INM13:*** *Candida tropicalis* ATCC 00956***; Fo7:*** *Fusarium oxysporum* ATCC 48112***; Tr1:*** *Trichophyton rubrum* ATCC 28188**; *Tm1*:** *Trichophyton mentagrophytes* ATCC 24198; **Afu8**: *Aspergillus fumigatus* ATCC 204305; **Afl6**: *Aspergillus flavus* ATCC 204304; **INM7**: *Aspergillus terreus* CDC 317; **ITZ**: Itraconazole; **AMB**: Amphotericin B; **TERB**: Terbinafine.

## Conclusions

The resultant data indicates that perylene bisimides (**2-4**) display wide spectrum and relevant antifungal activity. Compound **4** represents a new scaffold for the development of antifungal agents and warrant further structure activity relationship studies.

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## Experimental

*General Experimental Procedures*

Reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F-254 in 0.25 mm-thick plates. Purifications were performed by flash chromatography on Merck silica gel (230-400 mesh). Commercial reagent grade solvents and chemicals were used as received unless otherwise noted. Combined organic extracts were washed with brine, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure.

*Antifungal activity*

The antifungal activity of perylene bisimide derivatives was evaluated following the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing (AFST-EUCAST)[11] for yeasts and the Clinical and Laboratory Standards Institute M38-A (CLSI M38-A, 2002)[12] protocol for filamentous fungi. Yeast, *Candida albicans* (ATCC 10231), *C. parapsilosis* (ATCC 22019), *C.tropicalis* (ATCC 200956) and filamentous fungi, *Fusarium oxysporum*, *Aspergillus fumigatus* (ATCC 204305), *A. flavus* (ATCC 204304), *A. terreus* (CDC 317), *Trichophyton rubrum* (ATCC 28188), *T. mentagrophytes* (ATCC 24198), were used for these assays.

Briefly, duplicate samples (100 µL) of five serial dilutions of compounds were dispensed into 96-well microtitration plates (Becton Dickinson, New Jersey, USA) at final concentrations between 50-3.125 µg/mL. As positive controls, we included itraconazole and amphotericin B (Sigma-Aldrich, Co, MO, USA) at final concentrations of 0.031-16 µg/mL. Moreover, terbinafine (Recalcine Laboratories, Santiago de Chile, Chile) was included at final concentrations of 0.0078-4 µg/mL .One hundred microlitres of the fungal inoculum of 1-5 x $10^5$ CFU/mL and 0.2-2.5 x 105 CFU/mL for yeast and filamentous fungi, respectively, were added. For the AFST-EUCAST method, the minimum inhibitory concentration ($MIC_{90}$) was determined after 24 h of incubation at 35 °C and was defined as the lowest concentration that resulted in 90% of growth reduction. For the CLSI M38-A method, MIC were determined after 48 h of incubation at 35 °C (*F.oxysporum* and *Aspergillus* spp) and six days at 28°C (*Trichophyton* spp*).* In this assay, $MIC_{50}$ was defined as the lowest compound dilution that resulted in total inhibition of visible growth. Compounds were considered active when presented MIC values ≤ 50 µg/mL. The MIC values were expressed as geometric means (GM-MIC) of tests performed by duplicate in the three different assays against each fungi species.

## References


[1] M. H. Miceli, J. A. Díaz, S. A. Lee, *Lancet Infect. Dis.* **2011**, *11*, 142-151.
[2] M. A. Pfaller, D. J. Diekema, *Clin. Microbiol. Rev.* **2007**, *20*, 133-163.
[3] M. A. Pfaller, D. J. Diekema, *J. Clin. Microbiol.* **2004**, *42*, 4419-4431.
[4] C. Zafer, M. Kus, G. Turkmen, H. Dincalp, S. Demic, B. Kuban, *Sol. Energy Mater. Sol. Cells* **2007**, *91*, 427-431.
[5] T. Keskin, B. S. Isgor, Y. G. Isgor, F. Yukruk, *Chem. Biol. Drug Des.* **2012**, *80*, 675-681.
[6] G. Schnurpfeil, J. Stark, D. Wöhrle, *Dyes & Pigments* **1995**, *27*, 339-350.
[7] S. Rehm, V. Stepanenko, X. Zhang, T. H. Rehm, F. Würthner, *Chem. Eur. J.* **2010**, *16*, 3372-3382.
[8] M. Franceschin, C. M. Lombardo, E. Pascucci, D. D'Ambrosio, E. Micheli, A. Bianco, G. Ortaggi, M. Savino, *Bioorg. Med. Chem.* **2008**, *16*, 2292-2304.
[9] H. Icil, S. Icli, Ç. Sayil, *Spectroscopy Lett.* **1998**, *31*, 1643-1647.
[10] J. Baffreau, S. Leroy-Lhez, N. Vân Anh, R. M. Williams, P. Hudhomme, *Chem. Eur. J.* **2008**, *14*, 4974-4992.
[11] M. Cuenca-Estrella, C. Moore, F. Barchiesi, et al. *Clin. Microbiol. Infect.* **2003**, *9*, 467-474.
[12] CLSI. *Clinical and Laboratory Standards Institute*, Wayne, USA: **2008**.