# Fungal phospholipid metabolism for antifungal drug discovery




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**Invasive fungal infections often respond poorly to antifungal drugs. The fungal invasin phospholipase B (PLB) and/or its biosynthetic pathway are novel targets for drug development. Compounds with structural similarities to phosphatidylcholine, which is a preferred substrate of cryptococcal PLB1, were purchased or synthesised. For many, there was a correlation between antifungal and anti-PLB activity but not all demonstrated selectivity for fungal compared with mammalian phospholipase, and some were toxic to mammalian cells in culture. The most promising, a bis-pyridinium compound, is undergoing toxicity testing in mice. Miltefosine (MI), a stable phospholipid analogue used in the treatment of leishmaniasis also has broad spectrum fungicidal activity, but inhibition of PLB is not its major mode of action. To improve antifungal potency and reduce toxicity of MI, analogues of this alkyl phospholipid have been synthesised and are under investigation.**

Invasive fungal infections are a serious and escalating health problem. They are associated with a high morbidity, a mortality varying from 20% to more than 80% and a multibillion dollar economic burden, especially, but not only, in immunocompromised hosts. Current therapies are limited in safety and/or efficacy and resistant fungal pathogens are an emerging problem. Of the antifungal drugs marketed for systemic use, all except the echinocandins (which target the cell wall synthesis enzyme, glucan 3 synthase), act on the fungal membrane protein, ergosterol, or its biosynthetic pathways. Thus new drugs acting on novel targets, and which have activity against key fungal pathogens and favourable pharmacokinetic and safety profiles, are needed now.

Virulence factors, of which the multifunctional enzyme phospholipase B1 (PLB1) is an example, are potential targets for drug discovery. PLB1 is best characterised in the yeast *Cryptococcus neoformans* [1]. It contains three enzyme activities, PLB, lysophospholipase (LPL) and lysophospholipase transacylase (LPTA, Figure 1) and functions as a fungus-specific invasin [2]. The major mammalian cell wall phospholipid, phosphatidylcholine (PC) is a preferred substrate [3-4]. PLB is produced and secreted by many pathogenic fungi [5-9].

We synthesised and tested three classes of compound with structural similarities to PC as potential inhibitors. Two (alkyl-bis(biguanide) and alkyl-bis(phosphonium)) compounds inhibited both PLB activity and growth of *C. neoformans* and

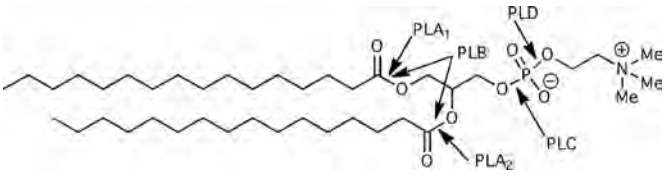


**Figure 1. Structure of a phospholipid, with sites of hydrolysis by different phospholipases shown by arrows. PLB1 has PLB activity but also lysophospholipase activity – a fatty acyl chain is cleaved from a lysophospholipid (structurally similar to the phospholipid shown but with only one fatty acyl chain in the *sn*-1 or *sn*-2 position on the glycerol backbone) and lysophospholipase transacylase (which re-acylates a fatty acyl chain on to the lysophospholipid). Phospholipase $A_2$ ($PLA_2$), phospholipase C (PLC) and phospholipase D (PLD) occur widely in mammalian cells.**

*C. albicans* [10]. This activity was not explained by a non-specific detergent-like action, since there was selectivity of effect between the LPL/LPTA and PLB activities of the enzyme. Analogues of the alkyl-bis(phosphonium) compounds were also active against filamentous fungi including *A. fumigatus, A. terreus* and *S. apiospermum,* with MICs similar to those of Amphotericin B [11]. Although promising, these early prototype compounds were not sufficiently selective for fungal PLB1 compared with mammalian secretory PLA2. We subsequently synthesised a novel structural class of bis-pyridinium compounds, which are completely selective for fungal PLB and not toxic to erythrocytes or nucleated mammalian cells [12]. They have broad spectrum antifungal activity and are currently being tested for toxicity in animals prior to efficacy studies in mice.

An additional compound class of interest is represented by the oral anti-leishmanial drug, miltefosine (hexadecylphosphocholine). This is a metabolically stable phospholipid analogue which we have shown has broad spectrum fungicidal activity *in vitro* against yeasts and moulds, including resistant species such as *Candida krusei, Candida glabrata, Scedosporium prolificans, Fusarium* spp. and Zygomycetes [13]. MICs are well below serum levels achieved by oral dosing with miltefosine and there is very preliminary evidence of effect in a mouse model of cryptococcosis and as combination salvage therapy in a child with severe, non-responsive, *S. prolificans* infection. Current investigation is directed to understanding the mode(s) of action of miltefosine in order to develop more potent, less toxic analogues. To date we have established that, although miltefosine inhibits cryptococcal PLB1, it does so only at high concentrations, indicating that this is not its major mode of action.

In conclusion, although the mature enzyme PLB1 is a potential target for antifungal drug development, inhibitor compounds to date appear to exert their antifungal effect through additional pathways. An alternative approach under active investigation is to seek to inhibit cellular synthesis and/or secretion of PLB1 [14].

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