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Original article

Synthesis of amino acid appended indoles: Appreciable anti-fungal activity and inhibition of ergosterol biosynthesis as their probable mode of action

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ABSTRACT

Rationally designed compounds consisting of mono- and di-peptide appendages on bis-indole template were synthesized in appreciable yield. Some of these compounds exhibited significant antifungal activities against *Candida albicans* with their MIC_{80} in μg/ml range. However, when used in combination with azoles, the antifungal activities of the azoles were considerably enhanced. The growth inhibition appeared to be specific to the fungal cells and mammalian cells were not affected by these compounds. It is shown that these compounds lower ergosterol levels in the fungal cells and probably act by targeting lanosterol 14α-demethylase, a key enzyme in the sterol biosynthetic pathway of *C. albicans*. The compounds do not appear to directly act on the fungal cell wall. Hence, the sensitivity of the fungal cells to these compounds cannot be attributed to cell wall damage and consequent accumulation of the compounds in the cell, though defects in cell wall due to defective sterol biosynthesis cannot be completely ruled out.

1. Introduction

Fungal infections and their treatment continue to be major challenges in the medical world today, not least due to the drug resistance that these organisms are known to exhibit upon prolonged treatment with antifungals [1,2]. Because of the eukaryotic nature of fungal cells and hence similarity of many of the essential pathways to mammalian cell, the identification of appropriate drug targets within these pathogens is highly complicated. However, fungi and yeast, unlike mammals, depend predominantly on ergosterol as the major membrane sterol while mammalian cells require cholesterol for their function. Additionally, fungal cells have a cell wall and the cell wall biosynthetic pathway is completely absent in mammalian cells. Thus, most of the drugs currently available for treatment of fungal infections either target membrane ergosterol or the ergosterol biosynthetic pathway or target the fungal cell wall [1]. Amongst the drugs of choice for treatment of *Candida* infections are the azoles such as ketoconazole, fluconazole and miconazole, all inhibitors of lanosterol 14α-demethylase (Erg11); terbinafine, an allylamine inhibitor of Erg1, the sqaulene epoxidase that functions upstream of Erg11; morpholines like amorolfine which inhibit Erg24 and Erg2 that function downstream of Erg11 in the same pathway; amphotericin B and nystatin, both polyenes that bind to membrane ergosterol and cause cytotoxic pores in the fungal cells [1,4]. More recently, echinocandins like caspofungin or micafungin have been used to inhibit β-glucan synthesis and thereby target the fungal cell wall [1,4,5] while nikkomycin Z is being viewed as a potent antifungal drug due to its ability to inhibit chitin synthases [4]. However, the many mechanisms that pathogenic fungi adapt for developing resistance to antifungals include overexpression of Erg11, a key enzyme of the sterol biosynthetic pathway, accumulation of point mutations in Erg11, overexpression of multidrug efflux pumps and reduction in drug import into resistant cells [1]. Of these, a very common reported mechanism in clinical drug resistant *Candida albicans* strains, is the overexpression of Erg11 or point mutations in it to counter the inhibition by azoles [1,3].

In a recent report, we showed that indole-based compounds can act as good antifungals [6]. It was also predicted therein, using homology and docking studies that these compounds may act by

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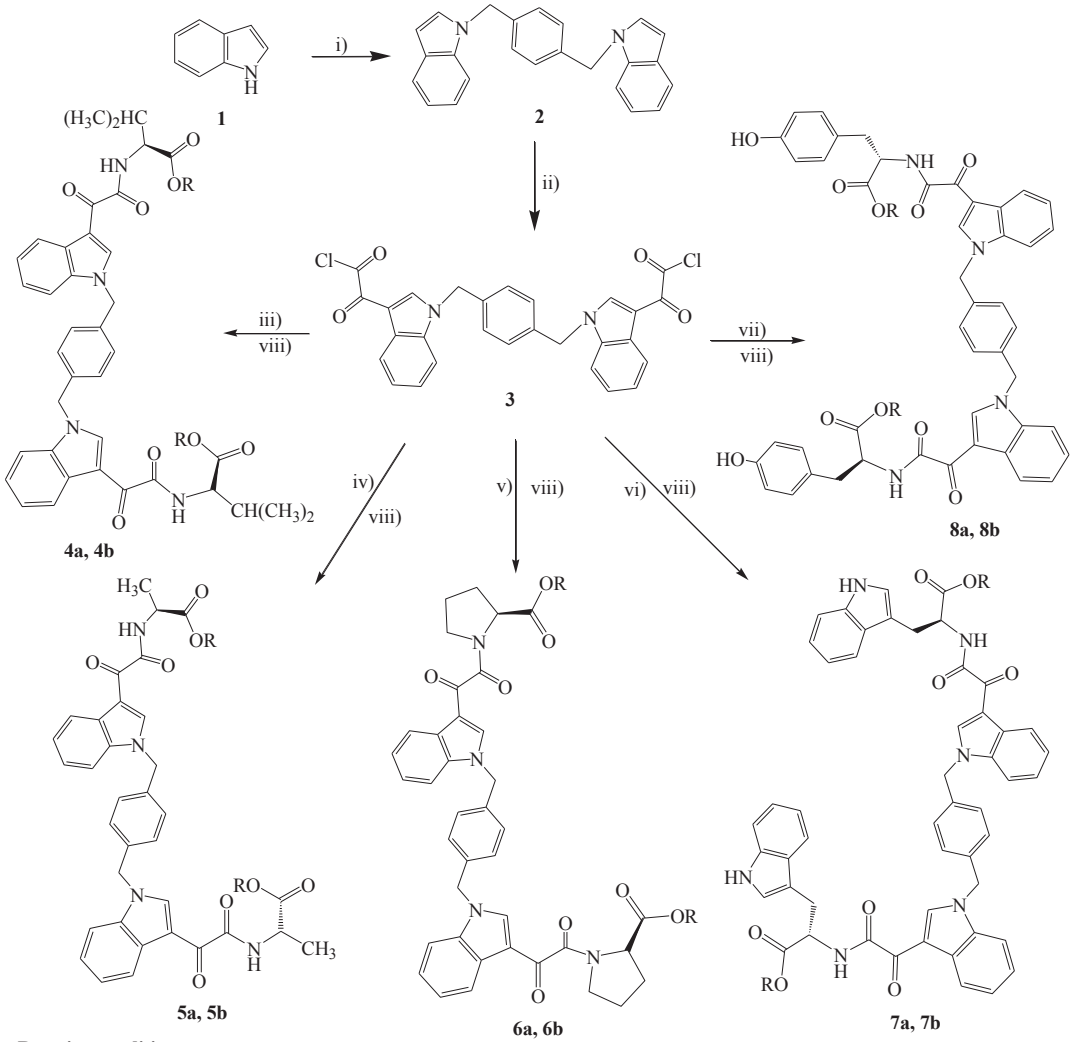
interacting at the catalytic site of lanosterol 14α-demethylase (molecular docking studies). Extending this work, another series of indole based compounds is explored to study their effect on growth of *C. albicans* and their probable mode of action. It is shown that a number of these compounds could work very well in combination therapies with azole drugs.

2. Results and discussion

2.1. Chemistry

Since antibiotics should be non-toxic to the host, killing only the pathogen; the biological acceptability of indole and amino acids was taken as the safe guard for non-toxicity of new compounds to human system. To induce selectivity in the molecules, chiral amino acids were suitably introduced on the indole to develop a library of compounds. Selection of linker group between two indole moieties was based on previous results where compounds with three carbon and xylidene spacers exhibited significant antimicrobial activities [6]. Compounds **2** and **9** were obtained by the reaction of indole with 1,4-bis(bromomethyl)benzene and 1,3-dibromopropane, respectively in the presence of KOH using DMSO as a solvent. Indole **2** and **9** were reacted with oxalyl chloride in dry ether to get corresponding oxoacetyl chlorides **3** and **10** (Schemes 1 and 2). Treatment of compound **3** with L-valine methyl ester hydrochloride/L-alanine methyl ester hydrochloride/L-proline methyl ester hydrochloride/L-tryptophan methyl ester hydrochloride/L-tyrosine methyl ester hydrochloride in acetonitrile in presence of K_2CO_3 provided amino acid appended indoles **4a**–**8a**. Hydrolysis of **4a**–**8a** with NaOH in acetone–water gave compounds **4b**–**8b** in 70–80% yields (Scheme 1).

Through a similar sequence of reactions as depicted in Scheme 1, compound **10** on reaction with methyl ester hydrochlorides of L-valine, L-alanine, L-proline, L-tryptophan and L-tyrosine gave compounds **11a,b**–**15a,b** (Scheme 2). Presence of amino acid residues in compounds **4a,b**–**8a,b** and **11a,b**–**15a,b** has considerably increased the solubility of these compounds in aqueous medium (observed during biological studies) as compared to those of previously reported compounds [6]. Further, compounds **4b**, **6b**, **11b** and **13b** carrying valine and proline were coupled with other amino acids.



Reaction conditions:
i) KOH, $BrCH_2(C_6H_4)CH_2Br$, DMSO;
ii) $(COCl)_2$, dry ether
iii) L-valine methyl ester HCl, K_2CO_3, dry CH_3CN;
iv) L-alanine methyl ester HCl, K_2CO_3, dry CH_3CN
v) L-proline methyl ester HCl, K_2CO_3, dry CH_3CN;
vi) L-tryptophan methyl ester HCl, K_2CO_3, dry CH_3CN
vii) L-tyrosine methyl ester HCl, K_2CO_3, dry CH_3CN;
viii) **for a (R=CH_3) to b (R=H)**: NaOH (3 eq), acetone - water (2:1)

Scheme 1. Synthesis of compounds **4**–**8**.