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Synthesis and Antifungal Activity of a Ferrocene–fluconazole Analogue

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Abstract—A novel ferrocene–fluconazole analogue was synthesized and its antifungal properties investigated against yeast strains of medical importance, including those intrinsically resistant to fluconazole. In vitro tests revealed a slight increase in fungal growth and a reversal of the effect of fluconazole at minimal inhibitory concentrations.

Introduction

Over the past few years, much interest has focussed on the synthesis and biological evaluation of active molecules modified by metallocene.[1] Metallocenic compounds, such as ferrocene, are stable, non toxic, and can cross cell membranes.[1,2] Recently, several organometallic compounds have been developed as interesting alternatives for the chemotherapy of drug-resistance in cancer and tropical diseases such as malaria.[3] A ferrocene-hydroxytamoxifen analogue (hydroxyferrocifen), in which a phenyl ring is replaced by a ferrocenyl group, was prepared as an oestradiol site-directed cytotoxic agent by Jaouen and co-workers.[4,5] This compound was tested against a human breast cancer cell-line and was found to be more cytotoxic than the parent compound tamoxifen.[4] Using a similar synthetic strategy, a ferrocene–chloroquine analogue (ferrochloroquine) was obtained by substituting the carbon chain of the antimalarial agent chloroquine with a ferrocenyl unit.[6–8] This organometallic compound was shown to be active against a chloroquine-resistant strain of *Plasmodium falciparum* in vitro as well as *P. berghei* N and *P. yoelii* NS in vivo.[8,9]

We applied a similar strategy to the antifungal agent fluconazole (**FCZ**). **FCZ** is a triazole derivative which inhibits specific steps in fungal sterol biosynthesis. It has been shown to be effective treatment against some systemic mycoses, in particular those caused by opportunistic yeasts of the genus *Candida*.[10] *Candida* species (i.e., *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. krusei*) are the fifth most common isolate in the hospital environment, and **FCZ** is considered to be a useful agent for managing patients at risk. However, several problems arise in clinical practice, in particular the presence of *Candida* species that are intrinsically resistant to **FCZ** (i.e., *C. krusei*) or species that rapidly acquire resistance (i.e., *C. glabrata*).[11,12] The molecular mechanisms leading to yeast resistance and their genetic regulation are currently the subjects of active research.[13] Iron overload is a serious risk factor for candidiasis and the growth of *Candida* is inhibited by iron deprivation. Exogenous iron inhibits fungistatic activity, but does not compromise the activity of **FCZ**, and may even increase candidacidal activity of monocyte-derived macrophages.[14] Given the avidity of *Candida* for free iron, and the absence of interference with **FCZ**, we proposed that the addition of iron to the **FCZ** molecule may be an effective way of overcoming **FCZ** resistance in yeasts. Furthermore, by coupling **FCZ** to ferrocene it might be possible to increase **FCZ** incorporation and targeting toward cytochrome P-450. Like **FCZ**, ferrocene also interacts with cytochrome P-450.[15] Thus, our approach was to substitute the phenyl ring of **FCZ** with a ferrocenyl unit, and to test the anticandida activity of the compound synthesized (Fig. 1).[16]

Chemistry

Synthesis of **FCZ**-ferrocene was carried out as shown in Scheme 1. First, ferrocene (**1a**) was metalated in the

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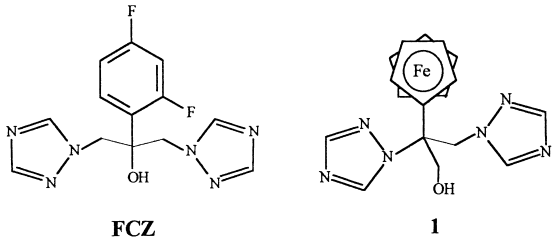
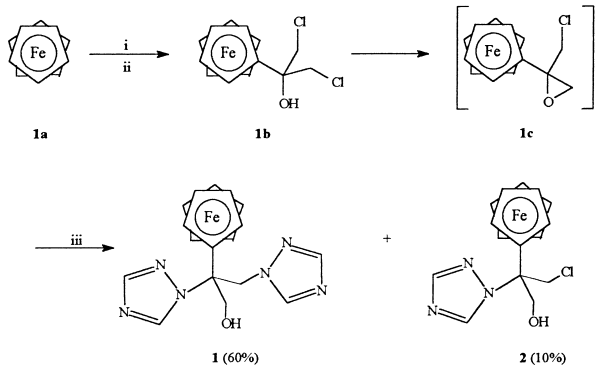


FCZ **1**

Figure 1. Structure of fluconazole (**FCZ**) and the ferrocene–fluconazole analogue (**1**).



Scheme 1. Reagents and conditions: (i) 0.83 equiv *t*-BuLi, anhydrous THF, O°C, 15 min; (ii) 1.3 equiv $(ClCH_2)_2CO$, anhydrous Et_2O, −78 °C, 30 min, then CH_3COOH, O°C, 15 min; (iii) 6 equiv 1,2,4-triazole, 4 equiv K_2CO_3, DMF, 70 °C, 19 h. Percentages indicate the yield of the reaction.

presence of *t*-BuLi in dried tetrahydrofuran at 0 °C under nitrogen.[17] The bis-halo material (**1b**) was then obtained by treating 1,3-dichloroacetone with ferrocenyllithium in anhydrous diethylether at −78 °C. The bis-halo and 1,2,4-triazole were heated together at 70 °C for 19 h in *N,N*-dimethylformamide in the presence of potassium carbonate.[18] The ferrocene–**FCZ** analogue (**1**) was purified (60% yield) by chromatography on silica gel using CH_2Cl_2:MeOH (95:5) as the eluant.[19]

Formation of the isomer, in which one of the triazole rings is attached to the adjacent methylene via the 4-position of the heterocyclic ring, was not observed. However, compound **2** resulting from incomplete condensation was recovered (10% yield).[20]

The relatively easy synthesis of ferrocene–**FCZ** was explained by the regioselectivity of the nucleophilic attack via the formation of oxirane compound **1c** and by the participation of the iron *d* orbitals.[21]

Results and Discussion

The in vitro activity of the organometallic compound **1**, fluconazole, and both products added simultaneously (1:1) was evaluated against 10 strains of four species of the genus *Candida*, selected for their different minimum inhibitory concentrations (MICs) to fluconazole.[22] Figs. 2–5 are representative examples of the results obtained with each yeast species.[23]

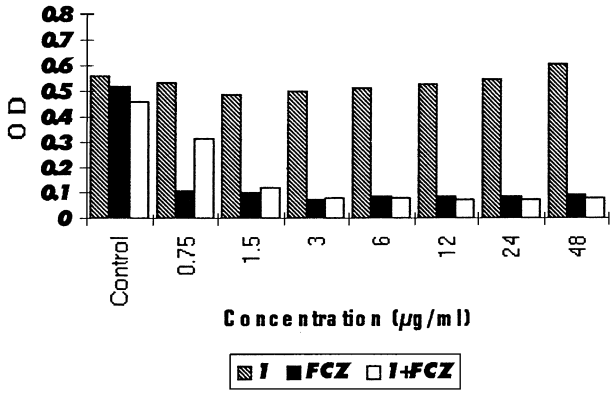


Figure 2. *C. albicans* strain (MIC <1.5 µg/mL). Spectrometric evaluation of fungal growth as a function of concentration of **1**, **FCZ**, **1** + **FCZ** (µg/mL).

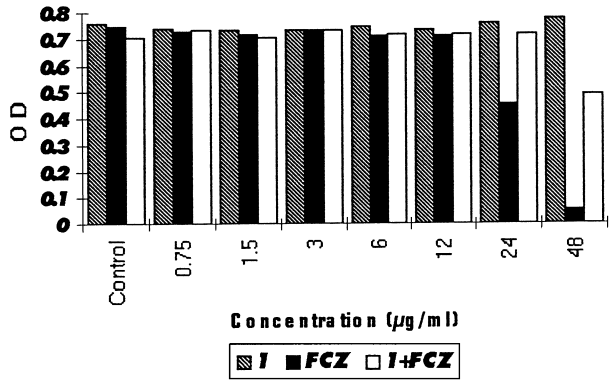


Figure 3. *C. glabrata* strain (MIC >48 µg/mL). Spectrometric evaluation of fungal growth as a function of concentration of **1**, **FCZ**, **1** + **FCZ** (µg/mL).

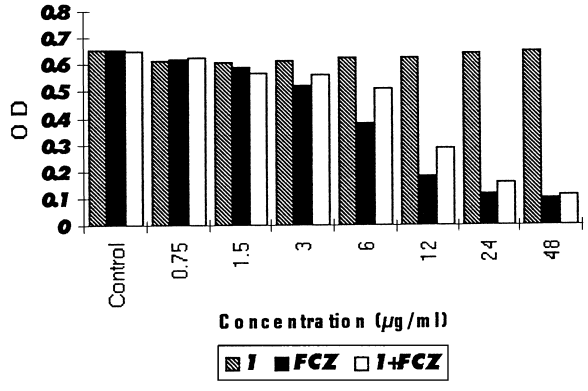


Figure 4. *C. parapsilosis* strain (MIC <12 µg/mL). Spectrometric evaluation of fungal growth as a function of concentration of **1**, **FCZ**, **1** + **FCZ** (µg/mL).

In comparison with **FCZ**, which generally induces dose-dependent growth inhibition (related to the MIC), the addition of the ferrocene analogue had no effect on fungal growth of any of the strains studied, except for a general trend towards stimulation of growth at high concentrations. Surprisingly, this stimulation of growth was more obvious for the three *C. krusei* strains which were highly resistant to **FCZ**.

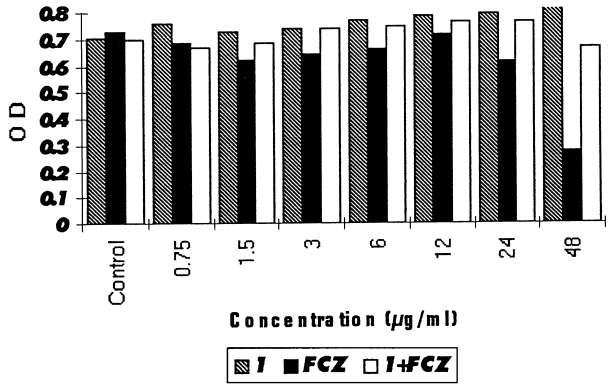


Figure 5. *C. krusei* strain (MIC >48 μg/mL). Spectrometric evaluation of fungal growth as a function of concentration of **1**, **FCZ**, **1** + **FCZ** (μg/mL).

Interestingly, at the first **FCZ** concentration leading to significant reduction in fungal growth, addition of **1** caused a reversion of the inhibitory effect of **FCZ**. This was observed for all strains and species tested, irrespective of their MIC.

Although the results of this study differ from the desired outcome, they nevertheless show that the hybrid compound has biological activity and should therefore be incorporated by the cell. This effect was related to at least one of the target molecules involved in **FCZ** efficacy, as the antifungal activity of **FCZ** was inhibited in the ferrocene-**FCZ** analogue.

These results suggest that the iron atom of ferrocene may be involved in the activity of the ferrocene–fluconazole analogue **1**. The challenge now is to restore the antifungal properties of the azole molecule.

Acknowledgements

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19. (Ferrocenyl)(1,2,4-triazole)(1,2,4-triazole-1-methyl)methanol **1** yellow oil. ^{1}H NMR ($CDCl_3/D_2O$; 95:5): δ 8.27 (s, 1H), 8.06 (s, 1H), 7.93 (s, 1H), 7.84 (s, 1H), 5.21 (d, J = 14.5 Hz, 1H), 4.89 (d, J = 14.5 Hz, 1H), 4.38–3.95 (m, 6H), 4.11 (s, 5H). MS (MALDI-TOF) dhb *m/e*: 378: $M^{\cdot+}$, 361:$(M-OH)^+$, 345, 242, 199. Anal. calculated for $C_{17}H_{18}N_6OFe$:C, 59.97; H, 4.76; N, 22.22; found:C, 60.01; H, 4.93; N, 22.51.
20. (Ferrocenyl)(1,2,4-triazole)(chloromethyl)methanol **2** yellow oil. ^{1}H NMR ($CDCl_3/D_2O$; 95:5): δ 8.05 (s, 1H), 8.00 (s, 1H), 4.40–4.18 (m, 8H), 4.20 (s, 5H). MS (EI) *m/e*: 347: $M^{\cdot+}$ ^{37}Cl, 345: $M^{\cdot+}$ ^{35}Cl, 345: $(M-H)^+$ ^{35}Cl, 279, 149, 121, 109, 95, 91, 77, 69, 55. Anal. calculated for $C_{15}H_{16}N_3OClFe$: C, 52.10; H, 4.63; N, 12.16; found: C, 52.32; H, 4.44; N, 12.30.
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23. Ten *Candida* isolates were used in this study according to their MIC, which was defined as the **FCZ** concentration leading to 80% growth inhibition. Three *C. albicans* strains (MIC <1.5 μg/mL), two *C. glabrata* strains (MIC >48 μg/mL), two *C. parapsilosis* strains (MIC <12 μg/mL) and three *C. krusei* strains (MIC >48 μg/mL).