When azoles meet silver: A chemistry power duo against fungi

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Abstract: Azole derivatives represent one of the most important classes of antifungal agents, acting as inhibitors of fungal cytochrome P450-dependent lanosterol 14*α*-demethylase, a key enzyme involved in ergosterol biosynthesis. The coordination of azoles with silver(I) ions has attracted growing attention as the resulting metal complexes can exhibit improved biological activity and therapeutic profiles compared to the parent ligands. This article reports on the synthesis, structural characterization, and biological evaluation of silver(I) complexes with selected azoles used in the treatment of fungal infections (clotrimazole, econazole, and voriconazole). The synthesized complexes were characterized using NMR, IR, and UV-Vis spectroscopy, mass spectrometry, and cyclic voltammetry, while their crystal structures were determined by single-crystal X-ray diffraction analysis. In most cases, the synthesized complexes showed enhanced anti-*Candida* activity compared to the parent antifungal azoles. Moreover, the corresponding silver(I) complexes demonstrated better activity in preventing hyphae and biofilm formation by *C. albicans* at subinhibitory concentrations, which is a highly desirable property for a new antimicrobial agent. In addition, these complexes were significantly more effective at inhibiting ergosterol synthesis in the cell membrane of *C. albicans* at 0.5 × MIC. Taken together, these findings suggest that compounds formed *via* the complexation of silver(I) ions with antifungal azoles merit further investigation as potential new antifungal agents, offering improved efficacy and safety over clinically used azoles in the treatment of life-threatening *Candida* infections.

Keywords: silver(I) complexes, azoles, fungal infections

1. Introduction

Invasive fungal infections remain a significant global health concern, particularly among immunocompromised patients, where they often lead to severe morbidity and mortality [1]. Azole derivatives, such as clotrimazole, econazole, and voriconazole, are among the most widely used antifungal agents due to their high efficacy and favorable pharmacokinetic profiles [2]. They act primarily by inhibiting the cytochrome P450-dependent enzyme lanosterol 14*α*-demethylase, thereby disrupting ergosterol biosynthesis and compromising fungal cell membrane integrity [2]. However, the emergence of resistance to azole drugs and the limited spectrum of activity highlight the urgent need for novel antifungal strategies. Among others, metal complexes with biologically active ligands have attracted considerable interest for their potential to overcome resistance mechanisms and enhance biological activity [3]. Silver(I) complexes are especially noteworthy because silver(I) ions possess intrinsic antimicrobial properties and can act synergistically with organic ligands. This article focuses on the synthesis, structural characterization, and antifungal evaluation of silver(I) complexes with selected azole ligands as potential next-generation antifungal agents.

2. Results and Discussion

2.1 Silver(I) complex with clotrimazole

The antifungal agent clotrimazole (ctz) was used for the synthesis of a mononuclear silver(I) complex, $[Ag(ctz)_2]SbF_6$ (Figure 1) [4]. In this complex, two ctz ligands are monodentately coordinated to the silver(I) ion through the nitrogen atom of the imidazole ring, resulting in a linear geometry. The $[Ag(ctz)_2]SbF_6$ complex exhibited significant antifungal activity against all tested *Candida* strains, particularly against *C. parapsilosis* (MIC = 0.01 μM) and *C. krusei* (MIC = 0.03 μM). Moreover, the activity of this complex was 9-fold (*C. glabrata*), 22-fold (*C. albicans*), 47-fold (*C. krusei*), and 1020-fold (*C. parapsilosis*) higher compared to clotrimazole. Notably, the toxicity of the $[Ag(ctz)_2]SbF_6$ complex on the MRC-5 cell line was 1.8 times lower compared to the parent azole.

The obtained results showed that the presence of the $[Ag(ctz)_2]SbF_6$ complex significantly reduced ergosterol concentration, while ctz caused a much smaller decrease in ergosterol levels compared to the control sample [4]. Furthermore, the $[Ag(ctz)_2]SbF_6$ complex completely inhibited *C. albicans* hyphae formation on solid medium, even up to 72 h. Besides that, this complex inhibited biofilm formation by *C. albicans* at 0.5 × MIC by 86%, while the corresponding azole inhibited biofilm formation by 79%, indicating a beneficial effect of the presence of the silver(I) ion.

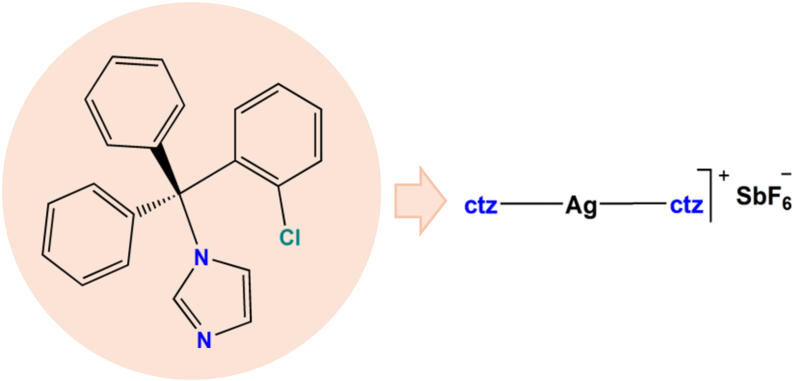

Figure 1. Silver(I) complex with clotrimazole (ctz) [4]

2.2 Silver(I) complexes with econazole

Three silver(I) complexes with econazole (ecz) of the general formula $[Ag(ecz)_2]X$ (X = SbF_6^-, $CF_3SO_3^-$, and PF_6^-; Figure 2) were synthesized, structurally characterized and biologically evaluated [4,5]. Spectroscopic and crystallographic data revealed that, in these complexes, the two ecz ligands are monodentately coordinated to the silver(I) ion through the nitrogen atom of the imidazole ring, forming a linear $[Ag(ecz)_2]^+$ cation, which is charge-balanced by the respective counterion X. The $[Ag(ecz)_2]X$ complexes exhibited the highest antifungal activity against *C. parapsilosis*, with MIC values ranging from 0.61 to 2.25 μM, and were more active than econazole (MIC = 3.9 μM). In addition, significant activity was observed for the $[Ag(ecz)_2]SbF_6$ and $[Ag(ecz)_2]PF_6$ complexes against *C. albicans* (MIC = 2.25 and 3.84 μM, respectively). The mechanism of action of the silver(I)-econazole complexes is likely related to the inhibition of ergosterol biosynthesis or direct interaction with this sterol, given that these complexes significantly reduced ergosterol content at subinhibitory concentrations (0.5 × MIC) [4,5].

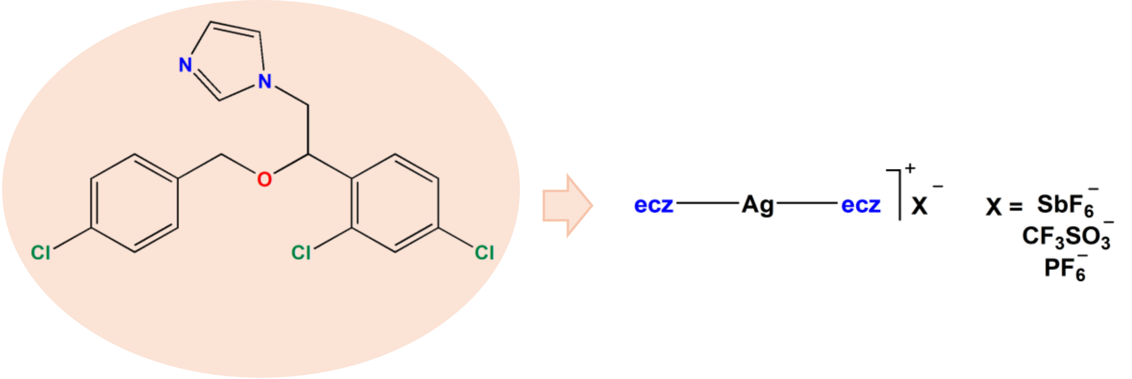

Figure 2. Silver(I) complexes with econazole (ecz) [4,5]

2.4 Silver(I) complexes with voriconazole

Four silver(I) complexes with voriconazole (vcz) were synthesized, namely $\{[Ag(vcz)_2]SbF_6\}_n$, $\{[Ag(vcz)(H_2O)]CH_3SO_3\}_n$, $\{[Ag(vcz)_2]BF_4\}_n$, and $\{[Ag(vcz)_2]PF_6\}_n$ (Figure 3) [4,6]. Crystallographic analyses revealed that in the solid state these complexes are polynuclear, with the silver(I) ions adopting distorted tetrahedral geometries. In contrast, DFT calculations indicated that the linear $[Ag(vcz)_2]^+$ species are thermodynamically favored in solution.

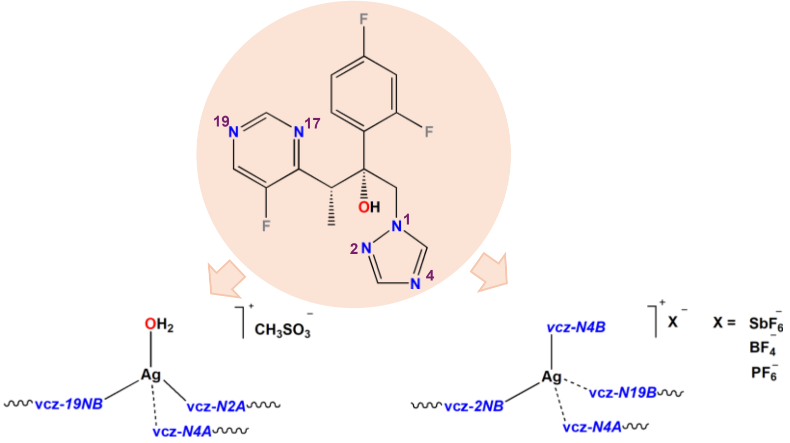

Figure 3. Silver(I) complexes with voriconazole (vcz) [4,6]

All these complexes demonstrated strong antifungal efficacy, with MIC values ranging from 0.01 to 1.05 μM. Notably, they were more active than vcz, especially against *C. albicans* and *C. glabrata*. For instance, $\{[Ag(vcz)_2]SbF_6\}_n$ was 9533-fold more active than vcz against *C. albicans*, and 75-fold more against *C. glabrata*, $\{[Ag(vcz)(H_2O)]CH_3SO_3\}_n$ and $\{[Ag(vcz)_2]BF_4\}_n$ showed 11440-fold increased activity against *C. albicans*, and 256- and 199-fold respectively against *C. glabrata*, while $\{[Ag(vcz)_2]PF_6\}_n$ displayed 545- and 105-fold higher activity against *C. albicans* and *C. glabrata*, respectively. All complexes completely inhibited hyphal formation in the *C. albicans* strain and, also significantly inhibited biofilm formation by this strain at subinhibitory concentrations by 87%, 79%, 80%, and 77%, respectively. In addition to inhibiting biofilm formation, the silver(I)-voriconazole complexes more effectively suppressed ergosterol biosynthesis compared to vcz itself.

3. Conclusions

The versatility of azole antifungals and their ability to coordinate to various metal ions, including silver(I), has led to the discovery of biologically relevant compounds that exhibit enhanced antimicrobial activity compared to the free azoles. The observable silver(I)-drug synergism represents a promising advancement in the ongoing fight against antimicrobial resistance, a growing global health concern. Continued exploration of such silver(I)-azole systems holds great potential for the development of next-generation antifungal agents capable of addressing current therapeutic limitations.

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