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Physicochemical characterization of the structure and desorption relationship of tioconazole-assembled gold nanoparticles investigated by density functional theory and Raman spectroscopy

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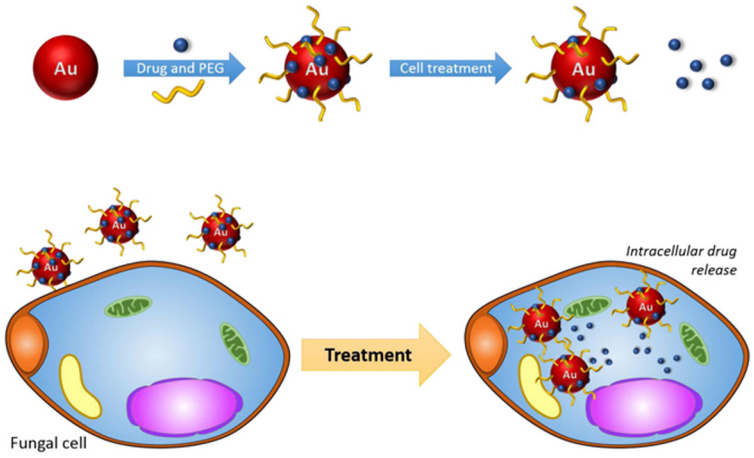
HIGHLIGHTS

- Surface-enhanced Raman scattering of tioconazole was performed on gold surfaces.
- Gold nanoparticles were characterized by electron microscopy and light scattering.
- Quantum calculations predicted the optimized structure of tioconazole on gold.
- Tioconazole adsorbates were supposed to release easily from gold via glutathione.
- Cell viability tests suggested enhanced killing for tioconazole-gold nanoparticles.

GRAPHICAL ABSTRACT



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ABSTRACT

The physicochemical characterization of the structure and desorption relationship in tioconazole (TN)-assembled gold nanoparticles (AuNPs) was studied using density functional theory (DFT) calculations and Raman spectroscopy. UV–vis absorption spectroscopy and surface-enhanced Raman scattering (SERS) were performed to examine the loading of TN on the metal nanoparticle surfaces. From the SERS spectra, TN appeared to adsorb on AuNPs mainly by way of its imidazole and 2-chlorothiophene unit. On the basis of the DFT calculations of the 6-metal cluster atom model, the binding energies of TN via the 2-chlorothiophene unit were predicted to be smaller than those for the imidazole coordination. The hydrodynamic diameters of TN-conjugated AuNPs were reduced to be in the range of ~165 nm after the PEGylation and to be suitable for the cellular uptake. The TN adsorbate was supposed to release from the AuNPs in the intracellular fungal component of glutathione after treatment with the fungi, consistent with the DFT calculations. Cell viability tests of *Penicillium digitatum*, *Penicillium expansum*, and *Aspergillus nidulans* before and after treatment with the TN-assembled AuNPs were examined to test

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the feasibility of developing an efficient drug delivery system. After 24 h treatment, TN-adsorbed AuNPs exhibited enhanced fungal cell killing according to a Student *t*-test ($p < 0.05$) in comparison to free TN at concentrations of 0.005–0.1 μM.

1. Introduction

Over the past decades, nanostructured materials have attracted significant interest due to their potential applications in water treatment [1], cancer therapy [2], and drug delivery [3,4]. The physicochemical characterizations of metal nanoparticles (NPs) have received great attention with respect to their light-induced transformations and spectroscopic properties [5]. Measurements of colloidal gold surface properties have been performed by various interfacial tools [6–8].

Surface-enhanced Raman scattering (SERS) has been utilized to investigate the interfacial behaviors on nanoparticle and Au surfaces [9–11]. There have been several SERS studies on gold nanoparticles (AuNPs) aiming to understand the surface reactions [12–14]. Density functional theory (DFT) calculations were also helpful in explaining the adsorption behaviors on metal colloid surfaces [15]. Despite the potentials with regard to drug delivery systems, the antifungal effect of metal NPs has received relatively little attention [16–20]. Considering that the introduction of NPs is helpful in treating drug-resistant cells [21,22], it would be informative to study the enhanced fungal cell killing behaviors of drug conjugates after surface modifications.

Azole compounds are known to involve the inhibition of ergosterol biosynthesis in fungal cells [23]. Tioconazole (TN), which goes by the brand names of Trosyd and Gyno-trosyd (Pfizer), with its thiophene and imidazole group, is used as a fungicide [24–26], TN shows efficacy and safety in topical preparations [27] and exhibits unusual fungicidal activity, as it kills yeast cells in the stationary phase [28]. The fungicidal action is related to direct membrane damage in ergosterol synthesis [29].

Penicillium digitatum is known to show resistance to several fungicides [30]. A sequence analysis of β-tubulin genes revealed that all TBZ-resistant isolates displayed a single transversion point mutation, resulting in a change at either amino acid 198 (glutamic acid → glutamine) or 200 (phenylalanine → tyrosine) [31]. *Penicillium expansum* is also known to have a drug resistance due to the mutation of β-tubulin [32]. A tioconazole resistant gene was also found in *Aspergillus nidulans* [33].

It is important to examine whether the drug molecules are released in the cell culture media or inside the cells to ensure an efficient delivery [34]. Glutathione (GSH) is known to exist in concentrations of up to 10 mM in yeast and filamentous fungi [35].

Table 1
Binding energies of the 6 metal atoms of TN and the Au–TN distances.[a]

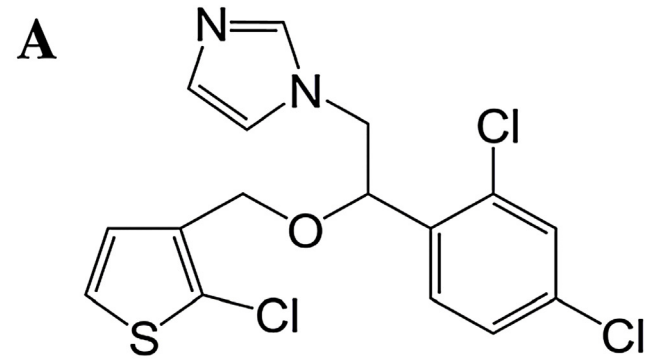
Configurations	B.E. (kcal/mole)	Au–TN distance (Å)
TN + Au_6···N (imidazole)	25.70	Au···N 2.17
TN + Au_6···S (2-chlorothiophene)	5.32	Au···S 2.86
GSH + Au_6···S (glutathione)	52.29	Au···S 2.48

[a] References [38,39].

Table 2
Hydrodynamic diameters of pristine and drug-conjugated AuNPs.[a]

AuNPs	Pristine AuNPs	AuNPs–TN-PEG
Average (±S.D.)	22.2 (±3.1)	165.5 (±3.0)

[a] Unit in nm.



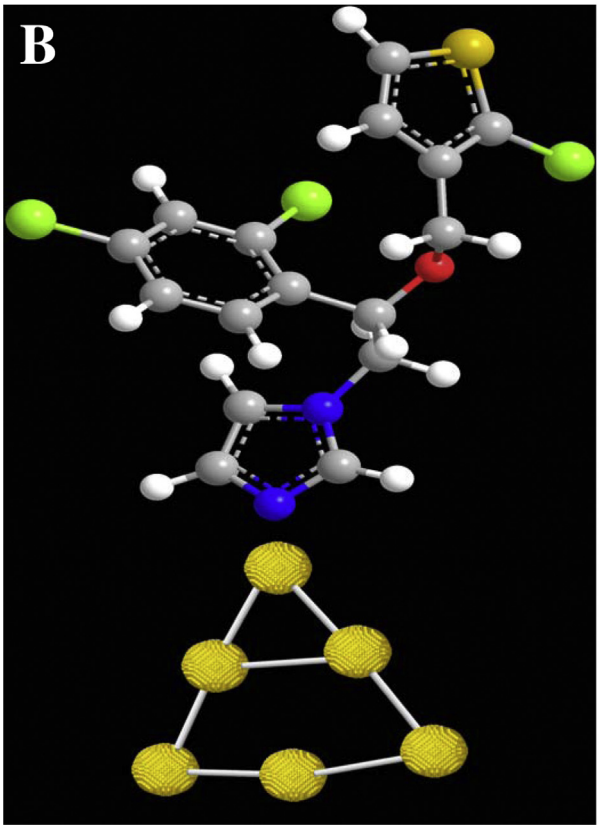


Fig. 1. (a) Molecular structure of tioconazole (TN). (b) Optimized geometries of the most stable TN on six Au atoms. The most stable coordination on Au is via the nitrogen atom of the imidazole group in TN.

Although TN has been used as an antifungal agent, to the best of our knowledge, no structural study has reported on TN-assembled on metal nanoparticles by means of vibrational spectroscopic tools. In this work, we present the synergistically enhanced antifungal activity of TN using AuNPs. We chose to use AuNPs to estimate potential effects on the fungal cell-killing capability.