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Photodynamic inactivation of mold fungi spores by newly developed charged corroles

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ABSTRACT

The photodynamic effect, originally used in photodynamic therapy (PDT) for the treatment of different diseases, e.g. of cancer, has recently been introduced for the inactivation of bacteria. Mold fungi, which provoke health problems like allergies and diseases of the respiratory tract, are even more resistant and their biology is also very different. This study presents the development of four new photosensitizers, which, in combination with low doses of white light, inhibit the germination of mold fungi spores. Two of them even cause lethal damage to the conidia (spores) which are responsible for the spreading of mold fungi. The photoactivity of the newly synthesized corroles was obtained by their application on three different mold fungi: *Aspergillus niger*, *Cladosporium cladosporoides*, and *Penicillium purpurgenum*. To distinguish between inactivation of germination and permanent damage, the fungi were first incubated under illumination for examination of photosensitizer-induced growth inhibition and then left in darkness to test the survival of the conidia. None of the compounds displayed dark toxicity, but all of them attenuated or prevented germination when exposed to light, and the positively charged complexes induced a complete damage of the conidia.

1. Introduction

Mold fungi cause not only esthetic problems and material damage to the wall surface of buildings, but also endanger human health [1–3]. Treatment by fungicides is quite efficient, but their increased use raises serious environmental problems [4]. Mold fungi are very successful survivalists with exceptional resistance to environmental stress like extremely high or low temperatures, drying-out, osmotic shock, or chemicals and ionizing radiation. This is due to their asexual life cycle: While growing they develop highly stress resistant conidia, which are able to stay in an inactive state for very long times. The conidia are easily spread by air and start the germination of new fungi mycelia under appropriate environmental conditions [5–8]. Due to the problems of using fungicides mentioned above, the development of new strategies for preventing fungi growth without side effects to human health and the environment is an important research goal.

Photodynamic therapy (PDT) where photosensitization (mainly type II: via singlet oxygen generation) has been investigated since the early 20th century and is of current use in oncology, ophthalmology and dermatology [9–11]. The use of the photodynamic effect for photodinduced inactivation of bacteria (PIB) has been under investigation for more than 10 years [12]. Since it could be introduced as a very promising alternative to antibiotic therapy, it is attracting the interest of many research groups worldwide [13–21]. This development raised our interest in testing if the photodynamic effect may also be used for inhibiting the growth of the even more treatment-challenging mold fungi that grow under different climatic conditions on outdoor surfaces.

In spite of the different structural composition, the cell walls of bacteria and fungi still share some fundamental characteristics. They protect the microbial cells from environmental influences, the viability of the microorganisms depends on the integrity of the cell walls, and both fungicidal and bacterial cell walls contain many charged macromolecules. The success in applying positively-charged photosensitizers (PS) for PIB [14,16] was the motivation for the current studies. Furthermore, it was shown before that charged porphyrins are able to damage conidia in suspension under illumination with high doses of white light [22–24].

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The hypothesis was that the charge of the PS molecules would allow for their electrostatic attachment to the outer cell wall of mold fungi and subsequent damage upon photoactivation. Previous investigations were carried out with other PSs like Methylene Blue, Toluidine Blue or charged porphyrins and in spore suspension. The photoxic effect was determined after short time illumination of the spore suspension with high intensity by counting colony-forming units [22–25].

In this work, we present for the first time the phototoxic effect of charged corroles to mold fungi and their conidia under growth conditions. It was possible to determine the inhibition of mold fungi conidia germination and growth, as well as the damage to the conidia. A special white light LED setup for irradiation was designed to simulate daylight conditions.

Two positively and two negatively charged metallocorroles were synthesized and investigated as potential PS for the photodynamic inactivation of mold fungi (Fig. 1). One specific feature of free base corroles and their chelates with non-transition elements is that their fluorescence quantum yield is generally higher than that of porphyrins and other macrocycles [26–29]. For instance, metallation of corrole by gallium and more so by phosphorus or aluminum leads to large increase in fluorescence quantum yield (Φ_{Fluo}) [30–32]. In fact, the Φ_{Fluo} of 0.76 [31] for aluminum corrole is a record value for oligopyrrolic macrocycles. On the other hand, the photophysical properties may be easily tuned by metallation of corroles with heavy non-transition metals, such as Sb and Sn [33,34] or by replacing the hydrogen atoms of corrole macrocycle via halogenations with bromide or iodide [35,36]. These modifications lower the fluorescence quantum yield (Φ_{Fluo}) and result in an increased phosphorescence quantum yield (Φ_P). In some cases the metallocorroles are phosphorescent even at room temperature. Aluminum corrole is a good example for that. It exhibits high Φ_{Fluo} and shows no triplet dynamics in time-resolved EPR experiments, while C–H by C–Br replacement increases the Φ_P/Φ_{Fluo} ratio and the brominated analog exhibits a rich time-resolved EPR spectrum [33]. Also, partial iodination of aluminum corroles skeleton leads to a compound with a long-lived triplet excited state and phosphorescence emission even at ambient temperature [36]. The ability of phosphorus corroles to efficiently produce singlet oxygen was confirmed by photoinduced oxygenation of thioanisoles and hydrocarbons by molecular oxygen, enabled by using antimony corroles as photosensitizer [37]. The results obtained by this catalyst outperform all previous reports in terms of absolute catalytic turnover numbers, selectivity, and catalyst stability. The potential of metallocorroles as PS was also demonstrated in several publications [38,39].

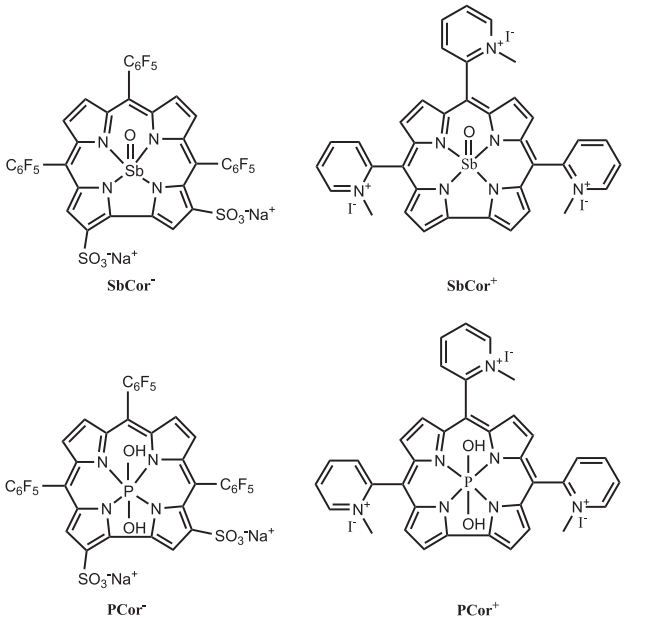

Fig. 1. Chemical structure of the photosensitizers SbCor$^-$, SbCor$^+$, PCor$^-$, and PCor$^+$.

2. Methods

2.1. Synthesis of the photosensitizers

The chemical structures of all investigated corroles are shown in Fig. 1. Their full names are listed in Table 1.

Preparation of PCor$^+$: *Preparation of compound* **2** (5,10,15-tris-(o-pyridyl)corrolato(trans-dihydroxo)phosphorus(V)): Based on previously published procedures [30,40], about 100-fold excess of $POCl_3$ (400 μl, 4.3 mmol) was added to the heated to reflux solution of the free base 5,10,15-tris-o-(pyridyl corrole) (**1**) [41] (40 mg, 76 μmol) in pyridine (5 ml) under an inert atmosphere. Reaction progress was monitored by UV–vis spectroscopy. The color of the reaction mixture immediately changed from deep green to green-purple. The solvent was evaporated after 40 min of reflux and the residue was dissolved in CH_2Cl_2 and passed through a short column of silica with methanol/pyridine (9:1) as eluent. A pink colored fluorescence band of complex **2** was collected, providing 28 mg of material (47 μmol, 63% yield). X-ray quality crystals of this complex **2** were obtained by slow recrystallization from methanol/benzene.

MS (MALDI-TOF) LD$^+$ (acetonitrile): m/z 574 [M – OH]$^+$. UV–vis (methanol): λ_{max}, nm (log ϵ) 412 (4.94), 588 (4.41).

^{1}H NMR (300 MHz, CD_3OD): δ_H, ppm
9.47 (1H, d, ^{3}J (H,H) = 4.40 Hz, pyrrole-*H*),
9.44 (1H, d, ^{3}J (H,H) = 4.40 Hz, pyrrole-*H*),
9.12 (1H, d, ^{3}J (H,H) = 5.10 Hz, pyrrole-*H*),
9.09 (1H, d, ^{3}J (H,H) = 5.10 Hz, pyrrole-*H*),
9.06 (1H, d, ^{3}J (H,H) = 4.30 Hz, pyrrole-*H*),
9.05 (1H, d, ^{3}J (H,H) = 4.30 Hz, pyrrole-*H*),
9.02–9.00 (3H, m, pyridine-*H*),
8.84 (1H, d, ^{3}J (H,H) = 5.10 Hz, pyrrole-*H*),
8.83 (1H, d, ^{3}J (H,H) = 5.10 Hz, pyrrole-*H*),
8.48 (2H, d, ^{3}J (H,H) = 7.60 Hz, pyridine-*H*),
8.19 (3H, t, ^{3}J (H,H) = 6.00 Hz, pyridine-*H*),
7.90 (4H, m, pyridine-*H*).
^{31}P {^{1}H} NMR (300 MHz, CD_3OD):
$\delta = -177.02$ ppm

Preparation of PCor$^+$: Compound **2** (28 mg) was dissolved in the minimum volume of DMF (about 4 ml), excess of methyl iodide (about 100 equivalents) was added, and the reaction mixture was stirred at room temperature overnight. About 3 ml of methanol and 10 ml of diethyl ether were added to the reaction mixture to precipitate the product, which was washed with additional portions of diethyl ether, dissolved in water, filtered and lyophilized

Table 1
IUPAC names of the photosensitizers.

Acronym	IUPAC names
SbCor$^-$	2,17-bis-sulfonato-5,10,15-tris (pentafluorophenyl) corrolato (oxo) antimony (V)
SbCor$^+$	5,10,15-tris-(N-methyl-o-pyridylium) corrolato (oxo) antimony (V)
PCor$^-$	2,17-bis-sulfonato-5,10,15-tris (pentafluorophenyl) corrolato (trans-dihydroxo) phosphorus (V)
PCor$^+$	5,10,15-tris-(N-methyl-o-pyridylium) corrolato (trans-dihydroxo) phosphorus (V)