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BRIEF REPORT

Repurposing auranofin as an antifungal: *In vitro* activity against a variety of medically important fungi

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ABSTRACT

Repositioning old drugs can significantly decrease the time and effort that it takes to develop novel antifungal therapeutics, which represents a pressing and unmet clinical need due to the devastating nature of fungal infections. We have previously described the activity of auranofin, a gold thiol compound used to treat rheumatoid arthritis, against *Candida albicans* biofilms. Here we evaluate its antifungal spectrum of action and describe its activity against a variety of medically important fungi.

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Text

The incidence of fungal infections has increased in the last decades, mostly as a consequence of advances in modern medicine which have led to an expanding population of immune- and medically-compromised patients; as such these infections constitute a growing public health threat.[1] In addition, a change and increase in the spectrum of pathogenic fungi has been observed. For example, infections due to yeasts other than *Candida* and molds other than *Aspergillus* are becoming increasingly frequent, and typically difficult to treat.[1] Unfortunately, dismal mortality rates associated with fungal infections remain high, pointing to major limitations of current antifungal therapy. Fungi are eukaryotic organisms and there is a paucity of targets for antifungal drug development; as a result the antifungal armamentarium is exceedingly limited, with polyenes, azoles and echinocandins representing the main classes of antifungal agents used in the clinics.[2,3] Moreover, toxicity (particularly in the case of polyenes) and the emergence of resistance (for azoles and echinocandins) pose additional challenges in the clinical management of fungal infections.[3] To make matters worse the antifungal pipeline is mostly dry.[4] All these facts underscore a dire and unmet need for new antifungal drugs. However, the development of an entirely new drug is a very expensive, time-consuming, and risky process, with high attrition rates, and having to undergo an arduous approval process by the FDA.

As an alternative for accelerated drug development, repurposing (or repositioning) old drugs with a new indication as antifungals may drastically reduce the effort, time and money required for moving drugs into clinical trials.[5] Drug repositioning involves the investigation of drugs that are already approved for the treatment of other diseases and/or whose mechanisms of action or targets are already known.[6] Along these lines, we have previously screened the Prestwick Chemical library, consisting of approximately 1,200 FDA-approved, off-patent drugs, and identified the potent antifungal activity of auranofin against *C. albicans* biofilms.[7] Also, the activity of Auranofin against *Cryptococcus* and *Candida* spp. has recently been reported.[8] Auranofin, consisting of a gold (I) center coordinated to a thiosugar and triethylphosphine (Fig. 1), inhibits several inflammatory pathways and has been in clinical use since 1985 as a disease-modifying antirheumatic drug used to slow down or stop the progression of this rheumatic disorder. Its oral bioavailability and reasonable systemic toxicity pave the way to its potential repositioning for new and different therapeutic uses. From a mechanistic point of view, inhibition of both inflammatory pathways and thiol redox enzymes

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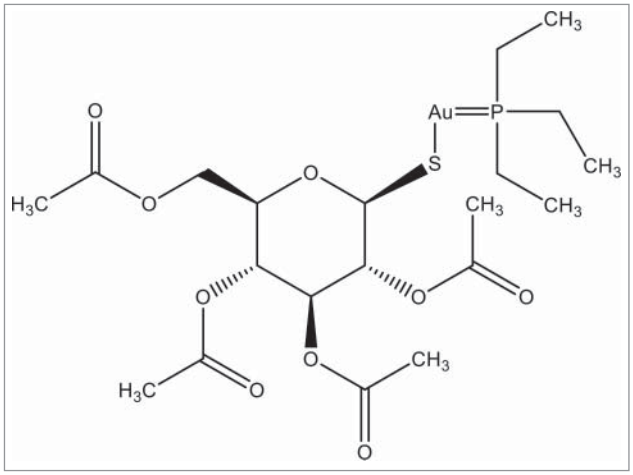

Figure 1. Chemical structure of Auranofin [2,3,4,6-tetra-o-acetyl-l-thio-β-D-glucopyrano-sato-S-(triethyl-phosphine) gold].

by auranofin makes it a new candidate for cancer therapy and for treating microbial infections.[9] Most recently several studies have indicated the efficacy of auranofin against multiple parasites and bacteria,[10-18] with the antimicrobial activity mostly due to reactive oxygen-mediated cell death.[10,12,13]

As a first step in the evaluation of its potential for the treatment of fungal infections, we sought to further characterize the in vitro antifungal activity of auranofin, compared to currently available antifungal agents, and to determine its antifungal spectrum of action. All clinical fungal isolates tested form part of the collection available in the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio. MICs were determined in accordance with the CLSI M27-A3 (for yeast) and M38-A2 (for filamentous fungi) reference standards for antifungal susceptibility testing.[19,20] For yeasts, in the case of *Candida* spp and *Cryptococcus neoformans* MICs for auranofin (determined at both 50% and 100% inhibition) and fluconazole (determined at 50% inhibition) were read at 24 and 72 hours respectively; whereas *Blastomyces dermatitidis* isolates were tested against voriconazole and auranofin using broth macrodilution methods, with MICs read as 80% inhibition at 72 – 96 hours compared to growth in control tubes. In the case of filamentous fungi, MICs for voriconazole and *Aspergillus fumigatus* (read at 48 hours), and *Scedosporium apiospermum* and *Lomentospora* (formerly *Scedosporium*) *prolificans* (read at 72 hours) were determined as 100% growth inhibition compared to growth controls, while MICs for auranofin were read at the same times and determined at both 50% and 100% inhibition. Tables 1 and 2 summarize the in vitro activity of auranofin against yeasts and molds.

Table 1. MIC values of auranofin against multiple clinical isolates belonging to different species of yeast, in comparison to fluconazole (for *Candida* and *Cryptococcus*) and Voriconazole (for *Blastomyces*). Values are in μg/ml.

Isolate	FTL #	Fluconazole	Voriconazole	Auranofin (50%)	Auranofin (100%)
C. albicans	CA-1	< 0.125	NT	1	>16
C. albicans	CA-2	8	NT	0.25	>16
C. albicans	CA-3	0.25	NT	1	>16
C. albicans	CA-4	<0.125	NT	1	>16
C. albicans	CA-5	>64	NT	0.25	>16
C. albicans	CA-6	16	NT	0.5	8
C. albicans	CA-7	<0.125	NT	0.5	>16
C. albicans	CA-8	<0.125	NT	0.5	>16
C. albicans	CA-9	<0.125	NT	1	4
C. albicans	CA-10	32	NT	0.5	16
C. albicans	CA-11	0.25	NT	0.5	8
C. albicans	CA-12	0.25	NT	1	16
C. albicans	CA-13	<0.125	NT	0.5	16
C. glabrata	CG-1	2	NT	2	>16
C. glabrata	CG-2	32	NT	16	>16
C. glabrata	CG-3	2	NT	1	>16
C. glabrata	CG-4	1	NT	8	16
C. glabrata	CG-5	1	NT	2	>16
C. glabrata	CG-6	1	NT	4	>16
C. glabrata	CG-7	2	NT	16	>16
C. glabrata	CG-8	2	NT	4	>16
C. glabrata	CG-9	2	NT	4	>16
C. glabrata	CG-10	32	NT	4	16
C. krusei	QC	16	NT	0.5	16
C. parapsilosis	QC	1	NT	>16	>16
C. parapsilosis	CP-1	0.25	NT	8	>16
C. parapsilosis	CP-2	0.25	NT	16	>16
C. parapsilosis	CP-3	2	NT	8	>16
C. parapsilosis	CP-4	0.25	NT	8	>16
C. parapsilosis	CP-5	0.5	NT	>16	>16
C. parapsilosis	CP-6	<0.125	NT	4	>16
C. parapsilosis	CP-7	0.5	NT	>16	>16
C. parapsilosis	CP-8	0.25	NT	1	>16
C. parapsilosis	CP-9	0.5	NT	8	>16
C. parapsilosis	CP-10	0.25	NT	8	>16
C. neoformans	CN-1	1	NT	1	>16
C. neoformans	CN-2	0.5	NT	1	>16
C. neoformans	CN-3	1	NT	2	>16
B. dermatitidis	BD-1	NT	<0.03	2 (80%)	–
B. dermatitidis	BD-2	NT	0.03	1 (80%)	–
B. dermatitidis	BD-3	NT	<0.03	2 (80%)	–

NT: not tested

As seen in Table 1, auranofin displays activity against different *Candida* spp. MIC values of $\leq$1 μg/ml (for 50% inhibition endpoints) were observed for all *C. albicans* isolates tested, and also for the *C. krusei* quality control strain (which displays high level resistance to fluconazole). Importantly, these inhibitory concentrations are several fold lower than the clinically achievable concentration of the drug in blood from patients treated with a conventional dosing regimen of Auranofin (3.5 μM).[12] Moreover, according to the package insert from the manufacturer, in *in vivo* toxicity studies, mice and rats tolerated doses up to 20 – 50 times higher than the normal human dose. Interestingly, auranofin remained active against many clinically relevant fluconazole-resistant *C. albicans* strains. However, we observed lower and more variable in vitro activity of

Table 2. MIC values of auranofin against multiple clinical isolates belonging to different species of molds, in comparison to voriconazole and/or posaconazole. Values are in μg/ml.

Isolate	FTL #	Voriconazole	Posaconazole	Auranofin (50%)	Auranofin (100%)
Paecilomyces variotii	QC	0.06	1.25	2	16
A. fumigatus	AF-1	1	NT	4	>16
A. fumigatus	AF-2	0.25	NT	4	>16
A. fumigatus	AF-3	0.25	NT	2	>16
R. oryzae	R-1	NT	0.5	>16	>16
R. oryzae	R-2	NT	0.25	>16	>16
R. oryzae	R-3	NT	0.25	16	>16
S. apiospermum	SA-1	1	NT	2	>16
S. apiospermum	SA-2	1	NT	2	>16
S. apiospermum	SA-3	0.5	NT	2	2
S. apiospermum	SA-4	0.5	NT	1	2
S. apiospermum	SA-5	1	NT	4	4
S. apiospermum	SA-6	>16	NT	4	8
S. apiospermum	SA-7	0.25	NT	4	4
L. prolificans	SP-1	>16	NT	2	>16
L. prolificans	SP-2	>16	NT	8	16
L. prolificans	SP-3	>16	NT	8	16
L. prolificans	SP-4	>16	NT	8	16
L. prolificans	SP-5	>16	NT	8	16
L. prolificans	SP-6	>16	NT	4	8

Note. NT: not tested.

Table 3. MIC ranges of auranofin at the 50% and 100% growth inhibition endpoints against various fungal species. Values are in μg/ml.

Species	50% Inhibition Endpoint	100% Inhibition Endpoint
C. krusei ATCC 6258	0.5	8
C. albicans (n = 13)	0.25 – 1	4 – >16
C. glabrata (n = 10)	1 – 16	16 – >16
C. parapsilosis (n = 10)	1 – >16	>16
C. neoformans (n = 3)	1 – 2	>16
A. fumigatus (n = 3)	2 – 4	>16
B. dermatitidis (n = 3)	1 – 2	>16
R. oryzae (n = 3)	16 – >16	>16
S. apiospermum (n = 7)	1 – 4	2 – >16
L. prolificans (n = 6)	2 – 8	8 – >16

auranofin against *C. glabrata* and, in particular, *C. parapsilosis* isolates (see also Tables 3 and 4). Auranofin seems to have excellent activity against *C. neoformans*, as MIC values against all *C. neoformans* isolates tested were $\leq$2 μg/ml, comparable to MIC values for fluconazole (Table 1). Similarly, as shown in Table 1, MIC values of $\leq$2 μg/ml were observed for all *B. dermatitidis* isolates tested.

Regarding molds, auranofin displays activity against *A. fumigatus, S. apiospermum* and *L. prolificans*, a mold resistant to all clinically available antifungals; but much more limited activity against *Rhizopus* (Table 2). MIC values of auranofin against *A. fumigatus* isolates ranged from 2 – 4 μg/ml using the 50% inhibition endpoint, although values of over 16 μg/ml were observed when read at 100% inhibition (see also Tables 3 and 4). Inhibitory concentrations against *R. oryzae* were generally high (>16 μg/ml), both when read at 50% and 100% inhibition, which seems to indicate an overall lack of activity against mucorales. Of note, auranofin displayed activity against *S. apiospermum* and *L. prolificans* (Table 2), which are remarkably recalcitrant to a majority of marketed antifungals.[21] Auranofin MICs values (50% inhibition endpoint) ranging from 1 – 8 μg/ml were determined for all isolates from these species tested (Tables 3 and 4), which compared quite favorably with their corresponding MIC values for voriconazole, particularly in the case of *L. prolificans* (>16 μg/ml against each isolate).

Overall, our in vitro findings substantiate the activity of auranofin against different pathogenic fungi, including common as well as resistant and emerging pathogens, and confirm the validity of repurposing (or repositioning) approaches so that "old" drugs can potentially be used with a new indication as antifungals in an expedited manner.[5] Together with its activity against *C. albicans* biofilms,[7] the fact that auranofin retains its activity against fluconazole resistant *C. albicans* clinical isolates indicate a potential use in refractory candidiasis. But perhaps most interesting is its activity against *S. apiospermum* and *L. prolificans*, since clinically available antifungal agents have modest to minimal activity against these organisms, a fact that has been also confirmed by suboptimal responses in the clinic and very poor outcomes in patients suffering from these devastating infections.[21] Future studies should focus on the characterization of the specific mechanism of action responsible for auranofin's antifungal activity and in

Table 4. Minimum inhibitory concentrations of auranofin (50% inhibition endpoint) and fluconazole or voriconazole against *Candida* and *Scedosporium/Lomentospora* species. MIC50 and MIC90: MICs against 50% and 90%, respectively, of isolates tested. GM MIC: geometric mean MIC. Values are in μg/ml.

Species	*C. albicans* (n = 13)		*C. glabrata* (n = 10)		*C. parapsilosis* (n = 10)		*Scedosporium/Lomentospora* (n = 13)	
Agent	Auranofin	Fluconazole	Auranofin	Fluconazole	Auranofin	Fluconazole	Auranofin	Voriconazole
MIC Range	0.25 – 1	0.125 – >64	1 – 16	1 – 32	1 – >16	0.125 – 2	1 – 8	0.25 – >16
MIC50	0.5	0.25	4	2	8	0.25	4	>16
MIC90	1	16	16	32	16	0.5	8	>16
GM MIC	0.587	0.766	4.287	2.828	7.464	0.354	3.595	5.222

vivo experiments. A caveat is that auranofin exerts anti-inflammatory effects,[9] so determining the optimal balance of immunosuppressive and antifungal activity to combat infections in different clinical settings will be of critical importance.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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