Gallium siderophores for the treatment of *Aspergillus fumigatus* lung infections

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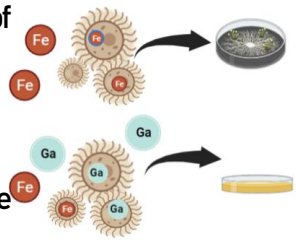
Introduction

25/10/222 - WHO report : *A. fumigatus* is one of the greatest threats to public health

Gallium: The displacement of Fe^{3+} by Ga^{3+}
→ Perturbation of Fe homoeostasis

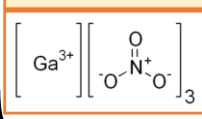
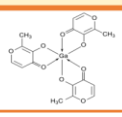
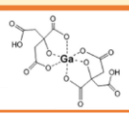
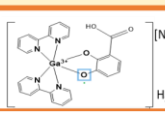
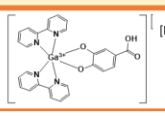
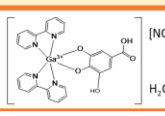


Siderophores: Sequester Fe from the environment forming Fe-siderophore complexes.

Aim of the Work

Assessment and characterisation of a gallium based dry powder for inhalation and solution for nebulisation for the treatment of cystic fibrosis patients infected by *Aspergillus fumigatus* (*A. fumigatus*).

Methods

Reference Compounds			Gallium-Siderophore Complexes		
GaN	GaM	GaC	GaS1	GaS2	GaS3
					

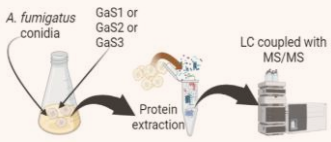
1. Toxicity and Efficacy Screening

1.1. Cytotoxicity on H441 lung cells
1.2. Microbiological screening on A. *fumigatus* (ATCC26933)

- ❑ Toxicity screening on *A. fumigatus* conc. range 4-500 µg/mL
- ❑ Zone of inhibition test
- ❑ Fungicidal/fungistatic test

Lead Compound Selection

2 Molecular mechanism of action (Proteomics)



3. Formulation Study

Dry powder for inhalation
- ❑ Morphology and solid-state nature (SEM and XRPD)
- ❑ Size (laser diffraction)
- ❑ *In vitro* deposition study (NGI)

Solution for nebulization
- ❑ Osmolality
- ❑ *In vitro* deposition study NGI

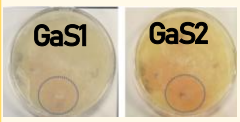
Efficacy test on *A. fumigatus*
Formulation toxicity screening on *A. fumigatus* conc. range 4-500 µg/mL

Results

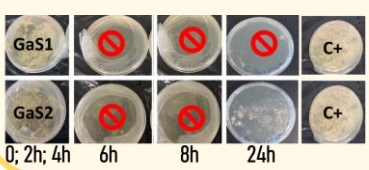
1. Toxicity and Efficacy Screening

	IC_{50} on *A. fumigatus*	IC_{50} on H441 cells
GaN	0.315 mg/mL	>0.5 mg/mL
GaM	>0.5 mg/mL	>0.5 mg/mL
GaC	0.358 mg/mL	>0.5 mg/mL
GaS1	**0.035 mg/mL**	**1.94 mg/mL**
GaS2	**0.035 mg/mL**	**2.06 mg/mL**
GaS3	**0.039 mg/mL**	**1.96 mg/mL**

✓ Ga-siderophores are selectively toxic on *A. fumigatus*



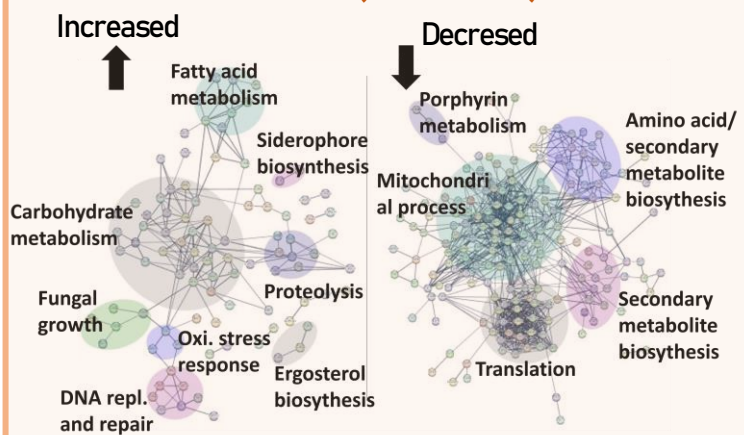
✓ Just GaS1 and GaS2 form a 2 cm zone of inhibition



✓ GaS1: fungicidal at 0.5 mg/mL

GaS1 Selected as Lead Compound

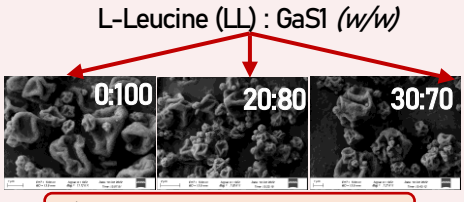
2 Determination of the Molecular Mechanism of Action (Proteomics)



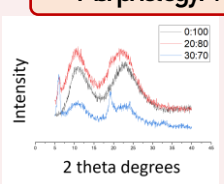
✓ Many fungal vital pathways affected

3. Formulation Study

Dry powder for inhalation
Three formulations obtained through spray drying
L-Leucine (LL) : GaS1 *(w/w)*



✓ Morphology: wrinkled surface



Solid state: Amorphous

Solution for nebulisation
5 mg/mL GaS1 in 0.9% NaCl 300 mOsm/Kg
Nebuliser: Aerogen® mesh nebuliser

	Laser Diffraction Results			NGI Results		
LL:GaS1	Dx10 (µm)	Dx50 (µm)	Dx90 (µm)	MMAD (µm)	FPF (%)	GSD
0:100	1.1 ± 0.1	2.9 ± 0.2	7.4 ± 0.4	3.3 ± 0.3	65% ± 6%	1.8 ± 0.1
20:80	1.0 ± 0.2	2.4 ± 0.2	4.9 ± 0.4	2.9 ± 0.1	76.1% ± 3.3%	1.7 ± 0.1
30:70	1.1 ± 0.1	2.7 ± 0.2	5.6 ± 0.4	2.4 ± 0.4	77.9% ± 2.5%	2.3 ± 0.1
Sol. For Neb				5.0 ± 0.4	53.8% ± 0.7%	2.9 ± 0.1

	IC_{50} on *A. fumigatus*
DPI, Sol for Neb	0.035 ± 0.007 mg/mL

Optimal characteristics for pulmonary drug delivery and efficacy against *A. fumigatus*

Conclusions: GaS1, a newly synthesized novel gallium-siderophore complex, was shown to be selectively toxic against *A. fumigatus* and was non-toxic to NCI-H441 cells. A siderophore-gallium based inhaled formulation, that can be efficiently delivered to the lungs, could be a promising adjuvant therapy for the treatment of *A. fumigatus* infected patients.