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# Synthesis and study of gallium complexes as potential antipseudomonal agents

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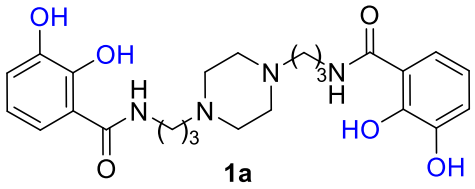
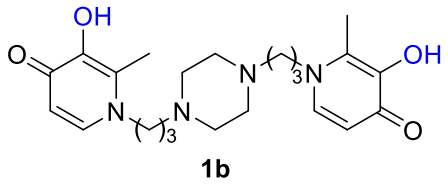
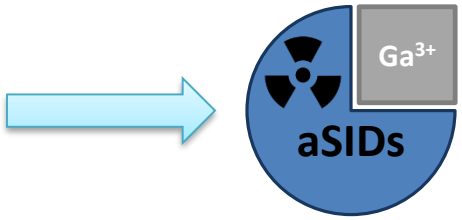
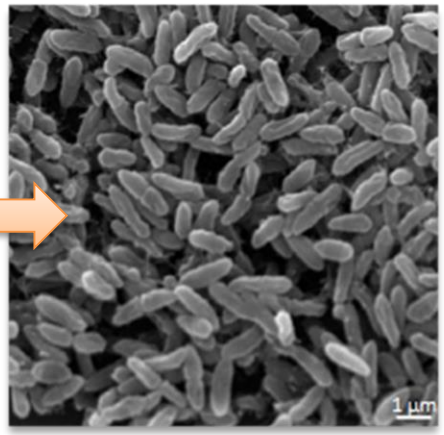
# SYNTHESIS AND STUDY OF GALLIUM COMPLEXES AS POTENTIAL ANTIPSEUDOMONAL AGENTS




*P. aeruginosa*

# ABSTRACT

Each year, antibiotic-resistant germs cause at least 700,000 deaths worldwide. Among them, *P. aeruginosa*, often involved in nosocomial infections, belongs to the first list of antibiotic-resistant "priority pathogens" described by the WHO. Alarmingly, the number of pan-drug-resistant specimen, untreatable with any of the antipseudomonal antibiotics available in the clinic, has increased. The double layered cell envelope of *P. aeruginosa* is responsible for a decreased penetration and low activity of many antibiotics.

An innovative idea to bypass this barrier relies on the siderophore dependent active iron uptake with a "Trojan Horse" strategy. These specific systems may allow the introduction of antibacterial agents such as toxic gallium complexes. Gallium has similar atomistic characteristics to iron and can be internalized by bacteria as siderophore analog-Ga(III) complexes competing with the corresponding siderophore analog-Fe(III) complexes. Once inside the bacteria, gallium, which cannot be reduced, blocks the bacteria's iron-dependent biological mechanisms.

A citrate-buffered gallium nitrate solution (FDA-approved, 1991) has shown promising results for the treatment of *P. aeruginosa* in patients with cystic fibrosis (phase 2 clinical trial, 2019). A low flow intravenous treatment must be used to prevent nephrotoxicity. Vectorization of gallium by siderophore analog might overcome this undesirable side effect.

Recently, we have synthesized two piperazine-based siderophore analogs bearing catechol and hydroxypyridinone ligands. In order to confirm their ability to take iron transport pathways, the physicochemical properties and the siderophore-like effect of the corresponding iron complexes have been evaluated on *P. aeruginosa* strains. Moreover, characterization and antipseudomonal activity were carried out for the corresponding gallium complexes.



**Keywords:** Gallium complexes ; *Pseudomonas aeruginosa* ; Trojan Horse ; Siderophore

# INTRODUCTION

## INFECTIOUS DISEASES

- 2nd cause of death :
  17 M deaths worldwide each year
- Steadily increasing bacterial resistance
- **Nosocomial Infections**
  - 750,000 cases/year
    4,000 deaths in France
  - ESKAPEE pathogens
    ***E****nterococcus faecium,*
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    opportunist pathogen ;
    1st list priority pathogens[1] ;
    pan-resistant strains[2]

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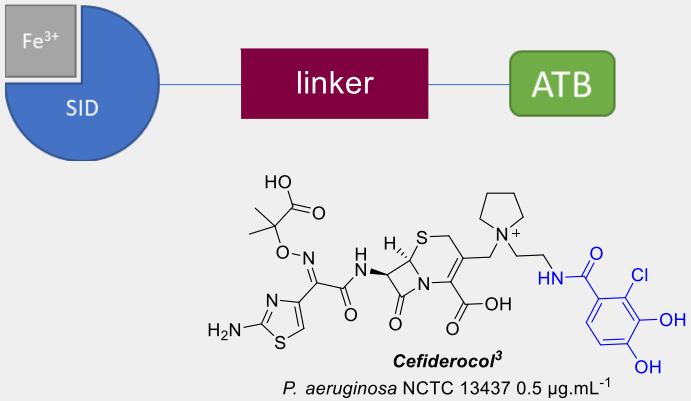


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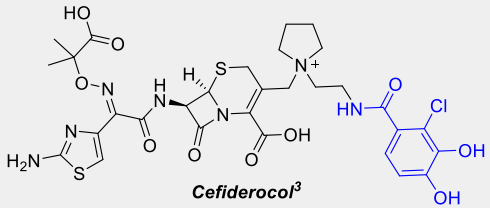
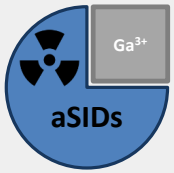
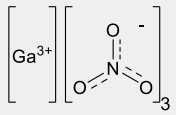


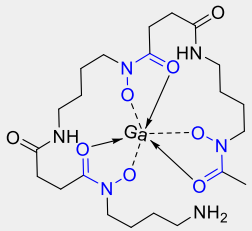
**Cefiderocol**[3]

*P. aeruginosa* NCTC 13437 0.5 µg.mL$^{-1}$



**GaN**
*Phase II*



**DFO-Ga(III)**

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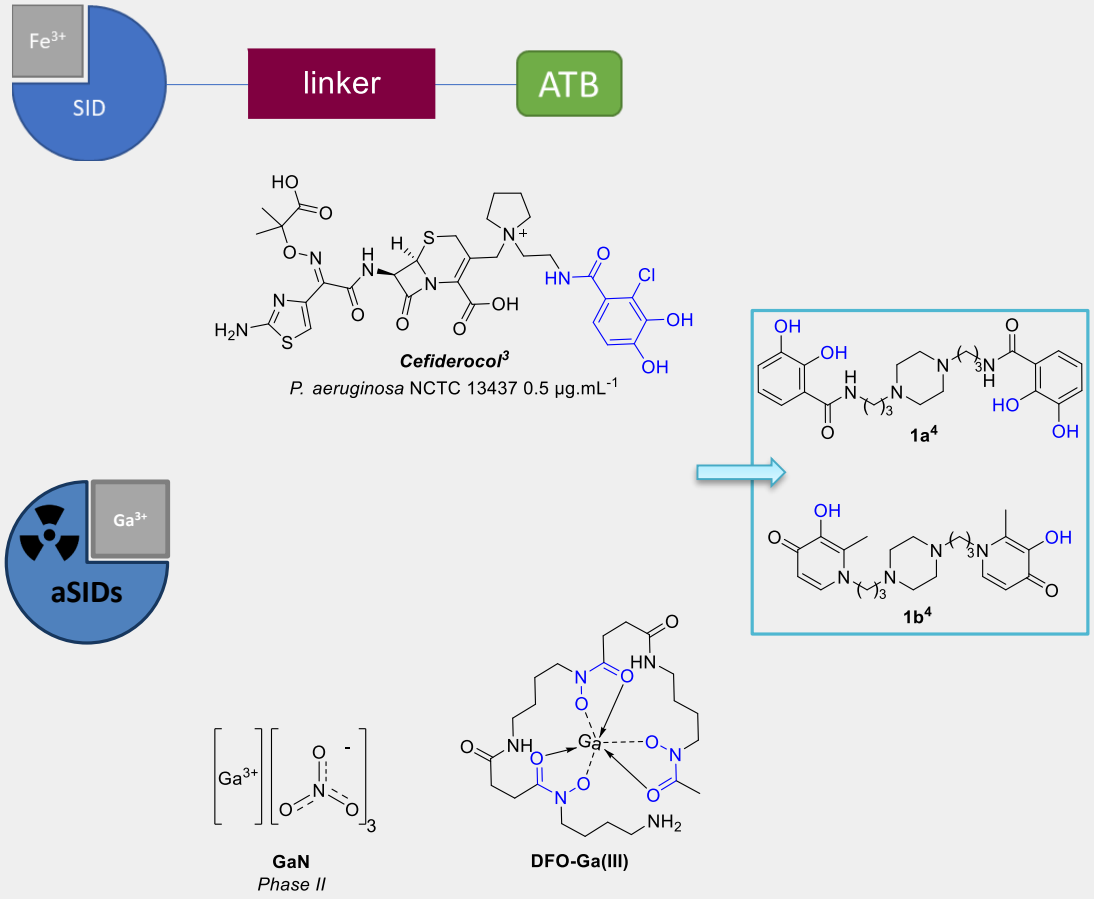


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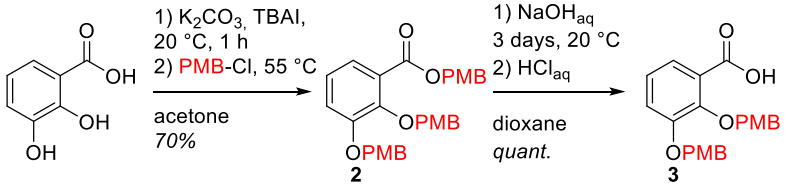
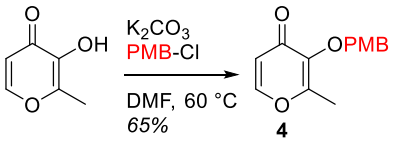
## 1,4-DISUBSTITUTED PIPERAZINES SYNTHESIS

- Bidentate ligands precursors **3** and **4**

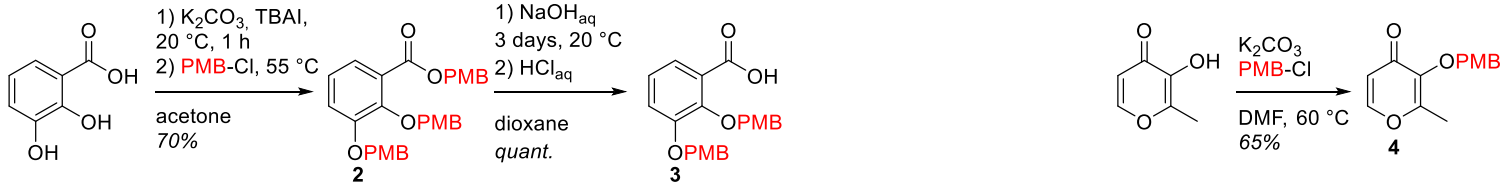


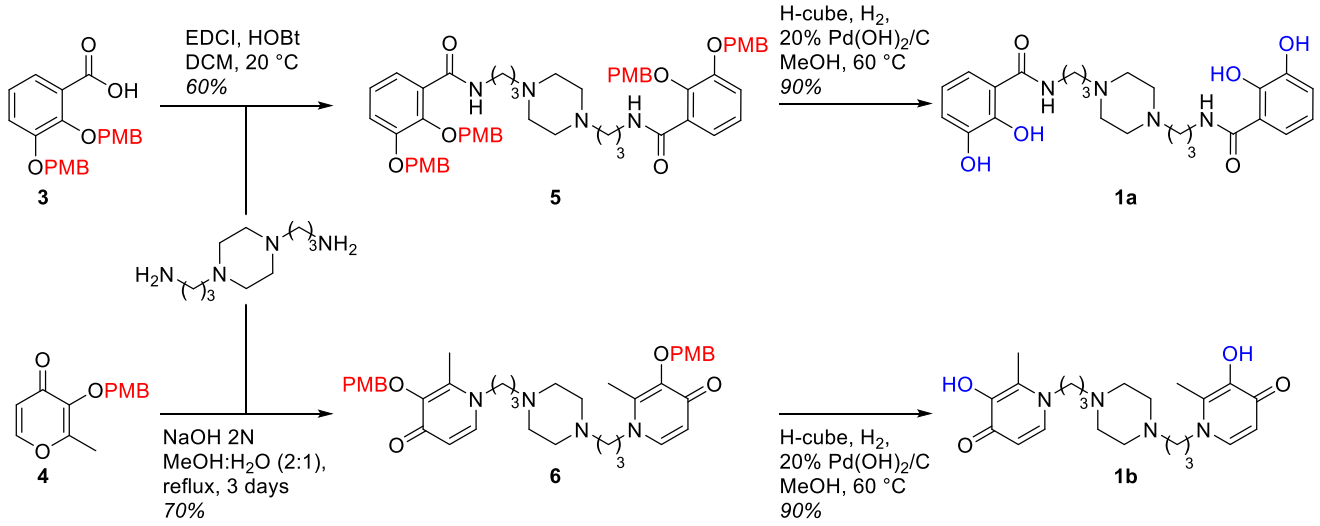
# Results and Discussion

## 1,4-Disubstituted Piperazines Synthesis

- Bidentate ligands precursors **3** and **4**



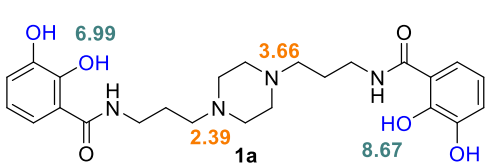

- 1,4-Disubstituted piperazines based siderophore analogs **1a** and **1b**.



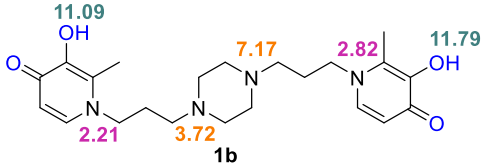

- **1a** and **1b** were, respectively, obtained in four and three steps with a 38% and 41% yield

## PHYSICOCHEMICAL STUDIES OF 1a AND 1b

- pKa values




1a

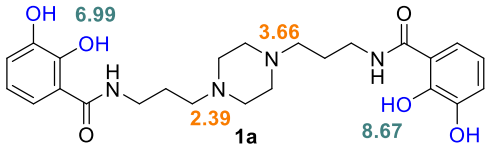


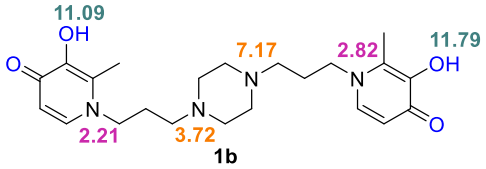

1b

# Results and discussion

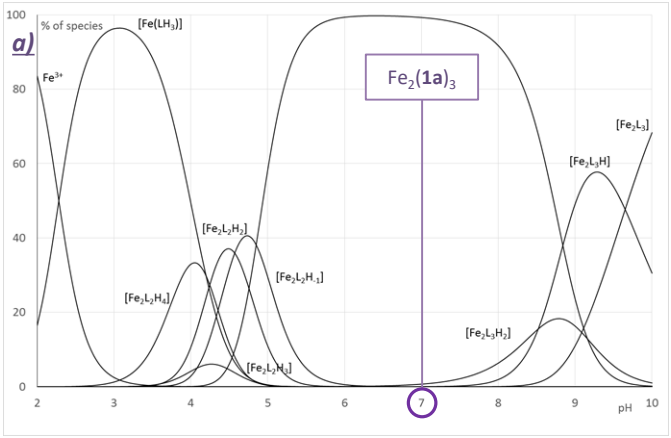
## Physicochemical Studies of **1a** and **1b**

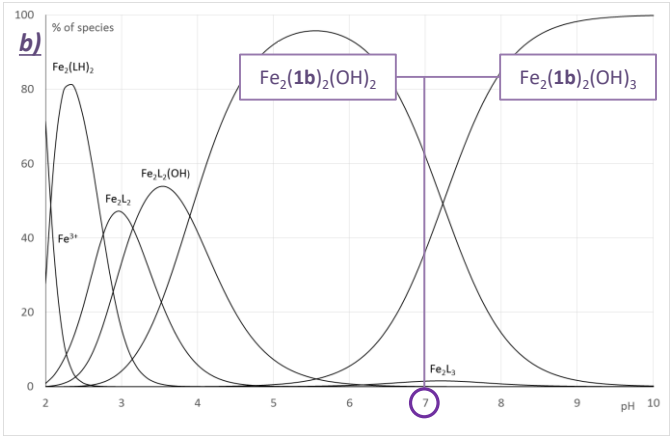
- pKa values




- Distribution curves



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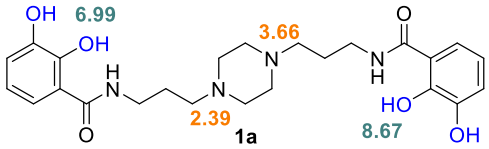


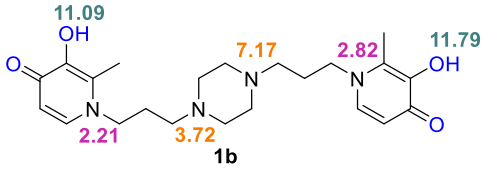


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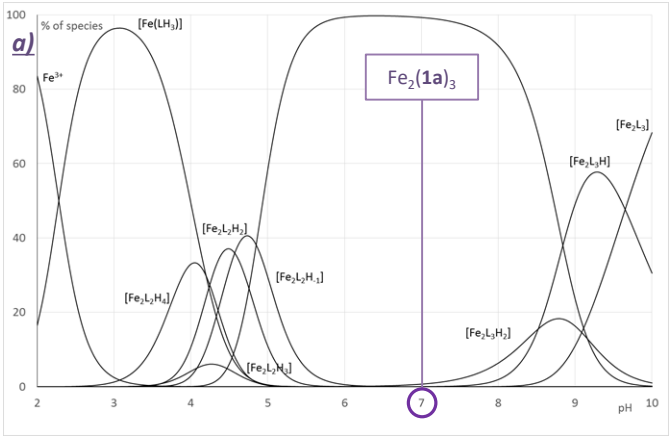


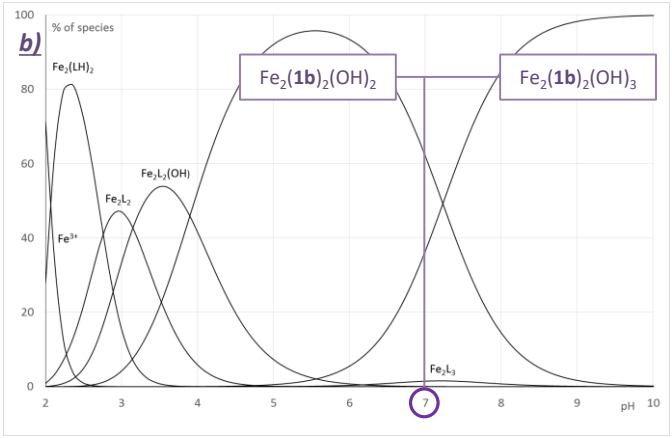


- pFe determination

**1a** : 28.04 and **1b** : 24.38 → meeting the criteria for an iron chelation therapy agent (pFe >20).

# RESULTS AND DISCUSSION

## BIOLOGICAL ACTIVITY AND « SIDEROPHORE-LIKE » EFFECT OF **1a** AND **1b** ON *P. AERUGINOSA*

- Intrinsic biological activity & Cytotoxicity
  - No intrinsic antipseudomonal activities
    → convenient for a "siderophore-like" activity.
  - Did not show cytotoxicity

| | Intrinsic Biological activity *P. aeruginosa* DSM 1117 | | Cytotoxicity HepG2 |
|---|---|---|---|
| **Compound** | **MIC – MMS (µg.mL$^{-1}$)** | **MIC – MH (µg.mL$^{-1}$)** | **$IC_{50}$ (µM)** |
| **1a** | >512 | >512 | >100 |
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- "Siderophore-like" effect

*P. aeruginosa* PAO1
(wild type)

*P. aeruginosa* PAD07
(Pvd-, Pch-)

- SMM without and with a known amount of $FeCl_3$.
- Controls: natural bacterial growth.

*: $p<0.05$ (*vs* control, Mann–Whitney Test)

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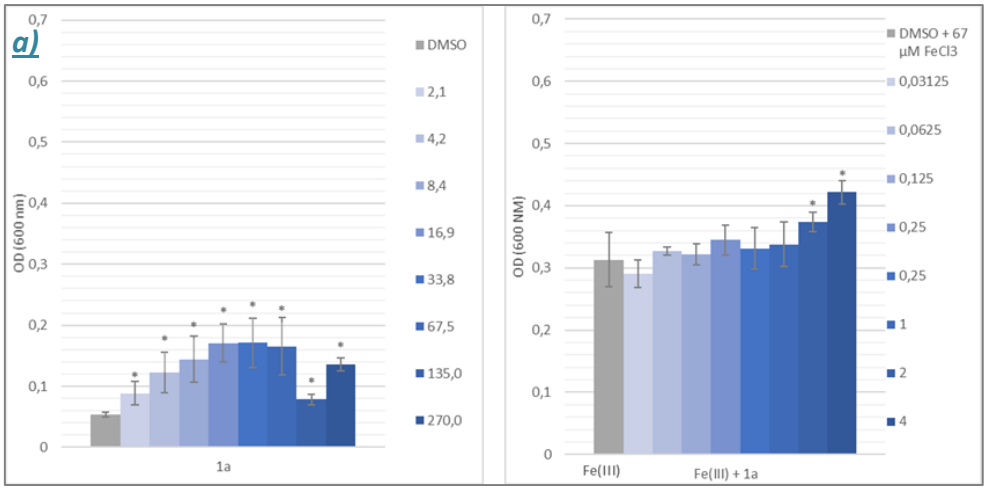


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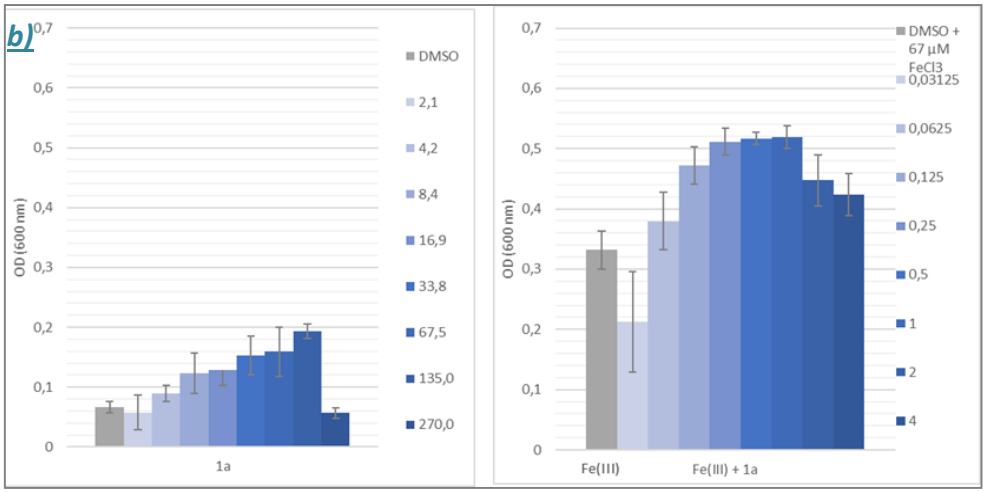
1a

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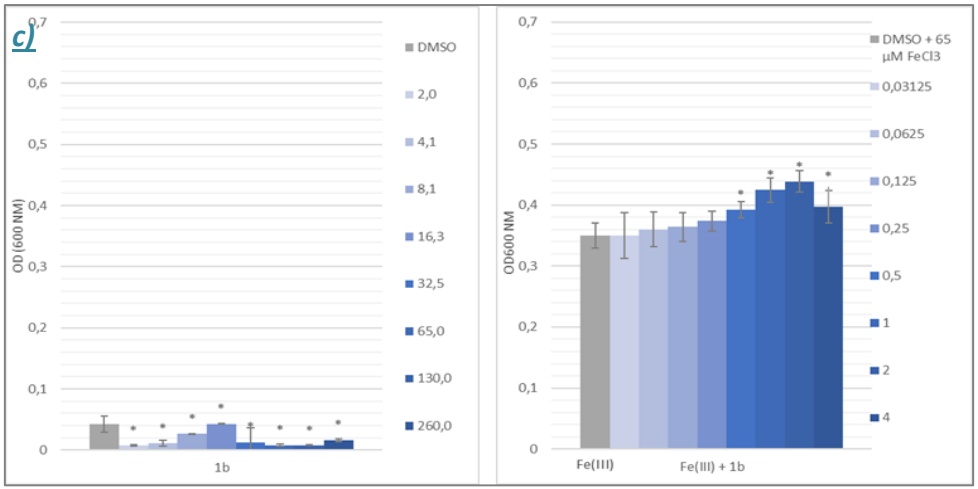
1b

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- Bacterial growth were lower in the presence of **1b** which could by explained by a lower recognition by the bacteria.

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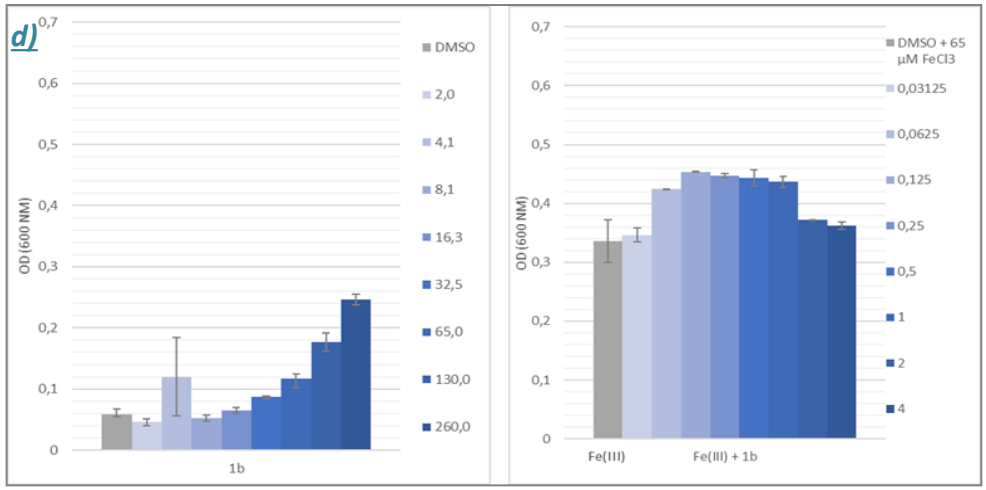
  - Did not show cytotoxicity

| | Intrinsic Biological activity *P. aeruginosa* DSM 1117 | | Cytotoxicity HepG2 |
|---|---|---|---|
| **Compound** | **MIC – MMS (µg.mL$^{-1}$)** | **MIC – MH (µg.mL$^{-1}$)** | **IC$_{50}$ (µM)** |
| **1a** | >512 | >512 | >100 |
| **1b** | >512 | >512 | >100 |
| Ciprofloxacin | 0.5 | 0.5 | ND |

- "Siderophore-like" effect

*P. aeruginosa* PAD07
(Pvd-, Pch-)

1b




*: p<0.05 (*vs* control, Mann–Whitney Test)

- In presence of **1a**, PAO1 bacterial growth is promoted suggesting a competition with the siderophore of *P. aeruginosa*.
- The growth of PAD07 shows that **1a** can be internalized by the bacteria.
- Bacterial growth were lower in the presence of **1b** which could by explained by a lower recognition by the bacteria.

# RESULTS AND DISCUSSION

## BIOLOGICAL ACTIVITY AND « SIDEROPHORE-LIKE » EFFECT OF **1a** AND **1b** ON *P. AERUGINOSA*

- Intrinsic biological activity & Cytotoxicity
  - No intrinsic antipseudomonal activities
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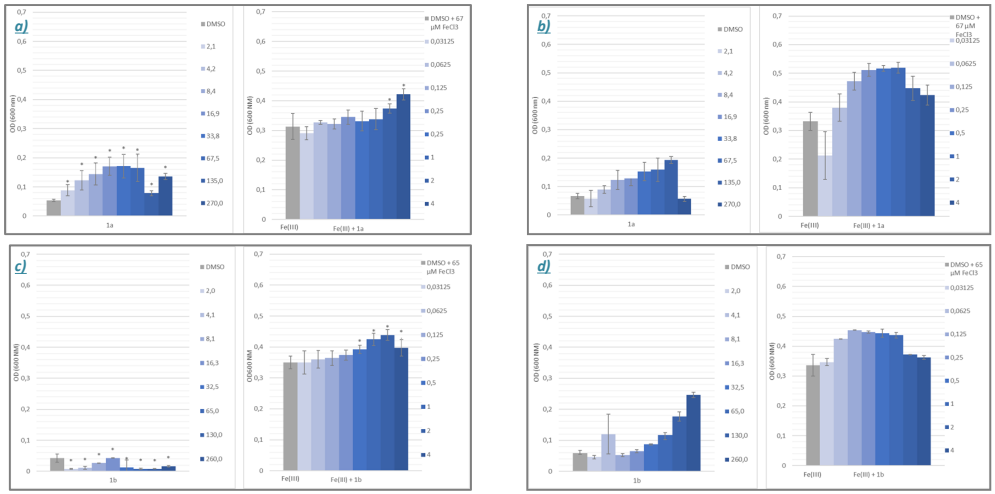
*P. aeruginosa* PAO1 (wild type) | *P. aeruginosa* PAD07 (Pvd-, Pch-)

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# Results and discussion

## Preparation and Study of Gallium Complexes

- Gallium complexes formation
  - Two gallium salts ($Ga(NO_3)_3$ or $Ga(acac)_3$)
  - Room temperature or 40 °C
  - Buffer Tris.HCl

| | Ligands | Gallium salts | Temperature Time | Buffers (pH 7) | Results (Mass Spectroscopy) |
|---|---|---|---|---|---|
| 1 | **1a** | $Ga(NO_3)_3$ | 20 °C 1 h | No | **1a** only |
| 2 | | $Ga(acac)_3$ | | | |
| 3 | | $Ga(NO_3)_3$ | 40 °C 3 h<br>20 °C 21 h | Tris. HCl | |
| 4 | | $Ga(acac)_3$ | | | |
| 5 | **1b** | $Ga(NO_3)_3$ | 20 °C 1h | No | Ga(**1b**), Ga(**1b**)$_2$, $Ga_2$(**1b**)$_3$ |
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| 7 | | $Ga(NO_3)_3$ | 40 °C 3 h<br>20 °C 21 h | Tris. HCl | Ga(**1b**)$_2$, $Ga_2$(**1b**)$_2$ |
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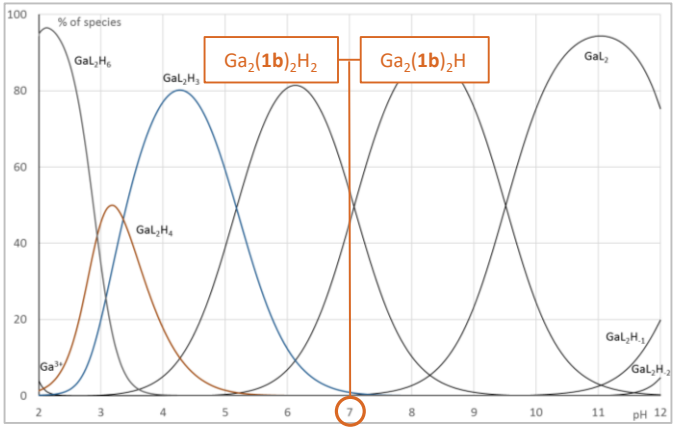
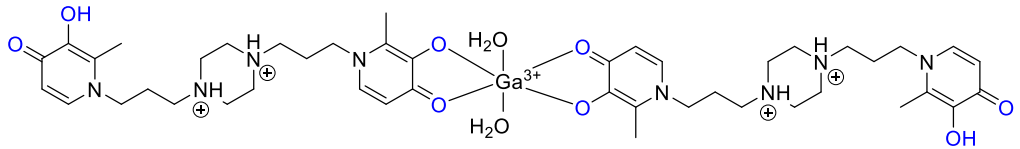
- Physicochemical studies of **1b**




- At physical pH : two species ($Ga_2(1b)_2H_2$ and $Ga_2(1b)_2H$)

- The pGa value obtained for **1b** was 26.85.
- 1b-Ga(III) has no antipseudomonal activity. This might be due to a lower recognition of the complex by the bacteria.




$Ga((1b)H_3)_2^{5+}$

# Conclusions

## 1,4-disubstituted piperazines 1a and 1b

- 3-4 steps synthesis with global yields in an average 40%
- pFe values 28.04 (**1a**) and 24.38 (**1b**) → meeting the criteria for an iron chelation therapy agent (pFe >20).
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→ The 1,4-disubstituted piperazine plateform could be used to vectorize antibiotics.

# ACKNOWLEDGMENTS

We would like to thank the DGA and Hauts-de-France for their financial support

and the ICMR laboratory for the physico-chemical evaluation of our compounds