Insights into the antibacterial activity of cyclam derivatives

Ana Beatriz Mendes [a,b], Sílvia A. Sousa [b], Stephanie Almada [a,c], Elisabete R. Silva [c], Jorge H. Leitão [b], Luís G. Alves [d,*]

[a] Centro de Química Estrutural, Instituto Superior Técnico, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal
[b] IBB-Instituto de Bioengenharia e Biociências, Instituto Superior Técnico, 1049-001 Lisboa, Portugal
[c] BioISI-Biosystems & Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[d] Centro de Química Estrutural, Associação do Instituto Superior Técnico para a Investigação e Desenvolvimento, Av. Rovisco Pais 1, 1049-003 Lisboa
[*] luis.g.alves@tecnico.ulisboa.pt

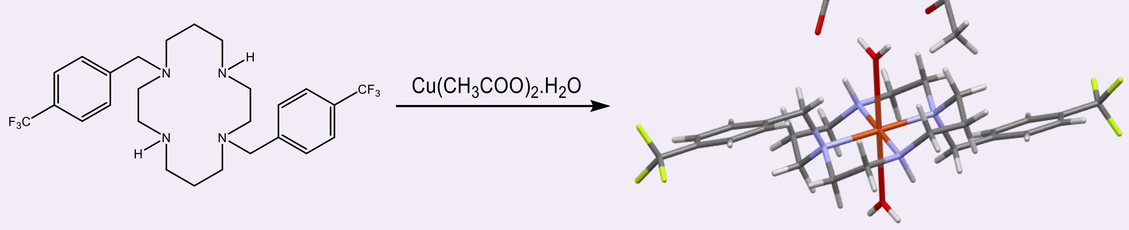
INTRODUCTION

Cyclam is a tetraazamacrocycle with applications in diverse fields of medicine[1]. In particular, the bis-cyclam derivative was found to be highly active and selective HIV inhibitor by interaction with the viral CXCR4 receptor[2]. More recently, cyclam derivatives and their metal complexes revealed anticancer[3-4], antimalarial[5], antischistosomal[6] and antimicrobial[7-9] properties. The *trans*-disubstituted cyclam salt $[H_4\{H_2(^{4\text{-}CF3}PhCH_2)_2Cyclam\}]Cl_4$ was found to be highly active against *E. coli* and *S. aureus*[8]. However, the molecular interactions between cyclams and bacteria remains unknown.

RESULTS AND DISCUSSION

Synthesis and characterization of $[(H_2(^{4\text{-}CF3}PhCH_2)_2Cyclam)Cu(H_2O)_2](CH_3COO)_2$

A new cyclam-based Cu(II) complex (**2**) was synthesized in solution and in the solid state by reaction of $H_2(^{4\text{-}CF3}PhCH_2)_2Cyclam$ (**1**) with one equiv. of $Cu(CH_3COO)_2.H_2O$.



(1) **(2)**

Compound **2** was chemically bonded to magnetic beads (Dynabeads Carboxylic acid®) and characterized by EDS and FT-IR (see Figure 1).

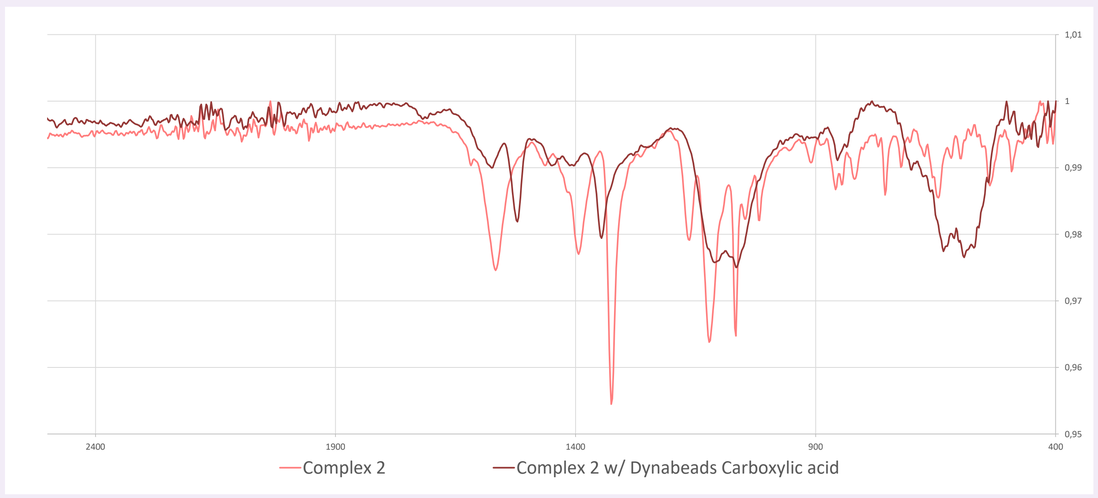
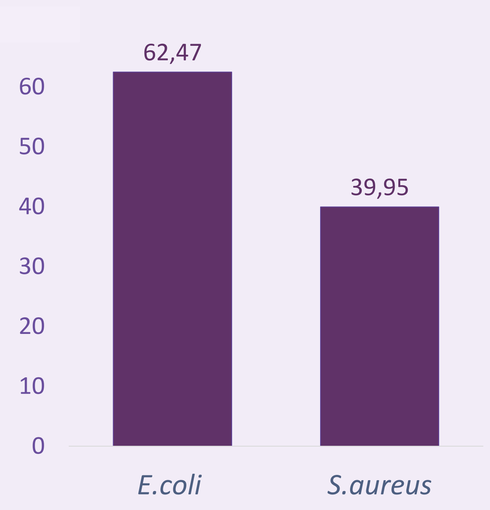


Figure **1** – FT-IR spectra of compound **2** (pink) and derivatized magnetic beads linked to **2** (red).

The FT-IR spectrum of **2** reveals the coordination of carboxylate groups to copper. The content of copper in the magnetic beads determined by EDS was 17.97 ± 6.85 %.

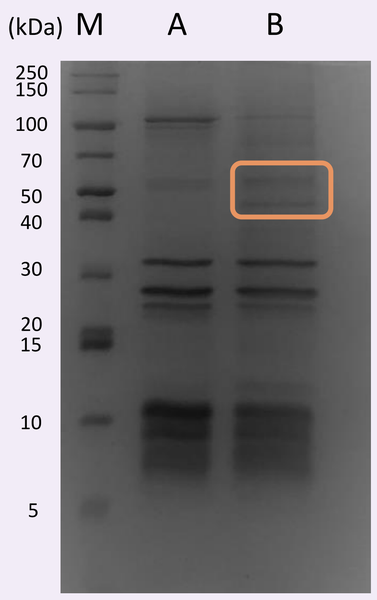
Antibacterial Activity



The antibacterial activity of compound **2** was determined based on the minimal inhibitory concentration (MIC) values towards *S. aureus* and *E. coli*. The results obtained are presented in Figure 2.

Figure **2** – MIC values (µg/mL) of compound **2** for *E. coli* ATCC25922 and *S. aureus* Newman.

- The copper content in *E.coli* grown in a subinhibitory concentration of the Cu(II) complex was analyzed by ICP-OES.
- A high accumulation of copper was verified in the cytoplasm.



Dynabeads® M-270 carboxylic acid **(A)** as well as derivatized magnetic beads with compound **2 (B)** were interacted with *E. coli* cytoplasmic proteins.

The significant difference between the protein profiles was observed around 50 and 40 kDa (see Figure 3). Profile **A** had less protein content than profile **B**. Both bands (in orange) were cut, and the identification of the corresponding proteins are currently being analysed by LC-MS.

Figure **3** - SDS-PAGE gel representing the elution sample profile of magnetic beads (**A**) and derivatized magnetic beads with compound **2** (**B**).

CONCLUSIONS AND PERSPECTIVES

A new cyclam-based Cu(II) complex (**2**) was synthesized, characterized and tested against *E. coli* and *S. aureus*. High accumulation of copper was verified in the cytoplasm when *E. coli* is grown in a subinhibitory concentration of the complex. Magnetic beads linked to **2** were used to separate interacting proteins. Disclosing proteins that interact with cyclam derivatives will be crucial to unveil the antibacterial mechanisms of this class of compounds.

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CQE Química Estrutural
iBB Institute for Bioengineering and Biosciences
BioISI Biosystems and Integrative Sciences Institute