

TITLE OF RESEARCH PROJECT AND KEYWORDS: Design, synthesis and studies of fluorescent systems with antitumor, tracing and theranostic potentialities

RESEARCH AREA: Area 03 Chemistry – General and Inorganic Chemistry

GENERAL PRESENTATION OF THE PROJECT AND STATE OF ART

In the realm of modern chemistry, a new and promising class of compounds is coming to light known as “bis-maltolpolyamines”. These particular molecules are characterized by a polyamine scaffold, defined by a linear or a macrocyclic skeleton, which plays the role of a spacer between two Maltol (3-hydroxy-2-methyl-4-pyrone) units. Attention has been paid to the hydroxypyrrone moiety given its high synthetic versatility, in addition to its capacity of binding metal ions, properties which lead to a potential use in the pharmaceutical field. Moreover, intriguing antineoplastic and antioxidant features are displayed by Maltol while anti-tumour activity are related to polyamine fragments. Among the previously synthesized molecules of this class, N,N'-bis[(3-hydroxy-4-pyrone-2-yl)methyl]-N,N'-dimethylethylenediamine (*Malten*) and 4,10-bis[(3-hydroxy-4-pyrone-2-yl)methyl]-1,7-dimethyl-1,4,7,10-tetraazacyclododecane (*Maltonis*) are the most promising (Figure 1), in terms of both binding and biological properties.

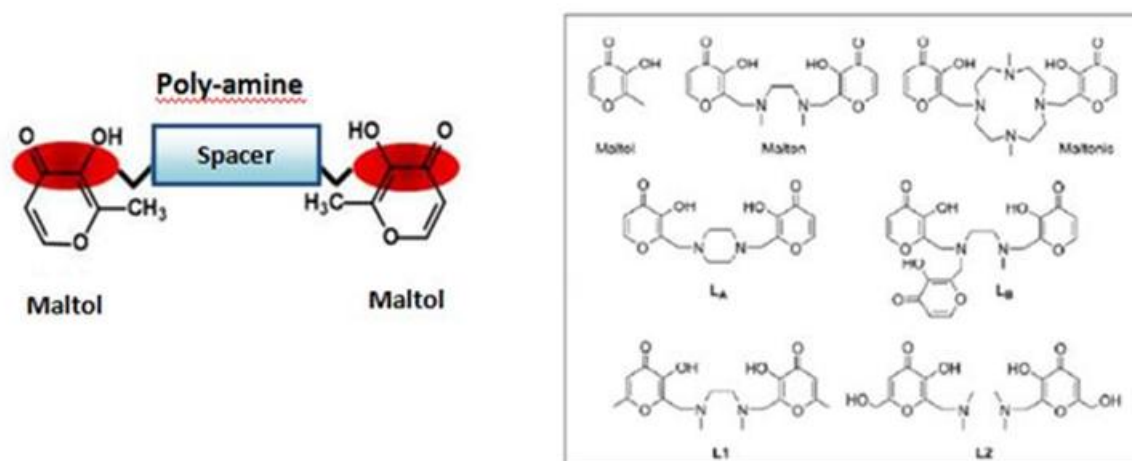


Figure 1. Scheme of bis-maltolpolyamines structure.

According to the biological tests conducted, the two molecules express an important anti-tumour function, provoked by a complex structural alteration of genomic DNA¹, activation of the apoptotic process and interference with chromatin. Malten shown capacity of activate programmed cell death in eight type of tumour cells, with a dose-dependent profile.^{2,3}

Concerning their binding activities, the two molecules feature peculiar properties. On one hand, they can be preorganized by the suitable transition metal ion, according to their geometry requirement. Such a condition can be achieved by using Co²⁺, in the case of Maltonis, that features a higher number of donor atoms, and Cu²⁺, in the case of Malten, that feature a lower number of donor atoms. On the other hand, the preorganization leads to the formation of an electron-rich area that is able to lodge hard species. Indeed, in aqueous solution Malten and Maltonis are able to accommodate alkali, alkaline-earth and rare earth metal ions^{4,5}, known for being difficult to bind in such medium. E.g. the Malten-Cu²⁺ complex can discriminate between Ca²⁺ and Mg²⁺ in water, a goal particularly difficult to achieve.⁶

Among RE ions, attention has been paid to gadolinium, characterized by incredible magnetic properties. Indeed its use spans many fields, such as industry or medicine, where it plays an important role in NMR imaging. Both Malten and Maltonis $\text{Cu}^{2+}/\text{Co}^{2+}$ complexes can bind the gadolinium in their electron-rich pocket: the coordination framework displays two $[\text{M}(\text{H}_2\text{L})]$ units (L = Malten, Maltonis; $\text{M} = \text{Cu}^{2+}$, for Malten and Co^{2+} , for Maltonis) that bind the Gd^{3+} ion through the oxygen atoms of the maltol rings ⁷ (Figure 2).

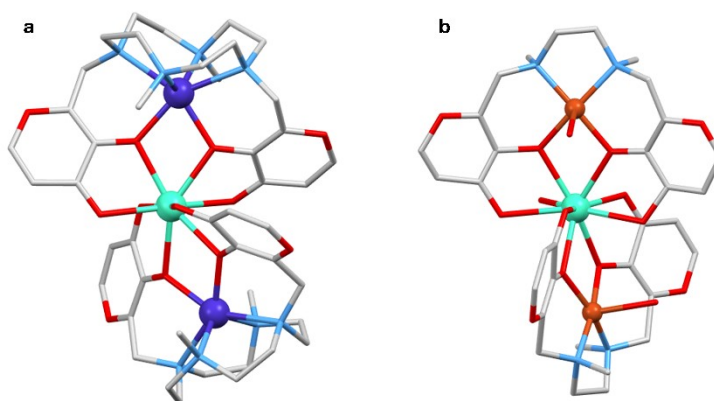


Figure 2. Trinuclear complexes of Maltonis- Co^{2+} (**a**) and Malten- Cu^{2+} (**b**) with Gd^{3+} .

Up to now, several derivatives of Malten have been developed⁸ by changing the polyamine scaffold, or substituting the Maltol ring at position 6, or replacing maltol with the 3-hydroxy-chromone unit, with the aim to optimize the biological activity and better understand the mechanism of action, or to change the binding properties, or to provide the molecule with fluorescent properties. The latter is a desirable goal to achieve, it would allow to obtain a fluorescent chemosensor usable for multiple applications, thanks to its characteristics, i.e. quick response time, high sensitivity and huge versatility.

Basically a chemosensor is made of i) a *receptor*, which recognizes a specific analyte, ii) a *signalling unit*, able to exhibit a response after the interaction between receptor and target, and iii) a *spacer*, important for maintaining of the distance between the two moieties (Figure 3). After binding of the guest, a fluorescent chemosensor can undergo either an enhancement or a quenching of fluorescence, i.e. it converts the chemical event into a photophysical signal.

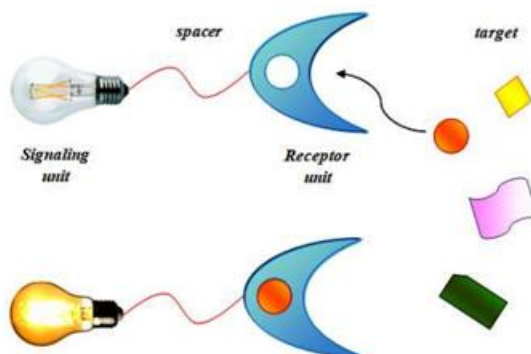


Figure 3. Scheme of a chemosensor.

A possible strategy to make Malten fluorescent could be the insertion of a photoactive unit to the molecule itself, through the easy functionalization of the ethylenediamine moiety (Figure 4).

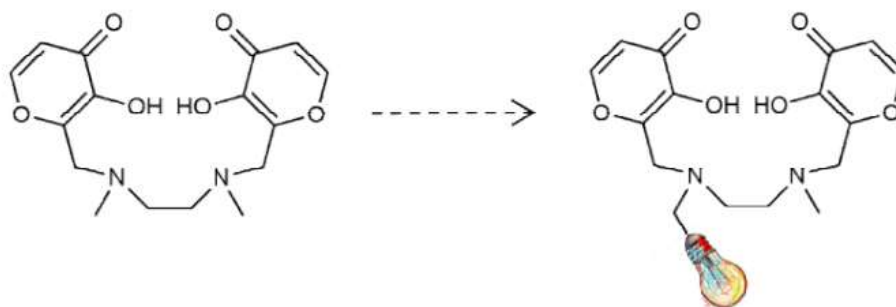


Figure 4. Scheme of derivatives of Malten containing fluorophore units.

A derivative of Malten containing an anthranilic moiety (L) has been already synthesized and partially characterized in aqueous solution (Figure 5).

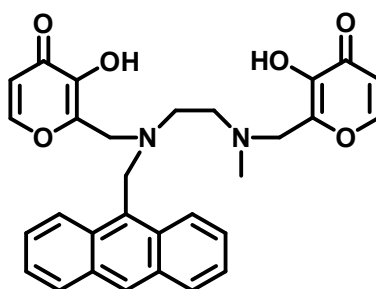


Figure 5. Derivative of Malten containing an anthranilic moiety (L).

The study of the acid-base properties by spectrophotometric and spectrofluorimetric analyses revealed a fluorescence emission mechanism based on the Photoinduced Electron Transfer (PET) effect, indeed L is switched-ON at acid pH values while it is OFF at more alkaline pH values. Coordination properties in aqueous solution towards transition, post transition and RE metal ions were also tested; even if all of them are bound by the ligand, none of them produced changes in the emission (416 nm). This could be likely due to the involvement in the coordination of the oxygen functions but not the amine groups, allowing for a residual PET effect that quenches the emission. Nevertheless, a peculiar behaviour was observed with Cd^{2+} and Zn^{2+} : in particular, a non-structured band, red-shifted compared to that of anthracene, was observed, probably ascribed to the formation of an excimer (470 nm). Indeed, measurements pointed at a 2:1 ligand to metal molar ratio in the complex formation. Due to the interest for RE ions, the behaviour of L-Cd^{2+} towards Gd^{3+} was investigated, revealing that probably the latter displaces Cd^{2+} from the complex and disrupts the excimer, shifting the emission towards that of the monomer (416 nm).

RESEARCH OBJECTIVES

The synthesis and study of **L** represents a successful first step towards the development of innovative fluorescent chemosensors belonging to the bis-maltol polyamine family; this Ph. D. project would be devoted to deepen the study of **L** and to the development of further related molecules to be used for multiple applications. To this purpose, similarly to anthracene, other moieties featuring fluorescent and/or intercalating properties would be appended to already studied active bis-maltol polyamine frameworks. The insertion of DNA intercalating moiety, on one hand, would allow to obtain more powerful antineoplastic agents, while the introduction of a photoactive unit, on the other hand, would allow to follow the molecule in the cellular environment and to better identify the mechanism of action of the biologically active compounds. Fluorescence properties could also be exploited in the recognition of metal ions, both in solution, for multiple purposes, as well as in a biological environment. Considering the proposal, the design of these systems will serve not only to widen the knowledge about the biological activity of the class but also could be used to develop multiple tasking structures as both environmental sensing systems, for detecting and recovering analytes, as well as in biology, for antitumor, tracing and theranostic applications.

METOLOGY AND EXPECTED RESULTS

During the Ph.D.project new compounds featuring enhanced biological activity and recognition and signalling abilities towards external guests will be developed, starting from previously studied molecules that show peculiar biological and binding activities, by inserting *ad hoc* photoactive and/or intercalating fragments. To reach the predetermined goals, multiple techniques would be used, such as UV-Vis absorption, fluorescence emission and NMR spectroscopies, potentiometry, X-ray diffraction, circular dichroism, confocal microscopy, just to name a few.

DESCRIPTION OF THE RESEARCH IN THE THREE-YEAR PERIOD (FEASIBILITY):

First year: Further studies on **L**, to better understand the fluorescence properties and to find out a possible biological activity. Bibliographic research supporting the development of new molecules containing *ad hoc* photoactive and/or intercalating fragments. Synthesis and of bis-maltol polyamine derivatives.

Second year: Study of the binding and fluorescence properties of the new compounds in solution. Synthetic variations on the bis-maltol polyamine derivatives, according the obtained results. Preliminary biological studies.

Third year: In depth biological studies on the developed molecules. Development of possible applications.

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