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METAL COMPLEXES AS MICROTUBULE- AND DUAL MICROTUBULE-R2 RNR-TARGETING DRUGS FOR CANCER TREATMENT - Metubin

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1. Introduction

Petru Poni Institute of Macromolecular Chemistry (ICMPP), Iasi, Romania is implementing the project "**Metal complexes as microtubule- and dual microtubule – R2 RNR-targeting drugs for cancer treatment** (Metubin), ID 99/31.07.2023, contract no. 760284/27.03.2024, project financed through the National Recovery and Resilience Plan, Component C9 – Support for the private sector, research, development and innovation, Investment I8: Development of a program to attract highly specialized human resources from abroad in research, development and innovation activities.

The **Metubin** project's *general objective* is to attract high-level staff from abroad, and develop a new research area in ICMPP, by implementing a project related to antiproliferative activity and tubulin polymerization inhibition of indolobenzazocine based Schiff bases, TSCs and their metal complexes, and therefore to support and increase the quality and capacity of the research, the development and innovation (RDI) activity. Although modern anticancer chemotherapy has made progress, there are still many problems caused by drug resistance and by the low selectivity of anticancer drugs generating serious side effects. Therefore, the search for new chemotherapeutics remains an essential challenge. Tubulin, the repeating subunit of microtubules (MTs), and R2 ribonucleotide reductase (RNR) are validated molecular targets for anticancer drugs, as they play crucial roles in cell proliferation and other vital cellular processes. MT-targeted agents (MTAs) and R2 RNR inhibitors lead to disruption of cell cycle progression and cell death via cell cycle arrest in G2/M and S-phase, respectively. The project leader's team recently discovered that Cu(II) compexes with indolobenzazocine-based Schiff bases show good antiproliferative activity and act as colchicine site inhibitors [1], while highly antiproliferative Cu(II) complexes with thiosemicarbazones (TSCs), sharing with the previous agents a similar tridentate binding motif, had both tubulin- and R2 RNR inhibitory activity [2] (Scheme 1). These latter compounds impaired different phases of the cell cycle and were without precedence in the literature.

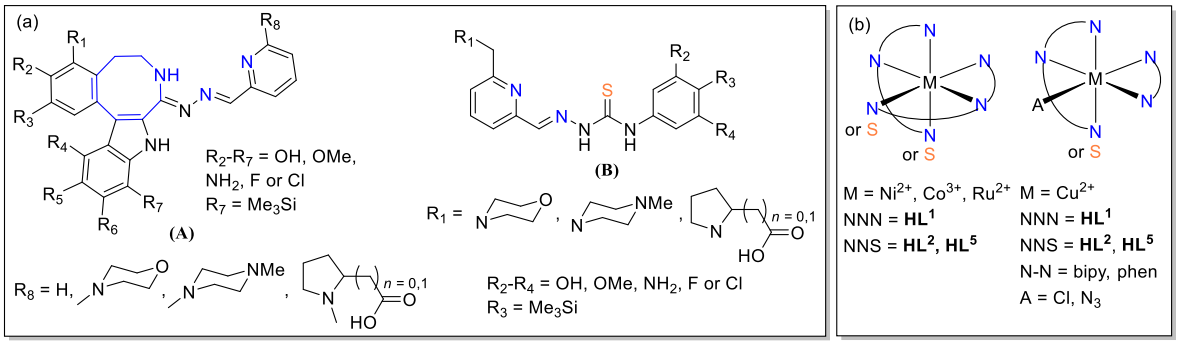
2. Project objectives

The aim of **Metubin** project is to further refine the electronic and geometric structure of the two prototype metal complexes [3,4] with modified indolobenzazocine and TSC ligands sharing a similar metal binding motif as inhibitor of tubulin polymerization or as dual agent with tubulin- and R2 RNR inhibiting properties endowed with high antiproliferative activity.

The *specific research objectives* (RO) of the project:
RO1. Preparation and characterization of the two prototype ligands bearing a trimethoxyphenyl group and copper(II) complexes thereof.

RO2. Design, synthesis and characterization of the second generation of the two modified prototype ligands bearing electron withdrawing and donating groups including Me_3Si and metal(II) complexes thereof.
RO3. Design, synthesis and characterization of the third generation of the two modified prototype ligands and metal complexes thereof.
RO4. Design, synthesis and characterization of the fourth generation of the two modified prototype ligands and metal complexes thereof.
RO5. Evaluation of stability and extended biological investigation of two lead Schiff bases and two metal complexes.

Based on the initial *in silico* screening, the two prototype molecules are structurally modified through four sequential iterations by attaching electron-withdrawing and donating groups, as well as solubilizing substituents (R_8 and R_1 in Scheme 1) The power of coordination chemistry to build structures with well-defined globular shapes, matching the 3D structure of the colchicine pocket, is exploited to complement the molecular diversity offered by purely organic scaffolds. Kinetic stability of the coordination sphere in biological media will be achieved by using both multidentate ligands and kinetically inert metal ions, e.g., Co(III), Ru(III). This approach was not used for the development of tubulin polymerization inhibitors so far, but proved to be successful for the development of efficient protein kinase inhibitors [3,4]. The synthesis and chemical modification will be followed by full analytical, spectroscopic and structural characterization, as well as biological evaluation of the isolated compounds. The lead drug candidate will be tested *in vivo*.



Scheme 1. Envisaged structural modification of the two main prototypes based on 5,6,7,9-tetrahydro-8H-indolo[3,2-e]benzazocin-8-one and TSCs and metal complexes thereof.

3. Project implementation and expected outcomes

The project objectives will be approached a competent, well-structured team from "Petru Poni" Institute of Macromolecular Chemistry, with top chemistry expertise and infrastructure, under the guidance of a recognized specialist in medicinal chemistry from the University of Vienna, and with strong external collaborative support. Thus, the design of specific compounds start with *in silico* calculations, which will be performed in collaboration with Dr. J. Reynisson (Keele University, UK), **EC1**. The substitution will be considered favorable if the docking scores (e.g., binding free energy) of the newly designed compounds, as well as their ADMET parameters (e.g., aqueous solubility, lipophilicity, physicochemical properties, Lipinski's rule of five), are not inferior to those of the prototype compounds and remain within the drug-like chemical space. The designed compounds that successfully go through this preliminary *in silico* screening will then be synthesized, fully characterized and tested for antiproliferative activity on several cancer cell lines (breast MCF-7, lung A549, ovarian CH1) and normal MRC-5 human embryonal lung fibroblasts and analyzed for effects on tubulin assembly. This theoretical preselection of relevant structures will make the synthesis work more effective and less time-consuming. Analysis of the results of this full cycle will allow the next iteration, which again will consist of design, molecular docking calculations with determination of molecular descriptors, synthesis, characterization and biological evaluation. The tests will

be performed within ICMPP (IntelCentre) and University of Belgrade, Serbia, and validated at NCI/NIH, USA, based on pre-established collaboration agreements.

The achievement of the scientific objectives is also expected to lead to broader goals (GO):
GO1: Development and progress of fundamental research in Romania through the establishment of a new RDI area at ICMPP conducted by an excellent research group led by a recognized specialist in medicinal chemistry from abroad.
GO2: Training of competent human resources and the formation of an interdisciplinary research group under the supervision of a highly qualified specialist, increasing the ICMPP international visibility.
GO3: Increase of the number of publications with high international impact and participation in the competitions of the European Union's Horizon Europe framework programme competitions.

4. Conclusions

This project aims to develop a new class of compounds based on indolobenzazocines and thiosemicarbazones and their copper(II) complexes, capable of selectively inhibiting tubulin and/or R2 RNR, offering an innovative alternative to existing anticancer agents. Through a structural optimization strategy guided by *in silico* screening, followed by full characterization and biological evaluation, the project will lead to the identification of promising candidates for preclinical development. The originality of the approach, combined with the expertise of the project team and cutting-edge research infrastructure, gives the project a high potential to contribute to the progress of medicinal chemistry and the discovery of new anticancer therapies.

Acknowledgements

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