Title of ANR project: **TOWARDS NEW ANTINFLAMMATORY AGENTS: DESIGN, SYNTHESIS AND EVALUATIONS OF MOLECULES TARGETING XIAP-BIR2 (ANR project)**

Goal: Designing new selective XIAP-BIR2 inhibitors using computer-aided rational methods and their biological evaluation.

Abstract: XIAP (X-chromosome-linked inhibitor of apoptosis protein) is involved in various cellular processes, such as apoptosis and the immune response. It also coordinates a series of events leading to the production of pro-inflammatory cytokines. Recently, the key role of XIAP in the NOD signalling pathway has been highlighted. This pathway is overexpressed in inflammatory diseases such as Crohn's disease and sarcoidosis. XIAP could therefore be a valuable target for developing new treatments for these NOD2-mediated inflammatory diseases. Indeed, it has been observed that the absence of XIAP in cells leads to defective ubiquitination of serine/threonine/tyrosine-protein kinase 2 (RIPK2), and reduces the production of pro-inflammatory cytokines, notably IL8. As the ubiquitination of RIPK2 is regulated by its binding to XIAP *via* its BIR2 domain, specific inhibitors of XIAP, binding to XIAP-BIR2, could disrupt XIAP/RIPK2 complex formation, and have therapeutic applications in chronic inflammatory diseases. The data in the literature also show that targeting XIAP *via* its BIR2 domain could result in more selective XIAP activity and avoid serious adverse effects.

The aim of this project is to design, synthesize, and evaluate synthetic small molecules that are both affine and selective for XIAP-BIR2. Preliminary results from CERMN have identified two promising candidates: a fragment (Hit 1) and a molecule (Hit 2), both of which exhibit first affinity and selectivity for XIAP-BIR2. Notably, Hit 2 demonstrated its ability to disrupt the interaction between XIAP-BIR2 and RIPK2 *in cellulo* studies. Structural studies of the interactions between Hits 1 and 2 and XIAP-BIR2 will be conducted by our colleagues in Lyon. Following this, an *in silico* drug design strategy will be initiated, led by the PhD student, to guide the synthesis of new compounds aimed at enhancing affinity and selectivity for XIAP. The PhD student will also be responsible for the in vitro evaluation of these compounds, assessing their affinity for XIAP-BIR2 and their selectivity by comparing their affinities for XIAP-BIR3 and cIAPs-BIR2. Throughout the project, we will conduct early druggability assessments, including solubility, logP, and membrane permeability. Finally, the synthesized molecules will be evaluated in cellular models by our colleagues in Lille to determine their potential to disrupt the XIAP/RIPK2 interaction and their anti-inflammatory effects.

The recruited PhD student will be actively involved in both molecular modeling and the biological evaluation aspects of this ANR project, with their research efforts evenly divided: 50% dedicated to molecular modeling and chemoinformatics, and 50% to the biophysical evaluation of compound affinities.

We are seeking a PhD candidate with background in one or both of the following areas:

- Biological/Biophysical Binding Studies: Experience with proteins in vitro, including techniques such as MST, ITC, polarization fluorescence, and AlphaScreen.
- Chemoinformatics: Proficiency in 3D pharmacophore modelling, docking, and molecular dynamics techniques.

<u>Contacts and other information :</u>

- Beginning of the thesis: November 2024 to October 2027
- Location of the thesis : UR 4258 - CERMN · Centre d'études et de recherche sur le médicament de Normandie, Université de Caen Normandie, bd Becquerel, 14032 CAEN Cedex, France.
- People to contact: Pr Jana Sopkova - de Oliveira Santos, jana.sopkova@unicaen.fr, phone : +33231566821