Progetto e Piano formativo per un assegno di ricerca dal titolo:

"Development of an analytical platform for the characterisation of Prolyl isomerase Pin1 inhibitors as novel drug candidates"

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Progetto di ricerca

The activity envisaged for the research fellowship falls within the scope of the project PRIN 2022 PNRR 202283YHXY titled "PRIN-UNO - Prolyl isomerase PIN1 inhibitors: a drug design strategy against aggressive tumors", Scientific Responsible of the Local Unit and of the funds: Dr. Michele Protti.

The mail goal of this project is to characterise from the analytical point of view new prolyl-isomerase 1 (Pin1) inhibitors, characterized up to preclinical level [1-4] where the best candidates will be evaluated for in vitro metabolic profile, pharmacokinetic features and and stability in biological fluids [5,6]. Analysis will be performed exploiting advanced miniaturized biosampling and sample treatment technologies coupled to original mass spectrometry (MS)-based methods in order to reduce sample volumes, solvents and reagents, in the general framework of sustainability [7-9].

In particular, hits identified in preliminary results and interesting molecules deriving from preliminary steps of the project will be evaluated for their metabolic stability based on in vitro studies in liver microsomes and will be investigated in vivo in terms of toxicity properties and half-life, to be compared with all-trans retinoic acid (ATRA), one of the most studied Pin1 specific inhibitor. Metabolic profiles and ADME properties of the selected compounds will be studied in depth by means of original miniaturised approaches for the collection and pretreatment of complex samples coming from both in vitro assays on liver microsomes and in vivo studies on animal models. The final aim will be the tailoring of a reliable sampling, pretreatment and MS-based analysis protocol characterised by a high degree of sustainability, which will be exploited to obtain reliable quali-quantitative data pertaining metabolic and pharmacokinetic assays, while granting affordable and logistically feasible workflows.

References

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[4] J. H. Yu, et al., Front Cell Dev Biol, 2020, 8, 120.

[5] E. Campaner, et al., Nat Commun, 2017, 8, 15772.

[6] Y. Chen, et al., Cell Death Dis, 2018, 9, 883.
[7] I. Varfaj, et al., J Chromatogr A, 2021, 1643, 462088.
[8] J. Millán-Santiago, et al., J Pharm Biomed Anal, 2023, 236, 115740.
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Piano formativo

The proposed activities for the research fellowship align with the objectives of project PRIN 2022 P2022ZWY8H entitled "Prolyl Isomerase PIN1 Inhibitors: A Drug Design Strategy Against Aggressive Tumors (PRIN-UNO)", under the scientific supervision of Dr. Michele Protti, who is responsible for both the local unit and the associated funds.

The planned activities within this project encompass specific training aimed at establishing an innovative mass-spectrometry-based platform dedicated to the characterization of novel drug candidates, specifically PIN1 inhibitors intended as potential antitumoral agents. This methodology involves the development, optimisation, and comprehensive validation in accordance with international standards, enabling its application to both standard solutions and complex samples.

The most promising PIN1 inhibitor candidates will undergo experimental investigation exploiting advanced mass spectrometry-based analytical platforms coupled with miniaturised yet robust sampling and pretreatment technologies, applicable to samples derived from both in vitro and in vivo assays.

Compound identification within complex samples will be carried out by means of high-resolution mass spectrometry (HRMS), while quantitation will be achieved through HPLC coupled with triple quadrupole tandem mass spectrometry (HPLC-MS/MS), and UHPLC-HRMS when enhanced sensitivity and selectivity are necessary. Rigorous assay design and execution will facilitate the development and optimisation of tailored analytical methodologies, subsequently validated for quali-quantitative analysis of the target compounds.

Additionally, microsampling technologies will be developed and rigorously validated following international guidelines for quantitative bioanalysis, with analytical performance assessed in terms of sensitivity, selectivity, precision, accuracy, and stability. Evaluation and selection of the most advanced volumetric microsampling technologies, based on absorptive, capillary, and microfluidic principles, will be carried out. Optimisation of parameters associated with microsampling, storage, processing, and analysis will ensure the generation of reliable quali-quantitative data.