MedChem and structural biology: tools and strategies for hit and lead optimization in the One Health perspective

Wednesday 27 September – Friday 29 September 2023

University of Siena, Siena, Italy

Students' collaborative report

Day One Report

Sandra Gemma's opening remarks not only extended a warm welcome to all of us but also emphasized the critical importance of taking into account the interplay between the environment, human beings, and animals in all our research endeavors. This overarching perspective falls under the philosophy of "One Health." Furthermore, she provided us with an insightful overview of what to expect in the upcoming days.

Alessandra Roncaglione's introductory lecture served as a pivotal starting point, offering us essential insights into "Computational Methods and Tools for Supporting Chemical Safety and Sustainability Assessment." She delved into the core components of this field, where Quantitative Structure-Activity Relationship (QSAR) emerged as the foundational pillar encompassing data preparation, mathematical modeling, statistical evaluation, validation procedures, and boundary definition. Moreover, Alessandra highlighted the crucial role of regulatory compliance, exemplified by REACH, in ensuring researchers remain informed about legal requirements.

Furthermore, she presented a comprehensive and engaging exploration of ToxRead, elucidating its significance in discerning chemical similarities and structural alerts. In addition, she adeptly covered the concept of Read Across, providing a thorough and interesting explanation of this methodology.

Simone Brogi's lecture delved deeply into the fundamentals and practical applications of "In silico tools for predicting the toxicity of natural compounds." Moreover, the insightful examples he presented underscored the critical relevance of these tools, offering valuable insights for our respective areas of interest. The discussion also encompassed the pivotal aspects of Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH).

It is noteworthy to highlight the incorporation of "Read Across" as a potential fourth "R," standing alongside Replace, Reduce, and Refine. This addition not only underscores its substantial influence but also reinforces the critical importance of its application within this particular context.

Valeria Tudino's lecture on "Drug Likeness and Optimization Approaches: Case Studies in Infectious Diseases" provided us with a valuable opportunity to either learn anew, refresh our knowledge, or contemplate, depending on our individual backgrounds, the fundamental significance of grasping basic concepts when interpreting the outcomes of in silico research. In this context, the inclusion of illustrative examples proved highly beneficial, serving to elucidate key points and enhance our retention of the material.

In the afternoon session, Alessandra Roncaglioni led a practical session, where she demonstrated the use of VEGA software & hands-on activities on the methods and tools in support of chemical safety and sustainability assessment.

We were trained on how to use the VEGA virtual models to evaluate the characteristics of chemical compounds within a global design.

We also used the VEGA QSAR models and rule-based expert systems to predict toxicities of chemical compounds on various subjects of interest including human toxicity endpoints such as carcinogenicity, mutagenicity, skin sensitization, and developmental toxicity). This platform also gave us an opportunity to examine the toxicities such as eco-toxicological endpoints which include acute aquatic toxicity on fish and Daphnia. The third was environmental properties (ready biodegradability and BCF). Finally, the physicochemical properties of chemical compounds such as LogKow.

We selected each of the above toxicities (human, eco-toxicological, environmental) and physicochemical properties after which we assessed the applicability domain of the predictions using an Applicability Domain Index (ADI) that had values ranging from 0 (worst case) to 1 (best case).

We also learned that most of the indices were based on the calculations of the most similar compounds contained in the test and the training set of the model was the basis for most of the indices we saw in the report. We learned that the software calculated this using a similarity index which considers the several structural aspects and the molecule's fingerprint.

Overall, it was an opportunity to learn how to use various models to identify the toxicities of chemical compounds.
Flash presentations from Selected Training School attendees were the icing on the cake! Several collaborations were established after each presentation as attendees exchanged contacts.

Day Two Report

LECTURE 1: LUCA POZZETI

HAMPERING PARASITIC SURVIVAL MECHANISMS: TRYPANOTHIONE REDUCTASE AS LEISHMANIA TARGET

Introduction about Leishmaniasis

Leishmania parasites

- Life cycle
- Phenotypic vs target-based drug discovery approaches
- Biological targets

Trypanothione reductase

- Polyamine -Trypanothiene pathway
- Structure and activity

Targeting TR

Why targeting TR

- Essential for survival of the parasite
- 3D X-ray structures available
- Absent in the host
- Druggable target

Drug discovery

Conclusions

- Future overlook
- 3 possible strategies

LECTURE 2: VALERIA TUDINO

GREEN CHEMISTRY AND SUSTAINABLE STRATEGIES TO COMBAT INFECTIOUS DISEASES

General concepts

The 12 principles of Green Chemistry

Case studies

- Preparation of newer fluoroquinolone derivatives
- Green synthesis of silver nanoparticles
- pH degradable polymers as impermanent antimicrobial agents for environmental sustainability
- Sustainable anti-trypanosomatid drugs

LECTURE 3: SANDRA GEMMA

FROM NATURAL COMPOUNDS TO SIMPLIFIED ANALOGIES: TOOLS AND STRATEGIES

The unique properties of natural compounds

Natural products chemical space

PCA analysis

High-throughput screening

Phenotypic screening

Finding novel compounds

Extension of NP Chemical space by synthesis

Antimalarial peroxides: From the natural compound dihydroplakortin to potent synthetic analogues

Natural products in antimalarialtherapy

- Use of artemisinin
- Dihydroplakortin (DHP) – simple structure

Chalcones as anti-Leishmania therapy

- Synthesis of Lophirone

LECTURE 4: DANIELE CASTAGNOLO

AN INTRODUCTION TO BIOCATALYSIS: A SUSTAINABLE TOOL FOR MEDICINAL CHEMISTS

Pioneering novel green and sustainable chemistry

Use of catalysis reagents

Biocatalysis – Use of natural enzymes or enzymes produced in situ from whole cells

Use of:

- Lipases
- Peroxy acid produced in situ
- Enzyme cofactors – organic or inorganic
- Monoamine oxidases
- Ketoreductases (KREDs)
- Various enzymes (Monooxygemases, Transaminases, Cytochromes P450, Immune reductases and reductive aminases (IRED and RedAm), Laccases, PETases – for plastic degradation

PRACTICAL WORK, DEMONSTRATION & GROUP ACTIVITIES ON BIOCATALYSIS

Between 14:30h – 18:30h all the attendees went to the Chemistry Laboratory to do two reactions catalysed by CALB. The attendees were organised in groups of two. The first experiment was the oxidation of methyl phenyl sulfide. The second experiment involved the selective catalytic cleavage of acetate from one of the enantiomers in a racemic mixture of phenylethyl acetate. This procedure may be helpful for separating enantiomers from a racemic mixture.TLC and HPLC were used to follow the course of the reactions, and filtration and liquid–liquid extraction were used to separate different components of the reactions.

Day Three Report

LECTURE 1: Integrative structure biology approaches for investigating macromolecular targets (Marco Mazzorana)

LECTURE 2: Practical aspects of permorming X-ray crystallographic experiments on biological macromolecules (Cecilia Pozzi)

LECTURE 3: Investigating enzyme-inhibitor complexes through X-ray crystallography (Cecilia Pozzi/ Marco Mazzorana)

In the morning session the lectures gave us an overview on structural biology approaches, focusing in particular on X-ray crystallographic experiments, from the DNA engineering to crystal formation.

Analysis of structural model for drug discovery purposes

Modeling through the use of the Coot software, exploring all the workflow of a crystallographer work. Also all the possible techniques of investigation of enzyme-inhibitor complexes through X-ray crystallography were explored.The practical sessions involved the use of Coot software, the use of PDB database

PRACTICAL WORK, DEMONSTRATION & GROUP ACTIVITIES ON PROTEIN CRYSTALLIZATION AND INVESTIGATION OF ENZYME-INHIBITOR COMPLEX

Between 14:30h – 18:30h the participants were divided into two groups. the first group in the computer room performed various protein-ligand manipulations on the coot (Crystallographic Object-Oriented Toolkit) software. *Coot* is for macromolecular model building, model completion and validation, particularly suitable for protein modeling using X-ray data.

Coot displays maps and models and allows model manipulations such as idealization, real space refinement, manual rotation/translation, rigid-body fitting, ligand search, solvation, mutations, rotamers, Ramachandran plots, skeletonization, non-crystallographic symmetry and more.

The second group proceeds with the lysozyme enzyme crystallization experiment with different precipitant solutions and different enzyme concentrations. The crystals formed were then observed under a microscope.