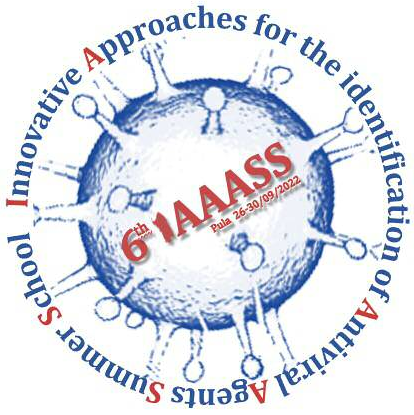

6th Innovative Approaches for Identification of Antiviral Agents Summer School

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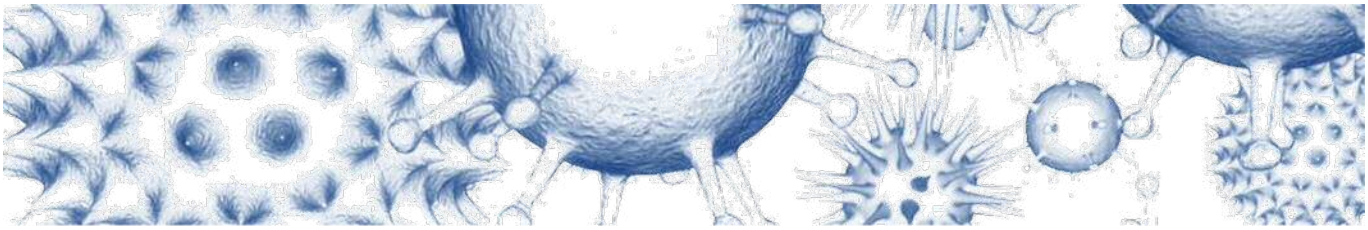

This pilot study shows that a significant proportion of Long COVID-19 cases are positive for HERV-W ENV expression along with a subgroup of ME/CFS samples from a pre-COVID pandemia collection, raising the question of whether the presence of HERV-W ENV protein, known to induce TLR4-driven immuno- and neuro-pathogenicity, could be a common factor to their overlapping symptoms. Being this the case, HERV-W ENV could constitute a future therapeutic target, following the steps of other neurologic or autoimmune diseases such as multiple sclerosis or diabetes type I. Particularly since ongoing clinical trials assaying HERV-directional therapies based on antiretroviral agents or monoclonal antibodies are showing promising results.

Poster 10

Therapeutic potential of parthenium hysterophorus plant extracts against hiv-1 rt and some microbial species

Jyotsna Jaiswal*[1] and Bechan Sharma[1]

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Parthenium hysterophorus possesses certain allelochemicals responsible for their medicinal effects. The presence of oils, polyphenols, alkaloids, terpenes, pseudoguaianolides, and histamines in P. hysterophorus has been shown to exhibit medicinal properties. However, the systematic biomedical properties of this plant are still unexplored. The extracts of leaves, stem, and flower of P. hysterophorus, both at low and high temperatures were prepared. The spectrophotometric and qualitative analysis of plant extracts demonstrated the presence of alkaloids, terpenoids, carbohydrates, and cardiac glycosides. The analyses of the free radical quenching potential of plant extracts were done by DPPH assay.
The total antioxidant capacity was determined by phosphomolybdate assay and the ferric reducing anti-oxidant power (FRAP) assay was used to determine the reduction potential of the extracts. The extracts prepared in hexane, ethylacetate, methanol, and water were resolved on TLC for the presence of phytochemicals. The occurrence of more than one Rf values for extracts determined by TLC indicated the presence of more than one phytochemical compound. The P. hysterophorus extracts contained strong antioxidant activity. These extracts exhibited strong antimicrobial activity against Staphylococcus epidermis, Salmonela typhi, Neisseria Gonococci or gonococci, Citrobacter, and Shigella flexineri. The evaluation of the antimicrobial potential of P. hysterophorus extracts was done by disc diffusion method. These extracts also showed significant inhibition against HIV-1 RT activity. The anti-HIV-1 RT activity was done using Roche Kit. The P. hysterophorus extracts displayed the presence of many phytochemicals with strong antioxidant, antimicrobial and anti-HIV-1 RT properties.

Poster 11

A Novel and Versatile Class of Coronavirus non-covalent Mpro Inhibitors based on 1,4,4-Trisubstituted Piperidines

Miguel Maldonado*[1], Sonia de Castro[1], Annelies Stevaert[2], Adrien Delpal[3], Julie Vandeput[2], Benjamin Van Loy[2], Cecilia Eydoux[3], Jean-Claude Guillemot[3], Etienne Decroly[3], Bruno Canard[3], Lieve Naesens[2], Federico Gago[4], María-José Camarasa[1], Sonsoles Velazquez[1]

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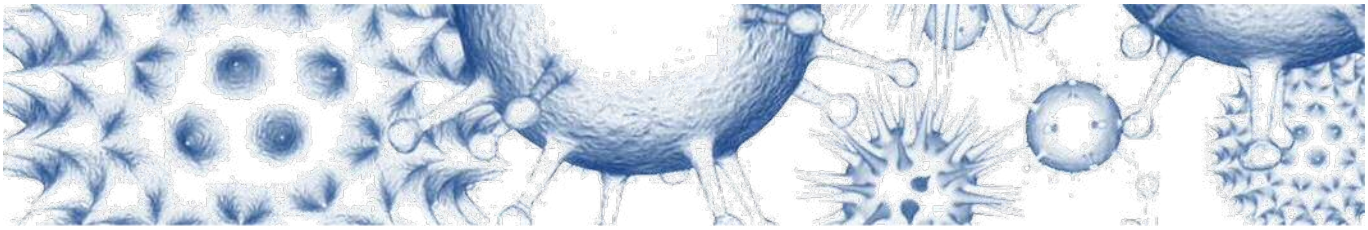
The COVID19 pandemia has greatly encouraged the development of vaccines and novel antivirals to control SARS-CoV-2 infection. Based on the promising anti-coronavirus activity observed for a class of anti-influenza H1N1 1,4,4-trisubstituted piperidines, developed in our goup, we performed a SAR analysis of these unique inhibitors that allowed to define the structural elements essential for anti-HcoV-229E activity.



Four of the best molecules were confirmed to be equally active against SARS-CoV-2. A TOA experiment indicated that these new CoV inhibitors interact at a post virus entry point lying at the stage of viral polyprotein processing and the start of viral RNA synthesis. Enzymatic assays were performed with different CoV proteins involved in these processes. The compounds clearly inhibited the nsp5 main protease (Mpro). Although the inhibitory activity was modest, the ability to bind to the catalytic site of Mpro was assessed by in silico studies.

The combination of results from TOA, enzymatic assays, resistance selection and in silico molecular modeling allowed us to conclude that the 1,4,4-trisubstituted piperidines represent a structurally novel and unique class of compounds that inhibit CoV Mpro inhibitors via a non-covalent mechanism, making these inhibitors fundamentally different from other Mpro inhibitors represented by the approved drug nirmatrelvir. The five points of diversity make these N-substituted piperidine-based compounds highly versatile and amenable to further rational optimization to maximize their activity and selectivity and gain full insight their antiviral mechanism.

Poster 12

Development of multiple assays to evaluate the inhibitory effect of small molecules against SARS-CoV-2 RNA dependent RNA polymerase (RdRp)

Paolo Malune[1], Francesca Esposito[1], Maura Masella[1], Enzo Tramontano[1]

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COVID-19, caused by the infection of SARS-CoV-2, has led to a worldwide pandemic started in 2019[1]. Despite the fast development of vaccines, monoclonal antibodies and antiviral drugs, the virus keeps on spreading and causing millions of deaths worldwide, evolving and accumulating mutations over time. Different strategies have been followed for the development of novel antiviral drugs, by targeting key viral enzymatic functions, such as the nsp12, the viral RNA-dependent RNA polymerase (RdRp)[2][3]. In order to characterize the potential inhibitory activity of repurposed or newly developed compounds, we set up different assays to assess the activity and the inhibition of the viral polymerase also in the presence of SARS-CoV-2 nsp12 cofactors nsp7 and nsp8. We developed strategies based on the measurement of the enzymatic activity, such as the detection of the elongated primers using double strand RNA intercalating agents, or by the introduction of fluorescent nucleotides into the nascent strand, by using polyA and heteropolymer templates. Known inhibitor Remdesivir-TP[4] was used as a positive control, with a calculated IC50 value of 0.69 ± 0.04 μM. These methods, with different advantages and disadvantages, will allow us to exploit different strategies to understand the molecular mechanisms of SARS-CoV-2 RNA replication and to characterize the inhibitory activity of antiviral small molecules, active against SARS-CoV-2 replication.

[1] Brant et al., Cell & bioscience (2021).
[2] Kabinger et al., Nature structural & molecular biology (2021).
[3] Lu et al., Antimicrobial agents and chemotherapy (2020).
[4] Gordon et al., The Journal of biological chemistry (2020).