OR07: Application of the PROTAC-based approach to trigger proteolysis of the Coxsackievirus B3 main protease

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The conservation of the main protease in viral genomes, combined with the absence of a homologous protease in humans, makes this enzyme family an ideal target for developing broad spectrum antiviral drugs with minimized host toxicity (1). GC-376, a peptidomimetic 3CL protease inhibitor, has shown significant efficacy against coronaviruses (2). Recently, we have shown that a GC-376-based PROTAC specifically targets and induces the proteasome-mediated degradation of the dimeric SARS CoV-2 3CL^{Pro} protein (3). Expanding this approach, we investigated the application of the GC-376 PROTAC to the 3C^{Pro} protease of enteroviruses, characterizing its interaction with Coxsackievirus B3 (CVB3) 3C^{Pro} through X-ray crystallography, NMR (Nuclear Magnetic Resonance) and biochemical techniques (4). The crystal structure of CVB3 3C^{Pro} bound to the GC-376 PROTAC precursor was obtained at 1.9 Å resolution. The crystallographic data show that there are some changes between the binding of CVB3 3C^{Pro} and SARS-CoV-2 3CL^{Pro}, but the overall similarity is strong (RMSD on C-alpha 0.3 Å). NMR backbone assignment of CVB3 3C^{Pro} bound and unbound to the GC-376 PROTAC precursor (80% and 97%, respectively) was obtained. This information complemented the investigation of the interaction of CVB3 3C^{Pro} with the GC-376 PROTAC and its precursor allowing us to define that the two molecules bind to the protease in a very similar mode, coherent to the SARS CoV-2 3CL^{Pro} binding. We are planning to characterize the interaction between novel PROTAC molecules and the enteroviral protease, demonstrating the extension of this promising strategy for the creation of cutting-edge antiviral treatments.

References: **1.** L. Zhang, D. Lin, et al, *J. Med. Chem*. 2020, 63, 4562–4578. **2.** C. Lin, Z. Zhum, et al. *J. Mol. Biol*. 2024, 436, 168474. **3.** D. Grifagni, E. Lenci, E., et al. *ACS Med. Chem. Lett*. 2024, 15, 250–257. **4.** A. De Santis, D. Grifagni, et al., *Biomolecules* 2024, 14, 1260.

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