Design and synthesis of peptides involved in the inhibition of influenza virus infection

PhD student: Dott. Maria Carmina Scala Tutor: Chiar.mo Prof. Pietro Campiglia

The main purpose of the present research project was the identification of peptide capable of exercising potent anti-influenza activity. We identified bovine lactoferrin (bLf) as drug target, a multifunctional glycoprotein that plays an important role in innate immunity against infections, including influenza.

Previously, by protein-protein docking calculations, it was demonstrated that different loops of bLf C-lobe, corresponding to the sequences 418-429 (SKHSSLDCVLRP, **1**), 506-522 (AGDDQGLDKCVPNSKEK, **2**), 552-563 (NGESSADWAKN, **3**), 441-454 (TNGESTADWAKN, **4**), 478-500 (KANEGLTWNSLKDK, **5**), 552-563 (TGSCAFDEFFSQSCAPGADPKSR, **6**), 619-630 (GKNGKNCPDKFC, **7**), 633-638 (KSETKN, **8**) and 642-659 (NDNTECLAKLGGRPTYEE, **9**) can contribute to the binding to HA.

These peptides were synthesized and tested for their ability to inhibit hemagglutination and cell infection. The results showed that peptide **1** binds to HA and neutralizes hemagglutination and cell infection of different strains of Influenza virus with a very high potency. Therefore, we considered peptide **1** as lead compound for the development of novel compounds with improved anti-influenza activity. To identify the shortest amino acid sequence needed for the peptide activity, we designed a new small library of peptides through addition and deletion of four amino acid residues at both the N- and C-terminals of this fragment (peptide **10-17**).

Three tetrapeptides, **14** (VLRP), **15** (SLDC) and **17** (SKHS), retained the inhibitory potency of the fragment 418-429, inhibiting the Influenza virus hemagglutination and cell infection in a concentration range of femto- to picomolar. Therefore, focused on peptide **14**, we evaluated the importance of the net positive charge (Arg^{428}) for the biological activity (peptides **18-23**). We then decided to apply to peptides **15** and **17** L-Alanine scanning approach, a classical chemical approach to check the relevance of side chains of each aminoacidic residue in the interaction with the target molecule (peptides **24-31**). Finally, in order to improve the pharmacokinetic properties of biologically active tetrapeptides **15** and **17**, we synthetized N-methyl peptides (peptides **32-41**) and peptoid analogues (compound **42-51**). We demonstrated that no compound was able to inhibit HA activity in a greater extent of peptides **15** and **17**. NMR spectroscopy analysis performed on compounds **1** showed a global turn conformation for this peptide and hypothesized the preferred bioactive conformation of our tetrapeptides.

Moreover, based on conformational analysis, we tried to stabilize 3D structure of peptide **1**, S[KHSSLD]CVLRP, through cyclization of the peptide backbone. To synthesize this cyclic peptide, we used the allyl ester (OAll) as orthogonal protecting group for aspartic acid side-chain.

Surprisingly, our approach to synthesize the peptide mentioned above, failed completely. In particular, after the deprotection of aspartic acid Fmoc group by piperidine (20% v/v in DMF) for 30 min at room temperature, the formation of unexpected side-products was observed.

Therefore, we studied this phenomenon and tried to obtain the desidered sequence with different approaches. We hypothesized that the aspartimide and pyperidinil derivative formation is conformation-dependent.

Finally, we synthetized the desidered peptide, using as β-protecting group of aspartic acid and lysine, β-2-phenylisopropyl ester and methoxytrityl, respectively. LC-MS analysis confirmed the presence of the desired cyclic peptide.