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Design and synthesis of "small molecules" as antiviral and radiotracer agents

Tutor
Prof. Paolo de Caprariis

PhD Student
Grazia Sellitto

Coordinator
Prof.ssa Nunziatina De Tommasi

ABSTRACT

The present Ph.D. project was divided into different work parts, in a way that helps to understand and define the goals of this project. In particular:

I) Design, synthesis and evaluation of antiviral activity of Arbidol analogs.

II) Evaluation of mechanism of Arbidol anti-influenza action.

III) Synthesis and characterization of a P.E.T. radiotracer for tumor hypoxia: *1- (5- [18F] Fluoro-5-deoxy-α-D-arabinofuranosyl) -2-nitroimidazole* or ^{18}F-FAZA.

The initial research activity concerned the design and synthesis of indole derivatives using as a lead compound Arbidol (ARB), a compound that exerts immunomodulatory, antioxidant, antiviral and antimetastatic effects[1]. ARB is a Russian-made potent broad-spectrum antiviral with demonstrated activity against a number of DNA and RNA viruses, enveloped and non-enveloped viruses, and pH-dependent and pH-independent viruses. It exhibits antiviral activity against a number of viruses including *influenza A (H1N1, H2N2 and H3N2), B* and *C viruses, respiratory syncytial virus (RSV), adenovirus type 7, coxsackie B3 virus, parainfluenza type 5* and *rhinovirus type 14, avian coronavirus, infectious bronchitis virus* and *Marek disease virus, hepatitis B virus* and *hepatitis C virus*. The wide spectrum of ARB's activity suggests that ARB targets common critical step(s) in virus – cell interaction. Several studies have shown that the affinity of ARB for lipid membranes could account for its antiviral actions, together with a differential level of interaction with key motifs in glycoproteins of different viruses. Its antiviral activity toward viruses is due probably to a direct effect of ARB on virus-cell membrane interactions where ARB intercalates into membranes and induces membrane alterations. This leads to excessive stabilization of cell membranes, which become resistant to virus fusion and in some cases (HCV) to virus replication[2-4]. The known biological properties of Arbidol led us to focus on its derivatives as potential antiviral agents. In order to maintain antiviral activity we preserved

the groups responsible of Arbidol interaction with membranes (indole ring, S-phenyl group, ester group and amino group) eliminating those that were not considered pharmacophores (*hydroxy* and *bromo* groups at the 5- and 6-positions of the indole ring). Moreover we introduced different substituents at the 2- and 5-position of the indole ring to investigate the influence of these variations on antiviral activity. The synthesis of Arbidol derivatives has been established through the validation of two synthetic schemes. Then, to evaluate anti-HCV and anti-HSV activity of synthesized compounds, biological assays were made. ARB derivatives showed antiviral activity comparable and, in some cases, even better than those of lead Arbidol, on both systems. In particular, it was shown that synthesized compounds are fusion inhibitors on both viruses and also non-selective inhibitors of HCV replication.

The second part of the present research project concerned the study of mechanism of Arbidol anti-influenza action. There are experimental evidences that ARB does not affect viral neuraminidase (NA, a surface protein of influenza virus) activity. It affects early post-adsorption stages of virus replication with possible involvement of the second surface viral protein, the haemagglutinin (HA). Arbidol could act increasing influenza virus HA stability and preventing low pH induced HA transition to its fusogenic state, thus blocking infection at the viral fusion stage[5]. To support this hypothesis, the interaction of Arbidol with the N-terminal hydrophobic fusion domain of haemagglutinin (HA) was evaluated. Therefore, the peptide host-guest (P20H6) was synthesized using techniques of Solid Phase Peptide Synthesis (SPPS) and *Circular dichroism* studies were made[6]. From these studies we demonstrated that Arbidol interacts with the haemagglutinin fusion domain at pH 5 and 7, through changes in the secondary structure of peptide.

At the end of present Ph.D. project, I spent six months at the University of Aberdeen where I worked to the synthesis of a PET (Positron Emission

Tomography) radiotracer for tumour hypoxia: the *[^{18}F]1-α-D-(5-Fluoro-5-deoxyarabinofuranosyl)-2-nitroimidazole*, known as ^{18}F-FAZA.

^{18}F-FAZA is currently the gold standard for PET Imaging of diseases characterized by hypoxia (solid tumours, ischemia, stroke)[7], but it is not routinely used and synthesized in Scotland. The work done is an important starting point for the introduction of ^{18}F-FAZA in Scotland with the aim of using it in clinical imaging and research. In particular, following a detailed bibliography research on this compound and its synthesis, that is not fully reported, and a subsequent optimization of the synthetic scheme used, the ^{18}F-FAZA precursor was obtained: *1-α-D-[5'-O-Toluenesulfonyl-2',3'-Di-Oacetylarabinofuranosyl]-2-nitroimidazole* (DAcTs-AZA).

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