SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Organic Chemistry

has the honor to invite you to the public defense of the PhD thesis of

Robin Van Den Hauwe

to obtain the degree of Doctor of Sciences

Title of the PhD thesis:

Design, Synthesis and Biological Evaluation of Apelin 13 Mimetics

Promotor:
Prof. dr. Steven Ballet (VUB)

The defense will take place on
Tuesday, June 28, 2022 at 16h in auditorium K.2.56

Members of the jury

Prof. dr. Ann van Eeckhaut (VUB, chair)

Prof. dr. Ulrich Hennecke (VUB, secretary)

Prof. dr. em. Dirk Tourwé (VUB)

Prof. dr. Patrick Vanderheyden (VUB)

Dr. Florine Cavelier (University of Montpellier)

Prof. dr. Pieter Van Der Veken (University of Antwerp)

Curriculum vitae

Robin obtained his MSc. Degree in Organic Chemistry with great distinction from the Vrije Universiteit Brussel in 2017. Immediately after, he started his PhD as a teaching assistant in 2018 in the Research Group of Organic Chemistry, under the promotorship of Prof. Dr. Steven Ballet. During his PhD, Robin was awarded a travel grant from the Fonds Wetenschappelijk Onderzoek and performed research for three months in the renowned lab of Prof. Dr. Eric Marsault and Prof. Dr. Philippe Sarret in Sherbrooke, Canada. Robin co-authored four scientific papers, published in peer-reviewed international journals, amongst which two as first author. Besides teaching activities, he also guided and supervised two master, two bachelor thesis students and one internship student.

Abstract of the PhD research

The apelin receptor (APJ) is a member of the GPCR superfamily. Its cognate ligand is apelin and together with the receptor it forms the so-called “apelinergic system”, a system which is widely distributed in the central nervous system and peripheral tissues, and which emerged as an important regulator of cardiovascular functions *via* its actions on the heart, vasculature, and kidneys. Even though patent activity around the apelinergic system has gone up over the past years, clinical validation remains problematic and no pharmaceutical targeting the apelinergic system is currently on the market.

As a basis of this thesis, it was assumed that modifications of the apelin peptide *via* application of χ-space constraints and/or modified amino acids, would give an additional handle over receptor signaling, stability, affinity etc. Herein, several strategies are presented by which the *C*-terminus of [Pyr^{1}]-apelin-13 has been chemically modified, *in casu* by the use of constrained dipeptides and modified amino acids. At first, the focus was placed on the synthesis of local constraints at both the Pro^{12} and the Phe^{13} positions of apelin-13. These ligands proved to possess subnanomolar binding affinity for the APJ receptor, as well as significantly increased *in vivo* stability and signaling potency. The *in vivo* results of our best first generation ligands (Aia^{12}-Phe^{13} and 1-Nal^{12}-Db$_z$G^{13}) demonstrated a hypotensive action. Next, the constrained library was expanded by ligands in which the side chains of neighboring amino acids were linked together. These ligands demonstrated high binding affinity, good signaling potency and improved plasma stability. As more rigid peptide analogues, these ligands are expected to provide new insights in the importance of side chain positioning upon binding.

Following the synthesis of local- and dipeptide constraints we aimed at the further exploration of the *C*-terminal pocket of apelin-13 by the incorporation of N_α-alkylated dipeptides. In a study containing around 20 ligands with different alkyl substituents, ranging in size from methyl to 4-benzyloxy-benzyl, improved binding affinities were obtained, as compared to the native ligand. As for the plasma half-life, this was now increased beyond 10 hours. Interestingly, a moderate bias favoring $G\alpha_{i1}$ activation over β-arrestin-2 recruitment was recorded for some analogues, which is to our knowledge the first linear apelin-13 analog without modification of the Phe^{13} side chain displaying a biased profile disfavoring the β-arrestin-2 pathway. *In vivo* evaluation showed a reduced capability of lowering blood pressure, as compared to the native ligand. In a later stage, the addition of amino acids bearing a positive charge at the *C*-terminus of apelin-13 was evaluated. Some analogs displayed unique biased agonism behavior favoring $G\alpha_{i1}$ over β-arrestin2 recruitment with β-arr2 E_{max} values below 50%. Incorporation of dipeptides in the apelin-13 *C*-terminus, having an alkylated N_α and bearing a positive charge, further improved binding affinity, stability and signaling potency. In conclusion, the incorporation of various unnatural amino acids and dipeptides at the *C*-terminus of the apelin-13 peptide has led to the synthesis of powerful apelin peptide mimetics which show high affinity, high plasma stability, biased agonism disfavoring β-arrestin2 recruitment and *in vivo* mean arterial blood pressure effects.