SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Organic Chemistry

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

Mathias Elsocht

to obtain the degree of Doctor of Sciences

Title of the PhD thesis:

Towards an improved treatment of Non-Small Cell Lung Cancer: Hit-to-Lead optimization of Spautin-1

Promotor:
Prof. dr. Steven Ballet (VUB)

The defense will take place on
Friday, April 22, 2022 at 17h00 in auditorium D.0.08 at the Campus Humanities, Sciences and Engineering of the Vrije Universiteit Brussel, Pleinlaan 2 - 1050 Elsene.

Members of the jury

Prof. dr. Ilse Rooman (VUB, chair)
Prof. dr. Ulrich Hennecke (VUB, secretary)
Prof. em. dr. Jacques De Greve (VUB)
Prof. dr. Charlotte Martin (VUB)
Dr. Frédéric Bihel (Université de Strasbourg)
Prof. dr. Patrick Eyers (University of Liverpool)

Curriculum vitae

Mathias Elsocht (°1994) graduated in 2017 as a Master of Science in Chemistry – Molecular and Macromolecular Design – from the Vrije Universiteit Brussel (VUB). Thereafter, he started his PhD within the Research Group of Organic Chemistry (ORGC) led by Prof. Dr. Steven Ballet and Prof. Dr. Ulrich Hennecke. The PhD research was funded by the Interdisciplinary Research Programme (IRP) of the VUB and the Wetenschappelijk Fonds Willy Gepts (WFWG).

His research has led to three first-author publications in peer-reviewed international journals (five scientific papers in total) and the work was presented at several (inter)national conferences. Besides teaching laboratory classes, he supervised one master and five bachelor thesis students, as well as one internship student.

Abstract of the PhD research

Worldwide, lung cancers account for the highest number of cancer-related deaths. Non-small cell lung cancer (NSCLC) represents 85% of all cases. Due to the high incidence and low survival rates associated with NSCLC, improvement of current treatments would have an immediate clinical impact. It is known that activating mutations in the Epidermal Growth Factor Receptor (EGFR), a tyrosine kinase receptor, leads to NSCLC. To date, three generations of EGFR tyrosine kinase inhibitors (TKIs) are clinically available and these typically show strong initial responses and improved quality of life in EGFR-mutant NSCLC patients. Nonetheless, EGFR TKI-based therapies are not fully curative and patients generally develop resistance within 9-12 months of treatment. Therefore, a combination therapy approach could obviate the occurrence of secondary resistance mechanisms by targeting pathways that cause innate insensitivity, thus improving the efficacy of EGFR-targeted therapies. Prior to this work, the Laboratory for Medical and Molecular Oncology at VUB showed that use of Spautin-1, a claimed inhibitor of ubiquitin specific protease 13 (USP13; a deubiquitinating enzyme implicated in the innate insensitivity system), in combination with an EGFR TKI (Afatinib or Osimertinib), strongly decreased the survival of NSCLC cells.

In light of the above, the aim of this thesis was to identify novel small molecule inhibitors which inactivate EGFR, by triggering its degradation, to be used alone or in combination with established EGFR kinase inhibitors (such as Afatinib). The search of promising inhibitors was started by the exploration of the chemical space around the quinazoline core of Spautin-1 and evaluation of the importance of the quinazoline core by means of a '*N*-screening' (i.e. a scaffold hopping approach). After evaluation of the newly synthesized compounds in an EGFR-mutant NSCLC cells viability screening, a first set of structure-activity relationships (SAR) was derived and revealed that *N*-(2-(substituted-phenyl)ethyl)-6-fluoro-4-quinazolinamines are more potent than Spautin-1 in the applied screening. Additionally, the quinoline and quinazoline scaffolds proved most promising, and the best analogues displayed submicromolar activities.

As an alternative to single pharmacophore utilization, to further improve the efficacy towards EGFR-mutant NSCLC cells, a merged and linked designed multiple ligand (DML) was developed, based on the abovementioned SAR and structural communalities with EGFR-TKIs. The merged DML was more potent than treatment with Spautin-1 analogues alone and, at a concentration of 50 nM, showed a reduction in phosphorylated EGFR. However, as compared to Afatinib monotherapy or the 'Afatinib plus Spautin-1 analogue' combination, it showed a lower efficacy. In contrast, the linked DML seemed completely inactive, indicating that the used design suffered from unfavourable sterics with the intended macromolecular targets.

As the most active '1st generation' analogues only possessed moderate half-maximal inhibitory concentrations (IC_{50}-values) around 300 nM, and because the combination of such analogues with Afatinib did not significantly improve the overall therapeutic efficacy, further exploration of the amine substituent led to the discovery of '2nd generation' Spautin-1 analogues with much lower 1-digit nanomolar activity towards EGFR-mutant NSCLC cells. These compounds displayed not only excellent potencies as a monotherapy, but also open pathways to an additive effect with Afatinib/Osimertinib, to potentially obviate or delay the occurrence of resistance.