Spring Meeting 2022

The Dutch Medicinal Chemistry & Chemical Biology landscape in the wake of the Corona pandemic

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Book of abstracts

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Medicinal Chemistry & Chemical Biology
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Discovery of quinazoline ligands for ACKR3 (CXCR7) utilizing a fragment-based approach

Rick Riemens, Reggie Bosma, Marta Arimont, Anna Konopka, Joep Wals, Maria Waldhoer, Mirjam Zimmermann, Susanne Roth, Nadine Dobberstein, Henry Vischer, Barbara Zarzycka, Maikel Wijtmans, Aurélien Rizk, Rob Leurs, and Iwan de Esch.

The atypical chemokine receptor 3 (ACKR3, also known as CXCR7) is a class A G protein-coupled receptor that is activated by the chemokines CXCL11 and CXCL12. Overexpression of ACKR3 in various cancers has been linked to poor disease progression and mortality. Moreover, small-molecule ligands targeting the ACKR3 have been found to be efficacious *in vivo* in the treatment of multiple inflammatory diseases as well as in several cancer models. Recent studies also show that ACKR3 scavenges a wide variety of opioid peptides, indicating additional clinical potential of ACKR3 ligands in, e.g., mood-disorders and addiction. We developed an assay that allows for a sensitive fragment library screen. Heteroaromatic fragment hits were optimized in a fragment growing campaign resulting in a series of high-affinity ligands that have been tested for their functionality resulting in the identification of VUF25449 as most potent ACKR3 modulator.

β-Sheet mimetics that Target the Transcriptional Coactivator β-Catenin

Alejandro Yeste Vazquez, VU Amsterdam

Protein complexes are defined by the three-dimensional structure of participating binding partners. Knowledge about these structures can facilitate the design of peptidomimetics which have been applied for example, as inhibitors of protein–protein interactions (PPIs). Even though β-sheets participate widely in PPIs, they have only rarely served as the basis for peptidomimetic PPI inhibitors, in particular when addressing intracellular targets. Here, we present the structure-based design of β-sheet mimetics targeting the intracellular protein β-catenin, a central component of the Wnt signaling pathway. Based on a protein binding partner of β-catenin, a macrocyclic peptide was designed and its crystal structure in complex with β-catenin obtained. The presented design strategy can support the development of inhibitors for other β-sheet-mediate.

Design and synthesis of photocaged antihistamines for histamine H_1R photopharmacology

Yang Zheng*, Zhiyong Wang, Ivana Josimovic, Tiffany K van der Meer, Andrea van de Stolpe, Hans Custers, Henry F. Vischer, Maikel Wijtmans and Rob Leurs

Amsterdam Institute of Molecular and Life Science (AIMMS), Division of Medicinal Chemistry, VU Amsterdam, The Netherlands.

Email: r.leurs@vu.nl

1. Introduction

- **G protein-coupled receptors** count for more than 30% of the FDA approved drugs.[1]
- The histamine receptor **H_1R** plays a role in important physiological processes, i.e. sleep cycles, food intake and allergic reactions, including itch.[2]
- Histamine H_1R antagonists, like **desloratadine**, **loratadine** and **olopatadine** have been successfully developed and used clinically.[3]
- **Photocaged ligands** are used as **pharmacological tool compounds** to yield **temporal** and **spatial** control of receptor signaling.[4,5]

2. Aim

Our aim is to develop **functionally photocaged hH_1R ligands** by combination of clinical H_1R antagonists with photo-responsive protecting groups based on their known Structure-Activity Relationships. These photocaged ligands can therefore be used to spatio-temporally modulate H_1R signalling in biological systems.

3. Design

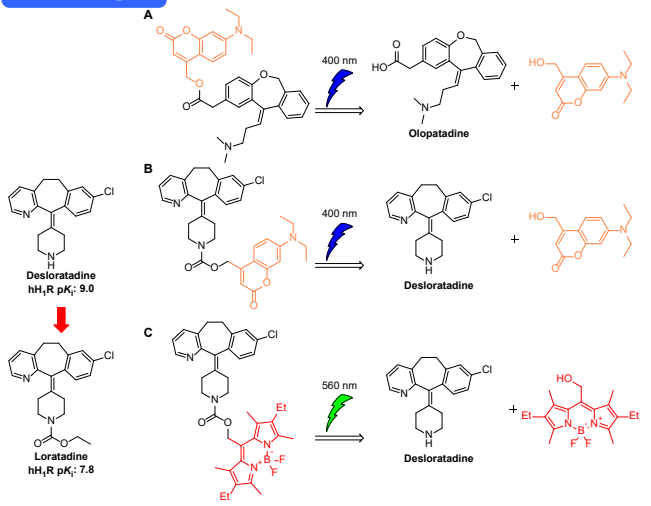
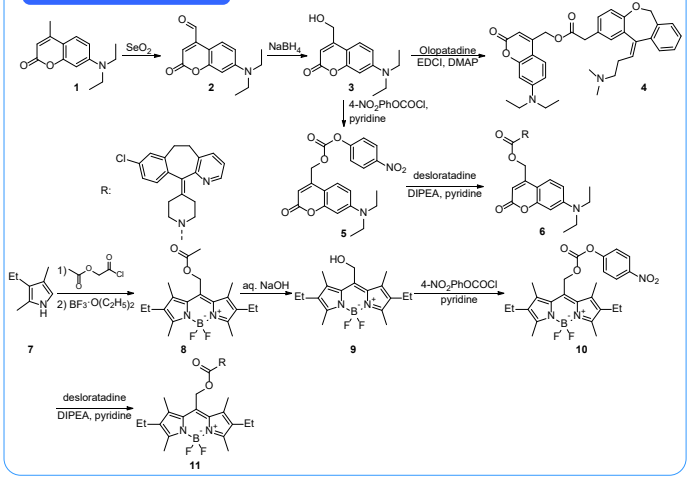


Figure 1. Proposed target compounds, photouncaging mechanism and postulated byproducts. (A) Coumarin-caged H_1R antagonist olopatadine; (B) Coumarin-caged H_1R antagonist desloratadine; (C) BODIPY-caged H_1R antagonist desloratadine.

4. Synthesis



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FLAG-ERA

5. hH_1R affinities

Compound No.	$pK_i \pm SD^a$	Minimum concentration of aggregation[b]
Olopatadine	8.3 ± 0.2	> $10^{-4.0}$ M
Desloratadine	9.0 ± 0.1	> $10^{-4.5}$ M
3	< 5.0[c]	> $10^{-5.5}$ M
4	8.6 ± 0.1	> $10^{-6.0}$ M
6	6.6 ± 0.1	> $10^{-6.5}$ M
11	< 6.0[d]	> $10^{-5.0}$ M

[a]Determined by competitive [^{3}H]mepyramine radioligand displacement on human H_1R (n=3). [b]The highest concentration at which no scattering was observed in nephelometry analysis. [c]Too low affinity for accurate determination. [d]The affinity could not be accurately determined due to precipitation at highest concentrations.

6. Photo uncaging

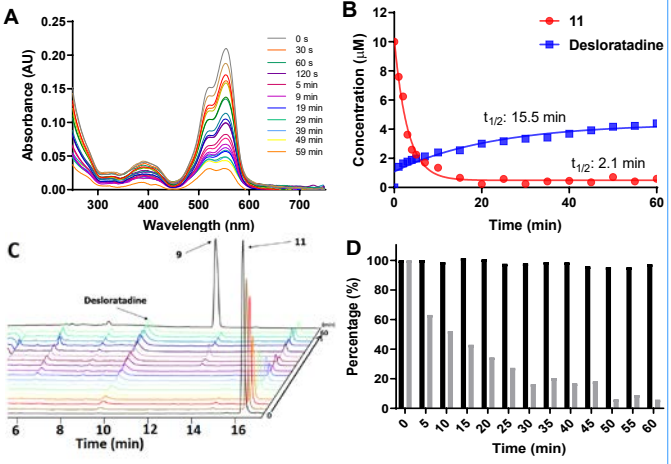
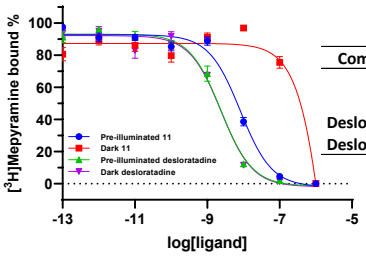


Figure 2. Photo-uncaging followed by UV-Vis and LC analysis. (A) **11** (10 µM) under 560 nm in 50 mM phosphate buffer (pH 7.4)/1% DMSO at room temperature; (B/C) **11** (10 µM) under green LED light in PBS at 37 °C; (D) Chemical stability of **11** under ambient light.

7. Affinity shift



Compound	Condition	$pK_i \pm SD^c$
11	Dark[a]	< 6.0
11	Pre-illumination[b]	8.5 ± 0.1
Desloratadine	Dark[a]	9.0 ± 0.1
Desloratadine	Pre-illumination[b]	9.0 ± 0.1

Figure 3. Pharmacological evaluation of desloratadine and caged desloratadine before and after illumination. [a]10 µM compound with 1% DMSO in phosphate buffer in the dark at room temperature for 60 min prior to analysis. [b]10 µM compound with 1% DMSO in phosphate buffer under green LED lamp at 37 °C for 60 min prior to analysis; [c]Determined by competitive [^{3}H]mepyramine radio ligand displacement on human H_1R (n=3).

8. Conclusions and future perspectives

- **Three photocaged antihistamine compounds were synthesised. Two of them 6 (VUF25245) and 11 (VUF25549) exhibited more than 2 log units lower affinity than their parent compounds.**
- **Release of desloratadine (44%) from the more red-shifted BODIPY-caged desloratadine (11) was studied quantitatively with LC analysis.**
- **Affinity assay results further confirmed the release of desloratadine.**
- ***In vivo* modulation of H_1R signaling in a mouse model will be performed.**

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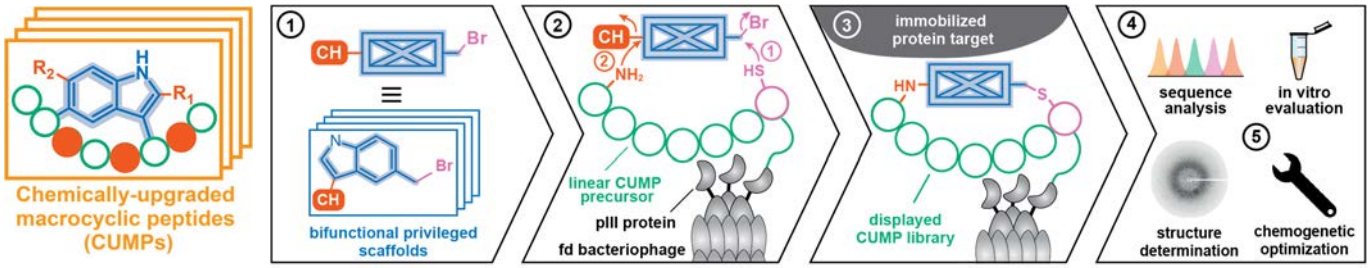
Selection of chemically-upgraded macrocyclic peptides by phage display

Titia Rixt Oppewal, Ivar Daniel Jansen, Johan Hekelaar and Clemens Mayer

Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 4, Groningen, The Netherlands, t.r.oppewal@rug.nl, c.mayer@rug.nl

Macrocyclic peptides (MPs) show great potential for the development of pharmaceuticals and chemical probes. Aided by *in vitro* selection strategies that enable the efficient culling of vast libraries, the number of approved MP drugs has steadily increased over the past decade.[1] However, man-made MPs are typically ill-suited for therapeutic intervention, as they cannot undergo the same *chemogenetic* optimization mechanisms that are key for ameliorating the properties of naturally-occurring MPs.[2] In nature, organisms take advantage of the evolutionary algorithm to fine-tune not only the amino acid sequence, but also posttranslational processes such as the introduction of non-peptidic moieties for peptide macrocyclization. As a result, mimicking such a chemogenetic optimization in the laboratory is desirable in order to improve the effectiveness (and pharmacological properties) of man-made MPs.[3]



OUR WORKFLOW: (**1**) synthesis of privileged scaffolds featuring distinct cyclization handles; (**2**) evaluate CUMP formation with synthetic peptides and linear precursors displayed on the phage coat; (**3-4**) select CUMP binders against drug targets; (**5**) characterization and chemogenetic optimization of CUMPs.

To this end, we present an efficient two-step cyclization strategy to access chemically-upgraded macrocyclic peptides (CUMPs) via the programmed modification of a unique cysteine residue and an N-terminal amine. We demonstrated that this approach yields MPs featuring asymmetric cyclization units from both synthetic peptides and when linear precursors were appended onto a phage-coat protein. Finally, we showcased that our cyclization strategy is compatible with phage-display protocols and enables the selection of CUMP-binders against a model target protein from a naïve library.[4] We anticipate that the future selection of CUMPs by phage display for clinically relevant targets will enable a comprehensive exploration of a previously-unexplored chemical space and provide unique opportunities for drug discovery.

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Tom Dekker, Vrije Universiteit Amsterdam

Fragment-based drug discovery (FBDD) has grown into a well-established drug discovery approach, as is evident from the six FDA-approved drugs and various clinical candidates it has produced up to January 2022. Compared to high-throughput screening, smaller molecules are screened which allows for the use of smaller libraries to sample the same degree of chemical space. Like most screening libraries, FBDD libraries are dominated by flat molecules and this has resulted in a growing interest in 3D fragments. We are developing new chemistry to generate novel 3D fragments libraries and have constructed an automated cheminformatics workflow for efficient 3D library design. This workflow uses the open source platform KNIME and selects novel and diverse fragments with suitable physicochemical properties and 3D character (using metrics such as PMI) from virtually generated libraries. The workflow can be used to design large screening libraries but is also applicable for diverse reagent selection in smaller and more focused chemistry programs.

R. Bosma[1]*, R. Riemens[1], M. Szpakowska[2], M. Zimmermann[3], M. Arimont[1], S. Mobach[1], N. Dobberstein[3], M. Wijtmans[1], H.F. Vischer[1], B. Zarzycka[1], I.J.P. de Esch[1], A. Chevigné[2] and R. Leurs[1]

1. Division of Medicinal Chemistry, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), Vrije Universiteit Amsterdam, The Netherlands.

2. Department of Infection and Immunity, Luxembourg Institute of Health (LIH), Luxembourg

3. InterAx Biotech AG, Villigen, Switzerland

***r.bosma@vu.nl**

Inactivation of the ACKR3 by a small-molecule inverse agonist

The atypical chemokine receptor type 3 (ACKR3) is frequently explored as a drug target for the treatment of various diseases, like cancer and cardiovascular disease. Until recently, all characterized small-molecule-ligands that bind the ACKR3 were agonists and it was shown that the ACKR3 had a high propensity for activation when compared to other chemokine receptors.[1,2,3]

We identified an ACKR3 antagonist in the patent literature, which is an analog of the clinical candidate ACT-1004-1239 of Idorsia.[1] The antagonist (VUF16840) was synthesized and its effect on the ACKR3 was characterized. VUF16840 is confirmed to antagonize chemokine induced receptor activation. It is additionally shown that this ligand stabilizes the inactive conformation of the ACKR3, as is evident from a dose-dependent inhibition of constitutive receptor signalling. Despite the distinct intrinsic activity of VUF16840 at the ACKR3, site-directed mutagenesis studies show a partial overlap in receptor binding interactions between VUF16840 and a small-molecule agonist. It is finally shown that VUF16840 has selectivity for the ACKR3 over all other human chemokine receptors. Some off-target activity was observed for the CCR3, which is the only other chemokine receptor that is modulated by VUF16840 at a concentration of 1 μM.

We therefore suggest that VUF16840 constitutes a valuable tool compound for probing the utility of ACKR3 as a drug-target. Moreover, the pharmacological characterization of ACKR3 binding site might facilitate further design of new agonists and antagonists.

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A Photoswitchable Ligand for the Atypical Chemokine Receptor 3

Sophie Bérenger, Justyna Adamska, Henry Vischer, Iwan de Esch, Maikel Wijtmans, Rob Leurs

Division of Medicinal Chemistry, Amsterdam Institute of Molecular and Life Sciences (AIMMS), Vrije Universiteit Amsterdam, The Netherlands; E-mail: m.wijtmans@vu.nl; r.leurs@vu.nl

Keywords: photoswitching, GPCR, ACKR3, photopharmacology, azobenzene

Photopharmacology allows the modulation of protein activity with light-responsive molecules. Photoswitchable tools compounds such as azobenzene derivatives can control the protein signalling in a spatial and temporal manner upon irradiation with light.[1] This strategy can be used to investigate the role of G protein-coupled receptors (GPCRs) in biological settings. The atypical chemokine receptor 3 (ACKR3) GPCR relies on β-arrestin recruitment. Targeting this receptor impacts the scavenging of endogenous CXCL12. Due to this signaling specificity and its implication on various immune diseases and cancers, this receptor is an interesting target for photoswitchable ligands.[2] In this work, we present the design and synthesis of a library of 12 photoswitchable compounds inspired by a patented ACKR3 agonist.[3] Structure-photochemistry, structure-affinity relationships and pharmacological properties will be discussed. The results of this analysis yielded a compound (VUF25471) with 10-fold potency shift between the dark and irradiated states. VUF25471 is shown to be a promising tool for ACKR3 photopharmacology.

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Photoswitchable small-molecule antagonists to optically modulate CXC chemokine receptor 3 activation

Justyna Adamska, Niels Hauwert, Tamara Mocking, Xavier Gómez-Santacana, Sabrina M. de Munnik, Sara Lopes-Van den Broek, Marta Arimont, Henry Vischer, Iwan de Esch, Maikel Wijtmans, Rob Leurs

Division of Medicinal Chemistry, Amsterdam Institute of Molecular and Life Sciences (AIMMS), Vrije Universiteit Amsterdam, The Netherlands; E-mail: m.wijtmans@vu.nl; r.leurs@vu.nl

CXC chemokine receptor 3 (CXCR3) belongs to the superfamily of G protein-coupled receptors and responds to chemotactic ligands, referred as chemokines. This receptor predominantly signals via $G\alpha_i$ protein family after stimulation with endogenous ligands CXCL9, CXCL10, CXCL11. CXCR3 plays a crucial role in T- cell function and is associated with inflammatory diseases.[1] In this study we developed functionally light-responsive ligands to optically modulate CXCR3 activity. Photoswitchable compounds were designed and synthesized based on the CXCR3 antagonist VUF11211.[2] Photoswitchable ligands VUF16338 and VUF16600 have been identified as a key compounds from a library of eleven compounds. It has been established that VUF16338 inhibits CXCL11-induced G protein activation by CXCR3 with 10-fold potency shift between dark and irradiated states. Moreover, a ten-fold CXCR3 affinity shift between dark and irradiated states of the fast-relaxing photoswitchable ligand VUF16600 was measured under continuous illumination using a LED-array plate. VUF16338 and VUF16600 are new complementary phototools to study the photopharmacology of the CXCR3 receptor.

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Jelle van den Bor, VU Amsterdam

Titel: NanoB^2: Combining nanobodies with nanoBRET technology

The single variable domains (VHH) from the heavy chain-only antibodies of camelids, often referred to as nanobodies, possess full antigen binding capacity and can serve as important research tools, diagnostics and therapeutics. Common assays to quantify the binding properties of nanobodies are ELISA BLI or surface plasmon resonance (SPR). SPR and BLI provide kinetic data, yet it required specialized equipment and can be challenging with membrane-bound target proteins.

In this study, using three G protein-coupled receptors (chemokine receptors CXCR4, ACKR3 & US28), we developed a new approach for assessing the target binding capacity of nanobodies against three G protein-coupled receptors (chemokine receptors CXCR4, ACKR3 & US28) using Bioluminescence Resonance Energy Transfer (BRET) technology.

We show that fluorescently labelled nanobodies in close proximity to its NanoLuciferase (NanoLuc)-tagged target GPCR in cells and membranes induce an increase in BRET, thereby directly visualizing the binding to the target protein. Furthermore, we show that this technique allows kinetic evaluation of binding of both fluorescently labelled nanobodies and unlabeled ligands in a high throughput manner. Altogether, our nanobody-BRET (NanoB^2) set up appears a promising alternative approach to SPR to assess ligand-receptor interactions in membrane context.

Selective targeting of ligand-dependent and -independent signaling by GPCR conformation-specific anti-US28 intrabodies

Nick Bergkamp, VU Amsterdam

While various G protein-coupled receptors (GPCRs), including the human cytomegalovirus-encoded chemokine receptor US28, display constitutive, ligand-independent activity, it remains to be established whether ligand-dependent and -independent active receptor conformations differ and can be selectively modulated. Previously, the agonist-bound conformation of US28 was stabilized and its structure was solved using the anti-US28 nanobody Nb7. Here we report the recognition of the constitutively active, apo-conformation of US28 by another nanobody VUN103. While the Nb7 intrabody selectively inhibits ligand-induced signaling, the VUN103 intrabody blocks constitutive signaling, indicating the existence of distinct US28 conformational states. By displacing $G\alpha_q$ protein, VUN103 prevents US28 signaling and reduces tumor spheroid growth. Overall, nanobodies specific for distinct GPCR conformational states, i.e. apo- and agonist-bound, can selectively target and discern functional consequences of ligand-dependent versus independent signaling.

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