Seek and Destroy
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Chapter 1
Perspective and Outline
1.1 Aim of the research described in this thesis

Medical imaging techniques such as Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET) and Optical Imaging (OI) form the cornerstone of cancer diagnosis and following the progress and staging of the disease during the treatment plan. Furthermore, the detailed spatial information derived from the medical imaging guides radiotherapy and supports decision making in surgery where to cut or not to cut. In contrast, for chemotherapeutic agents this spatial information about the disease cannot be used for local treatment, since after oral or intravenous administration the active drug is spread throughout the body by bloodstream and diffusion.

Most of the chemotherapeutic agents target biomolecules and cellular processes that are not exclusively found in tumor cells. For example, proteins are targeted that are in overexpression in tumor cells but are still expressed at low levels in healthy cells. This means that the drug will affect both tumor cells and healthy cells, which results in dose-limiting side effects. To overcome this limitation, external control over the activity of a drug is needed. By this, a drug can ultimately be administered in an inactive form and can be locally activated by an external stimulus in the proximity of tumor cells. An emerging external stimulus is light, since it is tolerated in biological systems and the intensity, wavelength, duration and location of irradiation can easily be controlled. This makes light a powerful tool for the control over the activity of small molecule drugs.

The field of photopharmacology develops bio-active molecules of which the activity can be controlled with light. By introducing a molecular photoswitch into the structure of a drug, a bio-active molecule is obtained that has two photo-isomers with both different biological properties. Light of a specific wavelength can be used to switch from one photo-isomer to the other and thereby changing the biological activity in a reversible manner, with spatial and temporal precision. Currently many photoswitchable compounds have been developed for a wide variety of human and pathogenic targets. However, most photoswitchable drugs still rely on UV-light photo-isomerization, which has low tissue penetration and high phototoxicity. Furthermore, the difference in the behavior of both photo-isomers of a drug in complex biological systems is not fully understood. For these reasons, the photopharmacology approach is not yet ready for clinical applications.

The work described in this thesis aims to develop tools and models to fundamentally understand photopharmacology and make a next step in the road to towards clinical applications. First, tools are needed for a better understanding of the molecular origin of the differences in biological activity between photo-isomers in order to improve future designs. Secondly, model compounds are needed to investigate the behavior of both photo-isomers in complex biological systems, with a focus on off-target activity profiles, pharmacodynamics and pharmacokinetics in vivo. Thirdly, expanding the repertoire of visible light photoswitches that operate under physiological conditions is needed to replace the current UV light operated photoswitches.
1.2 Thesis outline

Chapter 2 introduces photopharmacology as a method to control the activity of proteins with light. Besides its potential clinical applications of acquiring spatial and temporal control over the activity of drugs, there are many opportunities for bio-active molecules with light-controlled activity for mechanistic studies in bio-medical research. None of the available chemical and genetic tools for modulating protein activity give the opportunity of spatial and temporal control over protein activity in a reversible manner.\textsuperscript{12}

Chapter 3 shows the development of a glutamate transporter inhibitor with light-controlled activity. Based on a known inhibitor, a library of inhibitors containing an azobenzene photoswitch was synthesized and their biological activity was determined on proteoliposomes containing glutamate transporter Glt\textsubscript{Tk}. This transporter is thermally stable and serves as a model for structural and mechanistic studies.\textsuperscript{13,14} A 3.6-fold difference between photo-isomers was observed for the best inhibitor,\textsuperscript{15} paving ways for structurally understanding the differences in biological activity through X-ray crystallography.

Chapter 4 describes the development of a BRAF\textsuperscript{V600E} kinase inhibitor with light-controlled activity. Inspired by FDA-approved BRAF\textsuperscript{V600E} inhibitor Vemurafenib, eight inhibitors containing an azobenzene photoswitch were synthesized and their biological activity was tested on isolated BRAF\textsuperscript{V600E} using a western-blot based assay. An approximately 10-fold difference between photo-isomers was observed in the enzyme assay, however this difference could not be translated to differences in cytotoxicity in HeLa cells. Together with off-target screening several challenges for the development of photoswitchable kinase inhibitors were identified.\textsuperscript{16}

Chapter 5 reports the modification an earlier published HDAC2 inhibitor with light-controlled activity, with the aim of introducing a fluorine atom into the structure of the drug, while maintaining its biological and photochemical properties. Ultimately, the fluorine could be replaced by \textsuperscript{18}F, which enables to follow the HDAC2 inhibitor in either the \textit{trans} or \textit{cis} photo-isomer in a model organism using Position Emission Tomography (PET), which can be employed to acquire deeper understanding of the behavior of both photo-isomers \textit{in vivo} in a rodent model.

Chapter 6 describes the design, synthesis and photochemical evaluation of a new molecular photoswitch called Iminothioindoxyl (ITI).\textsuperscript{18} The ITI photoswitch is a fusion of photochromic dyes azobenzene and thioindoxyl. Azobenzene photoswitches have good band separation and operate in aqueous conditions, however require UV light for photo-isomerization. Thioindigo absorbs in the visible light region, however is poorly soluble in aqueous conditions and has poor band separation. The new fusion Iminothioindoxyl photoswitch has the best of both parents: ITIs are fully visible light switches that can operate in aqueous conditions and show a spectacular band separation of over 100 nm between both photo-isomers.

Chapter 7 explains how substituent patterns for the Iminothioindoxyl (ITI) photoswitch affect the photochemical properties. The unsubstituted ITI has a half-life at room
temperature of approximately 10 to 20 ms, which is too short to get a build-up of the \( E \) isomer and limits the applicability of ITI in photopharmacology. Ten new ITI photoswitches have been designed, with the aim of determining which positions are suitable to tune the thermal half-life of the \( E \) isomer. Substituents with different electronic properties on the thioindoxyl fragment only weakly influence the half-life. In contrast, the ortho positions on the imine fragment are sensitive, where electron withdrawing fluorine substituents decrease the rate of thermal re-isomerization. Altogether, the library of ITIs presented in this chapter paves the way for Iminothioindoxyl based light-controlled drugs.

Chapter 8 demonstrates the tuning of the photochemical properties of the Iminothioindoxyl (ITI) photoswitch by protonation. The presence of a nitrogen and its free electron pair in the isomerizable double bond results in short half-lifes of the \( E \) isomer, yet also provides the opportunity for protonation. By this approach is aimed to capture the electron pair of the \( E \) isomer and slow down the \( E \) to \( Z \) thermal re-isomerization process. Protonation of electron rich ITIs results in a red-light shift of the \( Z \) isomer and increased absorption, while the large band separation is maintained.

1.3 References


