Small changes for long term impact : optimization of structure kinetic properties : a case of CCR2 antagonists

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CHAPTER 1

GENERAL INTRODUCTION

ABOUT THIS THESIS

This thesis focuses on a new approach in drug discovery, the so-called drug-target residence time. Next to more traditional drug discovery efforts, which are based on structure-affinity relationships, this thesis describes the use of an additional parameter – the structure-kinetic relationships. Knowledge of this additional parameter at the early stages of drug discovery may help the pharmaceutical industry to generate better drug candidates. Additionally, it could save big expenses in the later stages of drug discovery by minimizing the attrition rate of drug candidates due to efficacy problems. In more detail, this thesis focuses on the synthesis of new high affinity and long residence time small molecule antagonists for the chemokine receptor CCR2 - a potential target for the treatment of various inflammatory diseases.

THE BEGINNINGS OF DRUG DISCOVERY

Already in the early days of human civilization men used different plants as healing agents. These early drugs were most probably discovered through trial and error experimentation by observing the reactions of human and animal as a result of consumption of such products. Folk medicines derived from these “clinical trials” were the only available treatments until recent times. Scientific techniques in drug discovery started to emerge only in the late 1800s. Oswald Schmiedeberg was one of the first scientists to describe the correlation between the chemical structure of substances and their effectiveness.[1] It was an early attempt to describe structure-activity relationships (SAR). At the beginning of the 1900s chemical methods were developed to produce synthetic drugs, marking the beginnings of pharmaceutical industry.

DRUG DISCOVERY AND DEVELOPMENT

With the increasing knowledge and understanding of diseases, scientists started to hypothesize that modulation of a specific biological target may have therapeutic value in the treatment of a disease. This was the beginning of a paradigm shift in drug discovery from

"trial and error experiments" to "rational drug design". In rational drug design, the biological target must fulfill two criteria. First, modulation of a given biological target must show evidence of therapeutic value. Second, the target must be "druggable", i.e. the target is able to bind to a small molecule and the small molecule can modulate the target's activity. When a suitable target is identified, the search for small molecules that bind to the target can begin. In pharmaceutical industry such small molecules ('hit' compounds) usually are found by screening libraries of potential drug compounds in high-throughput screening assays of the desired targets. Another option is to perform a virtual screening of libraries, if the structure of the target is available. Binding affinity is the main criterion in these screenings. Afterwards, the affinity of the initial hits is improved through further synthetic efforts and structure-affinity relationships are generated, a concept that was already used in the 19th century. After this 'lead' identification comes the lead optimization process in which a drug candidate is generated. Ideally, the drug candidate should not only have good binding affinity for the target, but also be "drug-like". It should possess properties that are predicted to lead to adequate chemical and metabolic stability, oral bioavailability and minimal toxicity, which in turn would result in a safe and efficacious drug. However, these characteristics are still difficult to optimize using rational drug design techniques. So, often scientists are forced to return to the "trial and error" experimentation in the late stages of the drug development process. A survey of phase II clinical trials in 2008 - 2010 done by Thomson Reuters[2] revealed that less than a fifth (18%) of phase II clinical trials were successful. Half of the reported failures were due to insufficient efficacy and another fifth due to safety concerns (Figure 1). This indicates that more predictive models in earlier stages of drug development are still needed.

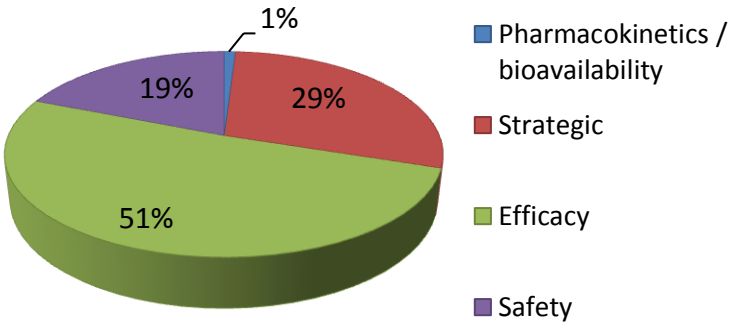

Figure 1. Phase II failures: 2008 – 2010. The failures are divided according to failure reasons. Adopted from Arrowsmith, 2011.[2]

RESIDENCE TIME IN DRUG DISCOVERY

As mentioned above, in current drug discovery the lead compounds are selected based on their affinity towards the desired target. Usually, affinity is determined using e.g., a radioligand displacement assay, which is an *in vitro* experiment based on binding equilibrium between a known radioligand and the compound in question. However, in the human body the concentrations of the drug and the endogenous competitor are constantly changing. Therefore, affinity that has been determined *in vitro* under equilibrium conditions does not necessarily translate in *in vivo* efficacy. To tackle the efficacy issue we need to improve and optimize the drug candidates on additional parameters. The past few years witnessed an increased interest in a new parameter, the so called drug-target residence time. More and more evidence suggests that drug efficacy may be associated with the time the drug remains on its target.[3, 4] Copeland et al[5] already demonstrated that the drug–target residence time is, in some cases, a better predictor of *in vivo* efficacy than the binding affinity. However, it depends on the physiological context and the nature of drug–target interactions whether a long or short residence time is needed to yield a better drug. Thus the additional knowledge of drug-target residence time already at the early stage of drug development could help to

address the afore mentioned efficacy problem, thus potentially decreasing the attrition rate of drug candidates in the more expensive phase II clinical trials.

CHEMOKINES, CHEMOKINE RECEPTORS

Chemokines (chemoattractant cytokines) are a family of small and structurally similar proteins of 70-120 amino acids (MW = 8-14 kDa).[6-8] They were first discovered in 1977[9] and initially identified as controllers of immune cell migration.[7] Chemokines are split into two main categories according to their biological actions – the inflammatory chemokines and homeostatic chemokines.[10] The inflammatory chemokines are released under pathological conditions. The concentration gradient of chemokines recruits the immune cells towards the site of inflammation. Homeostatic chemokines are continuously produced and are involved in housekeeping functions such as recruiting leukocytes during hematopoiesis.

The chemokine receptors are members of the G protein-coupled receptor (GPCR) superfamily. This protein family has proven to be one of the most fruitful target classes for therapeutics. It constitutes a class of membrane-bound proteins, with a conserved transmembrane heptahelical fold, which respond to a whole spectrum of different endogenous and synthetic agonist ligands ranging from light and cations, biogenic amines, fragrances, pheromones and lipids to peptides and globular proteins. The whole GPCR family comprises three major classes, termed A, B and C. The chemokine receptors belong to class A GPCRs, also known as rhodopsin-like receptors (after the visual pigment) whose members form the largest group of GPCRs. The axis of chemokines and chemokine receptors mediates a vast number of physiological effects making the receptors of great interest as possible drug targets. However, the development of chemokine receptor antagonists has been very challenging. Only two compounds have successfully completed phase III clinical trials and are now marketed: maraviroc, a CCR5 antagonist for the treatment of HIV/AIDS, and plerixafor, a CXCR4 antagonist for the mobilization of stem cells prior to collection and subsequent transplantation after chemotherapy.

CHEMOKINE RECEPTOR 2 AND THE RATIONALE FOR TARGETING IT IN DISEASE

In the last decade there has been profound interest in the chemokine receptor 2 (CCR2) and its endogenous ligand CCL2 (also known as MCP-1). CCR2 is the only characterized high affinity receptor for CCL2, although CCR2 can be activated by other chemokines (e.g. CCL7, CCL8 and CCL13),[11] albeit with lower affinity. Studies of CCR2- and CCL2-deficient animals have revealed the critical role of this axis in monocyte recruitment in a number of inflammatory conditions such as delayed-type hypersensitivity reactions, antigen-induced granuloma formation, non-infectious peritonitis and also in infectious diseases.[12, 13] Next to monocytes, CCR2 is located also on basophils, activated T cells, NK cells, and dendritic cells.[11, 14, 15] However, most of the interest in CCR2 antagonism was due to the intention to inhibit monocytes/macrophages and their products, which are thought to be involved in damaging effects of inflammation in a number of disease states (e. g. rheumatoid arthritis (RA), multiple sclerosis (MS), atherosclerosis).[16-18] Early studies showed TAK-779 (Figure 2), a small molecule CCR2 antagonist, inhibited collagen-induced arthritis.[19] Additionally, use of the CCR2 antagonist INCB3344 (Figure 2) showed improvement in adjuvant-induced arthritis in rats.[20] Recently Lebre et al.[21] described that blockade of CCR2 with an antibody prevented monocyte chemotaxis when it was induced by CCL2, however, it failed when chemotaxis was induced with synovial fluid, suggesting that CCR2 is the wrong target for the treatment of RA. Also in the case of MS, blockade of CCR2 drew a lot of enthusiasm. The preclinical data supported the role of the CCR2/CCL2 axis in a mouse model of MS with experimental autoimmune encephalitis.[17] However, CCR2 antagonist MK-0812 (Figure 2), which worked in the murine model, failed in a phase II clinical trial due to lack of efficacy.

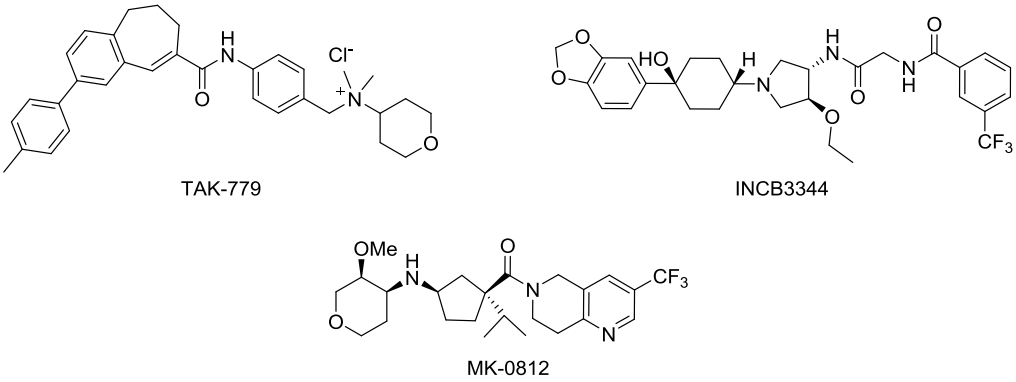

Figure 2. CCR2 antagonists.

Additionally, humanized anti-CCR2 antibody (MLN-1202) did not show efficacy in clinical trials of rheumatoid arthritis or multiple sclerosis.[22] Another victim of the efficacy problem was AZD-2423 (a potent, orally bioavailable, non-competitive, negative allosteric modulator of the CCR2 chemokine receptor) which failed in a clinical trial of posttraumatic neuralgia.[23] However, many other preclinical studies indicated promise in asthma,[24] metabolic disease,[25] fibrosis, pain,[26, 27] and COPD.[28]. Hence, the development of new efficacious CCR2 antagonists is warranted.

AIM AND CONTENT

In the previous sections, a short history on the evolution of the drug discovery process was presented with a further emphasis on the more recent challenges in the development of efficacious treatments for CCR2-related diseases. Modern drug discovery has evolved tremendously to understand the molecular causes of different diseases and how these can be treated. However, the 21st century drug discovery of new treatments is still mostly based on the principles suggested in the 19th and 20th century, for example the strict focus on target affinity. This is a very important parameter indeed as a drug can be effective only when it is bound to its target. It is, however, not the only criterion to yield a successful drug. Several CCR2 antagonists, all with high binding affinity, were discussed as examples of failures due to efficacy issues. These are only a few out of many failures that we cannot afford to have,

especially in the late and costly clinical stages of the drug discovery process. We made a plea to consider at least one other parameter, i.e. residence time. Thus, the aim of this thesis is to develop a path towards high affinity and long residence time (RT) CCR2 antagonists in the very early stages of drug discovery, thus helping to proceed in the next phases of drug development with potentially better drug candidates.

In **chapter 2** we review the literature on a chemical substructure that proved to be important in our work. It is the indane ring system and we analyzed its properties, preparation, and presence in ligands for GPCRs. We then describe our subsequent discovery of new indane-containing CCR2 antagonists (**chapter 3**). The additional knowledge of the binding kinetics of several known CCR2 antagonists allowed us to reevaluate and choose a structure for further optimizations that other researchers had abandoned due to it having only modest binding affinity. The decision to determine in parallel both affinity and RT yielded the discovery of new high affinity and long residence time CCR2 antagonists. Furthermore (**chapter 4**), the indane containing CCR2 antagonists went into another cycle of optimization based on affinity and RT and yielded compounds with even longer RT.

Chapter 5 describes the structure-kinetic relationship for CCR2 receptor antagonists based on derivatives of MK-0483, a CCR2 antagonist from Merck, Inc. with slow binding kinetics. The structure of MK-0483 was altered step by step to reveal the crucial structural components that yield the long RT for the CCR2 receptor.

Chapter 6 describes our medicinal chemistry efforts to explore chemical space even more and develop CCR2 antagonists based on a new piperidinediamide scaffold. Series of new structures were synthesized to evaluate SAR of the substituents on the N-piperidinediamide moiety revealing the specificity of substituents and spacer types needed for affinity in the case of this scaffold.

Finally, a general discussion of the results of this thesis and a future outlook of CCR2 antagonists as potential treatments is presented, concluding this thesis.

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