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

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CHAPTER 7

CONCLUSIONS AND FUTURE PERSPECTIVES

Inhibition of the CC chemokine 2 receptor (CCR2), the subject of my thesis, is an attractive strategy to combat inflammatory conditions. Hence, the pharmaceutical industry, both smaller and bigger actors, has paid considerable attention to this and related chemokine receptors. Currently, there are more than 45 different chemical classes known as CCR2 antagonists. However, despite the big diversity of antagonists and all the efforts in (pre)clinical pharmacology in the CCR2 research field there are still no marketed drugs targeting this receptor. In fact, all clinical trials targeting CCR2 have failed to show efficacy, which would suggest that CCR2 is the "wrong" target. However, the research described in this thesis provides a better understanding of the small-molecule antagonist interactions with the CCR2 receptor and might help to improve the efficacy of CCR2 antagonists in the future, and to resurrect CCR2 as a potential target for the treatment of various inflammatory diseases.

DEVELOPMENT OF NEW CCR2 ANTAGONISTS

All small-molecule CCR2 antagonists can be divided into two distinct classes based on their binding site in the CCR2 receptor. The bulk of antagonists bind to the same orthosteric binding site located at the interface between transmembrane domains and extracellular side of the receptor, where they are directly competing with the endogenous ligand CCL2. Recently we discovered another (allosteric) binding site, which is located on the intracellular side of the receptor. Until now, only a few scaffolds are known to bind to this intracellular binding site (unpublished data).

PHARMACOPHORES OF CCR2 ANTAGONISTS

Despite the substantial chemical diversity in the structures of the antagonists, the majority of the orthosteric ligands correspond to the same pharmacophore (Figure 1). In the pharmacophore one of the general features is the presence of a basic nitrogen in the center of the molecule. Although Bristol-Meyers Squibb developed BMS22[1] – a high affinity CCR2 antagonist without basic nitrogen -, a subsequent incorporation of an additional amine yielded a strong boost in affinity.[2] This is also in accordance with mutagenesis studies, which

suggest that the nitrogen atom, positively charged at physiological pH, forms a salt-bridge interaction with the receptor's glutamic acid E291. Another crucial feature is an aromatic ring on one side of the molecule connected via an amide-containing linker to the central nitrogen atom.

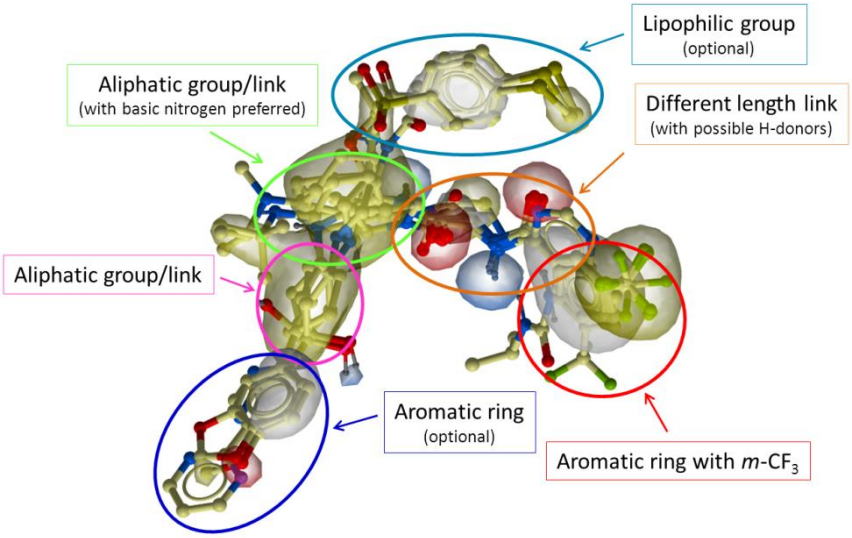

Figure 1. Pharmacophore based on superimposition of 7 CCR2 small-molecule antagonists. Important groups are indicated by the circles: aromatic ring with *m*-CF_3 group (red), amide containing linker (orange), aliphatic group/link with basic nitrogen (green), lipophilic group (light blue), aliphatic link (pink) between basic nitrogen and aromatic ring (blue).

Usually this aromatic ring is furnished with lipophilic, electron-withdrawing groups (preferentially CF_3) on the *meta* position. Interestingly, in the case of cyclopentylamines in the center of the molecule (see also **chapter 3**) any attempt to alter the location or characteristics of the substituent on this aromatic ring completely abolished affinity (unpublished data). The other side of the molecule is more versatile and can bear a wide range of different substituents as long as they fit the pharmacophore. Nevertheless, several criteria should be met before reasonable affinities can be achieved. This was evaluated in more detail with a piperidinediamide scaffold (**chapter 6**). This moiety is based on both pyrrolidine and azetidine scaffolds[3, 4] by expanding the central aliphatic ring to piperidine. Despite that this scaffold still

fits the pharmacophore, substituents that were very well tolerated in the pyrrolidine series[5] caused a complete loss of affinity. After extensive SAR studies it was concluded that, similarly to the azetidine series, a 4-aryl-cyclohexyl group is the preferred substituent for this scaffold.

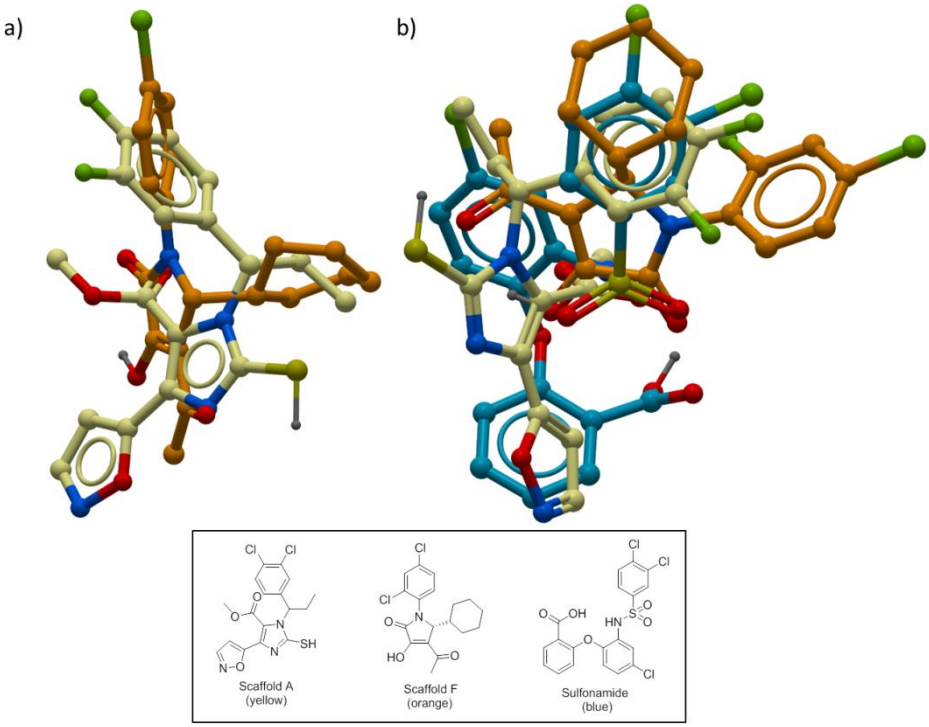

Figure 2. a) superimposition of allosteric CCR2 antagonists: Scaffold A (yellow) and Scaffold F (orange), which did not yield high affinity compounds; b) superimposition of docking poses (based on mutagenesis studies) of allosteric CCR2 antagonists: Scaffold A (yellow), Scaffold F (orange) and Sulfonamide (blue).

Structures of the allosteric (intracellular) binders (Scaffold A and Scaffold F) are similar and can be superimposed to generate another pharmacophore (Figure 2a). However, the new scaffolds (Figure 3) that were designed based on such a pharmacophore failed to yield compounds with better affinity than 10 µM towards the CCR2 receptor (unpublished data).

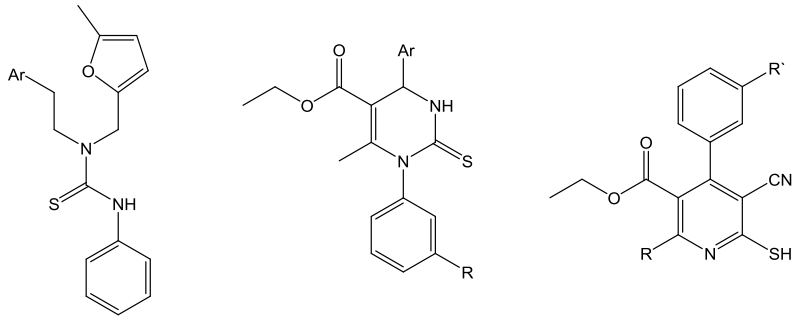

Figure 3. New core structures (thiourea, pyrimidine and pyridine) designed based on superimposition of scaffolds A and F (Figure 2a).

These failures suggested that there must be differences between the binding modes of the known allosteric antagonists. Later mutagenesis studies showed that, despite the structural similarity of the ligands (scaffold A and F), they only partially overlap in the binding site and this was also proposed by further docking studies (Figure 2b). To generate a valid pharmacophore of the allosteric binders I would suggest combining the knowledge of mutagenesis and docking studies with the SAR studies performed on known allosteric antagonists (Figure 2b). This could potentially reveal unexplored sub-pockets for interaction in the allosteric binding site and generate new CCR2 antagonists.

STRUCTURE-AFFINITY RELATIONSHIPS AND/OR STRUCTURE-KINETICS RELATIONSHIPS

Despite the big diversity and substantial amount of already known CCR2 antagonists and irrespective of all failures in clinical trials targeting CCR2, the same pharmaceutical companies file further patents almost on a yearly basis disclosing new chemical entities as potential drugs for the treatment of CCR2-related diseases.[6] Apparently, the general idea of *"If something doesn`t work, try something else"* also applies to drug discovery and development. Apparently it is tempting to generate huge amounts of structurally diverse compounds and to hope that one will eventually have the necessary properties in one compound to become an efficacious treatment. However, a more elaborate reevaluation of known hits may reveal important factors that were previously overlooked. Until now, the development of CCR2

antagonists has been based primarily on improving CCR2 affinity but it failed to yield efficacious medication. As described in the introduction of this thesis, for a CCR2 antagonist to exert its effect it should block its target for as long as possible. To be able to do this, first it needs to bind to the target (good affinity) and second it must stay bound to the target (long residence time) thus continuing to block it. In this thesis I describe the first attempts to use both properties (affinity and residence time) for the design of new high affinity and long residence time CCR2 antagonists. **Chapter 3** is a reflection of how the combined knowledge of structure-affinity relationships (SAR) and the drug-target residence time, which can be analyzed as structure-kinetics relationships (SKR), helped to reinvigorate a chemical structure that was previously discarded by other scientists due to its moderate affinity for the CCR2 receptor.[7, 8] Based on this structure, we designed and synthesized high affinity and long residence time antagonists. **Chapter 4** reports the follow-up on these structures and defines more detailed SAR and SKR through interrogation of the binding site of the CCR2 receptor by applying small changes to the ligand molecule in a step-by-step manner.[9] As I see it, this approach can already be utilized at the very beginning of the drug discovery cycle thus potentially improving the success rate of drug candidates while decreasing the expenses for the development of new drugs.

However, it will take time before principle of binding kinetics advocated in this thesis will become part of routine tests in early drug discovery. Until now, a possible correlation of binding kinetics with the efficacy of a drug has been shown only in hindsight. I hope the work described in this thesis and similar research will form the basis for the pharmaceutical industry to use binding kinetics in a more prospective manner. A next step would be to test the long residence time compounds in *in vivo* models and compare their efficacy with structurally similar high affinity, but short residence time CCR2 antagonists. Maybe the best choice for *in vivo* tests would be one of the derivatives of MK-0483 described in **chapter 5**. MK-0483 itself, stemming from the Merck Research Laboratories, is a high affinity dual CCR2/CCR5 antagonist and was developed as a potential clinical candidate with a good PK/PD profile and very slow binding kinetics at CCR2.[10] Despite the excellent properties of this compound, Merck decided to continue clinical trials with MK-0812 (thought to have fast

kinetics) while MK-0483 was kept as a backup candidate.[11] After the clinical failure of MK-0812 mentioned previously, the long residence time CCR2 antagonist MK-0483 never got a chance to enter clinical trials. However, if Merck would restore the CCR2 program in their pipeline, the best option for proof-of-concept would be to use MK-0483, due to its long residence time and dual antagonism (the value of dual antagonism will be discussed later).

SPECIFICITY, DUAL ANTAGONISM OR FUNCTIONAL SELECTIVITY – WHAT DO WE NEED?

To develop a new drug and get it to the market as an effective treatment, it must fulfill many requirements, one of which is being selective to avoid potential adverse effects via off-target binding. Usually, during the early stages of the drug discovery process, a counterscreen against a panel of different off-targets is used to determine the selectivity of a lead compound before advancing it into *in vivo* studies. However, in some cases, lack of selectivity over some targets can be tolerated or it can even be beneficial. Orthosteric CCR2 antagonists often have affinity for the CCR5 receptor too and are called dual antagonists, like the above-mentioned MK-0483. According to Pasternak et al.[10] it is unlikely that CCR5 blockade is a treatment liability for CCR2-related diseases. Zhao et al.[12] even suggested that BMS-A (a dual CCR2/CCR5 antagonist) offers a novel oral therapy for the treatment of autoimmune diseases. Additionally, CCR2/CCR5 dual inhibition could be beneficial in the treatment of Crohn`s disease and atherosclerosis.[13] However, in the case of rheumatoid arthritis (RA), Lebre et al. recently described that even dual antagonism of CCR2/CCR5 does not prevent monocyte migration when induced by synovial fluid (taken form RA patients), which contains many different chemokines.[14] Only the blockade of the CCR1 receptor in this assay resulted in inhibition of chemotaxis; however, CCR1 antagonists failed to show effect in clinical trials.[15] Apparently, the chemokine system in RA pathology is very versatile and one could argue that selective inhibition is doomed for failure as described above. To tackle this multi-level system, one should combine the separate benefits (observed in numerous *in vitro* studies) of the inhibition of CCR1, CCR2 and CCR5 in one treatment. This could potentially be achieved by a combination of an allosteric dual CCR1/CCR2 antagonist (such as the sulfonamide in Figure

2b)[16] with an orthosteric dual CCR2/CCR5 antagonist (e.g., MK-0483). Moreover, in radioligand binding studies it was observed that allosteric and orthosteric CCR2 antagonists enhance each other's binding.[17] Another possibility would be to develop an "all-in-one" antagonist, which most probably would be an allosteric binder as the intracellular binding site is more conserved between the different chemokine receptors. A good starting point could be the sulfonamide core (Figure 2b), which already has high affinity for CCR1/2 and sub-micromolar affinity for CCR4/5 receptors.[16]

Another peculiarity that should be taken into account when developing drugs that target the chemokine system is its intricate complexity. For example, the CCR2 receptor can be activated by multiple chemokines (e.g. CCL2, CCL7, CCL8, CCL11 and CCL13), while all these chemokines, except CCL2, can bind to multiple other chemokine receptors. Moreover, such receptor-ligand interchangeability is observed between almost all chemokine receptors and their ligands, rendering the whole chemokine system promiscuous. However, the distinct tissue expression of different chemokines points out that they are part of specific and fine-tuned immune system.[18] For example, the CCL2/CCR2 axis is important for monocyte migration from the bone marrow to the blood under normal homeostatic conditions. During inflammation, however, also CCL7, next to CCL2, plays an essential role in monocyte recruitment to the inflamed tissue.[19] Additionally, Berchiche et al. reported that CCR2 shows a degree of biased signaling when activated by different chemokines.[20] Although not entirely understood, this intricacy of the chemokine system must be taken into consideration when developing new ligands. For instance, lead compounds should not only be tested against the "primary" endogenous ligand but also all other endogenous ligands as they could bind differently and continue to activate the receptor.

HERG K^+ CHANNEL AS AN OFF-TARGET OF CCR2 ANTAGONISTS

Next to the challenge of developing efficacious drugs, they must be also safe. One of the major safety concerns for the pharmaceutical industry has become the human ether-à-go-go-related gene (hERG) potassium (K^+) channel. Blockade of the hERG K^+ channel can affect heart

rhythm by prolonging the QT interval, thereby increasing the risk of ventricular arrhythmias and fibrillation, potentially leading to torsades de pointes and sudden death.[21] Due to their binding to this off-target, many drugs have been withdrawn from the market or labeled with a 'black box' warning. Nowadays, screening for hERG K^+ channel interaction is an FDA prerequisite and a routine procedure in the drug discovery and development cycle. One of the reasons why so many drugs bind to this channel is the broad tolerance of the hERG binding pocket. In general, if a molecule contains a charged or basic nitrogen that can be protonated at physiological pH, linked to an aromatic group on one side of the molecule, and to a lipophilic group on the other, it is a potential binder to the hERG channel. Such description fits a considerable variety of molecules including the orthosteric CCR2 antagonists (Figure 1). However, medicinal chemists have developed different ways to synthesize out hERG affinity from lead compounds. One of the well-known methods is the introduction of an acidic group in the molecule. This approach was used in the development of MK-0483, where the introduction of a carboxylic acid moiety resulted in a 600-fold decrease in affinity for the hERG channel compared to its parent compound.[10] Another approach is to alter the pharmacophore by decreasing the pK_a of the basic nitrogen[22] or by exchanging an aromatic group to an aliphatic one.[23] However, such interventions often result in an affinity decrease for the primary target too, thus requiring additional lead optimization if possible. Redfern et al. have proposed a 30-fold margin be a minimum between free plasma concentration of the drug and its IC_{50} value for the hERG channel.[24] However, pharmaceutical companies should consider increasing this safety margin especially for drugs intended for non-debilitating diseases. Next to the affinity also binding kinetics to and binding configuration (i.e. binding to either the open or closed state of the channel) of the hERG channel should be taken into account. Recently, Veroli et al.[25] described that hERG inhibitors with similar affinities but different binding kinetics do not hold the same pro-arrhythmic risk. However, binding configuration (especially the closed state of the channel) had a much bigger influence on QT interval prolongation than binding kinetics, suggesting the need for additional screening for hERG binding configurations rather than potency alone.

FINAL NOTE

Drug discovery has come a long way in its evolution. Mankind journeyed from the prehistoric times when healers found and used medicinal herbs by trial and error of consumption of the local flora, throughout the birth of scientific understanding of chemistry and pharmacology in the 19th century, to current more rational design approaches in drug discovery. Throughout this journey, drug discovery and development have become more effective, safer and more complex and all these improvements have happened in evolutionary jumps. Usually these jumps accrued after discovering new methods, new relationships, or testing and proving new ideas that led to new understandings and paradigm changes. However, such breakthroughs usually came after facing a crisis and at the moment the pharmaceutical industry is thought to face a productivity crisis.[26] At the same time there is an increasing awareness that drug discovery cannot be successfully done anymore in 'splendid isolation'. Public-private partnerships, such as the Innovative Medicines Initiative of the EU and the Dutch TIPharma program (that made this thesis possible) are examples how the ideas and knowledge of academia come together with the practical expertise of the industry and in synergy can provide possible solutions to overcome the aforementioned crisis. This joint collaboration and awareness provides room for a further exploration of novel concepts in drug discovery, for which time was often lacking in industry alone. This is certainly true for the subject of my thesis, i.e. another paradigm shift in drug discovery – from "SAR" to "SAR/SKR", and I am happy that I could play a role in it.

REFERENCES

1. Cherney, R. J.; Mo, R. W.; Meyer, D. T.; Nelson, D. J.; Lo, Y. C.; Yang, G.; Scherle, P. A.; Mandlekar, S.; Wasserman, Z. R.; Jezak, H.; Solomon, K. A.; Tebben, A. J.; Carter, P. H.; Decicco, C. P. Discovery of disubstituted cyclohexanes as a new class of CC chemokine receptor 2 antagonists. *Journal of Medicinal Chemistry* **2008,** 51, 721-724.

2. Cherney, R. J.; Brogan, J. B.; Mo, R.; Lo, Y. C.; Yang, G.; Miller, P. B.; Scherle, P. A.; Molino, B. F.; Carter, P. H.; Decicco, C. P. Discovery of trisubstituted cyclohexanes as potent CC chemokine receptor 2 (CCR2) antagonists. *Bioorganic & Medicinal Chemistry Letters* **2009,** 19, 597-601.

3. Xue, C. B.; Wang, A.; Meloni, D.; Zhang, K.; Kong, L.; Feng, H.; Glenn, J.; Huang, T.; Zhang, Y.; Cao, G.; Anand, R.; Zheng, C.; Xia, M.; Han, Q.; Robinson, D. J.; Storace, L.; Shao, L.; Li, M.; Brodmerkel, C. M.; Covington, M.; Scherle, P.; Diamond, S.; Yeleswaram, S.; Vaddi, K.; Newton, R.; Hollis, G.; Friedman, S.; Metcalf, B. Discovery of INCB3344, a potent, selective and orally bioavailable antagonist of human and murine CCR2. *Bioorganic & Medicinal Chemistry Letters* **2010,** 20, 7473-8.

4. Zhang, X.; Hufnagel, H.; Markotan, T.; Lanter, J.; Cai, C.; Hou, C.; Singer, M.; Opas, E.; McKenney, S.; Crysler, C.; Johnson, D.; Sui, Z. Overcoming hERG activity in the discovery of a series of 4-azetidinyl-1-aryl-cyclohexanes as CCR2 antagonists. *Bioorganic & Medicinal Chemistry Letters* **2011,** 21, 5577-82.
5. Moree, W. J.; Kataoka, K.; Ramirez-Weinhouse, M. M.; Shiota, T.; Imai, M.; Sudo, M.; Tsutsumi, T.; Endo, N.; Muroga, Y.; Hada, T.; Tanaka, H.; Morita, T.; Greene, J.; Barnum, D.; Saunders, J.; Kato, Y.; Myers, P. L.; Tarby, C. M. Small molecule antagonists of the CCR2b receptor. Part 2: Discovery process and initial structure-activity relationships of diamine derivatives. *Bioorganic & Medicinal Chemistry Letters* **2004,** 14, 5413-5416.
6. Carter, P. H. Progress in the discovery of CC chemokine receptor 2 antagonists, 2009 - 2012. *Expert Opin Ther Pat* **2013,** 23, 549-68.
7. Vilums, M.; Zweemer, A. J.; Yu, Z.; de Vries, H.; Hillger, J. M.; Wapenaar, H.; Bollen, I. A.; Barmare, F.; Gross, R.; Clemens, J.; Krenitsky, P.; Brussee, J.; Stamos, D.; Saunders, J.; Heitman, L. H.; Ijzerman, A. P. Structure-Kinetic Relationships-An Overlooked Parameter in Hit-to-Lead Optimization: A Case of Cyclopentylamines as Chemokine Receptor 2 Antagonists. *Journal of Medicinal Chemistry* **2013,** 56, 7706-7714.
8. Kothandaraman, S.; Donnely, K. L.; Butora, G.; Jiao, R.; Pasternak, A.; Morriello, G. J.; Goble, S. D.; Zhou, C.; Mills, S. G.; Maccoss, M.; Vicario, P. P.; Ayala, J. M.; Demartino, J. A.; Struthers, M.; Cascieri, M. A.; Yang, L. Design, synthesis, and structure-activity relationship of novel CCR2 antagonists. *Bioorganic & Medicinal Chemistry Letters* **2009,** 19, 1830-4.
9. Vilums, M.; Zweemer, A. J. M.; Barmare, F.; Gracht, A. M. F.; Bleeker, D. C. T.; Yu, Z.; Vries, H.; Gross, R.; Clemens, J.; Krenitsky, P.; Brussee, J.; Stamos, D.; Saunders, J.; Heitman, L. H.; Ijzerman, A. P. When Structure–Affinity Relationships Meet Structure–Kinetics Relationships: An Example of 3-((Inden-1-yl)amino)-1-isopropyl-cyclopentane-1-carboxamides as CCR2 Antagonists. *Prepared Manuscript*.
10. Pasternak, A.; Goble, S. D.; Struthers, M.; Vicario, P. P.; Ayala, J. M.; Di Salvo, J.; Kilburn, R.; Wisniewski, T.; Demartino, J. A.; Mills, G. S.; Yang, L. Discovery of a potent orally bioavailable CCR2 and CCR5 dual antagonist. *Medicinal Chemistry Letters* **2010,** 1, 14-18.
11. Struthers, M.; Pasternak, A. CCR2 antagonists. *Current Topics in Medicinal Chemistry* **2010,** 10, 1278-98.
12. Zhao, Q. H.; Pang, J.; McIntyre, K.; Gilloolly, K.; Townsend, R.; Postelnek, J.; Grafstrom, R.; He, B.; Ford, G.; Conarello, S.; Xie, J.; Davies, P.; Barrish, J.; Trzaskos, J.; Cid, L. S.; Mckinnon, M.; Mandlekar, S.; Carter, P. A CCR2/CCR5-dual antagonist, BMS-A, offers a potential novel oral therapy for the treatment of autoimmune disease *The Journal of Immunology* **2009,** 182, 92.6.
13. Zhao, Q. H. Dual targeting of CCR2 and CCR5: therapeutic potential for immunologic and cardiovascular diseases. *J Leukocyte Biol* **2010,** 88, 41-55.
14. Lebre, M. C.; Vergunst, C. E.; Choi, I. Y. K.; Aarrass, S.; Oliveira, A. S. F.; Wyant, T.; Horuk, R.; Reedquist, K. A.; Tak, P. P. Why CCR2 and CCR5 Blockade Failed and Why CCR1 Blockade Might Still Be Effective in the Treatment of Rheumatoid Arthritis. *PLoS One* **2011,** 6.
15. Vergunst, C. E.; Gerlag, D. M.; von Moltke, L.; Karol, M.; Wyant, T.; Chi, X. D.; Matzkin, E.; Leach, T.; Tak, P. P. MLN3897 Plus Methotrexate in Patients With Rheumatoid Arthritis Safety, Efficacy, Pharmacokinetics, and Pharmacodynamics of an Oral CCR1 Antagonist in a Phase IIa, Double-Blind, Placebo-Controlled, Randomized, Proof-of-Concept Study. *Arthritis Rheum-Us* **2009,** 60, 3572-3581.
16. Peace, S.; Philp, J.; Brooks, C.; Piercy, V.; Moores, K.; Smethurst, C.; Watson, S.; Gaines, S.; Zippoli, M.; Mookherjee, C.; Ife, R. Identification of a sulfonamide series of CCR2 antagonists. *Bioorganic & Medicinal Chemistry Letters* **2010,** 20, 3961-4.
17. Zweemer, A. J. M.; Nederpelt, I.; Vrieling, H.; Hafith, S.; Doornbos, M. L. J.; de Vries, H.; Abt, J.; Gross, R.; Stamos, D.; Saunders, J.; Smit, M. J.; IJzerman, A. P.; Heitman, L. H. Multiple Binding Sites for Small-Molecule Antagonists at the CC Chemokine Receptor 2. *Molecular Pharmacology* **2013,** 84, 551-561.
18. Zlotnik, A.; Yoshie, O. The Chemokine Superfamily Revisited. *Immunity* **2012,** 36, 705-716.
19. Tsou, C. L.; Peters, W.; Si, Y.; Slaymaker, S.; Aslanian, A. M.; Weisberg, S. P.; Mack, M.; Charo, I. F. Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. *Journal of Clinical Investigation* **2007,** 117, 902-909.
20. Berchiche, Y. A.; Gravel, S.; Pelletier, M. E.; St-Onge, G.; Heveker, N. Different Effects of the Different Natural CC Chemokine Receptor 2b Ligands on beta-Arrestin Recruitment, G alpha(i) Signaling, and Receptor Internalization. *Molecular Pharmacology* **2011,** 79, 488-498.
21. Sanguinetti, M. C.; Tristani-Firouzi, M. hERG potassium channels and cardiac arrhythmia. *Nature* **2006,** 440, 463-9.

22. Vilums, M.; Overman, J.; Klaasse, E.; Scheel, O.; Brussee, J.; IJzerman, A. P. Understanding of Molecular Substructures that Contribute to hERG K+ Channel Blockade: Synthesis and Biological Evaluation of E-4031 Analogues. *ChemMedChem* **2012,** 7, 107-113.
23. Trujillo, J. I.; Huang, W.; Hughes, R. O.; Rogier, D. J.; Turner, S. R.; Devraj, R.; Morton, P. A.; Xue, C. B.; Chao, G.; Covington, M. B.; Newton, R. C.; Metcalf, B. Design and synthesis of novel CCR2 antagonists: investigation of non-aryl/heteroaryl binding motifs. *Bioorganic & Medicinal Chemistry Letters* **2011,** 21, 1827-31.
24. Redfern, W. S.; Carlsson, L.; Davis, A. S.; Lynch, W. G.; MacKenzie, I.; Palethorpe, S.; Siegl, P. K. S.; Strang, I.; Sullivan, A. T.; Wallis, R.; Camm, A. J.; Hammond, T. G. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovascular Research* **2003,** 58, 32-45.
25. GY, D. I. V.; Davies, M. R.; Zhang, H.; Abi-Gerges, N.; Boyett, M. R. hERG Inhibitors With Similar Potency But Different Binding Kinetics Do Not Pose the Same Proarrhythmic Risk: Implications for Drug Safety Assessment. *J Cardiovasc Electrophysiol* **2013**.
26. Pammolli, F.; Magazzini, L.; Riccaboni, M. The productivity crisis in pharmaceutical R&D. *Nature Reviews Drug Discovery* **2011,** 10, 428-438.