Development of assay panels and functional profiling for Chemokine receptors

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Introduction

Chemokines and chemokine receptors are implicated in a wide range of physiological and pathological functions (Bonecchi et al., 2009). They play a key role in auto-inflammatory diseases by acting on leukocyte adhesion, locomotion, diapedesis, chemotaxis, on viral infection, on cancer via control of cell proliferation, angiogenesis, metastasis and on development by regulating hematopoiesis, cardiogenesis, vascular and cerebellar development. Chemokine receptor modulators could prove to be useful therapeutics to target these diseases. However, with the exception of selective CCR5 antagonists for HIV (Dhami et al., 2009), the promise of obtaining new therapeutics related to chemokine receptors has not yet been realized, mainly due to the lack of predictability of animal models and redundancy of the chemokine signaling network. A cornerstone in further drug discovery programs is the access to a large panel of human and rodent chemokine receptor assays. Using these assays, several antagonists were profiled on all the human chemokine receptors and several so called specific antagonists revealed a large spectrum of activities on different chemokine receptors.

Fig. 1: Development of assays for human & mouse CCR2

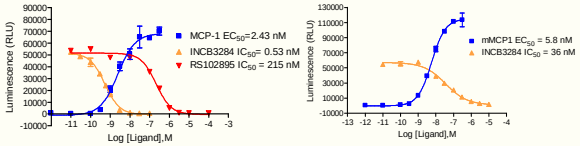


Fig. 2: Pharmacological characterization of human CXCR3a & CXCR3b variants

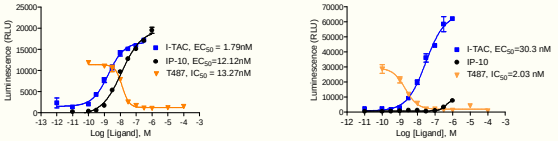


Fig. 3: Development of different functional assays for human CCR10

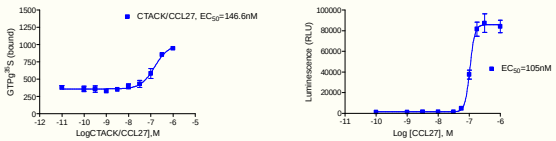


Fig. 4: Identification of high potent agonist for CCR5

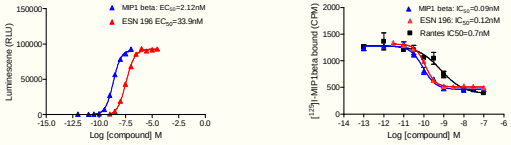
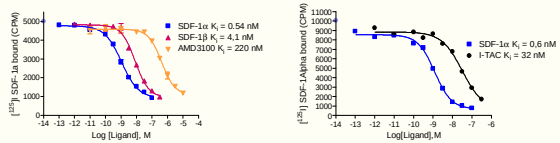


Fig. 5: Validation of different assays for SDF1α receptors: CXCR4 & CXCR7



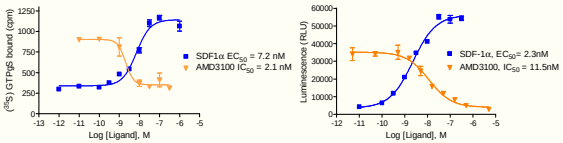
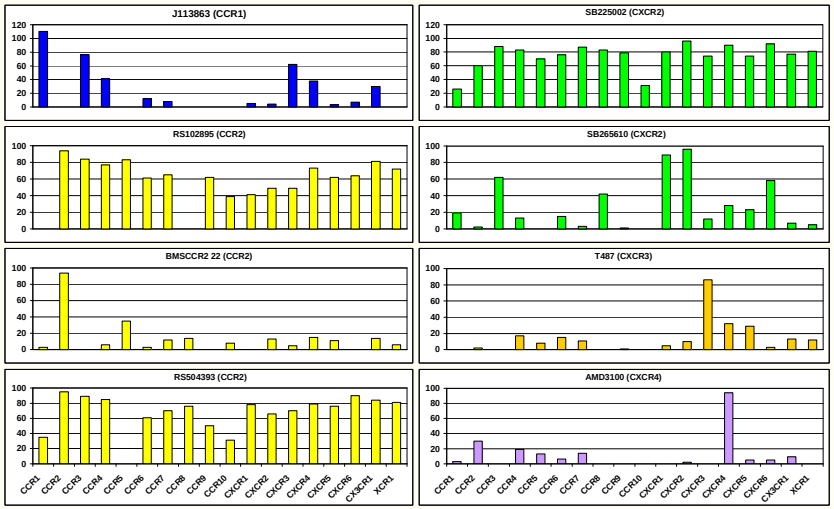


Fig. 6: Functional profiling on human chemokine receptors



Results & Conclusions

Functional and/or radioligand binding assays have been developed for all human chemokine receptors. Most of these receptors gave excellent data with aequorin assays except CCR1. GTPγS assay was also validated for most of these chemokine receptors, allowing to test compounds having intracellular sites of action or kinetics of action that are not suitable for a flash calcium assay like aequorin. Many mouse chemokine receptors have been also validated. Figure 1 shows aequorin assay for both human and mouse CCR2 (rat has been also validated). These data illustrates the importance of parallel testing for human and rodent receptors with a shift of 60fold for the reference antagonist. For rodent receptors, assays were developed in two months, including cDNA cloning.

Fig. 2 illustrates the difference in pharmacology observed for the two CXCR3 isoforms. I-TAC and IP-10 are potent agonists on CXCR3a whereas CXCR3b showed a shift in EC_{50} for I-TAC and no activity for IP-10. However T487 is very potent on both isoforms. Some receptors are less characterized or more challenging to develop robust assays, including CCR9, CCR10 and CXCR7. Fig. 3 shows the results obtained in two different functional assays for CCR10. Even if the potency is lower compared to most of chemokines for other receptors, the results were robust and reproducible.

All these assays allow to perform primary screening, molecular pharmacology and functional profiling. At Euroscreen, a drug discovery program was initiated to develop agonits for CCR5. Following a functional HTS, several chemotypes were selected and characterized using different assays (aequorin, GTPγS, internalization, chemotaxis and HIV infection). In a hit to lead program, ESN196 came out (Fig. 4) as a very potent small molecule agonist for CCR5 with activity similar to Maraviroc in a HIV infection test.

There are a number of recent data showing the critical role of SDF1α in cancer. The use of robust assays for its receptors, CXCR4 and CXCR7 is very important. Fig. 5 shows the radioligand binding assays for both CXCR4 and CXCR7 as well as aequorin and GTPγS assays for CXCR4. Using these functional assays, AMD3100 showed an 20-100fold increase in potency compared to radioligand binding assay, illustrating the importance of having different assays available for compound characterization.

For CXCR7, different functional assays were evaluated but neither aequorin, impedance, GTPγS or cAMP allowed to obtain positive results, illustrating a non conventional function for this receptor.

Using our panel of functional assays for chemokine receptors, we have profiled several reference antagonists against the full panel of human receptors, using aequorin assay or GTPγS for CCR1 (Fig. 6). Interestingly, some compounds used as reference antagonists were poorly specific like RS504393 and RS102895 for CCR2 that inhibited almost all receptors at 10µM. SB225002, a CXCR2 antagonist, was also poorly specific. Other ones were very specific like BMS CCR2 22 for CCR2 and T487 for CXCR3 showing that it is possible to obtain highly specific chemokine receptors antagonists.

References

Bonecchi et al. (2009) Front Biosci. 14:540-51

Dhami et al. (2003) J Clin Pharm Ther. 34:147-60

On Target