Small molecule drug design for biased antagonism of CCR3 in eosinophilic diseases

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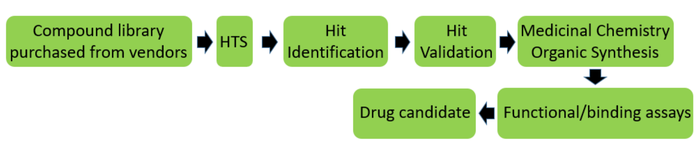
Introduction

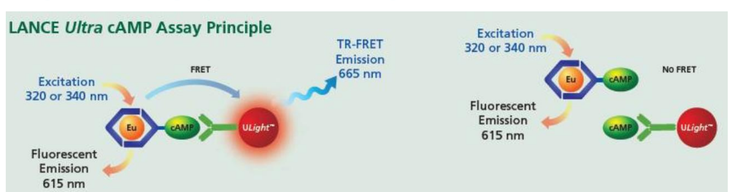
- Asthma is a major cause of morbidity and mortality that affects more than 25 million people in the US.
- Eosinophils play a dominant pathogenic role in allergic disorders, such as asthma and eosinophilic esophagitis.
- CCR3 is a G-protein-coupled receptor (GPCR) most highly expressed in eosinophils and interacts with inflammatory eotaxins like CCL11.
- Small molecule CCR3 antagonists have been unsuccessful in clinical trials likely due to their unbiased antagonism in both G-protein dependent signaling (chemotaxis) and β-arrestin dependent signaling (endocytosis).
- Professor Steven J. Ackerman, Vadim Gaponenko and their teams previously discovered R321 peptide as a potent and selective CCR3 biased antagonist that blocks eosinophil recruitment and airway hyperresponsiveness without losing β-arrestin-mediated receptor internalization.[1]
- We performed a High Throughput Screening (HTS) of 10,000 chemically diverse small molecules from the ChemDiv PPI library.
- We identified 75 compounds ranking in 4 tiers on activity and selected for secondary assays.
- Half-maximal inhibitory concentration (IC50) values of antagonists were determined.
- We describe HTS methods, secondary screenings, potencies of hits, and SAR strategy of **BA001** scaffold.

Methods

Biased CCR3 antagonist discovery pipeline



High Throughput Screening



cAMP detection assay: Perkin Elmer Lance Ultra cAMP Detection kit. The assay is based on the competition between the europium (Eu) chelate-labeled cAMP tracer and sample cAMP for binding sites on cAMP-specific monoclonal antibodies labeled with the ULight dye.

Results

Screenings identified biased CCR3 antagonists

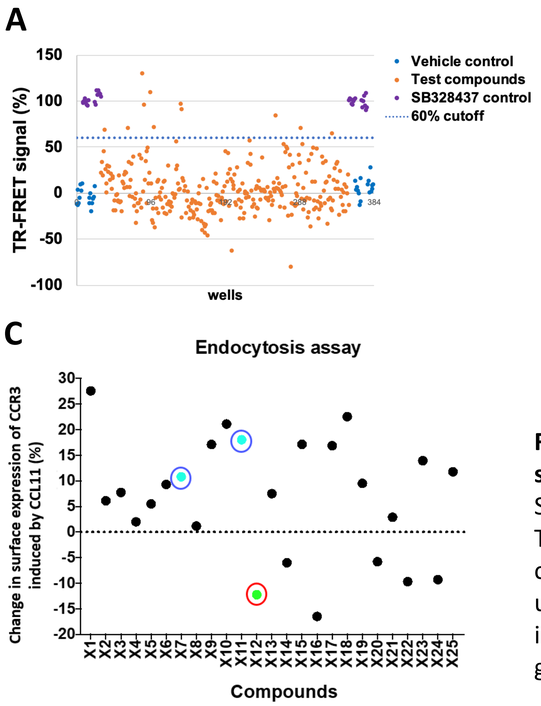


Figure 1. High throughput screening and secondary screenings were conducted. A. Screening of 10K compounds at 10 µM. **B.** Top 25 hits for inhibition of CCL11-induced chemotaxis assay (biased: green dot, unbiased: cyan dots). **C.** Top 25 hits for inhibition of endocytosis assay (biased: green dot, unbiased: cyan dots).

*Biased CCR3 antagonist **BA001**, a chromane derivative*

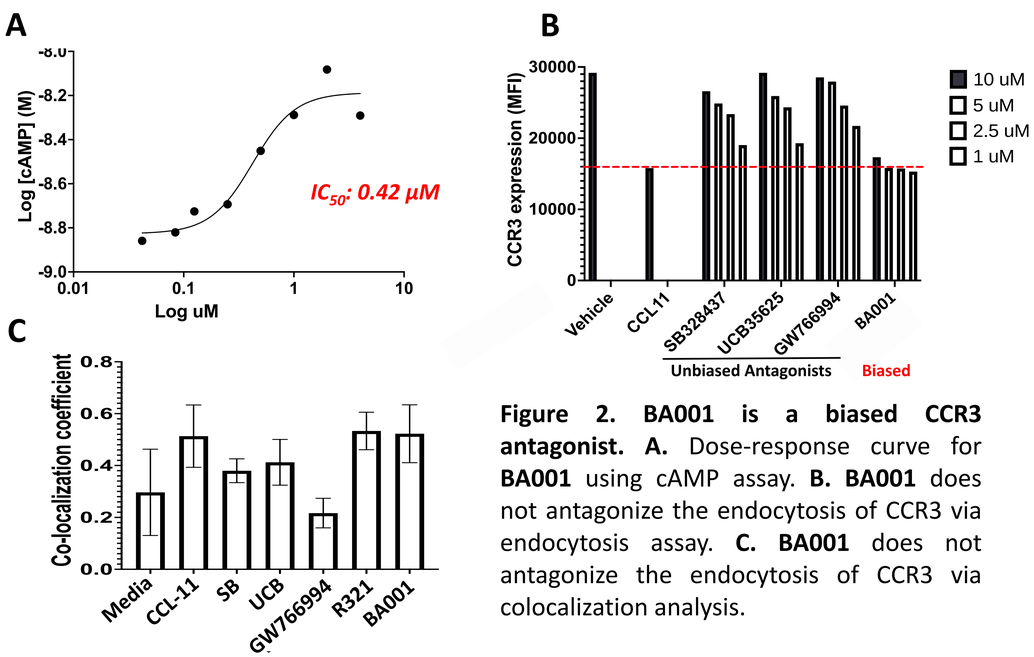
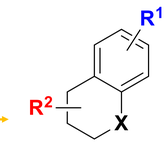


Figure 2. BA001 is a biased CCR3 antagonist. A. Dose-response curve for **BA001** using cAMP assay. **B. BA001** does not antagonize the endocytosis of CCR3 via endocytosis assay. **C. BA001** does not antagonize the endocytosis of CCR3 via colocalization analysis.

Pharmacophore based SAR strategy

Identified para-benzyl core offer different properties



Properly substituted alkyl amides

BA001 scaffold

Objective: Using structure-based design to enhance on-target potency, binding affinity, signaling bias, and safety profiles

Conclusions

- We identified biased CCR3 antagonist by high throughput screening and secondary screenings of 10K compounds.
- **BA001**, 0.42 µM IC_{50}, shows G-protein dependent signaling bias while it does not antagonize the β-arrestin-dependent pathway.
- Further improvement of **BA001** will have a huge impact for GPCR therapeutics in asthma and other eosinophilic diseases.

Literature cited

1. Grozdanovic, M.; Laffey, K. G.; Abdelkarim, H.; Hitchinson, B.; Harijith, A.; Moon, H.-G.; Park, G. Y.; Rousslang, L. K.; Masterson, J. C.; Furuta, G. T.; Tarasova, N. I.; Gaponenko, V.; Ackerman, S. J. *J. Allergy Clin. Immunol*. **2019**, *143*, 669-680.

Acknowledgements

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