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Discovery of indole inhibitors of chemokine receptor 9 (CCR9)

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ABSTRACT

Irritable bowel diseases (IBD) such as Crohn's disease (CD) and ulcerative colitis (UC) are serious chronic diseases affecting millions of patients worldwide. Studies of human chemokine biology has suggested C–C chemokine receptor 9 (CCR9) may be a key mediator of pro-inflammatory signaling. Discovery of agents that inhibit CCR9 may lead to new therapies for CD and UC patients. Herein we describe the evolution of a high content screening hit (**1**) into potent inhibitors of CCR9, such as azaindole **12**.

Irritable bowel diseases (IBD) such as Crohn's disease (CD) and ulcerative colitis (UC) affect 1.6 million people in the US alone.[1] As many as 70 thousand new cases of IBD are diagnosed each year.[1] IBD is characterized by chronic inflammation in the gastrointestinal (GI) tract with periods of flare and remission. IBD requires lifetime care and can cause significant morbidity. Surgery is often held as a last resort, with 40% of CD patients relapsing even after surgery.[4]

Utilizing steroids as first line treatment is effective in achieving short term remission, but long-term use leads to severe side effects and drug tolerance.[2] Immuno-modulators are often applied to maintain remission, but these have significant side effects, and are often ineffective in periods of disease flare. In the last decade, the standard of care in the US has become anti-TNFα monoclonal antibodies (e.g., infliximab) in combination with the immunosuppressant azathioprine which has been shown to initially benefit about two thirds of CD patients.[3] Of the patients that do respond, ~50% are still in remission after a year of therapy.[4] In addition, these biological agents are very expensive and may have immunosuppressive safety concerns. Thus, there remains a significant unmet medical need for those that either do not respond initially or do not maintain their remission.

The GI tract inflammation associated with IBD results from inappropriate recruitment and accumulation of leukocytes in the gut.[5–7] Chemokine receptor 9 (CCR9) is thought to be a mediator for pro-inflammatory T cell migration from the blood stream to the gut tissue.[8,9] The CCR9 ligand (CCL25) is believed to be expressed predominantly in the thymus and the small intestine. In CD patients, the chemokine CCL25 is overexpressed in the small intestine, and CCR9+ lymphocytes are reported to be significantly elevated.[10]

At the inception of our campaign, Chemocentryx/GSK had demonstrated efficacy of their CCR9 antagonist, vercirnon[11] (Fig. 1) in a phase II clinical trial of patients with moderate-to-severe Crohn's disease. In this study a single oral dose daily (500 mg) was consistently superior to placebo across multiple efficacy markers. Encouraged by preclinical biological links between CCR9, CCL25, and gut inflammation as well as apparent clinical validation we embarked on a campaign to discover novel CCR9 antagonists for IBD.[12,13]

A high content screening campaign of 357 K compounds from the Cubist in-house collection and Evotec's discovery library was conducted to identify small molecule antagonists of CCR9. Initial inhibition was assessed at 10 and 3 μM concentrations. Hits (>75% inhibition at 3 μM) were then confirmed with full IC_{50}s in our primary calcium (Ca^{2+}) FLIPR assay and an orthogonal GTPγS assay.[14] Thieno[3,2-*b*]pyrrole **1** (Fig. 1) emerged from this set as a potent antagonist of CCR9. It demonstrated 1000-fold selectivity for CCR9 over two important regulatory GPCRs, Par1 and M1.

Abbreviations: CCR9, chemokine receptor 9; CCL25, chemokine ligand 25.

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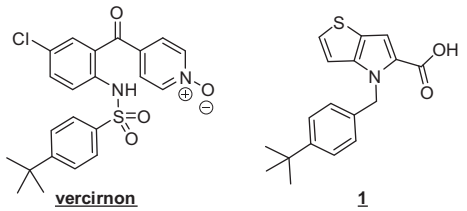


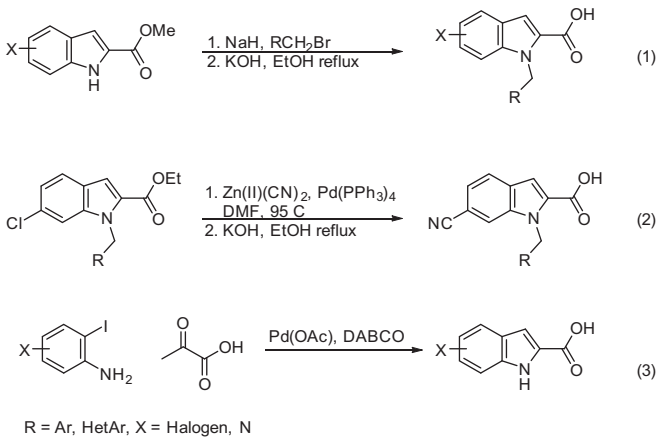
Figure 1. Vercirnon, Chemocentryx CCR9 Ph 3 candidate.

Proteasome activating receptor 1 (Par1), modulates inflammatory machinery and is expressed in the gut.[15] Muscarinic receptor (M1) is part of the parasympathetic signal system and thus involved in regulation of vasodilation, heart rate, and other critical physiological functions.[16]

Replacement of the fused thiophenepyrrole with a more drug-like heterocycle and reduction of log*P* were initially sought by screening structurally related compounds. Substituted indoles such as **2** were quickly identified as suitable replacements (Ca^{2+} IC_{50} = 230 nM). Further optimization focused on increasing cellular potency and improving microsomal stability and solubility. Efficiency metrics such as LipE, based on *c*log*D*, were utilized to prioritize design hypotheses.[17]

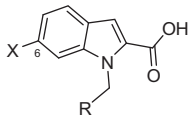
Indole and azaindole analogs were rapidly prepared from commercial materials via 2 step sequence described in Scheme 1. Direct alkylation of indole esters was accomplished with alkaline conditions and a suitable benzyl halide coupling partner. Saponification of the methyl or ethyl esters yielded desired analogs. Nitrile containing indoles were constructed from the corresponding indole chlorides via palladium catalyzed nitrile cross coupling (Scheme 1, Eq. 2). Less common indoles were prepared from corresponding iodo anilines via palladium mediated annulation (Scheme 1, Eq. 3) as described by Reider and co-workers.[18]

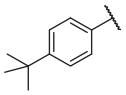
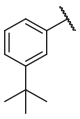
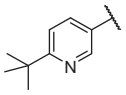
Our initial medicinal chemistry strategy involved surveying the indole core and aryl ring to identify opportunities to expand SAR understanding. Deletion of the chloride such as indole **3** (Table 1) had a dramatic and unexpected deleterious effect on potency (IC_{50} > 10 μM). Methyl substitution (**7**) however restored potency. Though not completely well understood, these data points initially suggested that polarizing or lipophilic groups were needed at this position. Towards this objective, bromide and methoxy (**5** & **6**) indoles were prepared and were shown to be potent (36/75 nM). The greatest improvement however, came with cyano[19] for chloride replacement **7** (Ca^{2+} IC_{50} = 8 nM), suggesting the potency



Scheme 1. Synthesis of functionalized indoles.

Table 1
In vitro profile of HTS follow up analogs



ID	X	R	Ca^{2+} IC_{50} (nM)	LipE[a]
2	Cl		232	3.9
3	H	"	>10,000	<1.8
4	Me	"	85	4.3
5	Br	"	36	4.5
6	OMe	"	75	5
7	CN	"	8	6.3
8	Cl		8400	2.3
9	Cl		29	5.8
10	CN	"	93	6.4
11	7-Cl	"	451	4.6

[a] LipE calculated based on log*D*.

was driven by directionality of the polar group and possibly an electronic effect on the indole nucleus rather than lipophilicity.

Diversification of the 4-*tert*-butyl benzyl group was examined next. The 3-*tert*-butyl analog **8** was significantly less potent (36X) suggesting a directionality requirement for the lipophilicity. Nitrogen introduction to the 3-position of the arene, indole **9**, improved potency 10-fold. Coupling this change to the cyano for chloro replacement in **7** however led to a decrease in potency (**10,** CCR9 Ca^{2+} IC_{50} = 93 nM, LipE 6.4), suggesting a need to balance polarity globally across the molecule.

We also investigated more dramatic alterations to the indole nucleus including migration of the chlorine to the 5 or 7 positions, substitution at the 3-position or introduction of a methyl group at the benzylic position to provide chiral indoles. In general, these types of modifications were not tolerated, with drops in potency from 2 to 15-fold (data not shown).

High protein binding of our series (>95%) directly impacted potency in our functional immune cell trafficking assay in the presence of serum (0.1% BSA). For example, despite single digit nanomolar potency in the FLIPR CCR9 Ca^{2+} assay, 6-cyanoindole **7** suffered a reduction in potency in our immune cell trafficking, chemotaxis, assay (IC_{50} = 320 nM). Attempts to modulate protein binding via carboxylic acid isosteres such as heterocycles, hydroxamates, substituted amides, (not shown) were not successful (CCR9 Ca^{2+} $IC_{50} \geqslant 5$ μM).

The ester variants of potent analogs as well as C3 migrated acids were also significantly less potent suggesting the importance of an ionizable group at the 2 position of the heterocyclic core (data not shown). The superior potency of 6-cyanoindole **7** over other indoles suggested introduction of polarity and electron deficiency to the indole nucleus may be beneficial. Thus a set of azaindoles were prepared (Table 2) that surveyed nitrogen incorporation at different indole positions.

From the parent indole **2**, 4- and 5-azaindoles **12** and **13** were identified as compounds of interest due to their nanomolar potency in primary FLIPR and secondary chemotaxis assays. Despite being more potent than 3-chloro indole **2** in the primary