CCR10 antagonist | BI-6901

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Summary

BI-6901 is the first small molecule inhibitor of the Chemokine receptor CCR10. It is a potent and selective compound suitable for *in vivo* validation.

Chemical Structure

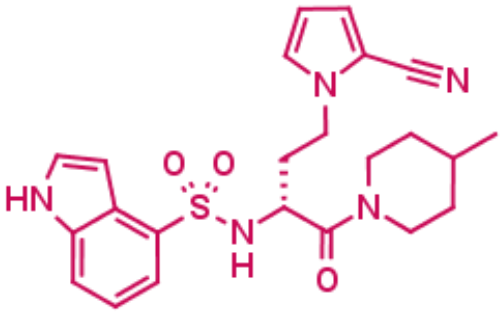

Figure 1: 2-D structure of BI-6901, a CCR10 antagonist

Absolute configuration (R) assigned in analogy to example #10 in Reference 1.

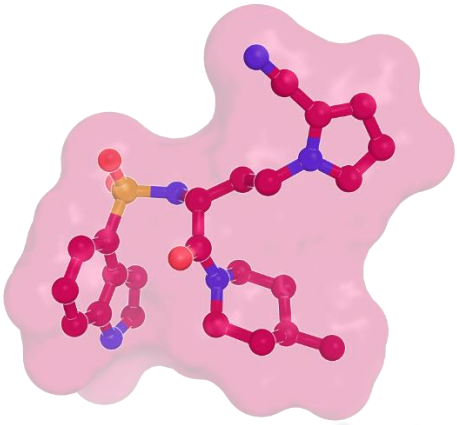

Figure 2: 3-D structure of BI-6901

Highlights

BI-6901 is the first small molecule inhibitor of the chemokine receptor CCR10.[1-3] The racemate of BI-6901 shows consistent potency in assays with various functional readouts and across different

cell backgrounds. No meaningful binding or activity was observed against 29 GPCR's, including 6 chemokine receptors. Although BI-6901 has a high clearance it was a suitable compound for *in vivo* validation. It is efficacious in a murine model of DNFB contact hypersensitivity.[1]

We also offer the optical antipode (BI-6902) as negative control which was inactive in the *in vivo* model.[1]

Target information

"The chemokine receptor CCR10 (GPR2)[4,5] and its two cognate ligands, CCL27 and CCL28 have been implicated in the regulation of epithelial immunity and related diseases. High expression of CCR10 has been noted in epithelia of skin, small intestine, colon, salivary glands, mammary glands, and fetal lung. In addition, other cell types have been reported to express high levels of CCR10, such as melanocytes, dermal fibroblasts, dermal microvascular endothelial cells and skin T cells, sub-populations of immune cells such as IgE-secreting B cells and IgA-secreting plasma cells in mucosal tissues."[3]

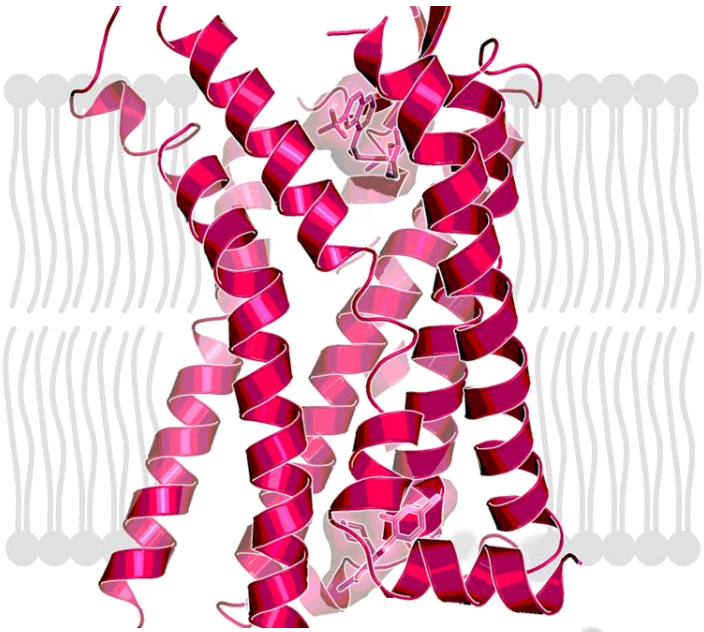

Figure 3: Complex of human CCR2 with orthosteric and allosteric antagonists (PDB code: 5t1a)

In vitro activity

BI-6901 inhibits the CCL27 dependent Ca^{2+} flux in CHO-K cells stably transfected with human CCR10 and aequorin with an pIC50 of 9.0. The optical antipode (BI-6902), which can be used as a negative control has a pIC50 of 5.5 in this assay. Additionally BI-6536 (the racemate of BI-6901 and BI-6902) was tested in assays with different functional readouts (Ca^{2+} flux, cAMP production GTP

binding and chemotaxis) and different cell backgrounds (CHO-K, HEK and Ba/F3) and gave highly consistent results (see *in vitro* activity table).

PROBE NAME / RACEMATE / NEGATIVE CONTROL	BI-6901 (EUTOMER)	BI-6539 (RACEMATE OF BI-6901 AND BI-6902)	BI-6902 (DISTOMER)
MW [Da]	454	454	454
FLIPR Ca^{2+} flux (hCCL27) pIC_{50}[a]	n.a.	9.4	n.a.
FLIPR Ca^{2+} flux (hCCL28) pIC_{50}[a]	n.a.	8.9	n.a.
Aequorin Ca^{2+} flux (hCCL27) pIC_{50}[a]	9.0	8.7	5.5
Aequorin Ca^{2+} flux (hCCL28) pIC_{50}[a]	n.a.	9.0	n.a.
cAMP (hCCL27) pIC_{50}[b]	n.a.	7.6	n.a.
GTP-Eu (hCCL27) pIC_{50}[c]	n.a.	8.0	n.a.
Chemotaxis (hCCL27) pIC_{50}[d]	n.a.	9.0	n.a.

Cell lines: [a] CHO-K (Aequorin, Gαq), [b] HEK, [c] HEK membrane prep, [d] Ba/F3

In vitro DMPK and CMC parameters

BI-6536 (the racemate of BI-6901 and BI-6902) shows high clearance in liver microsomes (LM): human LM >93% Q_H, murine LM > 91% Q_H, rat LM > 86% Q_H. The compound is highly bound to human plasma proteins: hPPB 99.4% bound. BI-6901 has a medium solubility across different pH ranges: (33 µg/mL @ pH 4, 38 µg/mL @ pH 7).

PROBE NAME	BI-6901	BI-6539 (RACEMATE OF BI-6901 AND BI-6902)
Solubility @ pH 7 [µg/ml]	38	34
Liver Microsome clearance [% Q_H] human / mouse	91 / n.a.	> 93 / > 91

Plasma protein binding human [%]	n.a.	99.4

In vivo DMPK parameters

Exposures of compound in 30% cremophore dosed in Balb-C mice: BI-6901, 100 mg/kg ip, 1 h: 7.6 ± 4.5 µM, 7 h: 0.2 ± 0.2 µM; 30 mg/kg ip, 1 h: 3.7 ± 0.4 µM, 7 h: not detected. BI-6902, 100 mg/kg ip, 1 h: 18 ± 2 µM; 7 h: not detected; 30 mg/kg ip, 1 h: 3.2 ± 0.8 µM, 7 h: not detected; 99% plasma protein binding for both compounds

In vivo pharmacology

High Clearance compound. High doses and *i.p.* administration needed to obtain sufficient levels.

The murine cellular potency and apparent specificity of BI-6901 qualified it as a tool to test the impact of CCR10 antagonism on dermal inflammation. BI-6901 was investigated for efficacy against 2,4-dinitrofluorobenzene murine contact hypersensitivity, with BI-6902 serving as a structurally related negative control.[1] The model captures a predominantly Tcell dependent inflammatory response of sensitized mice to topical DNFB challenge on the ear[6] Due to high clearance in mice a 100 mg/kg dose delivered intraperitoneally at 0 and 8 h was required to maintain plasma exposure near or above the murine IC50 of BI-6901 over the majority of the experiment (satellite exposures of the compounds see in vivo DMPK parameter section). Nonetheless, BI-6901 exhibited a dose-dependent anti-inflammatory response against DNFB stimulated ear swelling in sensitized mice. While the eutomer BI-6901 showed efficacy, the distomer BI-6902 demonstrated no activity, consistent with -the stereospecificity of CCR10 antagonism.1 The level of efficacy observed for BI-6901 was similar to that observed with anti-CCL27 antibody in the same model (60-85%).[7]

Selectivity

No meaningful binding or activity was observed against 29 GPCR's, including 6 chemokine receptors (see supplementary material).[1]

PROBE NAME	SELECTIVITY DATA AVAILABLE
Cerep®	No
Eurofins-Panlabs®	Yes
Invitrogen®	No

DiscoverX®	No
Dundee	No

Negative control

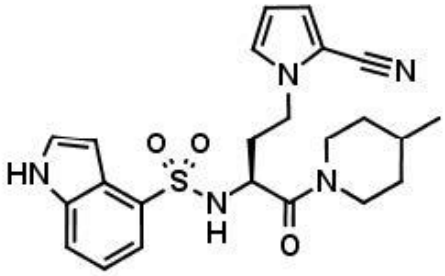

Figure 4: Chemical structure of the negative control BI-6902

BI-6902 is the optical antipode of BI-6901 and inhibits the CCL27 dependent Ca^{2+} flux in CHO-K cells stably transfected with human CCR10 and aequorin with an pIC_{50} 5.5. It was used as a negative control in *in vivo* pharmacology experiments (see *in vivo* pharmacology section).

Absolute configuration (S) assigned in analogy to example #10 in Reference 1.

Co-crystal structure of the Boehringer Ingelheim probe compound and the target protein.

Not available

Summary

Our compound is the first small molecule inhibitor of the Chemokine receptor CCR10.1-3 It is a potent and selective compound and suitable for in vivo validation. It showed efficacy in a murine model of DNFB contact hypersensitivity.[1] We also offer the optical antipode as negative control which was inactive in the *in vivo* model.[1]

These small molecules could be valuable in interrogating the role of CCR10 in dermatological or mucosal inflammation and cancer.[1]

Supplementary data

Selectivity data can be downloaded free of charge from this site.

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b)Asitha Abeywardane, Derek Cogan, Younggi Choi, Donghong A. Gao, Daniel R.Goldberg, Alexander Heim-Riether, Craig A. Miller, Philip D. Ramsden, Lana L.Smith Keenan, Roger J. Snow, Yang Yu Y., U.S. Patent 8,586,748, **2011**.

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