opnMe by Boehringer Ingelheim

CCR10 antagonist

BI-6901

Boehringer Ingelheim

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Summary

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

Chemical Structure

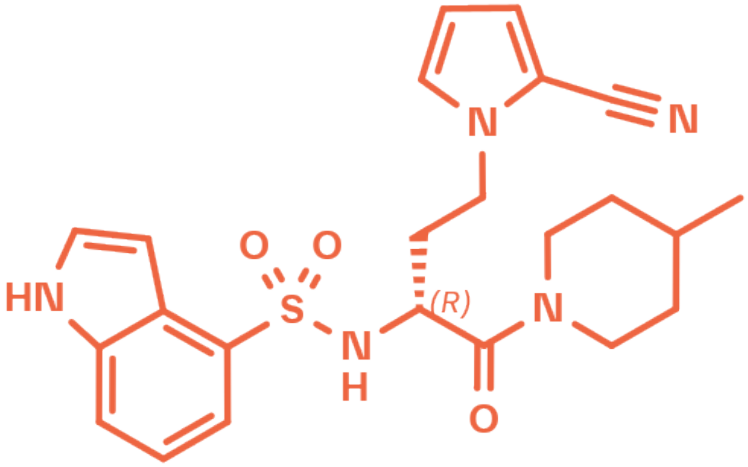

Figure 1: 2D structure of BI-6901, a CCR10 antagonist

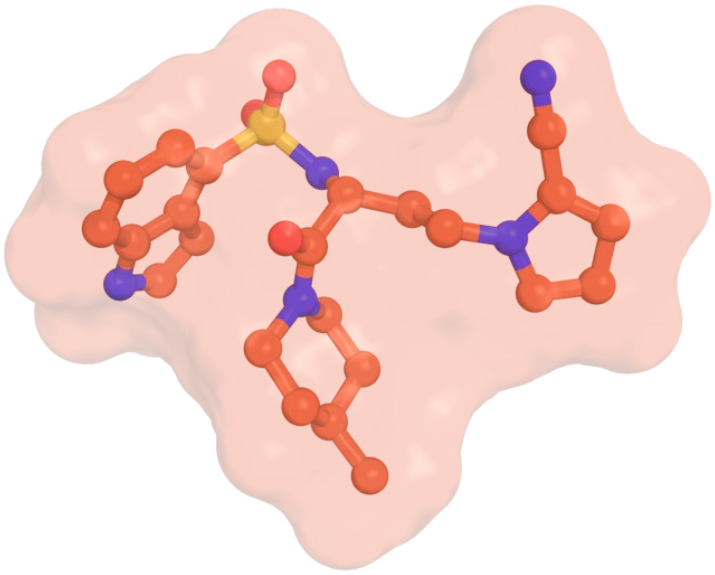

Figure 2: 3D structure of BI-6901

Highlights

BI-6901 is the first available small molecule inhibitor of the chemokine receptor CCR10 for *in vivo* studies[1-3]. Despite its high clearance, this potent and selective compound is suitable for *in vivo* studies if delivered intraperitoneally. In a murine model of DNFB contact hypersensitivity, it showed high efficacy with a dose-dependent anti-inflammatory response.

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)	RACEMATE OF BI-6901 AND BI-6902	BI-6902 (DISTOMER)
MW [Da, free base][a]	453.6	453.6	453.6
FLIPR Ca^{2+} flux (hCCL27) pIC_{50}[b]	n.a.	9.4	n.a.
FLIPR Ca^{2+} flux (hCCL28) pIC_{50}[b]	n.a.	8.9	n.a.
Aequorin Ca^{2+} flux (hCCL27) pIC_{50}[b]	9.0	8.7	5.5
Aequorin Ca^{2+} flux (hCCL28) pIC_{50}[b]	n.a.	9.0	n.a.
cAMP (hCCL27) pIC_{50}[c]	n.a.	7.6	n.a.
GTP-Eu (hCCL27) pIC_{50}[d]	n.a.	8.0	n.a.
Chemotaxis (hCCL27) pIC_{50}[e]	n.a.	9.0	n.a.

[a] For the salt form you will get, please refer to the label on the vial and for the molecular weight of the salt, please refer to the FAQs

Cell lines:

[b] CHO-K (Aequorin, Gαq),

[c] HEK,

[d] HEK membrane prep,

[e] Ba/F3

In vitro DMPK and CMC parameters

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	RACEMATE OF BI-6901 AND BI-6902
logD @ pH 11	3.4	n.a.
Solubility @ pH 7 [µg/mL]	38	34
Clearance (human/mouse) [% Q_H]	91 / n.a.	> 93 / > 91
Plasma Protein Binding human [%]	n.a.	99.4
Plasma Protein binding mice [%]	99.0	99.0

In vivo DMPK parameters

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

In vivo pharmacology

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 T cell 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].

Negative control

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).

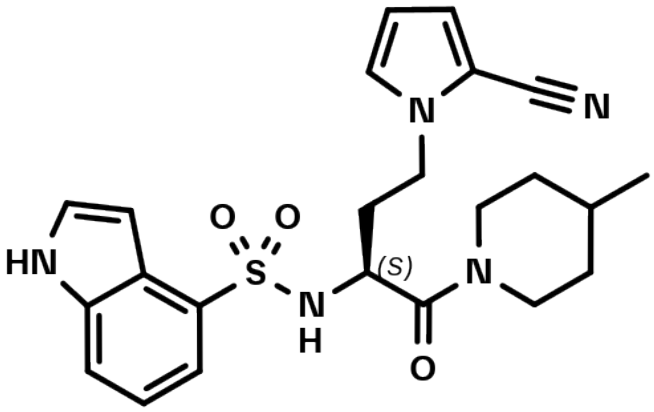

Figure 4: Chemical structure of the negative control BI-6902

Selectivity

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

SELECTIVITY DATA AVAILABLE	BI-6901	BI-6902
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

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

Not available

Supplementary data

2D structure files can be downloaded free of charge from opnMe.

References

1. Abeywardane A., Caviness G., Choi Y., Cogan D., Gao A., Goldberg D., Heim-Riether A., Jeanfavre D., Klein E., Kowalski J. A., Mao W., Miller C., Moss N., Ramsden P., Raymond E., Skow D., Smith-Keenan L., Snow R. J., Wu F., Wu J.-P., Yu Y. N-Arylsulfonyl-α-amino carboxamides are potent and selective inhibitors of the chemokine receptor CCR10 that show efficacy in the murine DNFB model of contact hypersensitivity *Bioorg Med Chem Lett* **2016**, 26(21), 5277–5283. DOI: 10.1016/j.bmcl.2016.09.047, PubMed.

2. Françoise Jung, Frank O. Gombert, Daniel Obrecht, Christian Bisang, Sophie Barthélémy, Alexander Lederer, Eric Chevalier U.S. Patent 8,754,038, **2014**.

3. Daubeuf F., Jung F., Douglas G. J., Chevalier E., Frossard N. Protective effect of a Protein Epitope Mimetic CCR10 antagonist, POL7085, in a model of allergic eosinophilic airway inflammation *Respir Res* **2015**, 16(1). DOI: 10.1186/s12931-015-0231-5, PubMed .

4. Jarmin D. I., Rits M., Bota D., Gerard N. P., Graham G. J., Clark-Lewis I., Gerard C. Cutting Edge: Identification of the Orphan Receptor G-Protein-Coupled Receptor 2 as CCR10, a Specific Receptor for the Chemokine ESkine *The Journal of Immunology* **2000**, 164(7), 3460–3464. DOI: 10.4049/jimmunol.164.7.3460, PubMed.

5. Homey B., Wang W., Soto H., Buchanan M. E., Wiesenborn A., Catron D., Müller A., McClanahan T. K., Dieu-Nosjean M. C., Orozco R., Ruzicka T., Lehmann P., Oldham E., Zlotnik A. Cutting edge: The orphan chemokine receptor G protein-coupled receptor-2 (GPR-2, CCR10) binds the skin-associated chemokine CCL27 (CTACK/ALP/ILC) *The Journal of Immunology* **2000**, 164(7), 3465–3470. DOI: 10.4049/jimmunol.164.7.3465, PubMed: 10725697.

6. Saint-Mezard P., Berard F., Dubois B., Kaiserlian D., Nicolas J.-F. The role of CD4+ and CD8+ T cells in contact hypersensitivity and allergic contact dermatitis *Eur J Dermatol* **2004**, 14(3), 131–138, PubMed: 15246935.

7. Homey B., Alenius H., Müller A., Soto H., Bowman E. P., Yuan W., McEvoy L., Lauerma A. I., Assmann T., Bünemann E., Lehto M., Wolff H., Yen D., Marxhausen H., To W., Sedgwick J., Ruzicka T., Lehmann P., Zlotnik A. Ccl27-CCR10 interactions regulate T cell-mediated skin inflammation *Nat Med* **2002**, 8(2), 157–165 DOI: 10.1038/nm0202-157. DOI: 10.1038/nm0202-157, PubMed: 11821900.