opnMe by Boehringer Ingelheim

LTB_4 receptor antagonist

BIIL315

Boehringer Ingelheim

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Summary

BIIL 315 is a highly potent LTB_4 receptor antagonist (K_i = 1.9 nM)[1]. It is the active metabolite of the *in vivo* tool compound BIIL 284 and is the ideal tool to study LTB_4 antagonism *in vitro*. Additionally, BIIS 035 is available as negative control for *in vitro* experiments.

Chemical Structure

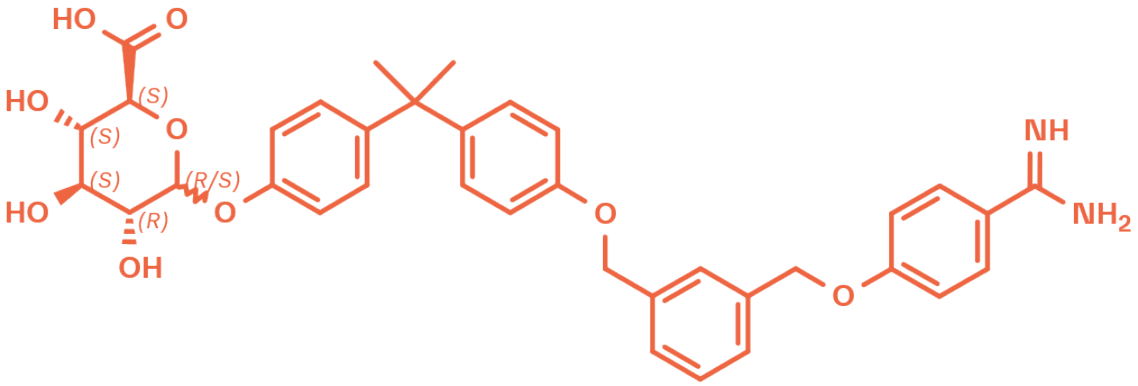

Figure 1: 2D structure of BIIL 315, a potent LTB4 antagonist.

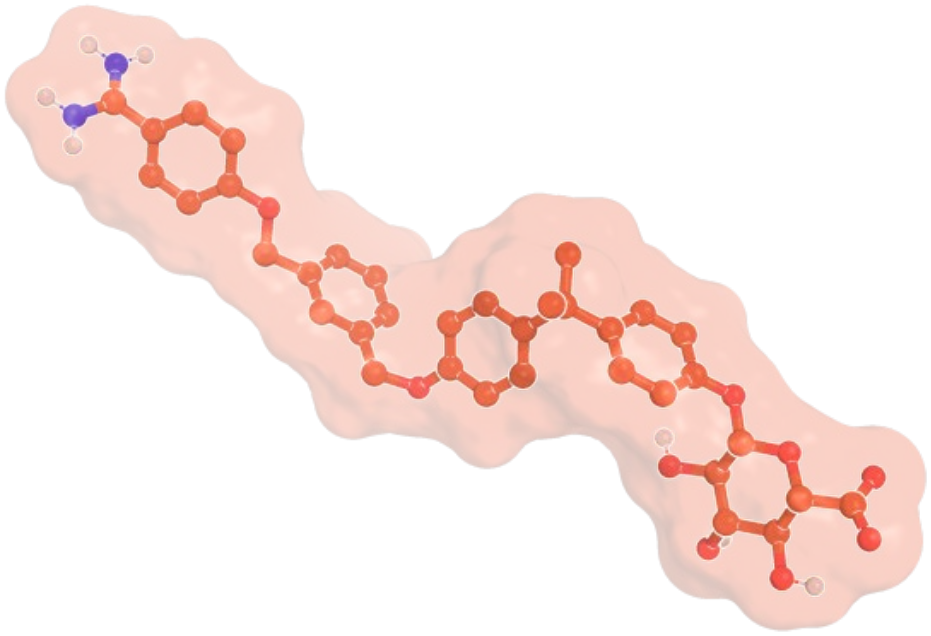

Figure 2: BIIL 315, 3D conformation.

Highlights

BIIL 315 is a highly potent and selective LTB_4 receptor antagonist. This compound binds with high affinity to the LTB_4 receptor (K_i = 1.9 nM) and shows good metabolic stability *in vitro*. It is an excellent tool to study LTB_4 signaling *in vitro*[1]. The prodrug BIIL 284, which is also available on opnMe.com, should be used for *in vivo* studies.

Target information

The LTB_4 receptor is a G-protein coupled receptor (GPCR) with high affinity specifically to Leukotriene B_4 (LTB_4), which is a dihydroxy fatty acid formed from arachidonic acid by the 5-lipoxygenase pathway[2]. LTB_4 is one of the most powerful mediators involved in inflammatory processes. Binding of LTB_4 to the receptor particularly activates neutrophilic leukocytes and triggers chemotaxis, degranulation and oxidative burst. In particular, neutrophilic leukocytes are readily attracted and activated by LTB_4, producing an accumulation of neutrophils and also macrophages, T lymphocytes and eosinophils at the site of inflammation. Thus, LTB_4 has been suggested to be an important participant in the pathophysiology of inflammatory processes of many human diseases with unmet medical need. The inhibition of LTB_4 has caused a reduction of inflammatory processes in various diseases models *in vivo*[1,2].

A crystal structure of the LTB_4 receptor was published in 2018 by Hori *et al.* [3].

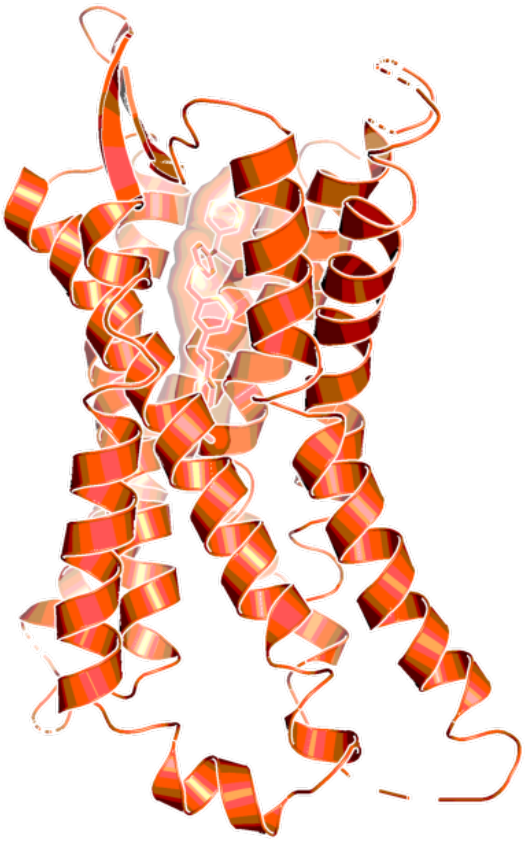

Figure 3: Crystal structure of LTB4 receptor with BIIL 260[3].

In vitro activity

Due to its prodrug character, BIIL 284 displays negligible affinity to the LTB_4 receptor (K_i = 230 nM). BIIL 315 (K_i = 1.9 nM), which is formed from BIIL 284, however, binds with high affinity to the LTB_4 receptor. BIIL 315 potently inhibits LTB_4-induced intracellular Ca^{2+} release in human neutrophils (IC_{50} value = 0.75 nM) as measured with Fura-2.[1] In the presence of 0.1% BSA, the inhibitory potency was reduced by 6-fold due to protein binding. Additionally, BIIL 315 (IC_{50} = 0.65 nM) potently inhibits LTB_4-induced chemotaxis of human polymorphonuclear leukocytes (PMNLs)[1]. LTB_4 receptor kinetic analysis of BIIL 315 revealed slow off-dissociation. The K_i value calculated from the kinetic is 4 pM for BIIL 315 on human neutrophil granulocyte membranes. Consequently, BIIL 315 is the dominating LTB_4 antagonist *in vivo* and a highly potent *in vitro* tool[4]. The negative control BIIS 035 did not display any binding affinity and can be used for *in vitro* experiments.

PROBE NAME	BIIL 284	BIIL 315	BIIS 035
MW [Da, free base][a]	538.6	642.7	610.7
LTB_4 receptor binding (K_i) [nM][b]	230[c] 221[d]	1.9[c] 1.1[d]	>1,000
Inhibition of LTB_4-induced Ca^{2+} (IC_{50}) [nM][b]	---	0.75[e] 4.3[f]	---
Inhibition of LTB_4-induced chemotaxis in human PMNLs (IC_{50}) [nM][b]	---	0.65	---

[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
[b] Assay conditions are described in reference 1
[b] intact cells (polymorphonuclear leukocytes (PMNLs)) used
[c] human neutrophil granulocyte cell membranes used
[d] without BSA
[e] with 0.1% BSA[1]

In vitro DMPK and CMC parameters

BIIL 315 displays good metabolic stability *in vitro*. However, it should not be used *in vivo*. Please use BIIL 284 for *in vivo* studies, which is also available on opnMe.

PROBE NAME	BIIL 315	BIIS 035
logD @ pH 2 / 7.4 / 11	2.14 / - / 2.16	4.2 / - / >6.0
Solubility @ pH 6.8 [µg/mL]	<1.0	<1
Caco-2 permeability AB @ pH 7.4 [$*10^{-6}$ cm/s]	<0.01	n.a.
Caco-2 efflux ratio	n.a.	n.a.
Microsomal stability (human/mouse/rat) [% Q_H]	- / <23 / <22	n.a.
Plasma Protein Binding (human/mouse/rat) [%]	99.7/99.9/99.8	n.a.

*Missing data will be generated soon and added to the profile.

In vivo DMPK parameters

In vivo studies revealed BIIL 315 as the dominating active metabolite in rats after p.o. administration of BIIL 284. For *in vivo* studies please use the *in vivo* prodrug BIIL 284, which is also available on opnMe.

BIIL 315	RAT
Clearance [ml/min/kg][a]	8.3
Mean residence time after *i.v.* dose [h][a]	0.31
t_{max} [h][b]	2.0
C_{max} [nM][b]	4,800
F [%][b]	7.5
V_{ss} [l/kg][a]	0.15
$AUC_{0-\infty}$ [ng*h/ml]	13,000
$t_{½}$[b]	3.1

[a] *i.v.* dose rat: 0.92 mg/kg
[b] *p.o.* dose rat: 70 mg/kg as a solution in Labrasol

In vivo pharmacology

The efficacy of BIIL 315 has been demonstrated in various *in vivo* LTB_4 models by *p.o.* administration of the *in vivo* prodrug BIIL 284. These models comprised

- Inhibition of LTB_4-induced mouse ear inflammation (ED_{50} = 0.0082 mg/kg *p.o.*)
- Inhibition of LTB_4-induced transdermal chemotaxis in guinea pigs (ED_{50} = 0.028 mg/kg *p.o.*)

For more details, please see reference 1[1].

In addition, BIIL 284 and its metabolite BIIL 315 have been investigated in disease models for asthma, rheumatoid arthritis and skin inflammation. The compound demonstrated significant efficacy in a collagen induced arthritis mouse model and reduced the antigen-induced eosinophilic bronchial influx in guinea pigs (asthma model). In the skin inflammation model a psoriasis like dermatitis LTB_4-induced skin effects can be antagonized with BIIL 284. Furthermore, BIIL 284 and its metabolite BIIL 315 counteracts with arachidonic acid induced skin inflammation in mice, however dermatitis is not blocked completely[4].

Negative control

BIIS 035 displays no affinity to the LTB_4 receptor and therefore can be used as negative control for *in vitro* experiments.

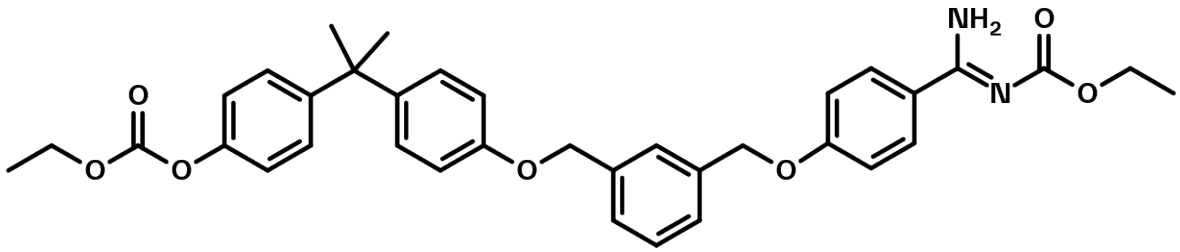

Figure 4 : BI-2853 which serves as a negative control

Selectivity

BIIL 315 is a selective LTB_4 antagonist with no relevant off-target effects in the Eurofins Safety Panel 44™.

SELECTIVITY DATA AVAILABLE	BIIL 315	BIIS 035
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.

A crystal structure of leukotriene B4 receptor in complex with BIIL 260 was published by Hori *et al.* (PDB code: 5X33)[3].

Metabolism of prodrug BIIL 284 to BIIL 260 and BIIL 315

BIIL 315 was identified as the major component in plasma after *p.o.* administration and appears to be the dominating LTB_4 antagonist of BIIL 284 *in vivo*. The major site of metabolism seems to be localized primarily in the gut wall. Thereby, ubiquitous esterases will convert BIIL 284 to BIIL 260, which will be further glucuronidated by UDP-glucuronyl-tranferases. Consequently, *p.o.* administration of BIIL284 will lead to BIIL 315 by fast metabolism. Intravenous administration of BIIL 284 and BIIL 260 will lead only to minor formation of the more potent BIIL 315 metabolite. Furthermore, BIIL 284 displays very low solubility causing crystallization of the compound in plasma. Combining these two aspects, BIIL 284 should only be used as *in vivo* tool using p.o. administration[4].

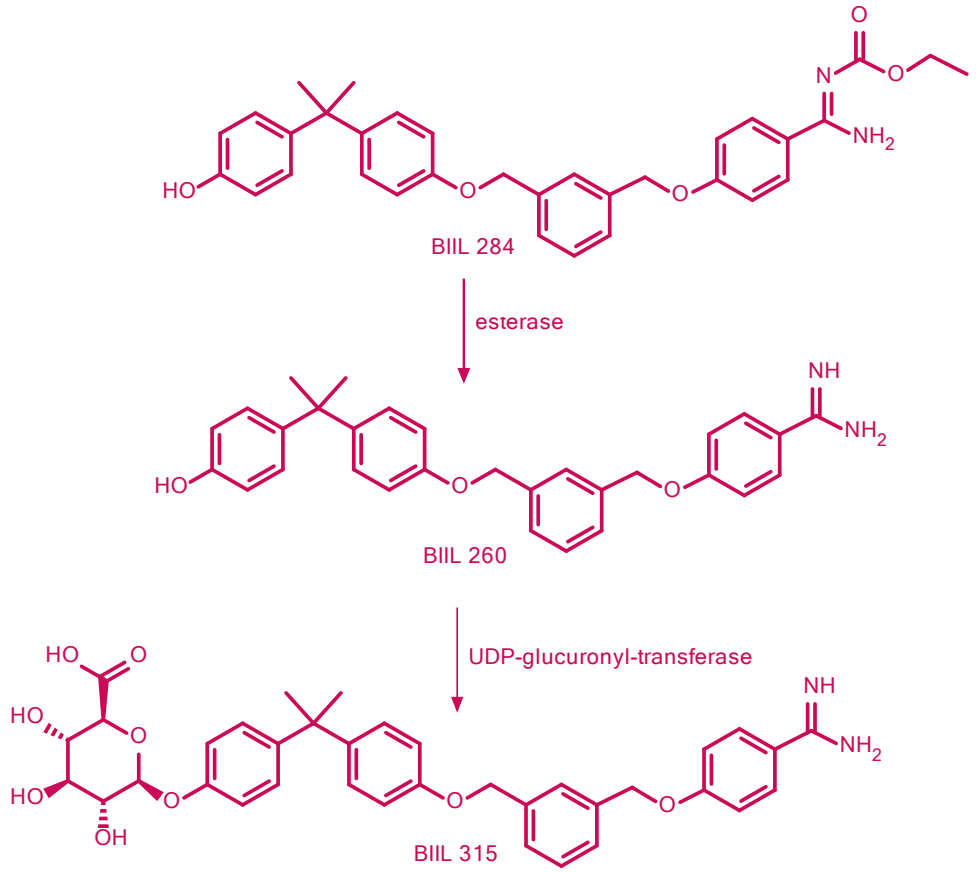

Figure 5 : Metabolism of prodrucg BIIL 284 to BIIL 315

Supplementary data

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

References

1. Birke F. W., Meade C. J., Anderskewitz R., Speck G. A., Jennewein H. M. In vitro and in vivo pharmacological characterization of BIIL 284, a novel and potent leukotriene B(4) receptor antagonist *J Pharmacol Exp Ther* **2001**, 297(1), 458–466, PubMed: 11259574.

2. Saeki K., Yokomizo T. Identification, signaling, and functions of LTB4 receptors *Semin Immunol* **2017**, 33, 30–36. DOI: 10.1016/j.smim.2017.07.010, PubMed: 29042026.

3. Hori T., Okuno T., Hirata K., Yamashita K., Kawano Y., Yamamoto M., Hato M., Nakamura M., Shimizu T., Yokomizo T., Miyano M., Yokoyama S. Na+-mimicking ligands stabilize the inactive state of leukotriene B4 receptor BLT1 *Nat Chem Biol* **2018**, 14(3), 262–269. DOI: 10.1038/nchembio.2547, PubMed: 29309055.

4. Raychaudhuri A., Kotyuk B., Pellas T. C., Pastor G., Fryer L. R., Morrissey M., Main A. J. Effect of CGS 25019C and other LTB4 antagonists in the mouse ear edema and rat neutropenia models *Inflamm Res* **1995**, 44 Suppl 2, S141-2. DOI: 10.1007/bf01778302, PubMed: 8548368.

5. Hicks A., Monkarsh S. P., Hoffman A. F., Goodnow R. Leukotriene B4 receptor antagonists as therapeutics for inflammatory disease: Preclinical and clinical developments *Expert Opin Investig Drugs* **2007**, 16(12), 1909–1920. DOI: 10.1517/13543784.16.12.1909, PubMed: 18042000.

6. Tong W.-G., Ding X.-Z., Hennig R., Witt R. C., Standop J., Pour P. M., Adrian T. E. Leukotriene B4 receptor antagonist LY293111 inhibits proliferation and induces apoptosis in human pancreatic cancer cells *Clin Cancer Res* **2002**, 8(10), 3232–3242, PubMed: 12374694.

7. Alten R., Gromnica-Ihle E., Pohl C., Emmerich J., Steffgen J., Roscher R., Sigmund R., Schmolke B., Steinmann G. Inhibition of leukotriene B4-induced CD11B/CD18 (Mac-1) expression by BIIL 284, a new long acting LTB4 receptor antagonist, in patients with rheumatoid arthritis *Ann Rheum Dis* **2004**, 63(2), 170–176. DOI: 10.1136/ard.2002.004499, PubMed: 14722206.