



LTB₄ receptor antagonist | BIIL 315

Table of contents

Summary	2
Chemical Structure	2
Highlights	3
Target information	3
<i>In vitro</i> activity	4
<i>In vitro</i> DMPK and CMC parameters	4
<i>In vivo</i> DMPK parameters	5
<i>In vivo</i> pharmacology	6
Negative control	6
Selectivity	7
Co-crystal structure of the Boehringer Ingelheim probe compound and the target protein.	7
Metabolism of prodrug BIIL 284 to BIIL 260 and BIIL 315	7
Supplementary data	8
References	8

Summary

BIIL 315 is a highly potent LTB₄ receptor antagonist ($K_i = 1.9 \text{ nM}$).¹ It is the active metabolite of the *in vivo* tool compound BIIL 284 and is the ideal tool to study LTB₄ antagonism *in vitro*. Additionally, BIIS 035 is available as negative control for *in vitro* experiments.

Chemical Structure

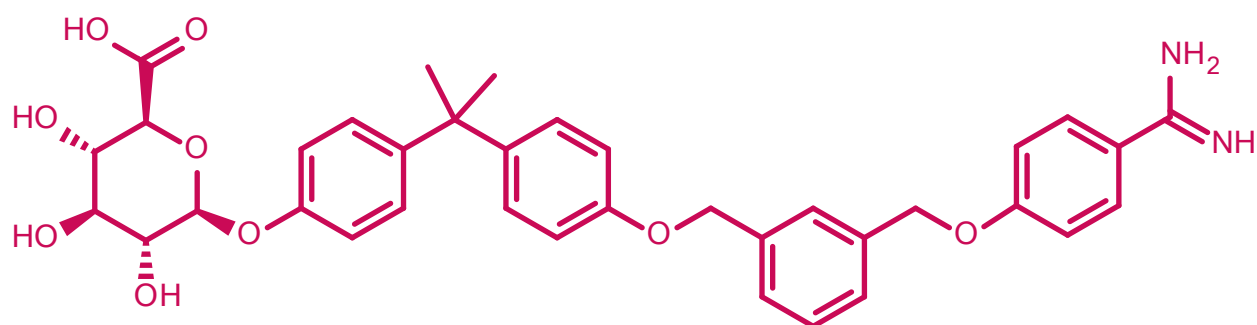


Figure 1: 2-D structure of BIIL 315, a potent LTB₄ antagonist.

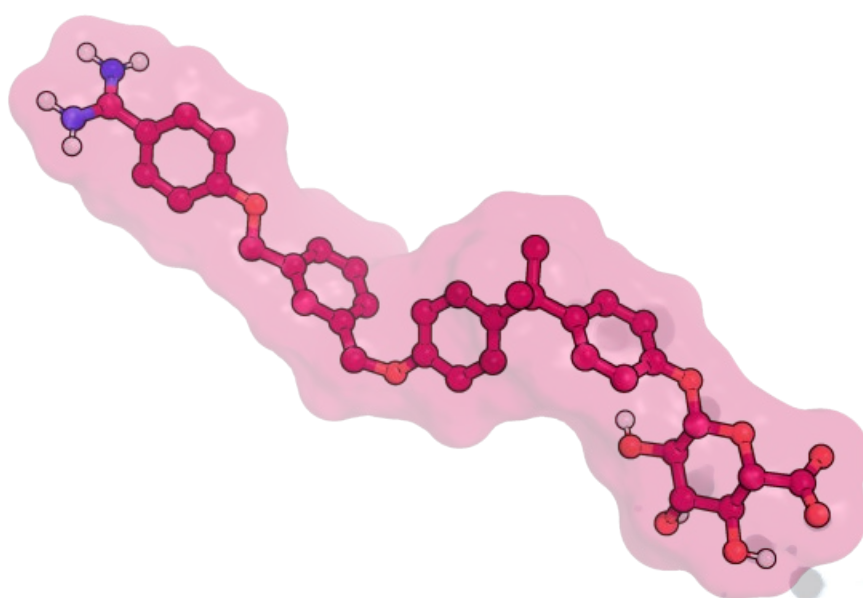


Figure 2: BIIL 315, 3D conformation.

Highlights

BIIL 315 is a highly potent and selective LTB₄ receptor antagonist. This compound binds with high affinity to the LTB₄ receptor ($K_i = 1.9$ nM) and shows good metabolic stability *in vitro*. It is an excellent tool to study LTB₄ 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 LTB₄ receptor is a G-protein coupled receptor (GPCR) with high affinity specifically to Leukotriene B₄ (LTB₄), which is a dihydroxy fatty acid formed from arachidonic acid by the 5-lipoxygenase pathway.² LTB₄ is one of the most powerful mediators involved in inflammatory processes. Binding of LTB₄ to the receptor particularly activates neutrophilic leukocytes and triggers chemotaxis, degranulation and oxidative burst. In particular, neutrophilic leukocytes are readily attracted and activated by LTB₄, producing an accumulation of neutrophils and also macrophages, T lymphocytes and eosinophils at the site of inflammation. Thus, LTB₄ 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 LTB₄ has caused a reduction of inflammatory processes in various diseases models *in vivo*.^{1,2}

A crystal structure of the LTB₄ receptor was published in 2018 by Hori *et al.*³

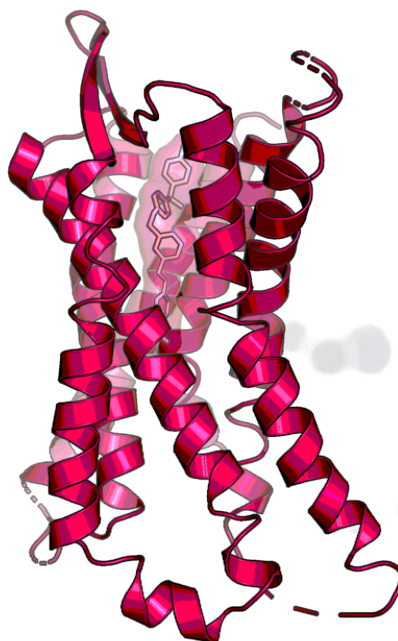


Figure 3: Crystal structure of LTB₄ receptor with BIIL 260.³

In vitro activity

Due to its prodrug character, BIIL 284 displays negligible affinity to the LTB₄ 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 LTB₄ receptor. BIIL 315 potently inhibits LTB₄-induced intracellular Ca²⁺ release in human neutrophils (IC₅₀ value of 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 (IC₅₀ = 0.65 nM) potently inhibits LTB₄-induced chemotaxis of human polymorphonuclear leukocytes (PMNLs).¹ LTB₄ 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 LTB₄ 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]	538.6	642.7	610.7
LTB ₄ receptor binding (K _i) [nM] ^a	230 ^b 221 ^c	1.9 ^b 1.1 ^c	>1,000
Inhibition of LTB ₄ -induced Ca ²⁺ (IC ₅₀) [nM] ^a	---	0.75 ^d 4.3 ^e	---
Inhibition of LTB ₄ -induced chemotaxis in human PMNLs (IC ₅₀) [nM] ^a	---	0.65	---

^a 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 [openMe](#).

PROBE NAME	BIIL 315	BIIS 035
LogD (pH2/pH7.4/pH11)	2.14/ - / 2.16	4.2/-/ >6.0

Solubility @ pH 6.8 [$\mu\text{g/ml}$]	<1.0	<1
CACO permeability @ pH 7.4 [$*10^{-6} \text{ cm/s}$]	<0.01	NA
CACO efflux ratio	NA	NA
Microsomal stability (human/mouse/rat) [% Q _H]	- / <23 / <22	NA
Hepatocyte stability (human/mouse/rat) [% Q _H]	NA	NA
Plasma protein binding (human/mouse/rat) [%]	99.7/99.9/99.8	NA
hERG [inh. % @ 10 μM]	NA	NA

*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 [openMe](#).

PROBE NAME	BIIL 315
	RAT
Clearance [ml/min/kg] ^b	8.3 ^c
Mean residence time after iv dose [h] ^b	0.31 ^c
t _{max} [h] ^a	2.0
C _{max} [nM] ^a	4,800
F [%] ^a	7.5

V_{ss} [l/kg] ^b	0.15 ^c
$AUC_{0-\infty}$ [ng*h/ml] ^a	13,000
$t_{1/2}$ ^a	3.1

^a Rat: 70 mg/kg of BIIL 284 was dosed orally as a solution in Labrasol.

^b Rat: IV administration of 0.92 mg/kg BIIL 284.

In vivo pharmacology

The efficacy of BIIL 315 has been demonstrated in various *in vivo* LTB₄ models by *p.o.* administration of the *in vivo* prodrug BIIL 284. These models comprised

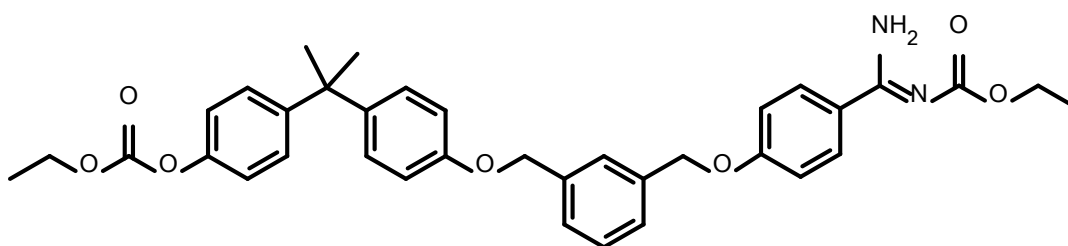
- Inhibition of LTB₄-induced mouse ear inflammation (ED₅₀ = 0.0082 mg/kg *p.o.*)
- Inhibition of LTB₄-induced transdermal chemotaxis in guinea pigs (ED₅₀ = 0.028 mg/kg *p.o.*)

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 LTB₄-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 LTB₄ receptor and therefore can be used as negative control for *in vitro* experiments.



Selectivity

BIIL 315 is a selective LTB₄ antagonist with no relevant off-target effects in the Eurofins Safety Panel 44™.

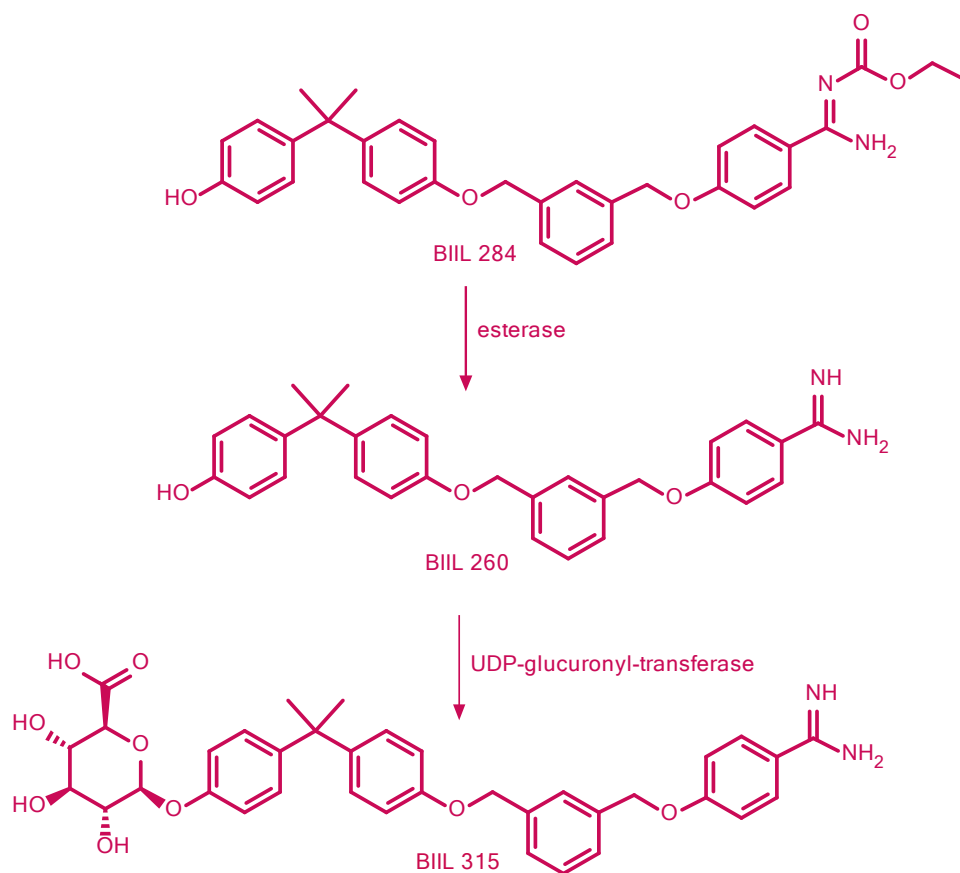
SELECTIVITY DATA AVAILABLE	BIIL 315	BIIS 035
SafetyScreen44™ with kind support of 	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

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 LTB₄ 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-transferases. Consequently, p.o. administration of BIIL 284 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



Supplementary data

2-D structure files can be downloaded free of charge from [opnMe](#).

References

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