opnMe by Boehringer Ingelheim

LTB_4 antagonist

BIIL 284

Boehringer Ingelheim

Table of contents

Summary

Prodrug BIIL 284 represents an excellent oral *in vivo* tool compound to study the effect of LTB_4 receptor antagonism *in vivo*. After *p.o.* administration, it is converted to the highly potent LTB_4 receptor antagonist BIIL 315 which was highly efficacious in various disease models[1]. Please note that BIIL 284 is shipped as a co-crystal of ethanol. The molecular weight of this co-crystal is 584.7 g/mol or Da.

Chemical Structure

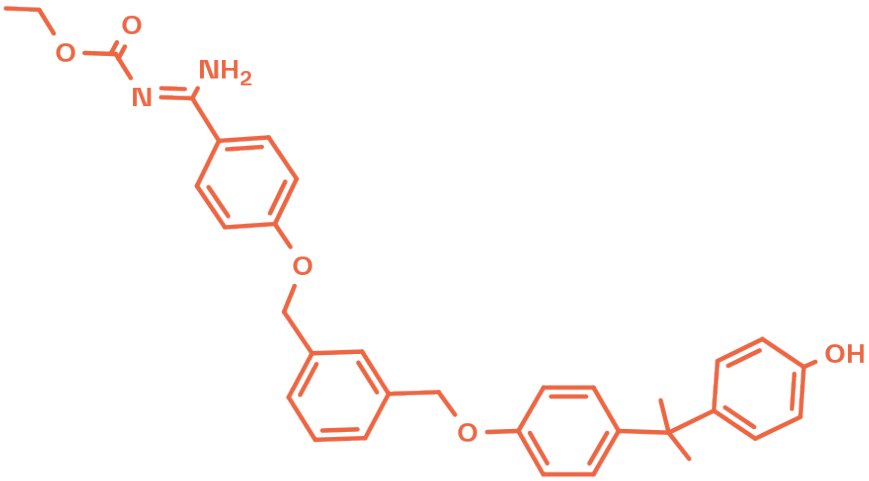

Figure 1: 2D structure of BIIL 284, a prodrug for the LTB4 receptor.

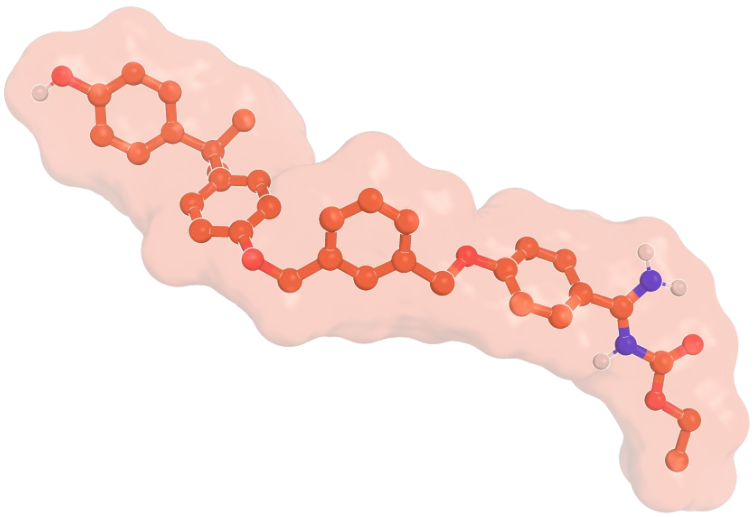

Figure 2: 3D conformation of BIIL 284.

Highlights

BIIL 284 is a Leukotriene B_4 (LTB_4) antagonist prodrug with negligible binding affinity (K_i = 230 nM) and represents an excellent tool for *in vivo* experiments to study LTB_4 receptor antagonism. After p.o. administration the molecule is metabolized to BIIL 315, which is a highly potent LTB_4 receptor antagonist (K_i = 1.9 nM). The compound should only be administered *p.o.* as the gut is the major side of metabolism[1]. Together with BIIL 284, we also offer BIIL 315 on opnMe.com and which is provided to study the effect of LTB_4 signaling *in vitro*.

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 (PDB code: 5X33)[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 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 (IC_{50} = 0.65 nM) potently inhibited 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*[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]	230 221	1.9 1.1	>1000
Inhibition of LTB_4-induced Ca^{2+} (IC_{50}) [nM][b]	---	0.75 4.3	---
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

In vitro DMPK and CMC parameters

BIIL 284 displays low solubility. It should only be administered *p.o.* as the gut is the major side of metabolism[1]. Metabolism is not present after *i.v.* injection of BIIL 284 and additionally causes the compound to crystalize in plasma due to low solubility.

PROBE NAME	BIIL 284	BIIS 035
logD @ pH 2 / 11	3.2 / 5.2	4.1 / >6
Solubility @ pH 7 [µg/mL]	0.2	<1.0
Microsomal stability (human/mouse/rat) [% Q_H]	81 / n.a. / n.a.	n.a.
Hepatocyte stability (human/mouse/rat) [% Q_H]	89 / 96 / n.a.	n.a.
hERG [inh. % @ 10 µM]	12.7	n.a.

**BIIL 284 is a prodrug and seems to be primarily metabolized in the gut wall before absorption.

In vivo DMPK parameters

In vivo studies revealed that BIIL 315 is the dominant active metabolite in rats after *p.o.* administration. BIIL 284 is only found in minor concentrations in plasma. Further studies indicated the gut to be the major site of metabolism. BIIL 284 is only poorly absorbed from standard aqueous suspension media. Administration with the addition of solubilizing agents was tolerated by rat and mini pigs (to a certain degree). Due to low solubility, intravenous administration of BIIL 284 should not be performed because the compound crystallizes out in the plasma[4].

PROBE NAME	BIIL 284
	RAT
Clearance [mL/min/kg][a]	41
Mean residence time after *i.v.* dose [h]	0.80
t_{max} [h][b]	1.0
C_{max} [nM]	24
F [%]	0.25
V_{ss} [L/kg]	1.9
$AUC_{0-\infty}$ [ng*h/ml]	69
$t_{½}$ [h]	1.0

[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 284 was demonstrated in various *in vivo* LTB_4 models such as

- 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 has been investigated in disease models for asthma, rheumatoid arthritis and skin inflammation. BIIL 284 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 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.

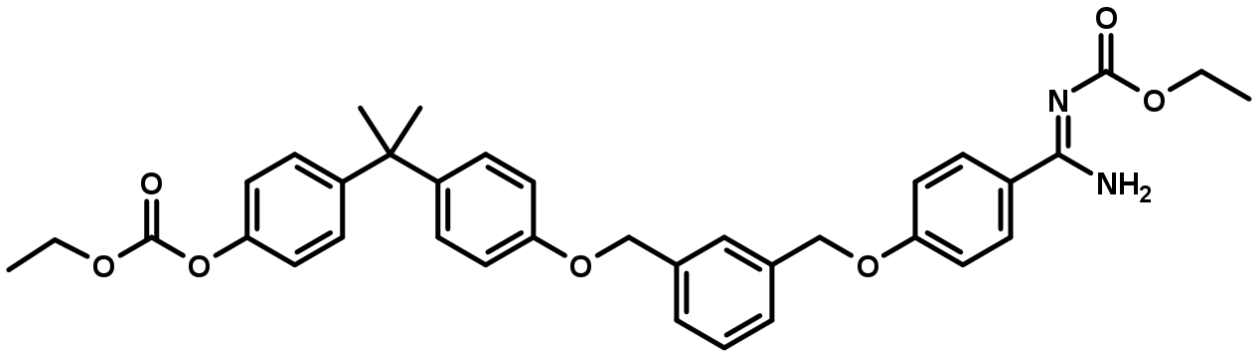

Figure 4: BIIS 035 can be used as a negative control

Selectivity

BIIL 284 is a selective LTB4 antagonist prodrug with no relevant off-target effects in the Eurofins Safety Panel 44™.

SELECTIVITY DATA AVAILABLE	BIIL 284	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 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 glucoronidated 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.

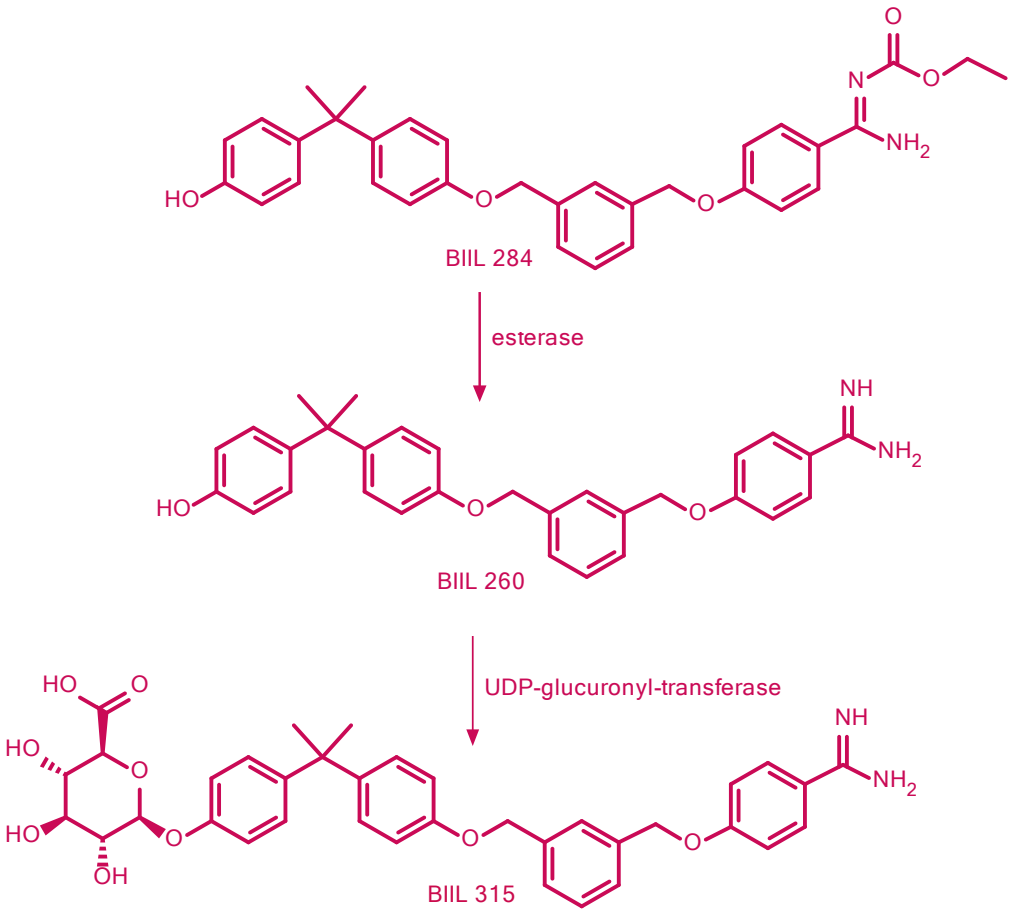

Figure 5 : metabolism of BIIL 284

Supplementary data

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

References

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