FLAP antagonist | BI 665915

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Summary

BI 665915 demonstrates nanomolar FLAP binding potency and is a molecule suitable for testing biological hypotheses *in vitro* and also *in vivo*.

Chemical Structure

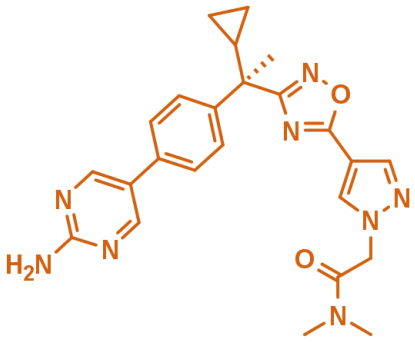

Figure 1: 2-D structure of BI 665915, an inhibitor of FLAP

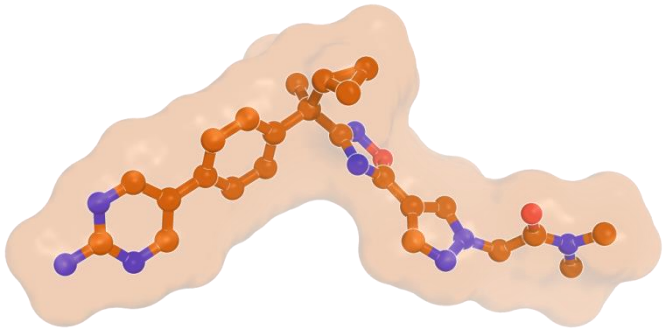

Figure 2: 3-D structure of BI 665915, an inhibitor of FLAP

Highlights

5-Lipoxygenase Activating Protein (FLAP) is an important protein in the Leukotriene pathway. BI 665915 demonstrates excellent FLAP binding potency with an IC_{50} of below 10 nM and potent inhibition of LTB4 synthesis in human whole blood with an IC_{50} of below 100 nM. It is an excellent molecule for testing biological hypotheses *in vitro* and also *in vivo*.[1] A favourable cross-species drug metabolism and DMPK profile and good selectivity make it an excellent molecule for testing

biological hypotheses *in vitro* and *in vivo*. The compound is also active in mice which differentiates it from related compounds as highlighted in a recent review by D. Pettersen et at.[2]

With BI-0153 we also offer a structurally similar molecule which can be used as negative control for *in vitro* experiments due to significant weaker potency (670 nM).

Target information

5-Lipoxygenase Activating Protein (FLAP) is an important protein in the Leukotriene (LT) pathway which acts as a partner of 5-lipoxygenase (5-LO) in the metabolism of arachidonic acid.[3]

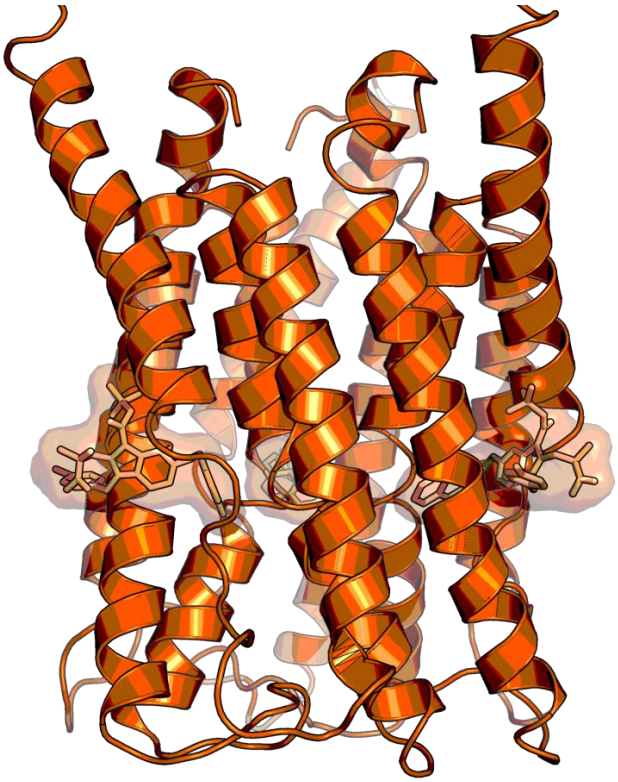

Figure 3: Human FLAP in complex with leukotriene synthesis inhibitors (PDB code: 2q7r)

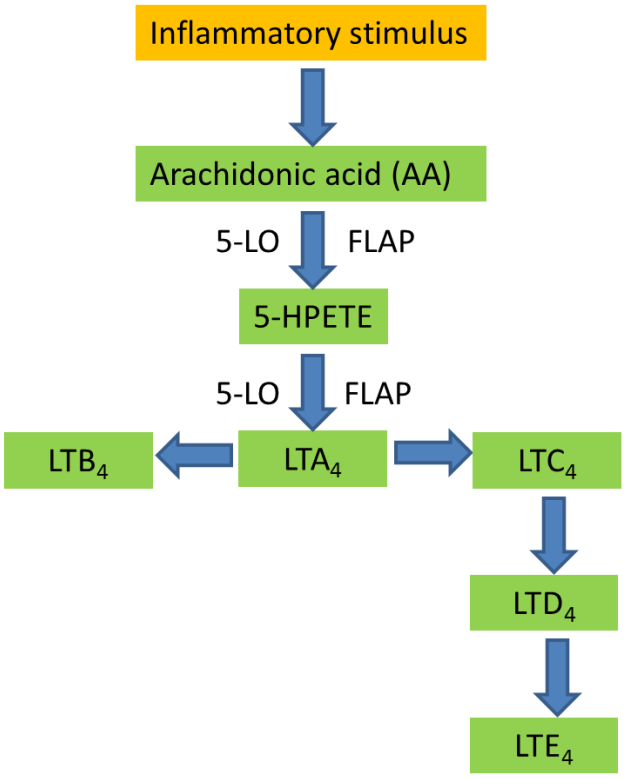

Figure 4: Leukotriene (LT) Pathway[1]

The membrane-attached 5-lipoxygenase activating protein (FLAP) binds to arachidonic acid (AA) and selectively transfers AA to 5-lipoxygenase (5-LO), which oxidizes AA to 5-hydroperoxyeicosatetraenoic acid (5-HpETE) followed by a dehydration to LTA_4.[1,3]

Leukotrienes (LTs) are a family of eicosanoid proinflammatory mediators that are biosynthesized from arachidonic acid (AA) *via* oxidative metabolism.[3] The leukotriene pathway constitutes a series of events underlying the inflammatory components of several diseases such as asthma, allergy, and atherosclerosis.[3,5,6]

More information about the target can be found in the following J. Med. Chem publication[1] by Hidenori Takahashi *et al.* and references cited therein.

In vitro activity

BI 665915 shows a high potency (IC_{50} = 1.7 nM in the FLAP binding assay).

PROBE NAME / NEGATIVE CONTROL	BI 665915	BI-0153[e]
MW [Da]	465	430
FLAP binding (IC_{50}) [nM][a]	1.7	670
FLAP Functional inhibition in human whole blood (IC_{50}) [nM][b]	45	> 5,000
FLAP Functional inhibition in mouse whole blood (IC_{50}) [nM][c]	4,800	n.d.

[a] Binding assay; geometric mean values (n ≥ 3), each determined from duplicate 10-point concentration–response curves; [b]Human whole blood assays; geometric mean values (n ≥ 3), each determined from duplicate 10-point concentration–response curves; [c]Mouse whole blood assaysperformed using the same protocol as that for the hWB assay; geometric mean values (n ≥ 3), each determined from duplicate 8-point concentration–response curves; [d] @ pH 7; [e] Please refer to the section negative control

In vitro DMPK and CMC parameters

PROBE NAME / NEGATIVE CONTROL	BI 665915	BI-0153[a]
Aqueous solubility @ pH 6.8 [µg/ml]	48	>43
CACO permeability @ pH 7.4 [*10^{-6} cm/s]	34	n.d.
CACO efflux ratio	1.9	n.d.
Human hepatocyte clearance [% Q_H]	41	n.d.
Plasma protein binding human [% Q_H]	95.3	n.d.

[a]Please refer to the section negative control

In vivo DMPK parameters

BI 665915 was evaluated in rats, dogs, and cynomolgus monkeys (see table). The compound showed low *iv* plasma clearance over the three species and a good bioavailability of 45 to 63%.

In mice high exposures were observed at a dose of 100 mg/kg ($AUC_{0\text{-}inf}$ = 436,000 nM*h).

In vivo DMPK parameters of BI 665915 in the rat, dog,and cynomolgus monkey[a]

BI 665915	rat	dog	monkey
CL [% Q_H][b,c]	7.0	2.8	3.6
Mean residence time after iv dose (l/kg)[b]	3.1	23	4.8
F [%][b]	63	58	45
V_{ss} [l/kg][b]	0.9	1.2	0.5

[a] Dose = iv, 1 mg/kg; dosing vehicle, 70% PEG; po, 10 mg/kg; dosing suspension vehicle, 0.5% methyl cellulose/0.015% Tween; all DMPK parameters were determined after 11-time point blood sampling (0, 5, 15, 30 min, 1, 2, 4, 6, 8, 12, and 24 h) per iv or po dose.

[b] Mean values (n = 3).

[c] Value represents the percentage of hepatic blood flow.

In vivo pharmacology

BI 665915 shows an attractive DMPK profile and therefore was tested in a mouse *ex vivo* model of mechanism engagement. Blood samples were stimulated with calcimycin, and the levels of LTB_4 were measured. BI 665915 demonstrated dose-dependent LTB_4 production inhibition in mouse whole blood, 2 h after a single oral dose.[1]

Negative control

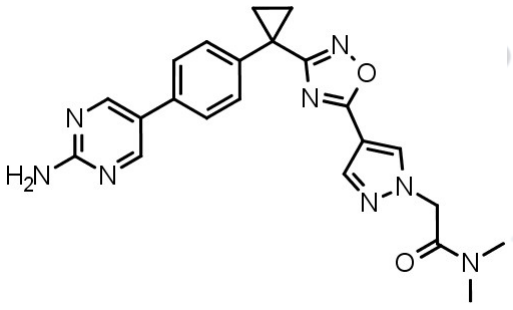

Figure 5: The closely related analogue BI-0153 can be used as an *in vitro* negative control

Selectivity

Extensive external screens covering 751 targets did not give strong hits (see supplementary data section)

Invitrogen panel: 546 kinases < 30% inhibition @ 3µM

Panlabs External screen covering 68 targets: @ 10 µM

Cerep External screen covering 137 targets: @ 20 µM

BI 665915	SELECTIVITY DATA AVAILABLE
Cerep®	Yes
Eurofins-Panlabs®	Yes
Invitrogen®	Yes
DiscoverX®	No
Dundee	No

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

No X-ray co-crystal structure available

Reference molecule(s)

For a recent review on FLAP inhibitors see Reference[2]

Summary

5-Lipoxygenase Activating Protein (FLAP) is an important protein in the Leukotriene pathway. The molecule BI 665915 demonstrates excellent FLAP binding potency with an IC_{50} of below 10 nM and potent inhibition of LTB4 synthesis in human whole blood with an IC_{50} of below 100 nM.[1] A favourable cross-species drug metabolism and DMPK profile and good selectivity make it an excellent molecule for testing biological hypotheses *in vitro* and *in vivo*. With BI-0153 we also can offer a structurally similar molecule as a negative control for *in vitro* experiments due to its

significant weaker potency of 670 nM. By providing this set of molecules we hope to further simulate research in the field.

Supplementary data

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

References

1. Hidenori Takahashi et al. Synthesis, SAR, and Series Evolution of Novel Oxadiazole-Containing 5-Lipoxygenase Activating Protein Inhibitors: Discovery of 2-[4-(3-{(R)-1-[4-(2-Amino-pyrimidin-5-yl)-phenyl]-1-cyclopropyl-ethyl}-[1,2,4]oxadiazol-5-yl)-pyrazol-1-yl]-N,N-dimethyl-acetamide (BI 665915) *J. Med. Chem.* **2015**, *58*, 1669-1690. **DOI:** 10.1021/jm501185j, PubMed.

2. Daniel Pettersen, Öjvind Davidsson, Carl Whatling Recent advances for FLAP inhibitors *Bioorg. Med. Chem. Lett.* **2015** *25* 2607-2612. DOI: 10.1016/j.bmcl.2015.04.090, PubMed.

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5. Jeffery M. Drazen, Elliot Israel, Paul M. O'Byrne Treatment of Asthma with Drugs Modifying the Leukotriene Pathway *N. Engl. J. Med.* **1999**, *340*, 197-206. DOI: 10.1056/NEJM199901213400306, PubMed.

6. Etty Osher, Gary Weisinger, Rona Limor, Karen Tordjman, Naftali Stern The 5 lipoxygenase system in the vasculature: Emerging role in health and disease *Mol. Cell. Endcrinol.* **2006**, *252*, 201–206. DOI: 10.1016/j.mce.2006.03.038, PubMed.

7. Lars Anders Bylock WO2013113799 A1