PAF receptor antagonist | Bepafant

Table of contents

Summary	2
Chemical Structure	2
Highlights	3
Target information	3
In vitro activity	4
In vitro DMPK and CMC parameters	6
In vivo PK parameters	7
In vivo pharmacology	8
Negative control	9
Selectivity	9
Reference molecule(s)	9
Summary	10
Supplementary data	10
References	10

Summary

Bepafant is a synthetic racemic platelet-activating-factor receptor (PAFR) antagonist based on the hetrazepine scaffold. It is a pharmacologically more potent derivative of Apafant. We also provide the PAFR antagonist, Apafant and the active enantiomer of Bepafant, S-Bepafant. The inactive distomer WEB2387 constitutes as a suitable negative control.

Chemical Structure

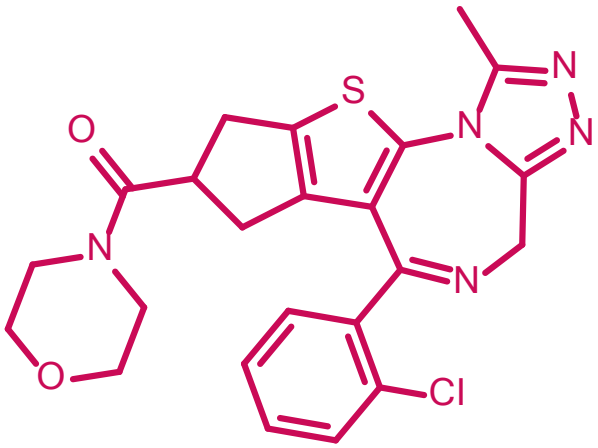

Figure 1: 2-D structure of Bepafant

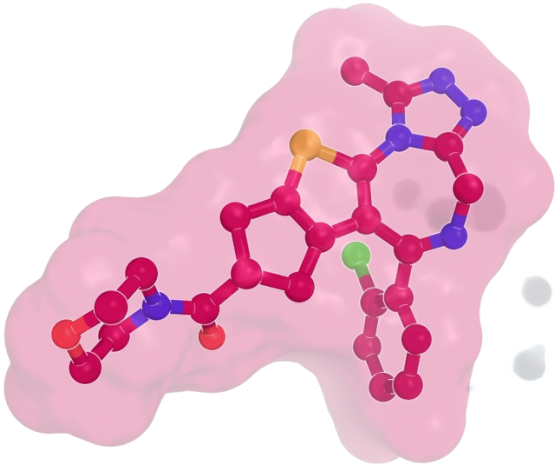

Figure 2: Bepafant, 3D conformation

Highlights

Bepafant is a potent and specific synthetic antagonist of the pro-inflammatory platelet activating factor (PAF) receptor. In competition experiments with [^{3}H]PAF, Bepafant displaces the natural ligand PAF with an equilibrium dissociation constant (K_D) of 16 nM, thereby inhibiting the signalling function of PAFR. PAF-induced human platelet and neutrophil aggregation is inhibited *in vitro* at IC_{50}'s of 310 and 830 nM, respectively. *In vivo*, extensive investigations using a range of animal models of human disease showed Bepafant to potently reduce bronchoconstriction, hypotension, microvascular leakage, and anaphylactic shock amongst many others. Bepafant represents a pharmacologically improved derivative of the previously described Apafant, showing higher potency in *in vivo* models. It is presented as a racemic mixture composed of the active eutomer *S*-Bepafant, and the inactive distomer WEB2387 [1-3,9-11,13].

Since its first disclosure in 1990, Bepafant has become a widely employed molecule for the *in vitro* and in *vivo study* of the PAF pathway, and has, as Apafant[4,5], been investigated in clinical studies.

Apafant and the active enantiomer of Bepafant, *S*-Bepafant are also available to order on opnMe.

Target information

The platelet-activating-factor receptor (PAFR) is a G-protein-coupled seven-transmembrane receptor that plays a profound role in stimulating inflammatory and thrombotic responses. PAFR is activated by platelet-activating-factor (PAF), which comprises a family of structurally related agonistic phospholipids that bind with high affinity to the receptor. PAFR stimulation mediates numerous cellular responses such as activation of the mitogen-activated protein kinase (MAPK) pathway, phosphoinositol turnover, platelet and granulocyte aggregation, and chemotaxis of leukocytes. PAF levels are elevated in disease tissues and fluids that lead to, amongst others, systemic hypotension, increased vascular permeability and thrombocytopenia. The interest in PAFR as a therapeutic target by inhibiting its function is underlined by its association with over 40 disease states that range from asthma to cancer. A number of diverse antagonists and inverse agonists of PAFR have been described that are either based on the original phospholipid structures or natural products, or entirely novel synthetic scaffolds. Bepafant represents a potent and well-characterised member of the latter class[3,6,7,8].

Figure 3: PAF receptor in complex with the ligand SR 27417, indicating the presumed binding location of Apafant and Bepafant, as determined by X-ray crystallography (PDB code 5ZKP, Nat Struct Mol Biol **25**: 488-495, **2018**)

In vitro activity

Bepafant binds with high affinity to the PAF receptor on human platelets, as determined by displacement of the natural ligand PAF from the PAFR receptor complex. Moreover, PAF-induced aggregation of both human platelets and neutrophils is inhibited by Bepafant in a dose-dependent manner. The interaction is specific as neither Bepafant (or Apafant) have significant effects on platelet or neutrophil aggregation in response to other aggregating agents[1,11]. Despite the structural similarity of thienotriazolodiazepines to the CNS-acting benzodiazepines, Bepafant only shows weak cross-reactivity to the central benzodiazepine receptor[2]. As with Apafant, both compounds display relatively low partition coefficients (logD, see below) resulting in low brain exposure[2], and the related Apafant did not induce observable benzodiazepine-like effects in humans[4].

	APAFANT	BEPAFANT	*S*-BEPAFANT	NEGATIVE CONTROL WEB2387
MW [Da]	455.97	467.97	467.97	467.97
Assay A: Receptor Binding (K_D) [nM], human	15[2]	16[9]	14[9]	660[9]
Assay B: Platelet aggregation (IC_{50}) [nM], human	170[1,11]	310[9,11]	350[9]	8790[9]
Assay C: Neutrophil aggregation (IC_{50}) [nM], human	360[1]	830[10]	n.d.	n.d.
Assay D: Benzodiazepine receptor inhibition (Ki) [nM], rat	388[2]	3495[2]	n.d.	n.d.

Assay A: Tritiated [^{3}H]PAF binding to human platelets was inhibited by addition of increasing concentrations of Bepafant, from which the K_D was determined. Refer to respective references for detailed methods.

Assay B: Platelet-rich plasma isolated from human venous blood was collected, and aggregation was induced by addition of PAF. The aggregation inhibitory effect of the antagonists was determined by adding various concentrations to the reaction mixture one minute prior to the addition of PAF. Refer to respective references for detailed methods.

Assay C: Human leukocytes were isolated from human venous blood. Aggregation was induced by addition of PAF, and the aggregation inhibitory effect of the antagonists was determined by adding various concentrations to the reaction three minutes prior to the addition of PAF. Refer to respective references for detailed methods.

Assay D: Selectivity to benzodiazepine receptors was tested through inhibition of [^{3}H]flunitrazepam binding to rat cortex synaptosomal membranes as a function of PAF antagonist concentration. Refer to respective references for detailed methods.

In vitro DMPK and CMC parameters

	APAFANT	BEPAFANT	*S*-BEPAFANT	NEGATIVE CONTROL WEB2387
Solubility at pH 2.0/6.8 [µg/ml]	55 / >100	33 / >100	51 / >100	44 / 86
logD at pH2/pH11	1.08 / 1.12	1.21 / 1.15	1.2 / 1.14	1.18 / 1.12
ClogP	0.98	0.87	0.87	0.87
Plasma protein binding (%) human/rat	degradation / 65	54 / 33	38 / 34	n.d. / n.d.
CACO permeability @ pH 7.4 [*10^{-6} cm/s]	3.2	11.8	7.1	15.1
CACO efflux ratio	14.5	6.4	4.9	6.8
Microsomal stability (human/rat) [% Q_H]	24.9/38.3	<23/25.4	<23/24.3	<23/25.1
MDCK permeability P_{app}a-b/b-a @ 1µM [10^{-6} cm/s]	0.25	1.1	0.94	0.72
MDCK efflux ratio	7	20.9	25.5	43.1
Hepatocyte stability (human/rat) [% Q_H]	20/54	7/55	<4/48	6/58
CYP 3A4 (IC_{50}) [µM]	>50	n.d.	>50	n.d.
CYP 2D6 (IC_{50}) [µM]	>50	n.d.	>50	n.d.

CYP 2C8 (IC_{50}) [µM]	>50	n.d.	>50	n.d.
CYP 2C9 (IC_{50}) [µM]	>50	n.d.	>50	n.d.
CYP 2C19 (IC_{50}) [µM]	>50	n.d.	>50	n.d.

In vivo PK parameters

CODE	APAFANT	BEPAFANT	*S*-BEPAFANT	NEGATIVE CONTROL WEB2387
t_{max} [h] rat (p.o.)	0.3	0.8	n.d.	n.d.
C_{max} [nM] rat (p.o.)	449 [a]	491 [b]	n.d.	n.d.
Clearance [ml/(min*kg)]]	n.d.	76 [c]	44 [d]	n.d.
Mean residence time after iv dose [h] rat	n.d.	0.38	0.5	n.d.
F [%]	n.d.	37	n.d.	n.d.
V_{ss} [l/kg]	n.d.	1.7	1.3	n.d.
$t_{1/2}$ [h], guinea pig, p.o.[11]	5.5	12.1	n.d.	n.d.
$t_{1/2}$ [h], rat, p.o.[1]	3.1	5.4	n.d.	n.d.

[a] 11 µmol/kg, [b] 10.3 µmol/kg, [c] 1.02 µmol/kg, [d] 2.08 µmol/kg

In vivo pharmacology

Acute bronchoconstriction induced by intravenously administered PAF is widely used to characterise PAF antagonists in animal models, where the antagonist efficacy is quantified by determining the recovery of respiratory flow and mean arterial pressure (MAP, a measure of hypotension).

Bepafant displays an ED_{50} of 0.021 and 0.007 mg/kg in guinea pigs when administered orally and intravenously, respectively, and the ED_{50} for MAP is comparable. Thus, despite the similar *in vitro* properties compared with Apafant, Bepafant displays superior *in vivo* potency[10]. This is likely caused by the increased $t_{1/2}$ of Bepafant (see table above)[11]. The eutomer of Bepafant (*S*-Bepafant) shows an additional slight increase in potency compared to the racemic Bepafant, while the distomer (WEB2387, negative control) shows a 40-80-fold reduction of *in vivo* potency compared to the *S*-Bepafant[12].

PROBE NAME / NEGATIVE CONTROL	APAFANT	BEPAFANT	*S*-BEPAFANT	NEGATIVE CONTROL WEB2387
Respiratory flow ED_{50} [mg/kg] p.o.	0.07	0.021	0.018	1.55
Respiratory flow ED_{50} [mg/kg] i.v.	0.018	0.007	0.004	0.081
Mean arterial pressure ED_{50} [mg/kg] p.o.	0.066	0.02	0.027	1.2
Mean arterial pressure ED_{50} [mg/kg] i.v.	0.016	0.006	0.005	0.086

Antigen-induced anaphylactic shock and bronchoconstriction was prevented by Bepafant (and Apafant) in guinea pigs co-treated with the antihistamine mepyramine, with 1.0 mg/kg Bepafant p.o. providing almost complete protection.

In a model of inflammation, both Apafant and Bepafant significantly attenuated PAF-induced paw edema in the rat, with Bepafant showing greater potency in this model.

Various additional pharmacology studies are reviewed in reference 2.

Negative control

WEB2387 is offered as a negative control. It is the distomer (inactive enantiomer) of racemic Bepafant. Thus, WEB2387 is an appropriate negative control for Bepafant and *S*-Bepafant and the structurally related Apafant.

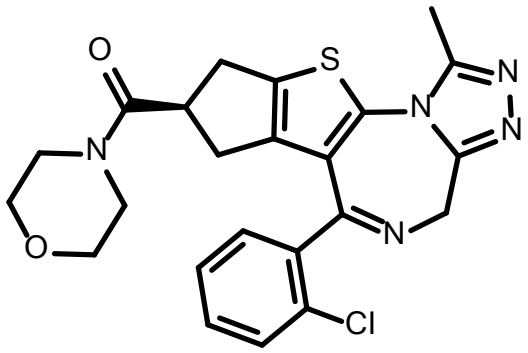

Figure 4: Structure of WEB2387, offered as an appropriate negative control

Selectivity

The SafetyScreen44™ panel has been measured for Bepafant and it showed no relevant off-target effects.

SELECTIVITY DATA AVAILABLE	APAFANT	BEPAFANT	*S*-BEPAFANT	NEGATIVE CONTROL WEB2387
SafetyScreen44™ with kind support of eurofins	Yes	Yes	Yes	Yes
Invitrogen®	No	No	No	No
DiscoverX®	No	No	No	No
Dundee	No	No	No	No

Reference molecule(s)

There are no reference compounds available.

Summary

Bepafant is a synthetic platelet-activating-factor receptor (PAFR) antagonist based on the thienotriazolodiazepine scaffold that has become a mainstay of *in vitro* and *in vivo* studies of the PAF pathway. Bepafant binds with low nanomolar affinity to PAFR, and by competing with the natural ligand PAF, the proinflammatory function of the receptor is inhibited. Bepafant has been intensively investigated in a range of disease models, and demonstrated superior *in vivo* potency compared to Apafant. Additionally, it has been subject to clinical studies in man.

Supplementary data

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

References

1) Casals-Stenzel J, Muacevic G, Weber KH (1987) Pharmacological actions of WEB 2086, a new specific antagonist of platelet activating factor. *J Pharmacol Exp Ther* **1987**, 241, (3), 974-81. PubMed.

2) Weber KH, Heuer HO (1989) Hetrazepines as antagonists of platelet activating factor. *Med Res Rev.* **1989**, Apr-Jun;9(2):181-218. PubMed, DOI: 10.1002/med.2610090204.

3) Hyland IK, O'Toole RF, Smith JA, Bissember AC (2018) Progress in the Development of Platelet-Activating Factor Receptor (PAFr) Antagonists and Applications in the Treatment of Inflammatory Diseases. *Chem Med Chem* **2018**, (13)18, 1873-1884. PubMed, DOI: 10.1002/cmdc.201800401.

4) Brecht HM, Adamus WS, Heuer HO, Birke FW, Kempe ER (1991) Pharmacodynamics, pharmacokinetics and safety profile of the new platelet-activating factor antagonist apafant in man. Arzneimittelforschung;41(1):51-9. PubMed.

5) Tamura G, Takishima T, Mue S, Makino S, Itoh K, Miyamoto T, Shida T, Nakajima S (1996) Effect of a potent platelet-activating factor antagonist, WEB-2086, on asthma. A multicenter, double-blind placebo-controlled study in Japan. *Adv Exp Med Biol* **2020**, 416:371-80. PubMed, DOI: 10.1007/978-1-4899-0179-8_60.

6) Kasperska-Zajac A, Brzoza Z, Rogala B (2008) Platelet-activating factor (PAF): a review of its role in asthma and clinical efficacy of PAF antagonists in the disease therapy. *Recent Pat Inflamm Allergy Drug Discov* **2008**, Jan;2(1):72-6. PubMed, DOI: 10.2174/187221308783399306.

7) Summers JB, Davidsen SK, Sheppard GS (1995) Platelet Activating Factor Antagonists Current Pharmaceutical Design 1, 161-190. ISSN 1381-6128

8) Dalmaso B, Silva-Junior IA, Fragel-Madeira L, Jancar S, Del Debbio CB (2021) Platelet activating factor in the eye: Physiological roles, diseases and future perspectives. *Prostaglandins Other Lipid Mediat* **2021**, Apr;153:106522. PubMed, DOI: 10.1016/j.prostaglandins.2020.106522.

9) Heuer H, Birke F, Brandt K, Muacevi G, Weber KH (1988) Biological characterization of the enantiomeric hetrazepines of the paf-antagonist web 2170. *Prostaglandins* **1988**, (35)5, 847. https://doi.org/10.1016/0090-6980(88)90257-2

10) Heuer HO, Casals-Stenzel J, Muacevic G, Weber KH (1990) Pharmacologic activity of bepafant (WEB 2170), a new and selective hetrazepinoic antagonist of platelet activating factor. *J Pharmacol Exp Ther* **1990**, 255(3):962-8. PubMed.

11) Casals-Stenzel J, Heuer HO (1990) Use of WEB 2086 and WEB 2170 as platelet-activating factor antagonists/at. *Methods in Enzymology* **1990**, (187), 455-465. PubMed, DOI: 10.1016/0076-6879(90)87052-5.

12) Heuer HO, Keller B, Urich K (1991) Action of the racemate and the isomers of the platelet-activating factor antagonist bepafant (WEB 2170) after oral administration to guinea-pigs and rats. *Naunyn Schmiedebergs Arch Pharmacol* **1991**, 343(5):546-50. PubMed, DOI: 10.1007/BF00169560.

13) Bechtel W-D, Casals-Stenzel J, Harreus A, Heuer H, Muacevic G, Stransky W, Walther G, Weber KH (1988), Hetrazepins and processes for their preparation (EP024245).