opnMe by Boehringer Ingelheim

PAF receptor antagonist

Apafant

Boehringer Ingelheim

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Summary

Apafant is a potent and specific synthetic antagonist of the pro-inflammatory platelet activating factor (PAF) receptor since its first disclosure in 1987. It is employed for the *in vitro* and *in vivo* study of the PAF pathway, and has been investigated in clinical studies for indications such as asthma[4,5]. It has been investigated in a range of disease models ranging from inflammatory disorders to cancer. The PAFR antagonist Bepafant, and its active enantiomer, S-Bepafant are also provided. The structurally related WEB2387 is used as negative control.

Chemical Structure

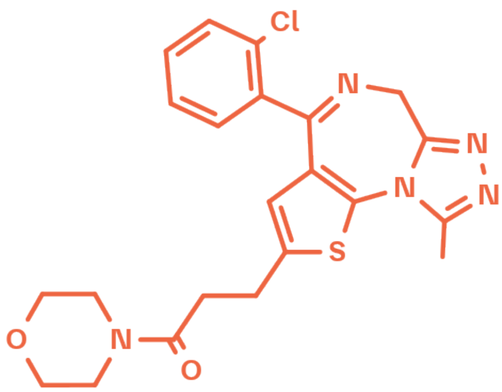

Figure 1: 2D structure of Apafant

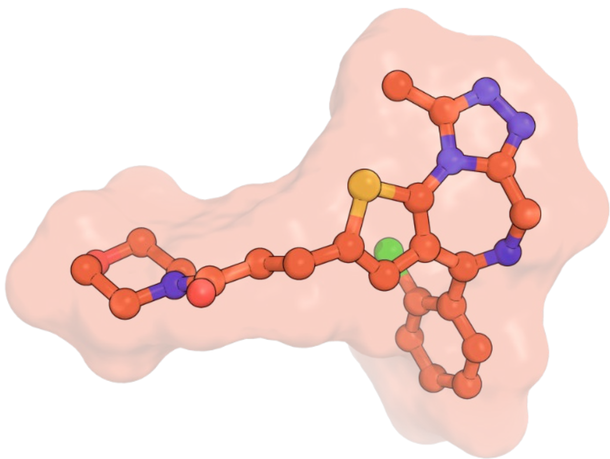

Figure 2: Apafant, 3D conformation

Highlights

Apafant is a potent and specific synthetic antagonist of the pro-inflammatory platelet activating factor (PAF) receptor. It is employed for the *in vitro* and *in vivo* study of the PAF pathway. The PAFR antagonist Bepafant, and its active enantiomer, S-Bepafant are also provided. The structurally related WEB2387 is used as negative control.

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. Apafant 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, as determined by X-ray crystallography (PDB code: 5ZKP[14])

In vitro activity

Apafant 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 Apafant in a dose-dependent manner. The interaction is specific as neither Apafant or Bepafant 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, Apafant shows only modest cross-reactivity to the central benzodiazepine receptor[2]. This activity is attenuated further (10-fold) in the otherwise equally potent Bepafant. Both compounds display relatively low partition coefficients (logD, see below) resulting in low brain exposure[2], and importantly, benzodiazepine-like effects were not observed at high doses in humans[4]. In competition experiments with [^{3}H]PAF, Apafant displaces the natural ligand PAF with an equilibrium dissociation constant (KD) of 15 nM, thereby inhibiting the signaling function of PAFR. PAF-induced human platelet and neutrophil aggregation is inhibited *in vitro* with an IC_{50}'s of 170 and 360 nM, respectively.

	APAFANT	BEPAFANT	*S*-BEPAFANT	NEGATIVE CONTROL WEB2387
MW [Da, free base][a]	456.0	468.0	468.0	468.0
Receptor Binding (K_D) [nM], human[b]	15[2]	16[9]	14[9]	660[9]
Platelet aggregation (IC_{50}) [nM], human[c]	170[1,11]	310[9,11]	350[9]	8790[9]
Neutrophil aggregation (IC_{50}) [nM], human[d]	360[1]	830[10]	n.a.	n.a.
Benzodiazepine receptor inhibition (Ki) [nM], rat[e]	388[2]	3495[2]	n.a.	n.a.

[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] Tritiated [^{3}H]PAF binding to human platelets was inhibited by addition of increasing concentrations of Apafant, from which the K_D was determined. In a reverse experiment, [^{3}H]Apafant was displaced by PAF and Apafant to the same degree. Refer to respective references for detailed methods.

[c] 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 adding various concentrations to the reaction mixture one minute prior to the addition of PAF. Refer to respective references for detailed methods.

[d] 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 adding various concentrations to the reaction three minutes prior to the addition of PAF. Refer to respective references for detailed methods.

[e] Selectivity to benzodiazepine receptors was tested through inhibition of $[^3H]$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 @ pH 2.0/6.8 [µg/mL]	55 / >100	33 / >100	51 / >100	44 / 86
logD @ pH 2 / 11	1.08 / 1.12	1.21 / 1.15	1.2 / 1.14	1.18 / 1.12
Plasma Protein Binding [%] human/rat	degradation / 65	54 / 33	38 / 34	n.a. / n.a.
Caco-2 permeability AB @ pH 7.4 [$*10^{-6}$ cm/s]	3.2	11.8	7.1	15.1
Caco-2 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_{appAB} @ 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.a.
CYP 2D6 (IC_{50}) [µM]	>50	n.d.	>50	n.a.
CYP 2C8 (IC_{50}) [µM]	>50	n.d.	>50	n.a.
CYP 2C9 (IC_{50}) [µM]	>50	n.d.	>50	n.a.
CYP 2C19 (IC_{50}) [µM]	>50	n.d.	>50	n.a.

In vivo PK parameters

CODE	APAFANT	BEPAFANT	*S*-BEPAFANT

t_{max} [h] rat	0.3 [a]	0.8 [b]	n.d.
C_{max} [nM] rat	449 [a]	491 [b]	n.d.
Clearance [mL/(min*kg)]]	n.d.	76 [c]	44 [d]
Mean residence time after *i.v.* dose [h] rat	n.d.	0.38	0.5
F [%]	n.d.	37[b]	n.d.
V_{ss} [L/kg]	n.d.	1.7[c]	1.3
$t_{1/2}$ [h], rat	3.1[a]	5.4[b]	n.d.

[a] *p.o.* dose: 5.3 mg/kg
[b] *p.o.* dose: 5.0 mg/kg
[c] *i.v.* dose: 0.48mg/kg
[d] *i.v.* dose: 1.0 mg/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).

In vivo, extensive investigations using a range of animal models of human disease showed Apafant to potently reduce bronchoconstriction, hypotension, microvascular leakage, and anaphylactic shock amongst many others[1,2,3,13].

Apafant displays an ED_{50} of 0.07 and 0.018 mg/kg in guinea pigs when administered orally and intravenously, respectively, and the ED_{50} for MAP is comparable. Despite the similar *in vitro* properties (see above), Bepafant displays enhanced potency (ED_{50} of 0.016 mg/kg for respiratory flow)[10]. This is likely caused by the increased $t_{1/2}$ for 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 *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

Further studies showed that Apafant inhibits PAF-induced vascular leakage (as measured by the extravasation of Evans blue dye) fully at 10 mg/kg *i.v.* in the guinea pig.

Antigen-induced anaphylactic shock and bronchoconstriction was prevented by both Apafant and bepafant 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 active, racemic Bepafant. Thus, WEB2387 is an appropriate negative control for Bepafant and *S*-Bepafant, and the structurally related Apafant.

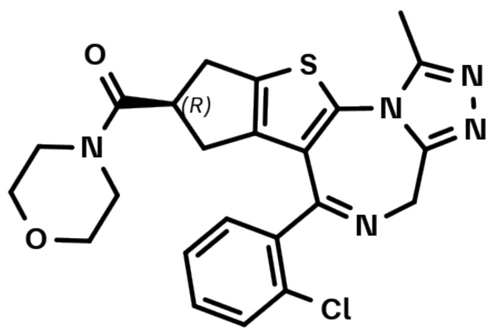

Figure 4: WEB2387 which serves as a negative control

Selectivity

The SafetyScreen44™ panel have been measured for all four compounds 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)

Apafant and analogues are considered reference molecules for PAFR antagonist.

Supplementary data

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

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