PAF receptor antagonist | *S*-Bepafant

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Summary

S- Bepafant is a synthetic platelet-activating-factor receptor (PAFR) antagonist based on the thieno-triazolodiazepine scaffold that has become a mainstay of *in vitro* and *in vivo* studies of the PAF pathway. S-Bepafant is offered as the most potent derivative of the thienotriazolodiazepine class. *S*-Bepafant represents the active isomer of Bepafant and displays somewhat enhanced *in vivo* potency compared to racemic Bepafant. As with racemic Bepafant, *S*-Bepafant binds with low nanomolar affinity to PAFR, and by competing with the natural ligand PAF, the proinflammatory function of the receptor is inhibited. We also provide the PAFR antagonists, Apafant and Bepafant. The inactive distomer WEB2387 is available as a negative control.

Chemical Structure

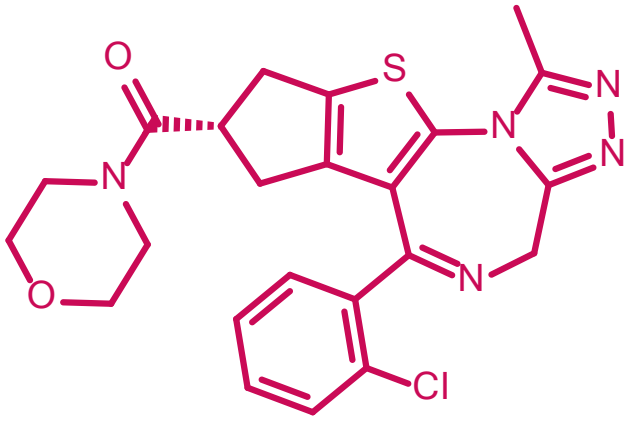

Figure 1: 2-D structure of *S*-Bepafant

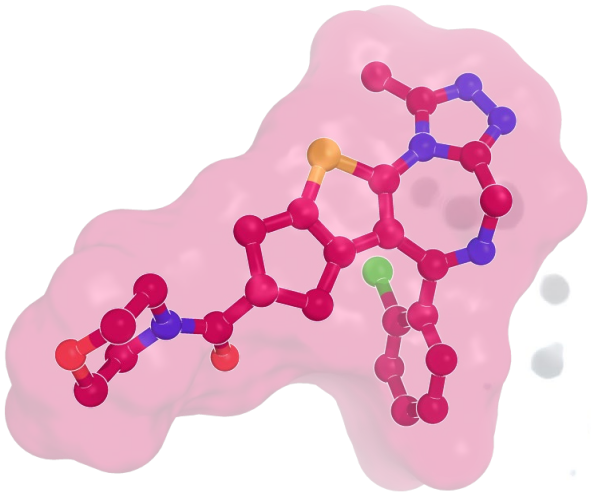

Figure 2: *S*-Bepafant, 3D conformation

Highlights

S-Bepafant, the eutomer of racemic Bepafan, is a specific synthetic antagonist of the pro-inflammatory platelet activating factor (PAF) receptor. A pharmacologically improved derivative of Apafant, is the most potent derivative of thienotriazolodiazepine scaffolds. Is used *in vitro* and *in vivo*, The negative control is the inactive distomer WEB2387.

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. *S*-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

S-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 is inhibited by *S*-Bepafant in a dose-dependent manner. Whilst not specifically tested, the advantageous properties observed for racemic Bepafant will likely also apply to *S*-Bepafant, such as a lack of activity towards the benzodiazepine receptors.

In competition experiments with [^{3}H]PAF, S-Bepafant displaces the natural ligand PAF with an equilibrium dissociation constant (KD) of 14 nM, thereby inhibiting the signaling function of PAFR. PAF-induced human platelet aggregation is inhibited *in vitro* at an IC_{50} of 350 nM.

	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).

In vivo, investigations using several animal models of human disease showed S-Bepafant to potently reduce bronchoconstriction and hypotension, where the efficacy exceeded that of racemic Bepafant. S-Bepafant and Bepafant represent pharmacologically improved derivatives of the previously described Apafant, showing higher potency in *in vivo* models[1-3,9-11,13]. *S*-Bepafant displays an ED_{50} of 0.018 and 0.004 mg/kg in guinea pigs when administered orally and intravenously, respectively, and the ED_{50} for MAP is comparable. Thus, the *in vivo* potency is similar or slightly improved compared to racemic Bepafant[12], and significantly enhanced compared to Apafant. By contrast, negative control WEB2387, the distomer of Bepafant, shows a 40-80-fold reduction of *in vivo* potency, supporting the argument for improved potency of *S*-Bepafant versus racemic Bepafant[12].

S-Bepafant is offered as the most potent derivative of the thienotriazolodiazepine class. The PAFR antagonists, Apafant and Bepafant are also available on opnMe.

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

Various additional pharmacology studies on racemic Bepafant are reviewed in reference 2.

Negative control

WEB2387 is offered as a negative control. It is the inactive *R*-isomer of Bepafant, and the mirror image of active *S*-Bepafant.

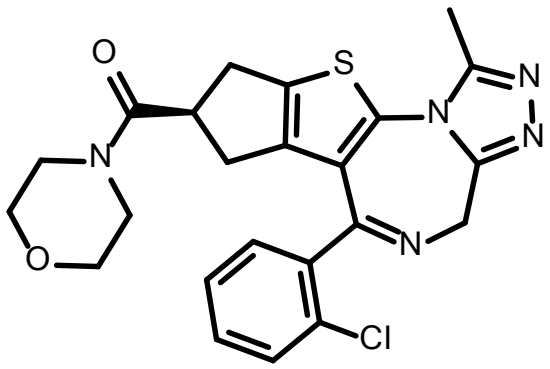

Figure 4: Structure of WEB2387, offered as an appropriate negative control

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.

Supplementary data

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

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