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ANACOR™ PHARMACEUTICALS

Developing Small-Molecule Therapeutics Derived From Our Boron Chemistry Platform.

AN6415: a Novel Highly Potent PDE4 Inhibitor with Oral Activity and Broad Spectrum Cytokine Suppression

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Introduction

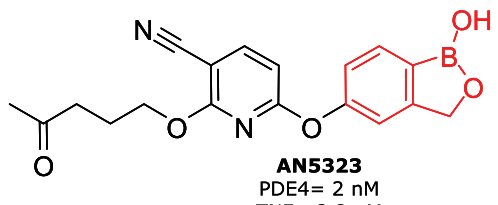
Anacor develops novel boron containing small molecular weight inhibitors of human cAMP specific phosphodiesterase-4 (PDE4) and other enzymes. An early PDE4 inhibitor, AN2728, has shown therapeutic benefit in the topical treatment of psoriasis in Phase IIb clinical trials. Synthetic chemistry was conducted with two aims: 1) improve affinity of PDE4 active benzoxaboroles; 2) improve the pharmacokinetics (PK) to match or exceed that of Roflumilast.

AN6415 was identified as a novel benzoxaborole with affinity and mouse PK parameters superior to Roflumilast and Apremilast, two leading catechol PDE4 clinical candidates.

Benzoxaborole (red)

AN6415
PDE4= 0.81 nM
TNF= 0.82 nM

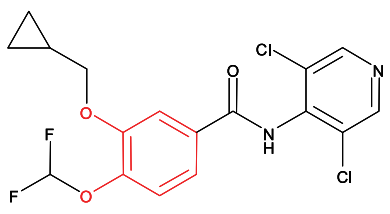
A close relative to AN5323



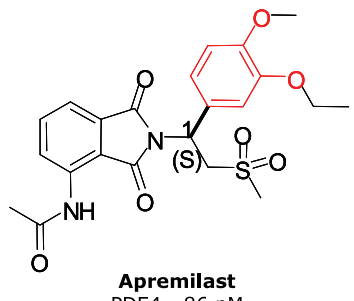
AN5323
PDE4= 2 nM
TNF= 3.2 nM

Catechol (red)

In Clinical trials

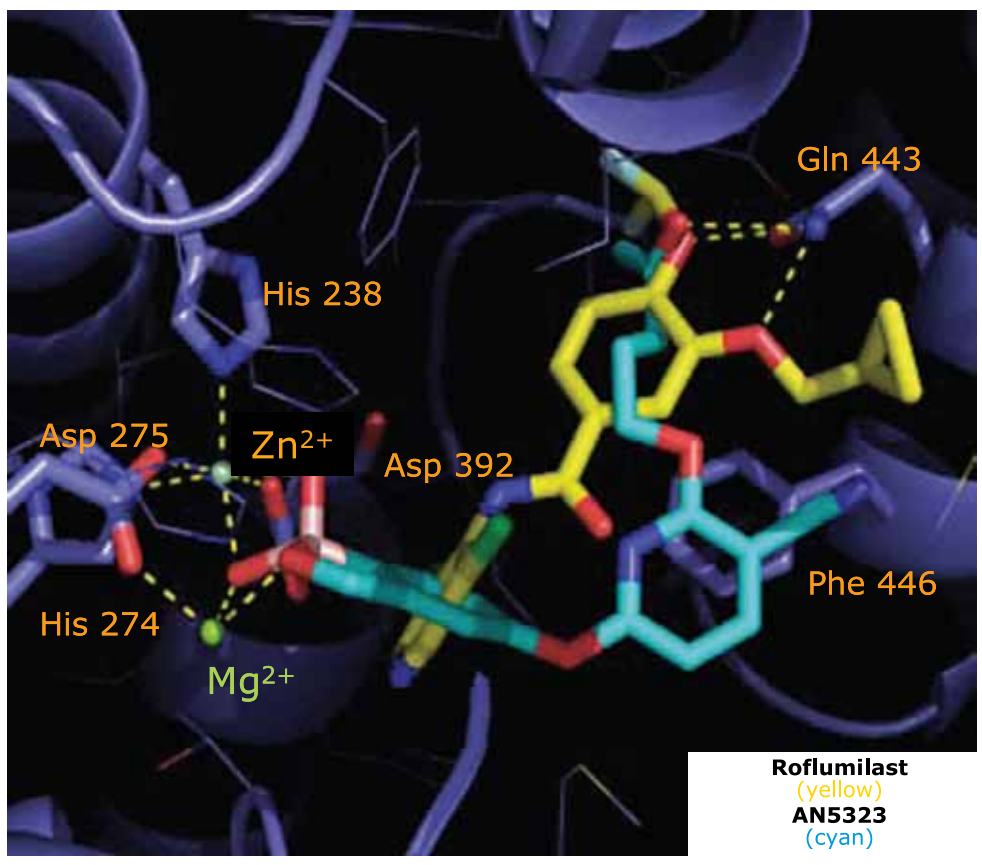


Roflumilast
PDE4= 1.7 nM
TNF= 4.5 nM



Apremilast
PDE4= 86 nM
TNF= 53 nM

Catechol PDE4 Inhibitors take advantage of a deep pocket where adenine binds – AN5323 takes advantage of the same pocket; it also interacts with the metal site, whereas Catechols do not



Roflumilast (yellow)
AN5323 (cyan)

AN6415 is an improvement over the best catechol in Mouse: Oral $t_{1/2}$ is long and bioavailability is high

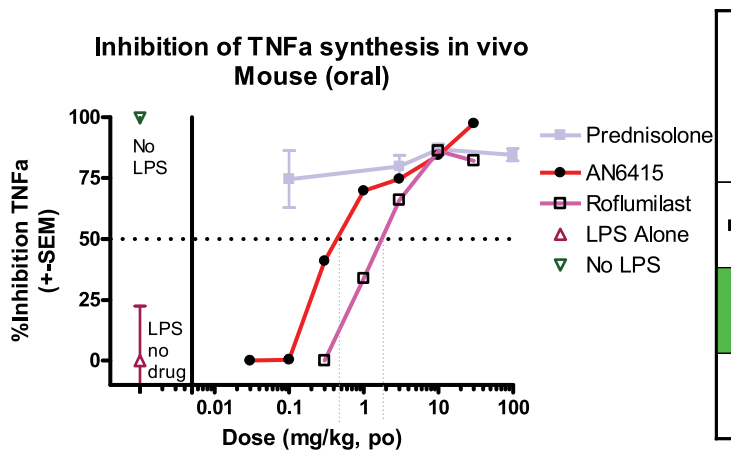
Mouse PK findings STRUCTURE	IV Findings @ 5mg/kg CL (mL/h/kg)	MRT (h)	Vss (mL/kg)	Oral Findings @ 10mg/kg T1/2 (h)	AUC (h*ug/mL)	F%
Apremilast	1335	2.67	3560	4.45	8.08	100
Roflumilast	1954	0.57	1091	1.19	1.14	21
AN6415	1472	0.652	960	7.68	7.22	96

AN6415 is a better Th1 Inhibitor compared to Dexamethasone, & Inhibits IL-23 and IL-4, unlike catechols

Inhibitor IC50 (nM)		Biochemical PDE4 U937 Cell Extract	Cellular Cytokine Secretion: Proinflammatory TNF-a PBMC with LPS	Proinflammatory IL-23 THP-1 with LPS +IFNg	Th1 IL-2 PBMC with PHA, 24h	Th1 IFN-g PBMC with PHA, 24h	Th2 IL-4 PBMC with PHA, 48h	Th2 IL-5 PBMC with PHA, 48h	Th2 IL-10 PBMC with PHA, 48h
AN6415	BENZOXABOROLE	0.805	0.821	98.9	1.67	2.58	708	4.66	NT
DEXAMETH-ASONE	STEROID	>10000	9.2	0.487	12	37	9.6	0.5	10
APREMILAST	CATECHOL	86.4	52.7	78000	27.2	29.9	>10000	202	27500
ROFLUMILAST	CATECHOL	1.7	4.47	>10000	1.73	1.41	>10000	10	49

- AN6415 is more potent than Roflumilast (green)
- AN6415 is more potent than Dexamethasone at blocking Th1 cytokine secretion (blue)
- AN6415 is the first PDE4 inhibitor to block IL-23 secretion at clinically relevant concentrations (pink)
- AN6415 is also the first PDE4 inhibitor to block IL-4 secretion (pink)

Oral administration of AN6415 shows 4-fold lower ED_{50} compared to Roflumilast



Compound	Estimated ED_{50} mg/kg
Prednisolone	<0.1
AN6415	0.5
Roflumilast	2

AN6415 is PDE isoform selective and likely PDE4 subtype (4A,B,D) non-selective

Recombinant PDE Isoform	AN5323 (a AN6415 relative): % Inhibition at 10x IC_{50} for PDE4 in U937 Extract (8nM)	AN5323: IC50	AN6415: %Inhibition at 10X IC50 PDE4 in U937 Extract (8nM)	AN6415: IC50
PDE1A	8%		-2%	
PDE2A	60%	76nM	23%	
PDE3A	15%		1%	>1000 nM
PDE4A			97%	
PDE4B		26 nM	95%	2.7 nM
PDE4D		2.5 nM		3.1 nM
PDE5A	34%	>10000 nM	5%	
PDE6	18%		20%	
PDE7A	77%	140nM	47%	200 nM
PDE7B	79%		52%	
PDE8A1	11%		3%	
PDE9A2	0%		-3%	
PDE10A1	13%		-1%	
PDE11A4	32%		-1%	

Methods

PDE4 assay

PDE4 was partially purified from human U-937 myeloid leukemia cells. Test article and/or vehicle was incubated with 0.2 mg of enzyme and 1 mM cAMP containing [^{3}H]cAMP. The reaction was terminated and the resulting AMP was converted to adenosine by the addition of snake venom nucleotidase. Unhydrolyzed cAMP was bound to AG1-X2 resin, and the remaining [^{3}H]Adenosine in the aqueous phase was quantitated by scintillation counting. Similar methods were used for recombinant forms of PDE, however, cGMP was substituted in the case of cGMP specific enzymes. Care was taken to assay each enzyme near the Km of cyclic-nucleotide for that isotype.

Cytokine assays

TNF-α was assayed using human peripheral blood mononucleocytes (hPBMCs), purified from whole blood by Ficoll separation. Cells were stimulated with 1 µg/mL LPS. Supernatants were harvested at 24 h, and tested using a multiplex assay. IL-23 was assayed using the human myelomonocytic THP-1 cells. Cells were stimulated with LPS (1 µg/mL) and IFN-γ (100 ng/mL). Supernatants were collected at 48 h. IL-23 was measured using an ELISA specific for the p19 subunit of IL-23 (R&D Systems).

In vivo TNFa

In vivo TNFa release was measured in Swiss Webster mice, 10/grp. Animals were dosed via oral gavage with drug in 1% CMC suspension at t=0min. Thirty minutes later LPS, 1.5mg/kg was administered by i.p. injection. Ninety minutes later, animals were sacrificed, blood was collected by exsangination and serum prepared for analysis. TNFa concentration was assessed by ELISA, LPS treated but non-drug treated animals had TNFa levels of 1000-3000pg/mL.

Pharmacokinetics studies

Pharmacokinetics was measured in female CD-1 mice, following an intravenous or oral dose. Plasma samples were collected and then analyzed by LC/MS/MS. Ten point calibration standards curves were prepared in fresh mouse plasma to which internal standard was added. Drug concentration was quantified based on peak area ratios of the compound to internal standard. PK was modeled using WinNonLin multi-compartment models.

Conclusions

1. AN6415 is a novel oxaborole with a superior ability to suppress Th1 and some proinflammatory cytokines compared to Dexamethasone.
2. AN6415 is a more potent PDE4 inhibitor than Roflumilast, likely by AN6415's making contacts both with the adenine pocket and bi-metal pocket.
3. AN6415 shows excellent oral availability, low clearance and long t1/2 in the mouse.
4. The mouse PK properties of AN6415 are better than Roflumilast, which is safe and effective in humans, thus we expect good human PK.
5. AN6415 is orally active and 4-fold more potent than Roflumilast.
6. AN6415 is a strong candidate therapeutic for inflammatory diseases.

Poster will be available at www.anacor.com