

# A Series of Potent Orally-Available Benzoxaborole PDE4 Inhibitors which Gain Potency by use of Novel Contacts Within the PDE4 Active Site

Kurt Jarnagin, Tsutomu Akama, Yvonne Freund, Chen Dong, Virginia Sanders, Yasheen Zhou, Liang Liu, Wei Bu, Charles Ding, Jake Plattner

Anacor Pharmaceuticals, Inc., Palo Alto, CA, USA

## Introduction

Anacor has discovered AN2728, a member of a novel class of benzoxaborole PDE4 inhibitors. Topical AN2728 has demonstrated tolerability and activity against psoriatic lesions in Phase 2 clinical trials. AN2728 has poor oral bioavailability and possesses modest PDE4 activity. To improve affinity, oral bioavailability and pharmacokinetics, we developed AN6414 and AN6415. Crystallography and modeling reveal that these compounds bind to PDE4, in a novel mode compared to other inhibitor classes, by use of the activated water and the bimetal center, along with other contacts. AN6414 has excellent PK properties in mice, rats and monkeys. These improvements will allow the use of benzoxaborole PDE4 inhibitors as a oral therapy for psoriasis, arthritis, or other autoimmune diseases.

### 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  $[^3H]cAMP$ . The reaction was terminated and the resulting AMP was converted to adenosine by the addition of snake venom nucleotidase. Unhydrolyzed cAMP was bound to AG-1-X2 resin, and the remaining  $[^3H]Adenosine$  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 the cyclic-nucleotide for that isotype.

#### Cytokine assays

TNF- $\alpha$  was assayed using human peripheral blood mononucleocytes (hPBMCs), purified from whole blood by Ficoll separation. Cells were stimulated with 1 mg/mL LPS for 24 h, to assay TNF $\alpha$  secretion, and with 20  $\mu$ g/mL PHA 48 h to measure IL-4 secretion. Supernatants were harvested, and tested using a multiplex bead based assay (BD Biosciences).

#### Pharmacokinetics studies

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

#### Crystallography

PDE4B-Cat was amplified by PCR and cloned in the pProExHTA vector. Cell pellets from 16 x 0.8 L were disrupted by nitrogen cavitation. After centrifugation, the supernatant was passed over a HisTrap column. Bound protein was eluted using a linear gradient of imidazole. After removal of the His-tag the protein was pooled, concentrated and further purified by gel permeation chromatography to yield ~17mg. Crystals of PDE4B in complex with AN2728/2898 were grown at 20 °C by hanging drop vapor diffusion. Crystals were obtained from the 14 mg/mL enzyme, 1 mM MgCl $_2$ , 2 mM AN2728/AN2898. In order to increase the occupancy of the ligand, crystals were soaked in 25 mM ligand for up to 7 days. Diffraction data were collected and data were integrated with XDS software.

**Table 2:** Among the PDE superfamily AN6415 is selective to PDE4, and displays low PDE4 isoform selectivity

Recombinant PDE Isoform	AN6415	
	Inhibition at 100 nM PDE4 in US37 Extract (nM)	IC50
PDE1A	<2%	
PDE2A	23%	
PDE3A	1%	>1000 nM
PDE4A	97%	2.7 nM
PDE4B	95%	3.7 nM
PDE4D	9%	3.3 nM
PDE6	20%	
PDE7A	47%	200 nM
PDE7B	52%	
PDE8A1	3%	
PDE8B	<1%	
PDE10A1	<1%	
PDE10A2	<1%	

## Results

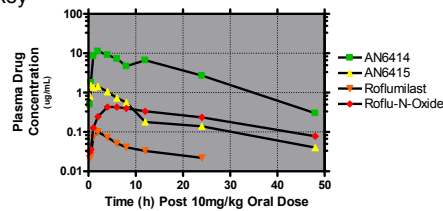
**Table 1:** Structures and activity of several key compounds along the development path to AN6415 compared to literature comparator compounds (pink)

Compound	Class	Biochemical	TNF $\alpha$	INF $\gamma$	IL-4
		hPDE4 US37 cell Extract	PBMC with LPS	PBMC with PHA 24hr	with PHA 48hr
		nM	nM	nM	nM
	Benzoxaborole	490	540	830	>10
	Benzoxaborole	60	160	220	>10
	Benzoxaborole	23.7	4.18	13.3	1.99
	Benzoxaborole	5.19	2.53	2.81	2.85
	Benzoxaborole	3.11	1.23	4.16	>10
	Benzoxaborole	0.805	0.438	2.58	0.788
	Catechol	0.616	0.564	1.5	>10
	Steroid	NT	8.67	37	0.0096

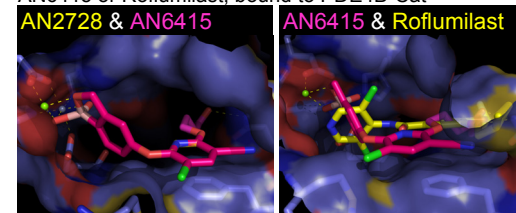
**Table 3:** AN6414 & AN6415 show generally better PK parameters in mouse, rats & monkey than Roflumilast

Compound ID	Species	I.v. Dose PK Parameters				Oral Dose PK Parameters			
		Cmax (ng/mL) (Scaled to 10mg/kg)	Clearance (mL/min/kg)	Volume of Distribution (mL/kg)	AUC(0-inf) (hr*ng/mL) (Scaled to 10mg/kg per capsule)	Cmax (ng/mL) (Scaled to 10mg/kg)	AUC(0-8h) (hr*ng/mL) (Scaled to 10mg/kg per capsule)	Bioavailability (%)	Terminal Half-life (hr)
AN6414	CD-1 Mice (F)	4.50	2160	1900	2.32	1.62	3.00	60	1.97
AN6415-arg	CD-1 Mice (F)	3.79	1510	3490	3.89	1.42	8.50	96	7.90
Roflumilast	CD-1 Mice (F)	6.08	1990	1001	2.96	0.78	1.14	31	1.50
AN6414	S.D. Rats (M)	8.70	737	1180	6.91	1.74	112	70	2.26
AN6415	S.D. Rats (M)	5.34	1260	1220	2.97	1.13	6.91	80	6.65
Roflumilast	S.D. Rats (M)	2.72	4030	3070	1.05	0.022	0.243	10	6.58
AN6414	Cyno Monkey (M)	37.4	780	880	59.9	0.96	78	75	6.52
AN6415	Cyno Monkey (M)	35.6	210	670	20.5	0.96	44.7	53	16.3
Roflumilast	Cyno Monkey (M)	20.5	178	670	32.2	0.108	1.68	2.3	19.3
Roflumilast-N-oxide	Cyno Monkey (M)	5.91	NA	NA	121	0.495	13.4	NA	15.4

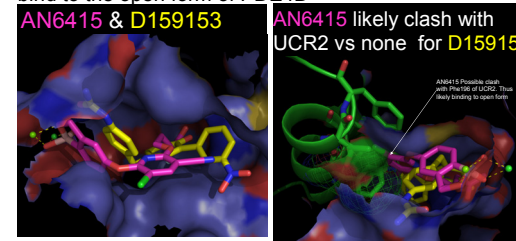
**Figure 1:** AN6414 shows higher plasma drug levels than AN6415 and Roflumilast/N-oxide in cyno. monkey



**Figure 2:** Comparison of the crystal structures of AN2728/AN2898 bound to PDE4B-Cat to models of AN6415 or Roflumilast, bound to PDE4B-Cat



**Figure 3:** Modeling of AN6415 into the D159153 co-crystal structure of PDE4D-fl suggests that AN6415 will bind to the open form of PDE4D



## Conclusions

- AN6415 inhibits PDE4 activity and TNF $\alpha$  secretion similarly to Roflumilast.
- AN6415 is phosphodiesterase super family selective, but PDE4 isoform non-selective.
- AN6414 has rat, mouse and monkey clearance and AUC(o-inf) notably better than Roflumilast.
- AN6414 in monkey exhibits higher blood levels than AN6415, and Roflumilast and its N-oxide.
- AN6415 makes new contacts in the adenine binding pocket as compared to AN2728.
- AN6415 binds to PDE4 using the activated catalytic water at the bimetal center; neither Roflumilast, a catechol, nor D159153, a methoxybiphenyl, bind in this mode.
- Modeling suggests AN6415 will bind to the open form of PDE4D and perhaps not the closed form.