



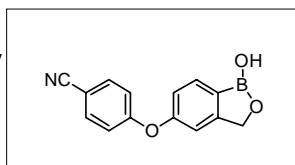
## In vitro activity and mechanism of action of AN2728, a novel oxaborole in development for treatment of psoriasis.

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### Introduction

AN2728 (5-(4-cyanophenoxy)-1-hydroxy-1, 3-dihydro-2, 1-benzoxaborole) is a broad spectrum anti-inflammatory compound currently under development for the topical treatment of plaque psoriasis. AN2728 inhibits TNF- $\alpha$  TH1 and TH2 cytokines, as well as IL-23. One potential mechanism of action of AN2728 is through inhibition of PDE4 enzyme activity. AN2728 inhibits all four PDE4 isoforms equally, as well as PDE1, 3 and 7.



**Table 1.** AN2728 inhibits LPS- and PHA-induced cytokine production by human PBMCs, as well as IL-23 production when human THP-1 monocytic cells are stimulated with IFN- $\gamma$  and LPS.

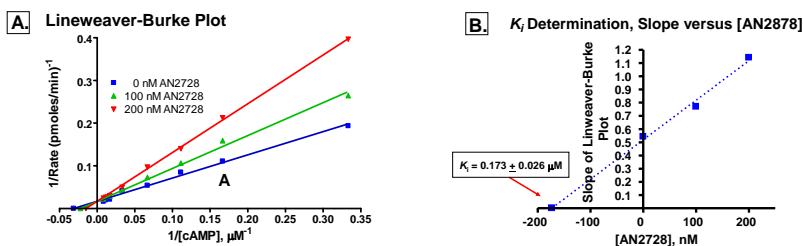
Compounds	IC <sub>50</sub> ( $\mu$ M)							
	Pro-inflammatory Cytokines				Th1 Cytokines		Th2 Cytokines	
	TNF $\alpha$	IL-1 $\beta$	IL-6	IL-23*	IL-2	IFN $\gamma$	IL-5	IL-10
AN2728	0.77	>30	>30	2.23	0.46	0.27	1.83	0.92
Rolipram	0.5	>30	NT	NT	0.15	0.28	0.79	0.63
Cilomilast	0.17	>30	NT	NT	0.14	0.2	0.89	4.52

\* Measured in THP-1 cells

**Table 2.** AN2728 inhibits PDE 4 and PDE7, to a lesser extent PDE1 and PDE3. Standards: 8-methoxymethyl-IBMX, Cilostazol, Rolipram and BRL-50481 respectively.

Compound	IC <sub>50</sub> ( $\mu$ M)			
	PDE1A3	PDE3 Cat	PDE4Cat	PDE7A1
AN2728	6.12	6.41	0.11	0.73
Standard	10	0.84	2.33	2.33

**Figure 1.** AN2728 is a competitive inhibitor of PDE4B with a  $K_i$  of  $0.173 \pm 0.026 \mu$ M.



### Conclusions

- AN2728 is a broad-spectrum inhibitor of TNF- $\alpha$ , IL-23 and Th1 and Th2 cytokines.
- AN2728 competitively inhibits PDE4 with a  $K_i$  of  $0.173 \mu$ M. AN2728 also inhibits PDE7 activity at sub-micromolar concentrations and to a lesser extent PDE1 and 3.
- Down regulation of TNF- $\alpha$  and IL-23 have been shown to be clinically efficacious in treatment of psoriasis, suggesting that AN2728 has potential for this indication.

#### Cytokine assay

Frozen human peripheral blood mononuclear (PBMC) were thawed and resuspended in fresh culture media (CM - RPMI 1640 and 10% FBS) in 96 well plates. Test article was dissolved in 100% DMSO to a 10 mM stock. All final dilutions contained <1% DMSO. Cytokine production was stimulated by  $1 \mu$ g/mL LPS for TNF- $\alpha$ , IL-1 $\beta$  and IL-6, or  $20 \mu$ g/mL PHA for IFN $\gamma$ , IL-2, IL-5 and IL-10. IL-23 was induced with  $100 \text{ ng/ml}$  IFN- $\gamma$  +  $1 \mu$ g/ml LPS, using THP-1 cells. Vehicle without inducer was used as a negative control. Cells were incubated at  $37^\circ\text{C}$ , 5% CO<sub>2</sub>. Supernatants were removed at 24 hours (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-2 and IFN- $\gamma$ ) or 48 hours (IL-5, IL-10 and IL-23), and stored at  $-20^\circ\text{C}$ . Supernatants were thawed and assayed for cytokine expression with fluorochrome-labeled cytokine-specific beads on a BD FACSAArray™. IL-23 was assayed using a commercial ELISA kit (R&D Systems).

#### PDE Isoform Profiling

Recombinant human PDE enzymes were expressed in a baculoviral system and assayed by the 2-step method of Thompson & Appleman (Biochem. 10:311-316, 1971). IC<sub>50</sub>s were generated from 11-point curves from duplicate points and analyzed using Prism software (GraphPad Inc.).

#### AN2728 Inhibition Kinetics

The inhibition kinetics of AN2728 were determined using the catalytic domain of PDE4B (Proteros Biostructures) and the [<sup>3</sup>H]-AMP reaction product was measured by precipitating with ZnSO<sub>4</sub>/Ba(OH)<sub>2</sub>. Enzyme inhibition was analyzed using the Lineweaver-Burke reciprocal transformation (Fig. 1A). Data from the Lineweaver-Burke plot was used to determine the  $K_i$  from the x-intercept of the graphed line in Fig. 1B.