

**P1318**

AN2898 Inhibits Cytokines Relevant to  
Topical Treatment of Psoriasis and Atopic  
Dermatitis

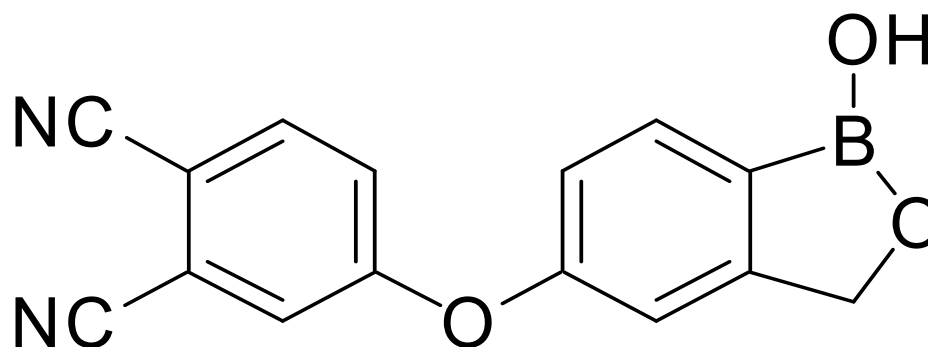
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# Introduction

AN2898 (Figure 1) is a broad spectrum anti-inflammatory oxaborole currently in development for the topical treatment of plaque psoriasis and atopic dermatitis, common skin diseases characterized by chronic inflammation. AN2898 selectively inhibits PDE4 and PDE7, enzymes present in pro-inflammatory cells with  $IC_{50}$ 's of 0.03 and 0.21  $\mu$ M, respectively. AN2898 is a competitive reversible inhibitor of PDE4 with a  $K_i$  of 65 nM. It has equal activity against the four PDE4 subtypes and does not significantly inhibit PDE 1,2,3,5, or 6. Additionally, AN2898 inhibits  $TNF\alpha$ , IL-12 and IL-23 release from stimulated PBMCs. IL-23 has been implicated in cutaneous inflammation (van Beelen, et al. 2007).

**Figure 1.** AN2898 (5-(3,4-dicyanophenoxy)-1-hydroxy-1,3-dihydro-2,1-benzoxaborole)



# AN2898 Is a Selective PDE4 and PDE7 Inhibitor

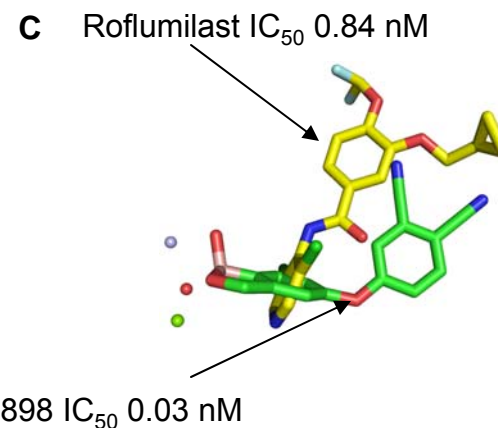
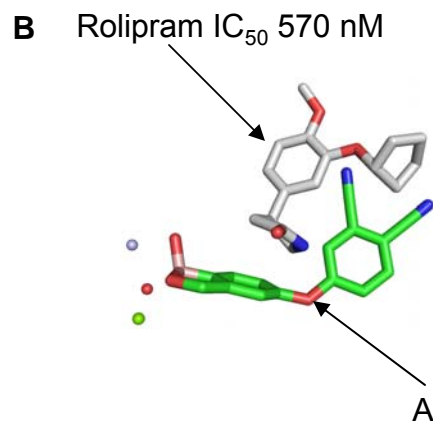
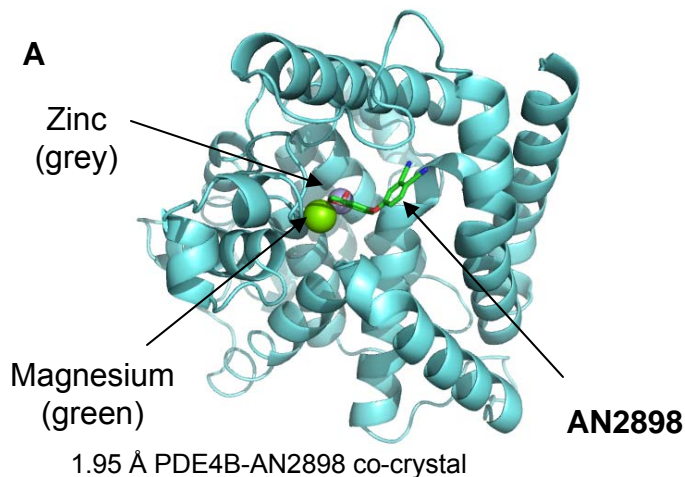
- AN2898 is not specific against the four PDE4 isoforms.

Percent Inhibition of PDE Enzymes (A) and PDE4 Isoforms (B) by AN2898 at 10  $\mu$ M

A	PDE1(IC <sub>50</sub> )	PDE2	PDE3 (IC <sub>50</sub> )	PDE4 (IC <sub>50</sub> )	PDE5	PDE6	PDE7 (IC <sub>50</sub> )	PDE8	PDE9	PDE10 (cAMP)	PDE11 (cAMP)
	85 (1.7 $\mu$ M)	68	66 (4.1 $\mu$ M)	99.8 (0.03 $\mu$ M)	25	25	88 (0.2 $\mu$ M)	72	0	57	67

B	PDE4A4	PDE4B2	PDE4C2	PDE4D3
	97	96	93	93

- The co-crystal structure of AN2898-PDE4B demonstrates AN2898 does not bind to exactly the same site classical PDE4 inhibitors Rolipram and Roflumilast



# AN2898 Inhibits a Broad Spectrum of Cytokines

- AN2898 inhibits the pro-inflammatory cytokines  $\text{TNF}\alpha$  and IL-23, as well as members of the Th1 and Th2 families.

Cytokine Inhibition $\text{IC}_{50}$ ( $\mu\text{M}$ )			
Pro-Inflammatory			
$\text{TNF}\alpha$	IL-1 $\beta$	IL-6	IL-8
0.16	>30	>30	>30

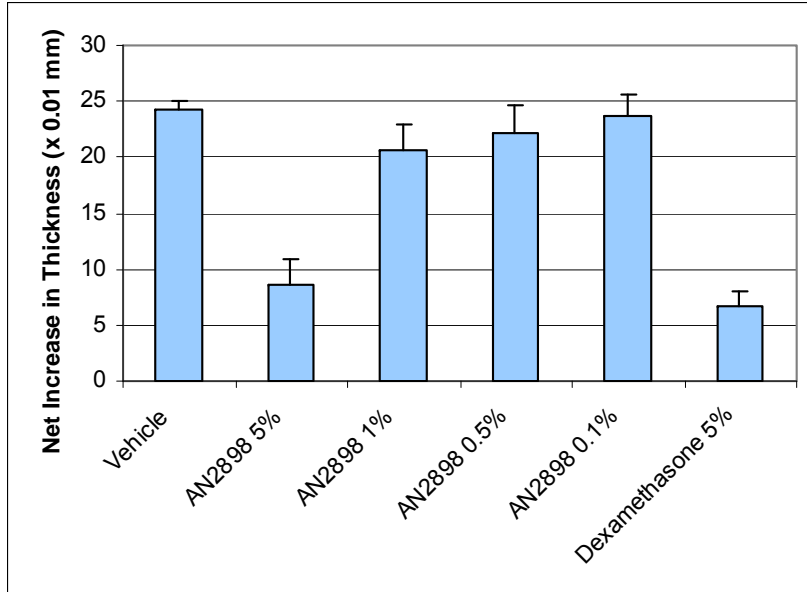
Th1		Th2		
IL-2	IFN- $\gamma$	IL-4	IL-5	IL-10
0.18	0.22	>10	0.2	1.5

IL-12	IL-23
0.02	1.1

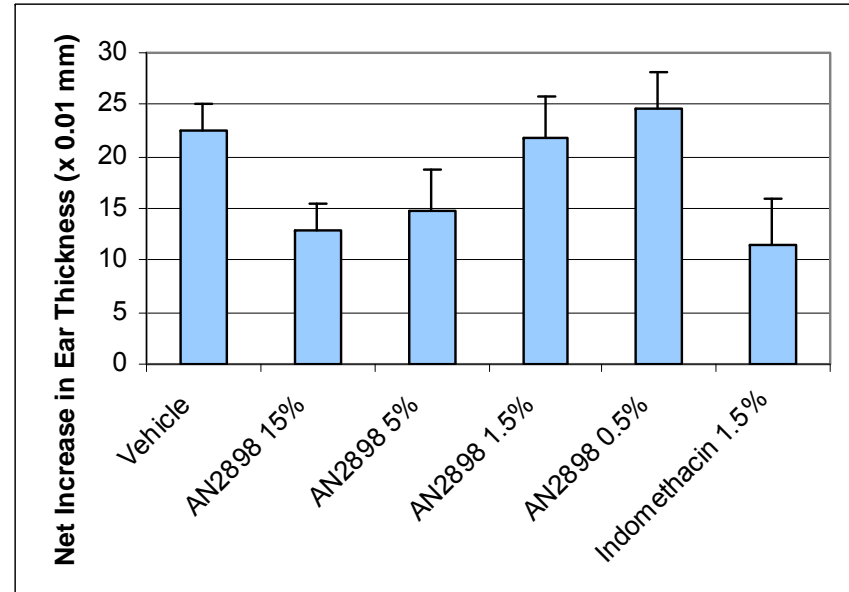
# AN2898 Inhibits Inflammation in Mouse Ear Edema Models

- Topically applied AN2898 inhibits inflammation in two different models of mouse ear edema

Acute Inflammation: AN2898 inhibits phorbol ester (PMA) induced ear edema. Dexamethasone is positive control



Delayed Type Hypersensitivity: AN2898 inhibits oxazolone induced ear edema. Indomethacin is positive control



## Conclusion

- AN2898 is a novel PDE4 and PDE7 inhibitor that has broad spectrum *in vitro* anti-inflammatory activity.
- It binds to the active site of the PDE4 enzyme in a manner different from traditional PDE4 inhibitors, i.e. rolipram.
- AN2898 also inhibits IL-23, a cytokine implicated in autoimmune/inflammatory disease
- Because of its anti-inflammatory profile, AN2898 has the potential to be useful in the topical treatment of psoriasis and atopic dermatitis

# Biological Assays

## **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  $\mu$ M cAMP containing 0.01  $\mu$ M [ $^3$ H]cAMP in Tris buffer (pH 7.5) for 20 minutes at 25 °C. The resulting [ $^3$ H]AMP is converted to [ $^3$ H]Adenosine by addition of snake venom nucleotidase and separated by AG1-X2 resin. An aliquot is removed and counted to determine the amount of [ $^3$ H]Adenosine formed. Test articles were tested at 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, and 0.001  $\mu$ M for IC<sub>50</sub> determination.

## **Cytokine assays**

Cytokines were assayed using human peripheral blood mononucleocytes (PBMC) in fresh culture media (CM) comprising RPMI 1640 and 10% FBS in 96 well plates. Test articles were diluted to 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, and 0.001  $\mu$ M in 200  $\mu$ L of CM (n = 3). Cells were stimulated with 1  $\mu$ g/mL LPS for TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-8, 20  $\mu$ g/mL PHA for IFN- $\gamma$ , IL-2, IL-4, IL-5 and IL-10, or 2  $\mu$ g/ml anti-CD3 for IL-12. Supernatants were harvested at 24 h (for TNF- $\alpha$ , IL-1 $\beta$ , IFN $\gamma$ , and IL-2) and 48 h (for IL-4, IL-5, IL-10, and IL12p70), and assayed using a multiplex assay. IL-23 was assayed using the human myelomonocytic THP-1 cell line. Cells were stimulated with LPS (1  $\mu$ g/mL) and IFN- $\gamma$  (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 Assays***

AN2898, dissolved in acetone/ethanol, was applied topically to ears of male CD-1 mice 30 minutes before and 15 minutes after phorbol 12-myristate 13 acetate (PMA) challenge. Ear swelling was measured with a Dyer micrometer gauge 6 h after PMA application as a measure of inflammation.

AN2898 dissolved in acetone/ethanol was applied topically to ears of oxazolone-sensitized male BALB/c mice 30 minutes before and 15 minutes after second oxazolone challenge. Ear swelling was measured with a Dyer micrometer gauge 24 h after oxazolone application as a measure of inflammation.