

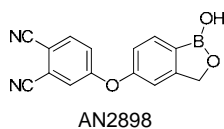
## Structure-Activity Studies of Novel Oxaborole Dual Inhibitors of PDE4 and IL-23 Release

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### Introduction

AN2898 (5-(3,4-dicyanophenoxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaborole) is a broad spectrum anti-inflammatory compound that is currently under development for the topical treatment of plaque psoriasis and atopic dermatitis. AN2898 inhibited phosphodiesterase 4 (PDE4) enzyme activity (IC<sub>50</sub> 0.060 μM) and the release of multiple cytokines including TNF-α (IC<sub>50</sub> 0.16 μM) from peripheral blood mononuclear cells (hPBMCs) stimulated by lipopolysaccharide (LPS) or phytohemagglutinin. AN2898 was also found to inhibit IL-23 release (IC<sub>50</sub> 1.0 μM) from THP-1 cells stimulated by LPS and IFN-γ. Investigation of the structure-activity relationship around this compound was carried out to identify a more potent dual TNF-α/IL-23 inhibitor.



### 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 0.01 mM [<sup>3</sup>H]cAMP in Tris buffer (pH 7.5) for 20 minutes at 25 °C. The reaction was terminated by boiling for 2 minutes and the resulting AMP is converted to adenosine by addition of 10 mg/ml snake venom nucleotidase and further incubation at 37 °C for 10 minutes. Unhydrolyzed cAMP is bound to AG1-X2 resin, and remaining [<sup>3</sup>H]Adenosine in the aqueous phase is quantitated by scintillation counting. Test articles were tested at 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, and 0.001 μM 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 μM in 200 μL of CM (n = 3). Cells were stimulated with 1 μg/mL LPS for TNF-α and IL-1β, and 20 μg/mL PHA for IFN-γ, IL-2, IL-4, IL-5, and IL-10. Supernatants were harvested at 24 h (for TNF-α, IL-1β, IFN-γ, and IL-2) and 48 h (for IL-4, IL-5, and IL-10), and tested using a multiplex assay. IL-23 was assayed using the human myelomonocytic THP-1 cells and human dendritic cells cultured from hPBMCs. 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).

### Results

**Table 1:** Structure-activity relationships of substituted cyanophenoxy benzoxaboroles

Cpd	R	IC <sub>50</sub> (μM)		
		PDE4	TNF-α	IL-23
1 (AN2898)	3'-CN	0.060	0.16	1.0
2	2'-OH	0.34	0.31	0.49
3	3'-OH	1.1	0.45	>10
4	2'-OMe	0.22	0.43	>10
5	3'-OMe	0.032	0.27	0.77
6	2'-Cl	0.082	0.27	1.5
7	3'-Cl	0.15	0.22	2.7
8	3'-CF <sub>3</sub>	0.090	0.23	5.5

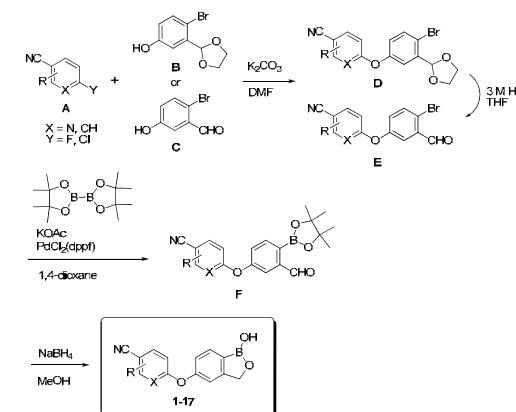
**Table 2:** Structure-activity relationships of substituted pyridinyloxy benzoxaboroles

Cpd	R <sup>1</sup>	R <sup>2</sup>	IC <sub>50</sub> (μM)		
			PDE4	TNF-α	IL-23
9	CN	H	0.18	0.36	3.2
10	CO <sub>2</sub> Me	H	0.084	0.26	>10
11	CO <sub>2</sub> Et	H	0.047	0.20	>10
12	CONHET	H	0.092	0.63	>10
13	CN	OMe	0.030	0.43	0.41
14	CN	OEt	0.012	0.060	0.35
15	CN	OPr	0.019	0.18	0.33
16	CN	NHMe	0.061	0.11	1.8
17	CN	NMe <sub>2</sub>	0.057	0.75	1.8

**Table 3:** IC<sub>50</sub> values (μM) of compound 14 against various cytokines

TNF-α	IL-23	IL-2	IFN-γ	IL-4	IL-5	IL-10
0.06	0.35	0.06	0.10	>10	0.08	0.34

**Scheme 1:** Synthesis of benzoxaboroles



### Conclusions

- Para-cyano, para-alkoxycarbonyl (**10**, **11**), and para-amido (**12**) derivatives showed inhibition against PDE4 enzyme and TNF-α release
- Para-cyano group was essential for the inhibition of IL-23 release
- Cyano-alkoxy pyridine derivatives (cpds **13–15**) showed potent inhibitory activity against the release of both TNF-α and IL-23
- Compound **14** also showed potent activity against other cytokines
- These compounds are expected to be promising leads for the topical treatment of plaque psoriasis