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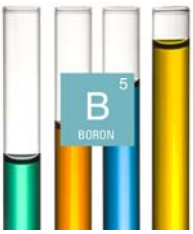

Structure-activity studies of AN2728 and AN2898, novel oxaborole compounds with anti-inflammatory activity

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Abstract

AN2728 is a novel boron containing compound currently under development for the topical treatment of plaque psoriasis. Since AN2728 was found to inhibit the phosphodiesterase 4 (PDE4) enzyme (IC_{50} 0.49 μM) as a part of its mechanism of action, further structure-activity relationship study was carried out to identify a more potent PDE4/TNF-α inhibitor. AN2898 was identified to show potent inhibition against PDE4 (IC_{50} 0.060 μM), while the IC_{50} of rolipram was 1.7 μM. AN2898 also inhibited the release of cytokines, such as TNF-α, IL-2, IFN-γ, IL-5, and IL-10. AN2898 was chosen for preclinical development.

Introduction

Psoriasis is a chronic skin disorder caused by inflammatory cell infiltration into the dermis and epidermis, and is accompanied by keratinocyte hyperproliferation. Once triggered, a strong T-cell response is mounted and a cascade of cytokines and chemokines is induced. Down-regulation of certain cytokines and chemokines is considered to be a good approach to treatment and indeed, the biologics targeting TNF-α demonstrate the effectiveness of this approach. Recently, small molecule PDE4 inhibitors are in development for skin diseases, such as psoriasis and atopic dermatitis. We have identified novel boron-containing compound AN2728 as an anti-inflammatory agent and PDE4 enzyme as its target molecule. This poster presents the structure-activity study that led to more potent PDE4/TNFα inhibitors.

Table 1: Structure-activity relationships of benzoxaboroles

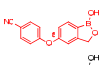
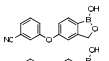
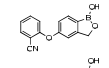
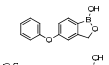
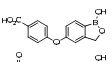
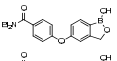
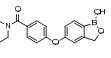
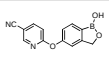
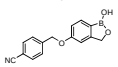
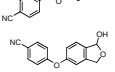
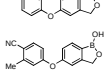
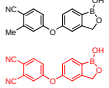
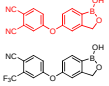
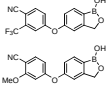
Compound	Structure	C_{50} (μM) PDE4	TNF-α	IL-2	IFN-γ	IL-5	IL-10
1 (AN2728)		0.49	0.77	0.46	0.27	1.8	0.92
2		4.4	8.6	3.3	3.4	4.7	8.9
3		3.7	8.5	4.0	4.1	7.8	8.0
4		6.8	>10	>10	>10	>10	>10
5		6.4	>10	>10	>10	>10	>10
6		1.5	1.3	0.43	1.4	4.3	2.0
7		0.57	0.44	0.17	0.33	1.9	0.37
rolipram		1.7	0.50	0.15	0.28	0.79	0.63

Table 1 (Cont.): Structure-activity relationships of benzoxaboroles

Compound	Structure	IC_{50} (μM) PDE4	TNF-α	IL-2	IFN-γ	IL-5	IL-10
8		0.18	0.36	0.30	0.14	1.4	0.62
9		1.7	7.7	2.6	1.9	3.8	10
10		>10	>10	>10	>10	>10	>10
11		0.14	0.23	0.63	0.45	0.86	>10
12 (AN2898)		0.060	0.12	0.14	0.050	0.22	0.31
13		0.090	0.23	1.1	0.61	0.12	>10
14		0.032	0.27	0.15	0.27	1.1	0.17

Structure-activity relationships

As shown in Table 1, AN2728 (**1**) showed potent inhibitory activity against both PDE4 (IC_{50} 0.49 μM) and cytokine expression (IC_{50} 0.27-1.8 μM), which was similar to rolipram. The regioisomers of the cyano group (**2** and **3**) were less active. Simple phenoxy derivative (**4**) and carboxy derivative (**5**) lost the cytokine inhibition, while amides (**6** and **7**) retained the activity. Pyridine analog (**8**) showed more potent activity than AN2728. When the boron atom was replaced with carbon (**10**), activity was completely lost, indicating the importance of the boron atom. Additional functional group besides the *para*-cyano group on the 5-phenoxy group significantly enhanced the potency especially against PDE4 enzyme (**11-14**). AN2898 (**12**) showed IC_{50} values of 0.060 μM against PDE4 and 0.050-0.22 μM against cytokines.

Conclusions

- **5-Phenoxy group bearing an electron-withdrawing group at the *para*-position was important for the activity**
- **Introduction of another functional group to the 5-phenoxy group enhanced the potency**
- **Compound 12 (AN2898) was identified to show potent inhibition against PDE4 enzyme and cytokine expression, and chosen for preclinical development**

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 [^{3}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 was converted to adenosine by addition of 10 mg/ml snake venom nucleotidase and further incubation at 37 ˚C for 10 minutes. Unhydrolyzed cAMP was bound to AG1-X2 resin, and remaining [^{3}H]Adenosine in the aqueous phase was 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_{50} determination.

Cytokine assay

Frozen human peripheral blood mononucleocytes (PBMC) were thawed and centrifuged. Cryopreservation media was aspirated off of the cell pellet, and the cells were resuspended in fresh culture media (CM) comprising RPMI 1640 and 10% FBS in 96 well plates. Test article was dissolved in DMSO to form a 10 mM sample (DMSO, 100%). The 10 mM samples were diluted to 100 μM in CM (DMSO, 1%), then further diluted to 10, 1, 0.1, and 0.01 μM final concentration (n = 3). Inducer (1 μg/mL LPS for TNF-α or 20 μg/mL PHA for IFNγ, IL-2, IL-5 and IL-10). plus vehicle (1% DMSO) was used as a control. Vehicle without inducer was used as a negative control. Cells were incubated at 37 ºC, 5% CO_2. Supernatants were removed at 24 hours (for TNF-α, IFNγ, and IL-2) or 48 hours (for IL-5, and IL-10), and stored at -20 °C. The supernatants were thawed, and assayed for TNF-α, IFNγ, IL-2, IL-5, and IL-10 expression using the fluorochrome-labeled cytokine-specific beads and the Becton Dickinson FACSArray™.

Related posters: #84, #86, #89, #90, and #367