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Discovery and structure–activity relationships of 6-(benzoylamino)benzoxaboroles as orally active anti-inflammatory agents

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ABSTRACT

Structure–activity relationships of 6-(benzoylamino)benzoxaborole analogs were investigated for the inhibition of TNF-α, IL-1β, and IL-6 from lipopolysaccharide stimulated peripheral blood mononuclear cells. Compound **1q** showed potent activity against all three cytokines with IC_{50} values between 0.19 and 0.50 μM, inhibited LPS-induced TNF-α and IL-6 elevation in mice and improved collagen-induced arthritis in mice. Compound **1q** (AN4161) is considered to be a promising lead for novel anti-inflammatory agent with an excellent pharmacokinetic profile.

The clinical success of various biologic therapeutics has demonstrated that several cytokines are important therapeutic targets for chronic inflammatory diseases. Therapeutics targeting tumor necrosis factor-α (TNF-α) are used to treat rheumatoid arthritis (RA), psoriasis, inflammatory bowel disease, for example.[1] Biologics targeting interleukin-1β (IL-1β) are approved for RA or cryopyrin-associated periodic syndrome and an interleukin-6 (IL-6) receptor monoclonal antibody is approved for RA, Crohn's disease, and Castleman's disease.[1] Although biologics have been highly effective and represent an important therapeutic advance, they have a number of limitations, including administration by injection, high cost, and poor or partial efficacy in considerable numbers of individuals.[2] Thus there is a need for more convenient, lower cost and effective medications.[3] An orally or topically administered small molecule which blocks multiple inflammatory cytokines would meet the needs for ease of administration, lower cost and potentially increased efficacy.

We have focused our efforts on discovery of new therapeutic agents by inclusion of boron into small molecule scaffolds. Using the benzoxaborole and related scaffolds as novel drug pharmacophores, we have discovered a number of novel anti-infective and anti-inflammatory agents.[4] As part of this research, we have created a library of boron-containing compounds which has proven to be productive in both biochemical and phenotypic assays. In the course of our research to identify novel anti-inflammatory agents, we sought inhibitors of the release of three pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) from lipopolysaccharide (LPS) stimulated peripheral blood mononuclear cells (PBMCs). During this screening campaign, we discovered compound **1a** (Fig. 1), which showed IC_{50} values of 2.1–7.2 μM against all three cytokines. Previously we reported another series of benzoxaborole analogs that polar substituents such as aminomethyl and carboxy groups were essential components for their activity in inhibiting cytokine release represented by AN3485 (Fig. 1).[5] Since compound **1a** is an uncharged and relatively hydrophobic molecule without such polar groups, this scaffold could have a different mechanism of action, which prompted us to explore the benzamide class. Here we describe the in vitro SAR of 6-(benzoylamino)benzoxaborole

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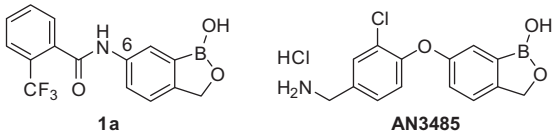
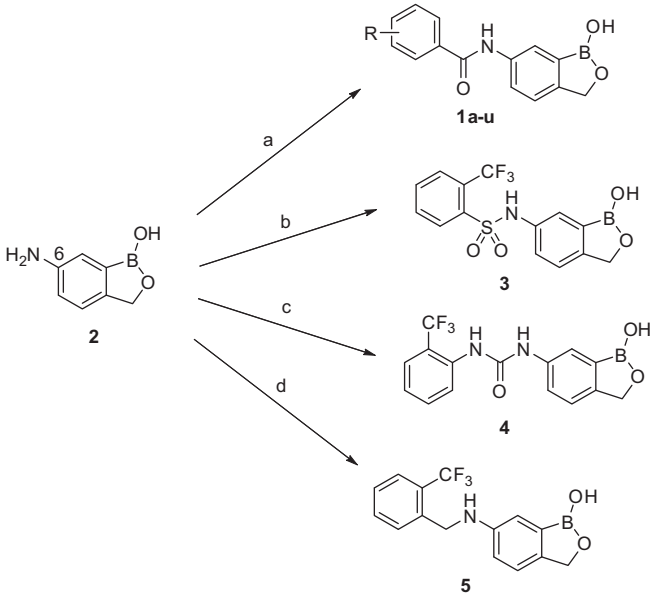


Figure 1. Chemical structures of compound **1a** and AN3485.

derivatives against the release of three cytokines, mouse pharmacokinetics, and in vivo activity of a selected compound (**1q**) against the LPS-induced production of TNF-α and IL-6 and collagen-induced arthritis model in mice.

The syntheses of 6-(benzoylamino)benzoxaborole analogs **1** and related analogs (**3**–**5**) are shown in Scheme 1. Reaction of 6-aminobenzoxaborole (**2**)[6] with benzoyl chlorides afforded the benzamide products (**1a**–**u**)[7] shown in Figure 2.[8] Sulfonamide **3** and urea **4**[8] were synthesized by treating **2** with sulfonyl chloride and isocyanate, respectively. Benzylamine analog **5** was obtained by alkylation of **2** with 2-trifluoromethylbenzyl bromide.

The compounds were tested for the inhibitory activity against the release of the three cytokines, TNF-α, IL-1β, and IL-6 as summarized in Table 1.[5] The unsubstituted benzamide analog (**1b**) was not active. Among the 2′-monosubstituted derivatives (**1a**–**h**), the trifluoromethyl analog (**1a**) was the only compound that showed significant inhibition against three cytokines. Relocation of the trifluoromethyl group of **1a** to the 3′- or 4′-positions resulted in loss of activity. Addition of fluoro group to the 6′- (**1k**) enhanced the potency against all three cytokines 6 to 12-fold, while the potency was slightly diminished by the addition of either 4′-fluoro (**1L**) or 5′-fluoro (**1m**) substitution. Having a fluoro group at the 2′-position and a trifluoromethyl group at either 3′- (**1n**), 4′- (**1o**), or 5′-position (**1p**) resulted in loss of activity. Addition of 5′-chloro decoration to **1k** to afford the trisubstituted analog **1q** provided further improvement in potency compared to **1k** and showed 0.19–0.50 μM IC_{50} values against all three cytokines. Bis(trifluoromethyl) analogs (**1r** and **1s**) showed similar levels of activity to **1a**. The 3′-chloro-2′-fluoro analog (**1t**) showed no activity, while the



Scheme 1. Synthesis of compounds **1a–u, 2–5**. Reagents and conditions: (a) corresponding benzoyl chloride, Et_3N, DCM, 0 °C to rt (34%–95%); (b) 2-trifluoromethylbenzenesulfonyl chloride, pyridine, MeCN, rt (74%); (c) 2-trifluoromethylphenylisocyanate, MeCN, rt (58%); (d) 2-trifluoromethylbenzyl bromide, $NaHCO_3$, MeCN, rt (25%).

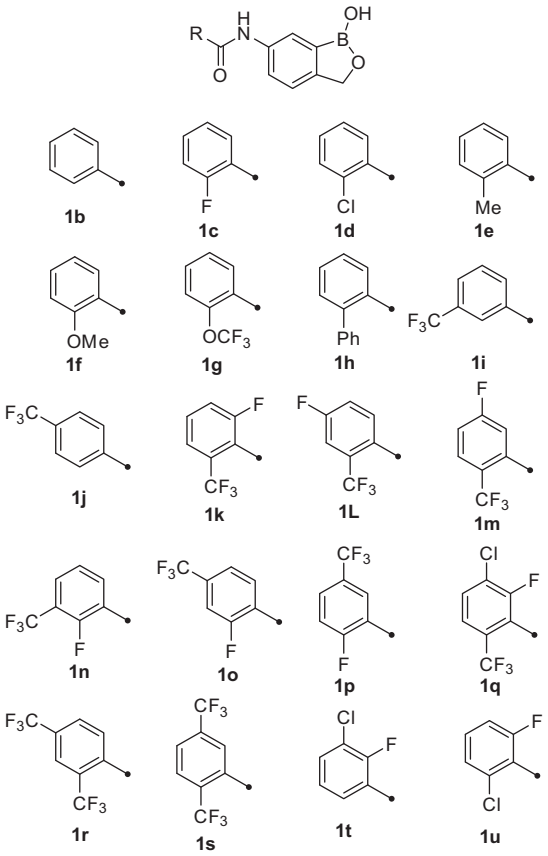


Figure 2. Chemical structures of 6-(benzoylamino)benzoxaboroles **1b–u**.

Table 1
In vitro IC_{50} results of compounds against three cytokines[a]

Compound	IC_{50} (μM) TNF-α	IC_{50} (μM) IL-1β	IC_{50} (μM) IL-6
1a	7.2	2.1	4.4
1b	>100	>100	>100
1c	>100	>10	>10
1d	>100	>100	>100
1e	>100	>100	>100
1f	36	>100	>100
1g	>100	>100	>100
1h	>100	>100	>100
1i	>10	>10	>10
1j	>100	>100	>100
1k	1.2	0.18	0.37
1L	13	3.2	5.6
1m	28	11	8.2
1n	99	>100	>100
1o	>100	>100	>100
1p	>100	>100	>100
1q	0.50	0.19	0.32
1r	10	5.3	8.3
1s	6.3	2.0	3.0
1t	>100	>100	>100
1u	15	3.1	4.9
3	>10	>10	>10
4	>10	>10	>10
5	>10	>10	>10
Clobetasol	0.0070	4.4	4.2
Dexamethasone	0.017	0.0095	43%[b]
SB-203580	1.0	0.34	>10

[a] IC_{50} values are calculated from means of at least two experiments.
[b] Inhibition% at 10 μM.