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Identification of a novel boron-containing antibacterial agent (AN0128) with anti-inflammatory activity, for the potential treatment of cutaneous diseases

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Abstract—A series of borinic acid picolinate esters were synthesized and screened for their minimum inhibitory concentration (MIC) against Gram-positive and -negative bacteria. Our lead compounds were then screened for anti-inflammatory activity. From these studies, we identified 3-hydroxypyridine-2-carbonyloxy-bis(3-chloro-4-methylphenyl)borane (**2g**, **AN0128**) as having the best combination of anti-bacterial and anti-inflammatory activities. This compound is now in clinical development for dermatological conditions.

Atopic dermatitis (AD), or eczema, is an inflammatory disease affecting around 10–20% of children and 1–3% of adults.[1] It is commonly characterized by flaky, itchy skin and sufferers will often scratch the affected area until it bleeds, compromising skin barrier function and causing bacterial infection. In 90% of cases there is a bacterial component involving the Gram-positive bacterium *Staphylococcus aureus*.[2] AD is caused by type 2 helper T (Th2) cells involved in the allergic response and is aggravated by allergens contacting the skin.[2] Depending on the severity of the condition, the primary treatment is management of environmental factors along with administration of topical or systemic corticosteroids to reduce inflammation.[3] However, steroids are known to have a number of side effects and their chronic use is not without risk. Other treatments include the calcineurin inhibitors tacrolimus (FK-506)[2] and picrolimus.[2] These drugs inhibit production of Th2 cytokines, but also Th1 cytokines, which are involved in immune response. Recently, the US-FDA issued a public-health advisory for these treatments.[4]

Since a bacterial component exists in most cases of AD, parallel treatment with antibacterial and anti-inflammatory agents produced an improved clinical response.[5] Therefore, it would be desirable to develop a single drug that possesses both these activities and has a superior safety profile.

We previously reported a new class of antibacterial agents, borinic acid quinoline esters (**1**).[6] In this report, we describe a related class, borinic acid picolinate esters (**2**) and the identification of a new antibacterial agent, 3-hydroxypyridine-2-carbonyloxy-bis(3-chloro-4-methylphenyl)borane (**2g**), which has additional activity against pro-inflammatory cytokines. This combination of activities is ideal for the treatment of AD and also acne, where the inflammatory response is mediated by the anaerobic bacterium, *Propionobacterium acnes* (Fig. 1).

Borinic acid picolinate esters (**2**), were synthesized as outlined in Scheme 1. Symmetrical borinic acids (**5**,

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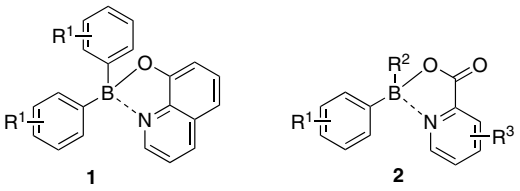
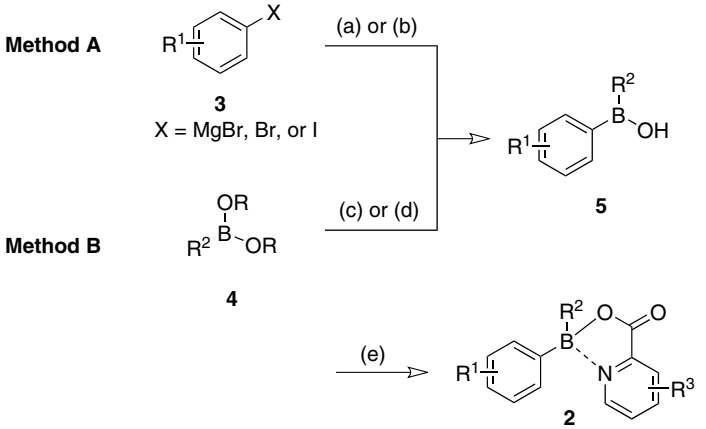


Figure 1. Diphenylborinic acid ester complexes.

$R^2 = R^1$–Ph) were synthesized by Method A. Two molar equivalents of an arylmagnesium bromide (**3**, X = MgBr) was treated with one molar equivalent of trimethylborate under anhydrous conditions. Alternatively, **5** ($R^2 = R^1$–Ph) was synthesized by treating two molar equivalents of an arylhalide (**3**, X = Br or I) with either butyllithium or isopropylmagnesium chloride followed by one molar equivalent of trimethylborate under anhydrous conditions to give the borinic acid (**5**, $R^2 = R^1$–Ph). Non-symmetrical borinic acids (**5**, $R^2 \neq R^1$–Ph) were prepared using Method B. Boronic acid esters (**4**, R ≠ H) were either purchased commercially or prepared by treating the corresponding boronic acid (**4**, R = H) with ethylene glycol and heating to reflux in THF or toluene to yield boronic acid ethylene glycol esters. If the boronic acid (**4**, R = H) was not commercially available then it was prepared by treating the arylanion of **3** with an equimolar amount of trimethylborate. The boronic acid esters of (**4**) were treated with Grignard reagents or aryllithiums to give the borinic acid (**5**). When R^2 of the borinic acid (**5**) was (substituted)pyridyl then butyllithium was added slowly to the corresponding bromopyridine in the presence of the glycol esters of **4** at −78 °C.[7] The borinic acids (**5**) were usually taken onto the final step as a crude mixture, but occasionally they were purified by column chromatography. In the final step the borinic acid (**5**) was heated to reflux with a picolinic acid in a mixture of ethanol/water to give the borinic acid picolinate esters (**2a**–**x**), which usually precipitated from the solution upon cooling.[8]

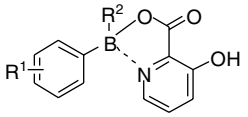
In general, these borinic acid picolinate esters (**2**) were found to be easy to handle and required no specialized equipment. We could purify the intermediate borinic acids (**5**) using standard column chromatography conditions and we found their esters (**2**) to be very stable under most conditions. These esters (**2**) can be stored at room temperature for prolonged periods.

Compounds were screened for their minimum inhibitory concentration (MIC) against Gram-positive and -negative bacteria. The results are given in Table 1. We identified two initial lead compounds. Both possessed a symmetrical borinic acid moiety containing a 3- or 4-chloro-substituent on each ring and a 3-hydroxy group on the picolinic acid unit giving **2a** or **2b**, respectively. These had reasonable activity against all pathogens. The bis(4-chlorophenyl) derivative was slightly more active against *Staphylococcus epidermidis*, *P. acnes*, and *Bacillus subtilis*, however, the bis(3-chlorophenyl) derivative was more active against *S. aureus* (Table 1). We first set out to determine the SAR of the diphenyl borinic acid unit by substituting R^2 with various groups. Substitution of one chlorophenyl group of either **2a** or **2b** with a pyridin-3-yl group to give **2c** and **2d**, respectively, essentially eliminated all activity against Gram positive bacteria, even when the chloro group was introduced back in the same place on the pyridine ring (**2e**). Interestingly, when R^2 was thiophen-3-yl (**2f**) activity was lost only against *S. aureus* and *S. epidermidis*. When methyl groups were added to the 4-position of **2a**, to give **2g**, activity was increased against most strains except *Haemophilus influenzae*, where activity was lost (Table 1). Since activity against Gram-positive pathogens was most important to us, **2g** became our new lead compound. We investigated the effect of adding alkyl groups at R^2 and synthesized the methyl (**2h**) and phenethyl (**2i**) derivatives of **2g**. The methyl derivative (**2h**) was less active but showed the similar activity profile to the thiophene derivative (**2f**). The phenethyl derivative showed remarkably similar activity to **2g**, however, stability studies found that these alkyl derivatives were not as stable as the diphenyl borinic acid picolinate ester



Scheme 1. Synthesis of borinic acid picolinate esters (**2**). Reagents and conditions: (a) $B(OMe)_3$ (0.5 equiv), THF, 0 °C to rt (when X = MgBr); (b) BuLi, $B(OMe)_3$ (0.5 equiv), THF, −78 °C to rt (when X = Br); (c) **3** (X = MgBr), THF, 0 °C to rt; (d) **3** (X = Br), BuLi, THF, −78 °C to rt; (e) R^3-picolinic acid, EtOH, water, reflux.

Table 1. Minimum inhibitory concentration (MIC, μg/mL) results for compounds **2** containing a 3-hydroxypicolinic acid moiety



Compound	Method of synthesis[a]	R^1	R^2	*S. aureus*	*S. epidermidis*	*P. acnes*	*B. subtilis*	*H. influenzae*
Erythromycin				0.5	0.15	0.1	0.1	4
2a	A	3-Cl	3-Cl–Ph	⩽0.125	8	10	16	16
2b	A	4-Cl	4-Cl–Ph	4	1	1	1	16
2c	B	3-Cl	Pyridin-3-yl	16	32	nt	nt	32
2d	B	4-Cl	Pyridin-3-yl	64	32	nt	nt	16
2e	B	4-Cl	2-Cl-pyridin-5-yl	32	32	nt	nt	32
2f	B	3-Cl	Thiophen-3-yl	32	32	10	16	32
2g	A	3-Cl–4-Me	3-Cl–4-Me–Ph	1	0.5	0.3	1	>64
2h	B	3-Cl–4-Me	4-Me	32	32	10	16	32
2i	B	3-Cl–4-Me	Phenethyl	0.5	1	1	1	>64
2j	A	3-F	3-F–Ph	>64	>64	>100	>64	>64
2k	B	3-Cl	3-SMe–Ph	8	8	3	4	>64
2l	B	3-Cl	2-Me–Ph	8	8	3	4	>64
2m	A	3-Cl–4-F	3-Cl–4-F–Ph	1	8	3	8	4
2n	A	3-Cl–4-OEt	3-Cl–4-OEt–Ph	2	2	1	2	>64
2o	A	3-Cl–4-NMe2	3-Cl–4-NMe_2–Ph	32	32	nt	64	>64
2p	B	3-Cl–4-Me	4-Me–Ph	4	2	3	2	>64
2q	A	4-Cl–2-Me	4-Cl–2-Me–Ph	4	2	0.3	0.5	16

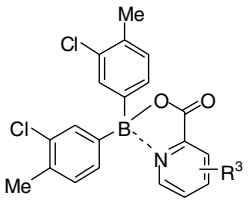
[a] Refer to Scheme 1; nt, not tested.

(**2g**) (unpublished data) and so this substitution was not considered for further development. From these results we concluded that a phenyl group at R^2 would be the best for activity.

Next, we investigated the SAR of substituents on the diphenylborinic acid moiety, using the bis(3-chlorophenyl) (**2a**) and bis(3-chloro-4-methylphenyl) (**2g**) derivatives as leads. Initially, we replaced the 3-chloro- groups of **2a** with fluoro groups, to give **2j**, however, this simple, homologous substitution led to a total eradication of antibacterial activity against all strains tested. Replacement of one 3-chloro group of **2a** with either 3-methylthiol group, to give **2k**, or 2-methyl, to give **2l**, resulted in reduced activity against *S. aureus* and *H. influenzae*, but increased activity against *P. acnes* and *B. subtilis* by 3- to 4-fold. Turning our attention to the bis(3-chloro-4-methylphenyl) derivative (**2g**), we replaced the methyl groups with fluoro groups to give **2m**. This showed an 8- to 16-fold reduction in activity against *S. epidermidis*, *P. acnes*, and *B. subtilis* but, interestingly, this compound had greatly increased activity against the Gram-negative bacterium *H. influenzae*. Replacement of the 4-methyl group of **2g** with ethoxy groups, to give **2n**, showed only a marginal decrease in activity, while replacement with dimethylamino groups, to give **2o**, rendered the molecule almost inactive. Removal of one 3-chloro group of **2g**, to give **2p**, showed reduction in activity of 2- to 10-fold, confirming the importance of both chloro groups. Finally, moving the methyl- and chloro- groups to the 2- and 4-positions, respectively, to give **2q**, showed an increase in activity against *B. subtilis* and *H. influenzae*, at the expense of activity against *Staphylococcus* species, which decreased 4-fold. From this study we concluded that one methyl and one chloro substituent on both phenyl rings gave the most potent activity.

In a final study, we investigated the importance of the substituent on the pyridine ring of picolinic acid. The results are shown in Table 2. We kept the bis(3-chloro-4-methyl)borinic acid unit constant while modifying the 3-hydroxy group on the picolinic acid part of **2g**. Removal of the 3-hydroxy group, to give **2r**, led to a loss of activity except against *S. aureus*, where the activity remained approximately equal. Since the 3-hydroxy group of **2g** can form a 6-membered hydrogen bonded ring with the carbonyl group at the 2-position, we synthesized the 3-acteoxy derivative (**2s**) to disrupt this potential ring structure and to determine if a proton donor was important for activity. This derivative was approximately as active as the lead compound (**2g**), implying that this hydrogen bonding and the proton on the 3-hydroxy group may not be important for activity. Next we increased steric bulk at the 3-position with a benzoyl group to give **2t**. This modification was not tolerated by most bacteria tested with a loss of activity of 30-fold or greater. Placement of a 3-amino group also led to a dramatic loss in activity except against *P. acnes* and *B. subtilis*. Lastly, we were able to determine the importance of the position of the substituent using a carboxylic acid group as a probe. The 3-carboxy derivative (**2v**) showed better activity than **2g** against *S. aureus*, and also showed surprisingly good activity against *H. influenzae*, however, it was not so effective against *S. epidermidis* and *P. acnes*. As the carboxy group was moved to the 4- and 5-positions, giving **2w** and **2x**, respectively, activity was approximately equivalent to **2v**. We concluded that the 3-hydroxy group was optimal for

Table 2. Minimum inhibitory concentration (MIC, μg/mL) results for compounds **2** containing a bis-(3-chloro-4-methylphenyl) borinic acid moiety



Compound	R^3	*S. aureus*	*S. epidermidis*	*P. acnes*	*B. subtilis*	*H. influenzae*
2r	H	0.5	>64	nt	nt	nt
2g	3-OH	1	0.5	0.3	1	>64
2s	3-OAc	2	1	1	0.5	>64
2t	3-COPh	0.5	32	30	64	>64
2u	3-NH_2	>64	>64	1	2	>64
2v	3-CO_2H	0.125	4	3	8	8
2w	4-CO_2H	2	4	3	nt	nt
2x	5-CO_2H	0.5	8	3	8	8

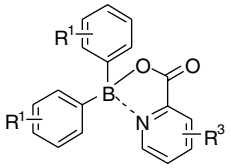
nt, not tested.

activity against the major cutaneous bacterial pathogens *S. aureus* and *P. acnes*.

In order to test for concomitant anti-inflammatory activity, compounds **2g**, **2s**, **2q**, and **2x** were evaluated for their ability to inhibit release of inflammatory cytokines from human peripheral blood mononuclear cells (PBMCs). Either lipopolysaccharide (LPS, 1 μg/mL), Concanavalin A (1 μg/mL) or phyto-hemagglutinin (PHA, 2 μg/mL) was used to induce the release of cytokines from the PBMCs. These compounds were screened at a concentration of 10 μM. ELISA kits were used to measure for two pro-inflammatory cytokines, TNF-α and IL-1β, a Th1 cytokine, IFN-γ, and a Th2 cytokine, IL-4. The inhibition of cytokine release for each compound was recorded as a percent of untreated control[9] and the results are shown in Table 3. Compound **2g** showed strong inhibition of the release of pro-inflammatory cytokines but no inhibition of IFN-γ or IL-4 release. Compound **2s** showed no inhibition of IL-1β release, so this was not tested for inhibition of IFN-γ or IL-4 release. Compounds **2q** and **2x** showed a similar profile to that of compound **2g**. By comparison, the antibiotic erythromycin showed no inhibition of pro-inflammatory cytokines. From these studies, compound **2g** was shown to have the best combination of anti-bacterial and anti-inflammatory activities.

In conclusion, we report the identification and structure–activity relationship of a new class of antibacterial agents, diphenylborinic acid picolinate esters. These compounds primarily show activity against Gram-positive bacteria and SAR showed the diphenyl borinic acid moiety was essential for activity. Potency was increased with the positioning of methyl- and chloro-substituents on the diphenyl borinic acid moiety in combination with a 3-hydroxyl group on the pyridine ring. The most potent derivative from this effort was **2g**, which was also found to inhibit the production of pro-inflammatory cytokines. As a result, **2g** (**AN0128**) was selected as a clinical candidate and is currently in clinical development for the treatment of dermatological diseases

Table 3. Percent inhibition of cytokine release from PBMCs, stimulated with either LPS, Concanavalin A, or PHA, by selected borinic acid picolinate ester screened at 10 μM



Compound	R^1	R^3	TNF-α[a]	IL-1β [a] (%)	IFN-γ[b] (%)	IL-4[c] (%)
	Erythromycin		22	−32	nt	nt
2g	3-Cl–4-Me–	3-OH	100	99	−20	−21
2s	3-Cl–4-Me–	3-OAc	101	−49	nt	nt
2q	4-Cl–2-Me–	3-OH	101	103	15	57
2x	3-Cl–4-Me–	5-CO_2H	100	80	24	9

nt, not tested.
[a] LPS stimulation (1 μg/mL) for 24 h (%).
[b] PHA stimulation (2 μg/mL) for 24 h.
[c] Concanavalin A stimulation (20 μg/mL) for 48 h.

including acne and atopic dermatitis. Results of these trials will be described in future publications.

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8. Typical procedures for the synthesis of borinic acid picolinate esters: (a) Preparation of 3-hydroxypyridine-2-carbonyloxy-bis(3-chloro-4-methylphenyl)borane (**2g**, **AN0128**): 2-chloro-4-iodotoluene (50 g, 200 mmol) was dissolved in THF (250 mL) at 0 °C and treated with isopropyl magnesium chloride (122 mL, 2.0 M in THF, 244 mmol) for 5 h. Trimethylborate (9.7 g, 93 mmol) was added and the reaction mixture was stirred overnight allowing to warm to room temperature. The reaction was quenched with 3 N HCl (100 mL) and the resulting mixture was extracted into ethyl acetate giving the crude intermediate, bis(3-chloro-4-methylphenyl)borinic acid, as a white solid in quantitative yield. A portion of this intermediate (14.6 g, 52.5 mmol) was dissolved in ethanol (120 mL) and heated to reflux. 3-Hydroxypicolinic acid (5.83 g, 42 mmol) was added in portions to the hot solution. The reaction mixture was stirred at reflux for 15 min after the addition of the last portion of 3-hydroxypicolinic acid and then cooled to room temperature. The reaction mixture was concentrated by removal of some ethanol and the product precipitated from the solution. The solid was removed by filtration and recrystallized from ethanol to give the title product (**2g**) as white crystals (13.4 g, 64%): mp 165.0–166.5 °C; ESI-MS (*m*/*z*) = 400, 402, 404 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 2.25 (s, 6 H), 7.08–7.11 (d, J = 7.5 Hz, 2H), 7.19–7.21 (m, 4H), 7.91–7.92 (d, J = 3.3 Hz, 2H), 8.56 (t, J = 2.9 Hz, 1H). (b) Preparation of 3-hydroxypyridine-2-carbonyloxy- (3-chloro-4-methylphenyl)(*p*-tolyl)borane (**2p**): 3-chloro-4-methylphenylboronic acid (5.0 g, 29.3 mmol) was heated to reflux with ethylene glycol (1:1 molar ratio) in toluene using a Dean–Stark trap. Rotary evaporation of the solvent gave 3-chloro-4-methylphenylboronic acid ethylene glycol ester (5.42 g, 27.6 mmol, 94%), which was then treated with *p*-tolylmagnesium bromide (28 mL, 1.0 M THF solution, 28 mmol) in anhydrous THF (100 mL) at −78 °C. The cooling bath was removed and the reaction mixture was stirred for 1 h before a room temperature water bath was placed for additional 2 h of stirring. The reaction was quenched with 6 M HCl and the resulting solution was rotary evaporated. The residue was extracted with ethyl acetate and purified by flash column chromatography over silica gel eluted with a mixed solvent of ethyl acetate and hexane (1:5) giving the desired (3-chloro-4-methylphenyl)(*p*-tolyl)borinic acid as a yellow liquid (6.35 g, 26 mmol, 94%). This material was mixed with 3-hydroxypicolinic acid (3.07 g, 22.1 mmol) in pure ethanol (150 mL) and stirred overnight at room temperature. The precipitates formed were collected by filtration and washed with hexane to give **2p** as a cream solid (6.99 g, 87%): mp 192–194 °C; ESI-MS (*m*/*z*) = 366 $(M+H)^+$, 364 $(M-H)^-$; ^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) 2.22 (s, 3H), 2.25 (s, 3H), 7.03 (d, J = 7.5 Hz, 2H), 7.09–7.13 (m, 3H), 7.19 (d, J = 6.0 Hz, 2H), 7.90 (d, J = 3.0 Hz, 2H), 8.48–8.49 (m, 1H), 12.10 (br s, 1H). (c) Preparation of 3-hydroxypyridine-2-carbonyloxy-(3-chlorophenyl)(3-pyridynyl)borane (**2c**): 3-chloro-phenylboronic acid (1.00 g, 6.09 mmol) was heated to reflux with ethylene glycol (0.340 mL, 6.09 mmol) in toluene using a Dean–Stark trap. Rotary evaporation of the solvent gave 3-chlorophenylboronic acid ethylene glycol ester (3.66 g, 20.0 mmol 91%), which was mixed with 3-bromopyridine (2.64 g, 16.7 mmol) and THF (100 mL), and treated with *n*-BuLi (1.6 M, 12 mL) over 4 h at −78 °C. The mixture was stirred overnight and allowed to warm to room temperature. Water was added and the pH was adjusted to 7 with hydrochloric acid. The mixture was extracted with Et_2O several times. The organic extracts were combined and dried on anhydrous $MgSO_4$. The solvent was removed under reduced pressure to give the crude borinic acid (2.05 g), which was used for the next step without purification. To a heated solution of the borinic acid (2.05 g, 9.43 mmol) in EtOH (10 mL) was added 3-hydroxypicolinic acid (1.04 g, 7.50 mmol) in EtOH (7 mL) and water (5 mL). The mixture was stirred for 4 h while cooling down to room temperature. Precipitates formed were collected by filtration and dried to give **2c** (1.56 g, 28% overall) as a tan solid: mp > 250 °C; ESI-MS (*m*/*z*) 339, 341, 343 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 7.07 (m, 1H), 7.16 (dd, J = 7.9, 7.2 Hz, 1H), 7.3–7.4 (m, 4H), 7.86 (dd, J = 7.6, 5.6 Hz, 1H), 8.01 (dd, J = 3.9, 1.9 Hz, 1H), 8.49 (d, J = 7.9 Hz, 1H), 8.6–8.7 (m, 2H).
9. Dexamethasone was used as a positive control for inhibiting release of TNF-α, IFN-γ, and IL-4, and cyclohexamide was used as a positive control for inhibiting release of IL-1β.