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Letters

Discovery of a New Boron-Containing Antifungal Agent, 5-Fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (AN2690), for the Potential Treatment of Onychomycosis

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Abstract: A structure−activity relationship investigation for a more efficacious therapy to treat onychomycosis, a fungal infection of the toe and fingernails, led to the discovery of a boron-containing small molecule, 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (AN2690), which is currently in clinical trials for onychomycosis topical treatment.

One of the most common fungal infections remains one of the most difficult to treat.[1,2] Onychomycosis is a fungal infection of the toenails and fingernails and has an incidence of 14% of the population in the U.S.[3] In diabetic and elderly patients the incidents ranges from 33% to 50%.[3,4]

Onychomycosis is caused mainly by dermatophytes, a class of fungus that dwells on skin, hair, and nails and is the cause of other cutaneous fungal infections such as athlete's foot.[5] The major dermatophytes involved are *Trichophyton rubrum* and *Trichophyton mentagrophytes*, accounting for approximately 60−90% of all cases.[1,6,7] There are few treatments available, and these include oral terbinafine, oral itraconazole, topical ciclopirox, and topical amorolfine. However, topical treatments have poor clinical efficacy and oral treatments have a high level of recurrence and concerns of systemic safety.[8−15]

Previously, we reported the development of borinic acid quinoline ester compounds as novel antibacterials (**1**, Figure 1).[16] Further structural modification of these boron-containing compounds led to the 1,3-dihydro-2,1-benzoxaborole class as illustrated by **2**. Focused screening of our library revealed these dihydrobenzoxaboroles had good antifungal activity against *C. albicans*. Further screening against yeast, filamentous fungi, and dermatophytes showed these compounds had broad spectrum activity against all these fungal pathogens including the major dermatophytes that cause onychomycosis, *T. rubrum* and *T. mentagrophytes*. Herein, we report the focused medicinal chemistry effort to develop a first-in-class boron-containing antifungal agent for the topical treatment of onychomycosis.

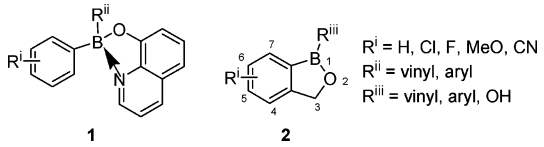

Figure 1. Boron-containing small-molecule library.

The dihydrobenzoxaborole class (**2**) emerged as a result of a directed effort to expand the chemical diversity of the borinic acid quinoline esters (**1**), all of which contained a boron−nitrogen dative bond.[16]

Dihydrobenzoxaboroles bearing aryl, heteroaryl, or vinyl substituents at the 1-position (**9**−**15**) were synthesized starting from 2-bromo-5-fluorobenzaldehyde (**3**) or 2-bromobenzyl alcohol (**4a**) as shown in Scheme 1. The hydroxy groups of **4a** and **4b** were protected as the methoxymethyl ether to give **5a** and **5b**, respectively. Compounds **5** were treated with butyllithium at −78 °C, and the anion formed was trapped by a boronic acid ethylene glycol ester (**7**), prepared from the corresponding boronic acid and ethylene glycol, to give the borinic acid (**8**). For **11** and **15**, intermediate **5b** was converted into the glycol ester **6**, which was reacted with Grignard reagents to give **8.** Finally, the protecting group was removed under acidic conditions and the free alcohol spontaneously cyclized to give the target compounds (**9**−**15**).

1-Hydroxydihydrobenzoxaboroles (**19b**−**m**) were synthesized as shown in Scheme 2. The protected *o*-bromobenzyl alcohol derivative (**18**), prepared from **16** or **17**, was converted into the corresponding phenylboronic acid. Deprotection of the methoxymethyl ether using hydrochloric acid followed by spontaneous cyclization gave the target compounds **19b**−**m** (**19c**, R^v = Me, was prepared as a racemate). When compounds have functional groups sensitive to butyllithium, such as a nitrile group, an in situ trap method was applied.[18]

The 7-fluoro derivative (**19n**) was synthesized through directed ortho metalation of 3-fluorobenzyl alcohol (**20**) (Scheme 3). [19]

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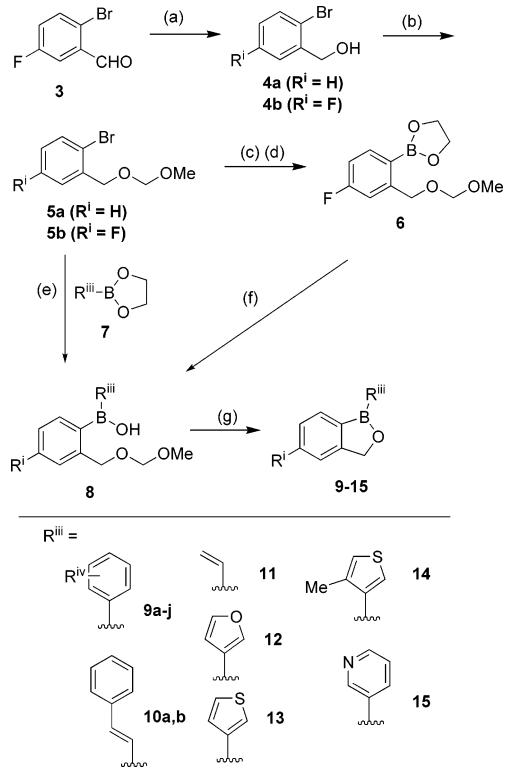
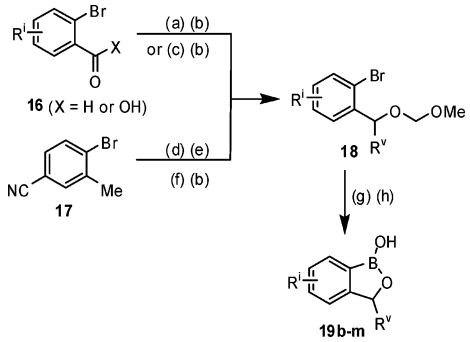
Scheme 1[a]



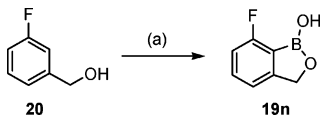
[a] Conditions: (a) $NaBH_4$, MeOH, room temp; (b) $MeOCH_2Cl$, *i*-Pr_2NEt, CH_2Cl_2, room temp; (c) *sec*-BuLi, $(MeO)_3B$, THF, −78 °C to room temp; (d) ethylene glycol, THF or toluene, reflux; (e) *n*- or *t*-BuLi, **7**, THF, −78 °C to room temp; (f) 3-bromopyridine, *i*-PrMgCl, THF, 0 °C (ref 17), or vinylmagnesium bromide, THF, −78 °C to room temp; (g) 6 N HCl, THF, room temp.

Scheme 2[a]



[a] Conditions: (a) $NaBH_4$, MeOH, room temp (when X = H), or BH_3−THF, THF, room temp (when X = OH); (b) $MeOCH_2Cl$, *i*-Pr_2NEt, CH_2Cl_2, room temp; (c) MeMgBr, THF, −78 °C to room temp; (d) NBS, AIBN, CCl_4, reflux; (e) NaOAc, DMF, 70 °C; (f) NaOH, MeOH, reflux; (g) *n*-BuLi, (*i*-PrO)$_3$B, THF, −78 °C to room temp; (h) 6 N HCl, THF, room temp.

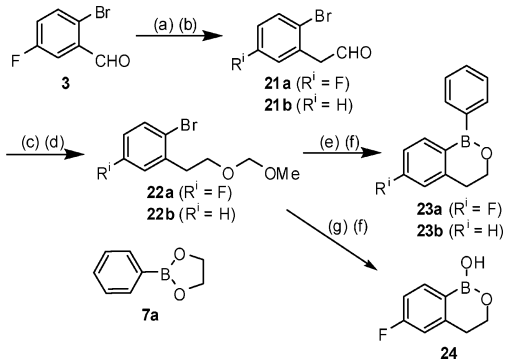
Scheme 3[a]



[a] Conditions: (a) *sec*-BuLi, (*i*-PrO)$_3$B, THF, −78 °C to room temp, then HCl.

Six-membered benzoxaborin analogues were synthesized as shown in Scheme 4. For 6-fluoro analogues, the benzaldehyde (**3**) was subjected to a Wittig reaction and the resulting enol ether was hydrolyzed to give the phenylacetaldehyde (**21a**). Reduction of the carbonyl group, followed by protection of the resulting alcohol gave the methoxymethyl ether (**22a**), which was converted to the final products **23a** and **24** using the same chemistry described previously. The unsubstituted derivative (**23b**) was synthesized from commercially available 2-bromophenylacetaldehyde (**21b**).

Scheme 4[a]



[a] Conditions: (a) Ph_3PCH_2OMeCl, *t*-BuOK, DMF, 0 °C to room temp; (b) 6 N HCl, THF, reflux; (c) $NaBH_4$, MeOH, room temp; (d) $MeOCH_2Cl$, *i*-Pr_2NEt, CH_2Cl_2, room temp; (e) *n*-BuLi, **7a**, THF, −78 °C to room temp; (f) 6 N HCl, room temp; (g) *n*-BuLi, (*i*-PrO)$_3$B, THF, −78 °C to room temp.

Table 1. Minimum Inhibitory Concentration (μg/mL) of Boron-Containing Compounds Compared to Ciclopirox

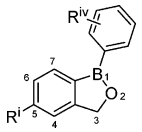
	R^i	other	*T. rubrum*	*T. mentagrophytes*	*C. albicans*	*C. neoformans*	*A. fumigatus*
ciclopirox			0.5	0.5	0.5	0.5	1
9a	H	R^{iv} = H	4	4	4	8	4
9b	5-F	R^{iv} = H	1	2	0.5	2	2
10a	H		8	8	2	4	4
10b	5-F		≤1	2	0.5	0.5	1
11	5-F		4	2	1	4	2
12	5-F		4	4	4	4	4
13	5-F		1	4	1	1	1
14	5-F		1	2	1	1	1
15	5-F		16	32	4	16	16
19a	H	R^v = H	8	4	2	1	2
19b	5-F	R^v = H	1	1	0.5	0.25	0.25
19c	5-F	R^v = Me	32	16	16	32	16
23a	6-F		8	8	8	8	8
23b	H		8	8	8	16	16
24	6-F		32	32	64	>64	32

To determine the antifungal activity of these compounds, we screened for their minimum inhibitory concentrations (MIC) against the major dermatophytes that cause onychomycosis, *T. rubrum* and *T. mentagrophytes*, and against the yeasts and molds *C. albicans*, *C. neoformans*, and *A. fumigatus* to test for their broad spectrum activity. The antifungal agent ciclopirox, currently in use for the topical treatment of onychomycosis, was used as a reference.

Our initial lead compound was the 1-phenyldihydrobenzoxaborole (**9a**) (Table 1). This showed modest broad spectrum activity with MIC values of 4−8 μg/mL. One of the first modifications made was to install a 5-fluoro group, giving **9b**. This substitution led to a 2- to 8-fold increase in potency against the strains tested. Subsequently, most of the following analogues synthesized contained this fluoro substitution.

We set out to determine the effect of replacing the 1-phenyl group of **9** with various substitutions. The 1-styryl-substituted dihydrobenzoxaboroles **10a** and **10b** led to approximately equivalent activity to our leads **9a** and **9b**, respectively, against all fungi tested (Table 1). Again, when R^i = 5-F (**10b**), potency was improved 4- to 8-fold. Replacing the 1-phenyl group of **9b** with 1-vinyl (**11**) or 1-(furan-3-yl) (**12**) led to an approximate

Table 2. Minimum Inhibitory Concentration (μg/mL) of 1,3-Dihydro-1-phenyl-2,1-benzoxaborole Compounds (**9**)



compd	R^i	R^{iv}	*T. rubrum*	*T. mentagrophytes*	*C. albicans*	*C. neoformans*	*A. fumigatus*
9a	H	H	4	4	4	8	4
9b	F	H	1	2	0.5	2	2
9c	H	3′-Cl	16	8	8	4	8
9d	H	3′-F	8	16	4	4	8
9e	H	4′-F	8	4	4	2	2
9f	F	3′-Cl	4	8	0.25	0.5	2
9g	F	3′-F	1	2	0.5	1	1
9h	F	4′-F	4	4	1	1	2
9i	F	3′-Me	2	4	1	0.5	0.5
9j	F	4′-Me	2	2	0.5	0.5	0.5

2- to 8-fold decrease in activity, while replacement with 1-(thiophen-3-yl) (**13**) or 1-(4-methylthiophen-3-yl) (**14**) led to approximately equal activity against all fungi (Table 1). Interestingly, replacement of the 1-phenyl group of **9b** with 1-(pyrid-3-yl) (**15**) showed selectivity toward nondermatophyte strains; there was a 16- to 64-fold decrease in activity against the dermatophytes *T. rubrum* and *T. mentagrophytes* but no change in activity against *C. albicans* and only a 4-fold reduction in activity against *C. neoformans* and *A. fumigatus* (Table 1).

In another modification to enhance hydrophilicity, we replaced the 1-phenyl group of **9a** and **9b** with a 1-hydroxy group to give **19a** and **19b**, respectively. Compounds **19a** and **19b** proved to have a more broad spectrum profile than **9a** and **9b**, respectively. Both **19a** and **19b** showed an 8-fold increase in activity against *C. neoformans*, and **19b** showed an 8-fold increase in activity against *A. fumigatus*. (Table 1).

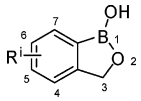
In an effort to understand the effect of the 3-substiution on the oxaborole ring, we added a methyl group to the 3-position to give **19c**. However, this modification led to an 8- to 32-fold decrease in activity (Table 1).

We then increased the ring size from a five-membered oxaborole of **9a**, **9b**, and **19b** to the corresponding six-membered oxaborin, giving **23b**, **23a**, and **24**, respectively. The results of these are shown in Table 1. The 1-phenyl substituted oxaborin **23b** was only approximately 2-fold less active than the oxaborole **9a**. In contrast, the 5-fluoro-1-phenyloxaborin **23a** was 4- to 16-fold less active than the corresponding oxaborole **9b**, showing that in this case, the 5-fluoro group gave no advantage on potency over the unsubstituted oxaborin **23b**. Finally, the 1-hydroxyoxaborin **24**, was 32- to 256-fold less active than the corresponding oxaborole **19b**.

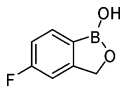
The results shown in Table 1 led us to conclude that a five-membered benzoxaborole ring, with no substitution at the 3-position, was optimum for activity. Furthermore, we found that the 1-phenyl or 1-hydroxy substituents gave the best potency.

Next, we focused our attention on the 1-phenyldihydrobenzoxaborole scaffold (**9**) to determine the effect of substitutions on the 1-phenyl ring, and examples of these are shown in Table 2. As before, in all cases, compounds with $R^i = F$ were more potent than compounds with $R^i = H$. Compounds **9f**−**j** showed activity similar to that of the lead compound **9b**; however, because of in vitro cytotoxicity of **9f**−**j** compared to **9a** (data not shown), these compounds were not considered for further development.

Table 3. Minimum Inhibitory Concentration (μg/mL) of 1,3-Dihydro-1-hydroxy-2,1-benzoxaborole Compounds (**19**)



compd	R^i	*T. rubrum*	*T. mentagrophytes*	*C. albicans*	*C. neoformans*	*A. fumigatus*
19b	5-F	1	1	0.5	0.25	0.25
19d	5-Cl	1	2	1	2	1
19e	5-Me	8	4	2	8	2
19f	5-CF_3	8	8	16	16	8
19g	5-NC	16	16	8	8	16
19h	5-MeO	64	32	>64	>64	>64
19i	5-$HOCH_2$	64	64	>64	>64	>64
19j	6,7-benzo	4	2	32	32	32
19k	5-F-6-F	4	4	4	2	2
19l	4-F	16	16	64	32	32
19m	6-F	16	32	16	32	8
19n	7-F	16	16	32	32	4



19b

Figure 2. Structure of AN2690, currently in clinical trials for onychomycosis.

In a final study, we synthesized various analogues of 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (**19b**) to determine the structure−activity relationship of this scaffold. The results of this study are shown in Table 3. We first substituted the 5-F group with other groups, giving **19d**−**i**, to determine the optimum substituent for this position. The results showed that the 5-F compound **19b** remained the most potent with only the 5-Cl analogue **19d** approaching similar activity. The 5-methyl, 5-trifluoromethyl, and 5-cyano analogues **19e**, **19f**, and **19g**, respectively, showed only weak activity, while 5-methoxy and 5-hydroxymethyl analogues **19h** and **19i**, respectively, were inactive. Interestingly, the 6,7-benzo analogue (**19j**) showed moderate activity toward the dermatophytes but was effectively inactive against the nondermatophyte strains.

In another modification, we found that addition of a second fluoro group at the 6-position, giving **19k**, effectively offset the additional potency provided by the 5-fluoro substituent. In a final modification we moved the fluoro group to other positions around the benzo ring, giving **19l**−**n**, and found that the optimum position for the fluoro group remained at the 5-position.

From the results shown in Tables 2 and 3, we concluded that the 1-phenyl- and 1-hydroxy- 5-fluoro-1,3-dihydro-2,1-benzoxaborole compounds **9b** and **19b**, respectively, were the most active against fungi and especially against the dermatophytes *T. rubrum* and *T. mentagrophytes*, the primary fungal pathogens causing onychomycosis. To evaluate the ability of the dihydrobenzoxaborole antifungals to treat onychomycosis by topical application, we have examined these compounds in a number of in vitro experiments including nail penetration and keratin binding (results to be reported elsewhere). From these preclinical studies, 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (**19b**) (Figure 2) was identified as having a unique profile of in vitro antidermatophyte activity, maintenance of this activity in the presence of keratin, and exceedingly good penetration of human nails. This compound is currently undergoing clinical trials for the treatment of onychomycosis.

In conclusion, we present a novel class of boron-containing compounds with broad spectrum antifungal activity. We report the synthesis and describe the structure−activity relationship for in vitro antifungal activity. One member of this new family of antifungal compounds, **19b**, was identified as our clinical candidate for onychomycosis. Future reports from our labs will describe other preclinical studies, mechanism of action, application to systemic diseases, and clinical trial results for onychomycosis.

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Supporting Information Available: Experimental procedures and characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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