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Preliminary investigations into the synthesis and antimicrobial activities of boron-containing capsaicinoids

Sharwatie Ramsaywack, Allyson Bos, Christopher M. Vogels, Christopher A. Gray, and Stephen A. Westcott

Abstract: This preliminary study reports on the synthesis of two new boron-capsaicin derivatives containing either a short or long chain aliphatic tail group using an iridium catalyzed hydroboration reaction with pinacolborane. The boronate ester groups reside on the terminal position of the tail group and are necessary for the bioactivity of these compounds. Indeed, both compounds showed considerable activity against two Gram-positive bacteria, including Vancomycin-resistant *Enterococcus*. Vancomycin is considered the last resort medication for the treatment of septicemia, and new antibacterial agents that can treat sepsis are of paramount importance. The more lipophilic boron compound with the longer aliphatic chain also showed antifungal activity against *Saccharomyces cerevisiae*.

Key words: antimicrobial, boron, capsaicin, hot peppers.

Résumé : Cette étude préliminaire fait état de la synthèse de deux nouveaux dérivés de la capaïcine à base de bore comprenant un groupe « de queue », composé d'une chaîne aliphatique soit courte ou longue, au moyen d'une hydroboration avec le pinacolborane catalysée par l'iridium. Les groupes d'esters boroniques se trouvent à la position terminale du groupe de queue et sont nécessaires à la bioactivité de ces composés. En effet, les deux composés ont présenté une activité considérable contre deux bactéries à Gram positif, dont un entérocoque résistant à la vancomycine. La vancomycine est considérée comme *le* médicament de dernier recours pour traiter la septicémie, et les nouveaux agents antibactériens capables de traiter un sepsis sont d'une importance capitale. Le composé de bore plus lipophile, doté de la chaîne aliphatique la plus longue, présente également une activité antifongique contre *Saccharomyces cerevisiae*. [Traduit par la Rédaction]

Mots-clés : antimicrobien, bore, capsaïcine, piments forts.

Introduction

Although α-aminoboronic acids have been known for several decades to be potent inhibitors of serine proteases,[1] boron-containing compounds have been largely ignored by the pharmaceutical industry until the discovery that the boropeptide Velcade® (aka Bortezomib, Fig. 1 compound (*a*) with the α-aminoboronic acid fragment shown in blue) showed significant promise for the treatment of multiple myeloma (a cancer of the plasma cells)[1a] and mantle cell lymphoma (a cancer of the lymph nodes).[1b] Indeed, Velcade® has been approved by the FDA as the first ever boron-containing pharmaceutical molecule. Since this remarkable discovery, the second-generation derivative Ninlaro® (aka Ixazomib citrate, Fig. 1 compound (*b*) with the α-aminoboronic acid fragment shown in blue) has been approved for the treatment of relapsed multiple myeloma. Although Velcade® must be administered by injection, Ninlaro® has a huge marketable advantage in that it can be taken orally.

Other small molecule boron compounds that do not contain α-aminoboronic acid fragments have also shown promise for their potential use as pharmaceuticals. For instance, diazaborines are a privileged class of heterocyclic compounds that have potent antimicrobial properties (a representative example of a thienodiazaborine is shown in Fig. 1 compound (*c*)).[2] The mechanism of action of diazaborines is believed to involve the inhibition of fatty acid biosynthesis in *Escherichia coli*, where the boron compound inhibits its maturation of rRNAs for the large ribosomal subunit.[2f,2j] Although no diazaborines have yet to reach clinical trials, another small boron molecule, Kerydin® (aka Tavaborole, Fig. 1 compound (*d*)), has been developed for the treatment of onychomycosis, a fungal infection of the nail and nail bed.[3] This simple compound inhibits the essential fungal enzyme, leucyl-tRNA synthetase, leading to termination of cell growth and ultimately cell death, thus completely eradicating the fungal infection.

As part of our ongoing program for generating bioactive boron compounds,[2o,2t,4] we have begun to design new compounds based on the structural motif of capsaicin. Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) is the spicy and active ingredient in hot chili peppers and its biological activities are well-known to include, but not limited to, anticancer and anti-inflammatory properties and show promise in the treatment of Alzheimer's disease.[5] The chemical structure of capsaicin consists of two major groups; (*i*) an aromatic head group with an amide linkage, and (*ii*) an aliphatic hydrocarbon tail (Fig. 2). In this preliminary study, we have decided to prepare a small family of capsaicinoids in an effort to incorporate a boronate ester fragment into the tail group, with one representative short chain and one long chain

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Fig. 1. Structures of (*a*) Velcade®, (*b*) Ninlaro®, (*c*) a representative thienodiazaborine, and (*d*) Kerydin®. [Colour online.]

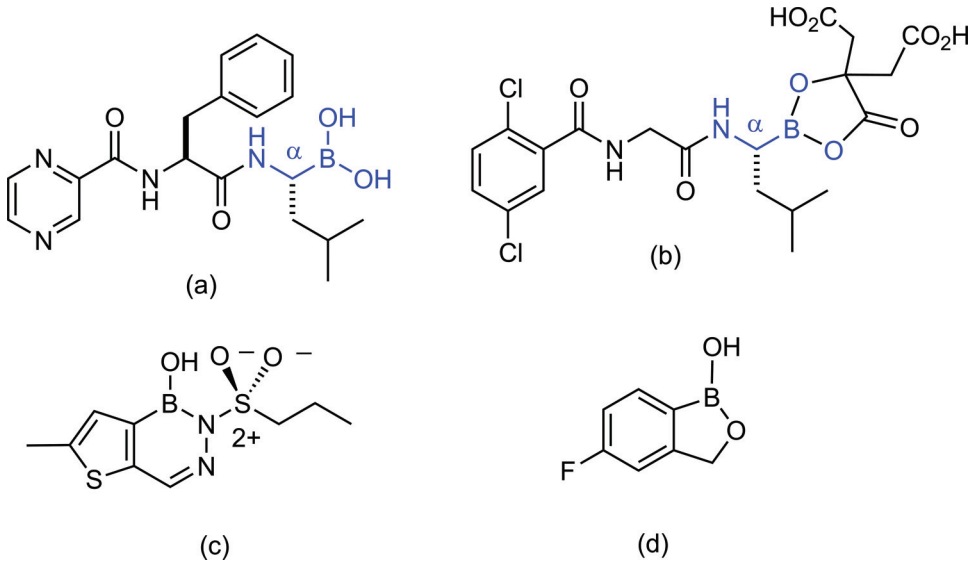
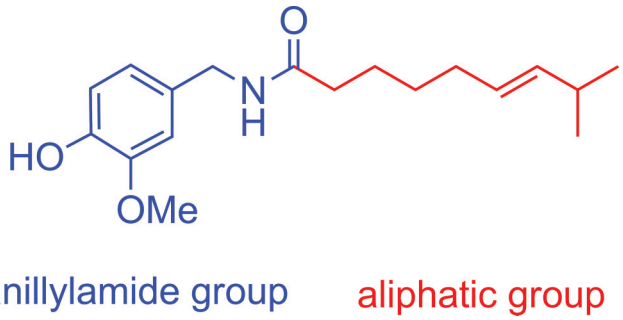


Fig. 2. The molecular structure of capsaicin. [Colour online.]



example, to see if the boron group could augment biological activities in these compounds, the results of which are presented herein.

Experimental

Materials and methods

Reagents and solvents used were obtained from Sigma-Aldrich. $[Ir(cod)Cl]_2$ (cod = 1,5-cyclooctadiene),[6] 4-(benzyloxy)-3-methoxybenzonitrile,[7] and (4-(benzyloxy)-3-methoxyphenyl) methanamine and its hydrochloride salt[8] have been synthesized previously. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL JNM-GSX400 FT NMR (^{1}H: 400 MHz; ^{11}B: 128 MHz; ^{13}C: 100 MHz) spectrometer. Chemical shifts (δ) are reported in ppm (relative to residual solvent peaks (^{1}H and ^{13}C) or external $BF_3{\cdot}OEt_2$ (^{11}B)). Multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint), multiplet (m), broad (br), or overlapping (ov) with coupling constants (*J*) reported in hertz. Fourier transform infrared (FTIR) spectra were obtained with a Thermo Fisher Scientific Nicolet iS5 FTIR spectrometer in attenuated total reflections (ATR) mode and are described as strong (s), medium (m), or weak (w) and are reported in cm^{-1}. Melting points were measured uncorrected with a Stuart SMP30 apparatus. Elemental analyses for C, H, and N were carried out at Laboratoire d'Analyse Élémentaire de l'Université de Montréal (Montréal, QC). Column chromatography was carried out using silica gel (230–400 mesh). Thin layer chromatography (TLC) used pre-coated glass-backed plates covered with 0.25 mm silica gel containing fluorescent indicator UV 254 nm; visualization by UV light or $KMnO_4$ stain. Reactions were performed in oven-dried glassware under an inert atmosphere unless otherwise stated.

General procedure for the synthesis of acyl chlorides

Mixtures of excess $SOCl_2$ (10 equivalents) and the unsaturated carboxylic acid derivatives (5 mmol) were heated at reflux for 2 h in THF (10 mL). Removal of excess $SOCl_2$ under vacuum afforded the corresponding acyl chloride compounds (as confirmed by ^{1}H NMR spectroscopy), which were used without further purification.

Synthesis of *N*-(4-(benzyloxy)-3-methoxybenzyl)pent-4-enamide (1)

A solution of compound (4-(benzyloxy)-3-methoxyphenyl) methanamine·HCl (160 mg, 0.57 mmol) and NEt_3 (69 mg, 0.68 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C was treated with a solution of the appropriate acyl chloride (92 mg, 0.78 mmol) in anhydrous CH_2Cl_2 (5 mL). Aqueous workup, removal of solvents, and column chromatography (silica gel, EtOAC/hexanes, 1:2 to 2:1) gave compound **1** as a white solid following removal of the solvents under vacuum. Yield: 160 mg (86%); mp: 108–109 °C. R_f = 0.34 (EtOAc/hexanes, 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.41 (d, *J* = 6.9 Hz, 2H, Ar), 7.35 (ov dd, *J* = 7.3, 6.9 Hz, 2H, Ar), 7.28 (t, *J* = 7.3 Hz, 1H, Ar), 6.81 (d, *J* = 1.8 Hz, 1H, Ar), 6.80 (d, *J* = 8.2 Hz, 1H, Ar), 6.72 (dd, *J* = 8.2, 1.8 Hz, 1H, Ar), 5.81 (ov ddt, *J* = 17.4, 10.1, 6.9 Hz, 1H, CH_2CH=CHH), 5.70 (br s, 1H, N*H*), 5.13 (s, 2H, OCH_2Ph), 5.06 (d ov dt, *J* = 17.4, 1.8, 1.4 Hz, 1H, CH_2CH=CH*H*), 4.98 (d ov dt, *J* = 10.1, 1.4, 1.4 Hz, 1H, CH_2CH=C*H*H), 4.30 (d, *J* = 5.7 Hz, 2H, $NHCH_2Ar$), 3.86 (s, 3H, OMe), 2.40 (ov tddd, *J* = 7.8, 6.9, 1.8, 1.4 Hz, 2H, CH_2CH_2CH=CH_2), 2.28 (td, *J* = 7.8, 0.9 Hz, 2H, $C(O)CH_2CH_2$). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ: 172.1, 149.9, 147.6, 137.1, 137.0, 131.5, 128.7, 127.9, 127.3, 120.1, 115.8, 114.0, 111.7, 71.1, 56.1, 43.5, 36.0, 29.7. IR: 3297 (m), 2929 (w), 1638 (m), 1511 (m), 1259 (s), 1226 (s), 1135 (s), 1005 (m), 916 (m), 855 (m), 696 (s). Anal. calcd for $C_{20}H_{23}NO_3$ (325.40 g/mol) (%): C 73.82, H 7.12, N 4.30; found: C 73.86, H 7.20, N 4.27.

Synthesis of *N*-(4-(benzyloxy)-3-methoxybenzyl)undec-10-enamide (2)

Under ambient conditions, NaOH (1.20 mL of a 1.0 N solution, 1.20 mmol) was added to a solution of (4-(benzyloxy)-3-methoxyphenyl) methanamine·HCl (0.33 g, 1.18 mmol) in H_2O (10 mL) at RT. The resulting mixture was allowed to stir for 15 min before $CHCl_3$ (20 mL) was added and the organic layer separated. The aqueous layer was extracted with $CHCl_3$ (2 × 20 mL) and the combined organic extracts sequentially washed with distilled water and

brine and dried with anhydrous $MgSO_4$. The $MgSO_4$ was separated by gravity filtration and the solvent was removed under vacuum to give free amine (4-(benzyloxy)-3-methoxyphenyl)methanamine as a light yellow oil (0.25 g, 1.03 mmol, 87%), which was used in the subsequent step without further purification. Under an inert atmosphere, the corresponding acyl chloride (0.21 g, 1.04 mmol) in anhydrous CH_2Cl_2 (5 mL) at 0 °C was added to an anhydrous CH_2Cl_2 (10 mL) solution of (4-(benzyloxy)-3-methoxyphenyl) methanamine (0.25 g, 1.03 mmol) and NEt_3 (0.11 g, 1.09 mmol). Upon warming to room temperature, the reaction was allowed to proceed for 4 h, at which point, the reaction was concentrated under vacuum and purification by flash column chromatography (silica gel, EtOAc/hexanes, 1:1) gave **2** as a white solid after removal of solvents. Yield: 0.33 g (78%); mp: 98–99 °C. R_f = 0.51 (EtOAc/hexanes, 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.41 (d, *J* = 6.9 Hz, 2H, Ar), 7.34 (ov dd, *J* = 7.3, 6.9 Hz, 2H, Ar), 7.29 (t, *J* = 7.3 Hz, 1H, Ar), 6.81 (d, *J* = 1.8 Hz, 1H, Ar), 6.80 (d, *J* = 8.2 Hz, 1H, Ar), 6.72 (dd, *J* = 8.2, 1.8 Hz, 1H, Ar), 5.79 (ov ddt, *J* = 16.9, 10.1, 6.4 Hz, 1H, CH_2CH=CHH), 5.64 (br t, *J* = 6.0 Hz, 1H, N*H*CH_2Ar), 5.13 (s, 2H, OCH_2Ph), 4.97 (d ov dt, *J* = 16.9, 1.8, 1.4 Hz, 1H, CH_2CH=CH*H*), 4.91 (d ov dt, *J* = 10.1, 1.4, 1.4 Hz, 1H, CH_2CH=C*H*H), 4.34 (d, *J* = 6.0 Hz, 2H, NHCH_2Ar), 3.86 (s, 3H, OMe), 2.17 (t, *J* = 7.5 Hz, 2H, C(O)CH_2CH_2), 2.01 (ov td, *J* = 7.8, 6.4 Hz, 2H, CH_2CH=CH_2), 1.63 (quint, *J* = 7.1 Hz, 2H, $CH_2CH_2CH_2$), 1.36–1.24 (ov m, 10H, –(CH_2)$_5$–). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ: 173.0, 149.9, 147.6, 139.3, 137.1, 131.6, 128.7, 127.9, 127.3, 120.1, 114.3, 114.0, 111.7, 71.1, 56.1, 43.5, 36.9, 33.9, 29.4 (3C), 29.2, 29.0, 25.9. IR: 3310 (m), 3066 (w), 2926 (s), 2853 (m), 1637 (s), 1542 (m), 1516 (m), 1466 (m), 1236 (m), 1138 (m), 1012 (w). Anal. calcd for $C_{26}H_{35}NO_3$ (409.56 g/mol) (%): C 76.25, H 8.61, N 3.42; found: C 76.36, H 8.61, N 3.43.

Synthesis of (*E*)-*N*-(4-(benzyloxy)-3-methoxybenzyl)but-2-enamide (3)

A solution of compound (4-(benzyloxy)-3-methoxyphenyl) methanamine·HCl (0.42 g, 1.50 mmol) and NEt_3 (0.18 g, 1.78 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated with a solution of the appropriate acyl chloride (0.18 g, 1.72 mmol) in anhydrous CH_2Cl_2 (5 mL). Aqueous workup, removal of solvents, and column chromatography (silica gel, EtOAC/hexanes, 1:2 to 2:1) gave compound **3** as a white solid after removal of solvents under vacuum. Yield: 0.44 g (94%); mp: 122–123 °C. R_f = 0.38 (EtOAc/hexanes, 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.41 (d, *J* = 7.3 Hz, 2H, Ar), 7.34 (ov dd, *J* = 7.3, 6.9 Hz, 2H, Ar), 7.29 (t, *J* = 6.9 Hz, 1H, Ar), 6.84 (ov dq, *J* = 15.1, 6.9 Hz, 1H, HC=C*H*CH_3), 6.81 (d, *J* = 1.8 Hz, 1H, Ar), 6.80 (d, *J* = 8.2 Hz, 1H, Ar), 6.72 (dd, *J* = 8.2, 1.8 Hz, 1H, Ar), 5.77 (dq, *J* = 15.1, 1.4 Hz, 1H, *H*C=CHCH$_3$), 5.64 (br s, 1H, N*H*CH_2), 5.13 (s, 2H, OCH_2Ph), 4.40 (d, *J* = 5.5 Hz, 2H, NHCH_2Ph), 3.86 (s, 3H, OMe), 1.84 (dd, *J* = 6.9, 1.4 Hz, 3H, CH=CHCH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ: 165.9, 149.8, 147.4, 139.9, 137.1, 131.8, 128.6, 127.9, 127.3, 125.1, 120.1, 114.1, 111.8, 71.1, 56.0, 43.3, 17.7. IR: 3288 (m), 3035 (w), 2916 (w), 1671 (m), 1625 (s), 1540 (m), 1511 (s), 1385 (m), 1224 (s), 1009 (m), 971 (m), 795 (s). Anal. calcd for $C_{19}H_{21}NO_3$ (311.37 g/mol) (%): C 73.29, H 6.80, N 4.50; found: C 73.29, H 6.82, N 4.48.

Synthesis of *N*-(4-(benzyloxy)-3-methoxybenzyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pentanamide (4)

A solution of $[Ir(cod)Cl]_2$ (12 mg, 0.018 mmol) and 1,2-bis (diphenylphosphino)ethane (16 mg, 0.040 mmol) in THF (2 mL) was stirred for 10 min and to this mixture was sequentially added a THF (2 mL) solution of **1** (100 mg, 0.31 mmol) and a THF (2 mL) solution of HBpin (95 mg, 0.74 mmol). After 24 h, the reaction mixture was concentrated and the mixture purified by flash column chromatography (silica gel, EtOAc/hexanes, 1:1) to provide **4** as a colourless waxy solid after removal of solvents. Yield: 132 mg (94%); mp: 74–75 °C. R_f = 0.23 (EtOAc/hexanes, 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.41 (d, *J* = 6.9 Hz, 2H, Ar), 7.34 (ov dd, *J* = 7.3, 6.9 Hz, 2H, Ar), 7.28 (t, *J* = 7.3 Hz, 1H, Ar), 6.81 (d, *J* = 1.8 Hz, 1H, Ar), 6.79 (d, *J* = 8.2 Hz, 1H, Ar), 6.71 (dd, *J* = 8.2, 1.8 Hz, 1H, Ar), 5.77 (br s, 1H, N*H*), 5.12 (s, 2H, OCH_2Ph), 4.30 (d, *J* = 5.5 Hz, 2H, NHCH_2Ar), 3.86 (s, 3H, OMe), 2.19 (t, *J* = 7.5 Hz, 2H, C(O)CH_2CH_2), 1.64 (quint, *J* = 7.5 Hz, 2H, C(O)$CH_2CH_2CH_2$), 1.43 (quint, *J* = 7.5 Hz, 2H, $CH_2CH_2CH_2$Bpin), 1.20 (s, 12H, pin), 0.78 (t, *J* = 7.5 Hz, 2H, CH_2CH_2Bpin). ^{11}B NMR ($CDCl_3$, 128 MHz) δ: 33 (br). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ: 172.9, 149.9, 147.5, 137.2, 131.7, 128.6, 127.9, 127.3, 120.1, 114.0, 111.7, 83.1, 71.1, 56.1, 43.4, 36.7, 28.4, 24.9, 23.7, 11 (br, *C*B). IR: 3291 (w), 2924 (s), 2854 (s), 1728 (m), 1637 (m), 1459 (m), 1379 (m), 1223 (m), 1138 (m). Anal. calcd for $C_{26}H_{36}NBO_5$ (453.38 g/mol) (%): C 68.88, H 8.00, N 3.09; found: C 68.62, H 8.16, N 3.01.

Synthesis of *N*-(4-(benzyloxy)-3-methoxybenzyl)-11-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)undecanamide (5)

A mixture of $[Ir(cod)Cl]_2$ (10 mg, 0.015 mmol) and dppe (dppe = 1,2-bis(diphenylphosphino)ethane) (12 mg, 0.030 mmol) in THF (2 mL) was stirred for 10 min, at which point, a THF (1 mL) solution of **2** (98 mg, 0.24 mmol) and a THF (1 mL) solution of HBpin (46 mg, 0.36 mmol) were added sequentially. After 24 h, the reaction mixture was concentrated and purified by flash column chromatography (silica gel, EtOAc/hexanes, 1:1) to provide compound **5** as a colourless waxy solid after removal of solvents. Yield: 120 mg (93%); mp: 74–76 °C. R_f = 0.53 (EtOAc/hexanes, 1:4). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.41 (d, *J* = 7.3 Hz, 2H, Ar), 7.34 (ov dd, *J* = 7.3, 6.8 Hz, 2H, Ar), 7.28 (t, *J* = 6.8 Hz, 1H, Ar), 6.81 (d, *J* = 1.8 Hz, 1H, Ar), 6.80 (d, *J* = 8.2 Hz, 1H, Ar), 6.72 (dd, *J* = 8.2, 1.8 Hz, 1H, Ar), 5.67 (br t, *J* = 5.5 Hz 1H, *H*NCH_2Ar), 5.13 (s, 2H, OCH_2Ph), 4.34 (d, *J* = 5.5 Hz, 2H, NHCH_2Ar), 3.86 (s, 3H, OMe), 2.17 (t, *J* = 7.5 Hz, 2H, C(O)CH_2CH_2), 1.62 (quint, *J* = 7.5 Hz, 2H, C(O)$CH_2CH_2CH_2$), 1.37 (quint, *J* = 7.5 Hz, 2H, $CH_2CH_2CH_2$Bpin), 1.30–1.20 (ov m, 24H, -(CH_2)$_6$- and pin), 0.74 (t, *J* = 7.5 Hz, 2H, CH_2CH_2Bpin). ^{11}B NMR ($CDCl_3$, 128 MHz) δ: 33 (br). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ: 173.0, 149.9, 147.6, 137.1, 131.6, 128.6, 127.9, 127.3, 120.1, 114.0, 111.7, 82.9, 71.1, 56.1, 43.5, 37.0, 32.5, 29.7, 29.6, 29.5, 29.4, 25.9, 24.9 (2C), 24.1, 11 (br, *C*B). IR: 3307 (m), 2924 (s), 2854 (s), 1639 (w), 1462 (s), 1377 (m), 1145 (w). Anal. calcd for $C_{32}H_{48}NBO_5$ (537.54 g/mol) (%): C 71.50, H 9.00, N 2.61; found: C 71.52, H 9.06, N 2.57.

Stability testing of compounds

In NMR tubes, compounds **1–5** were dissolved in DMSO-d_6 and analyzed by ^{1}H NMR spectroscopy. The solutions were stored at 37 °C for 2 d, at which point, the compounds were reanalyzed by ^{1}H NMR spectroscopy. No significant decomposition of the compounds was observed over this time period.

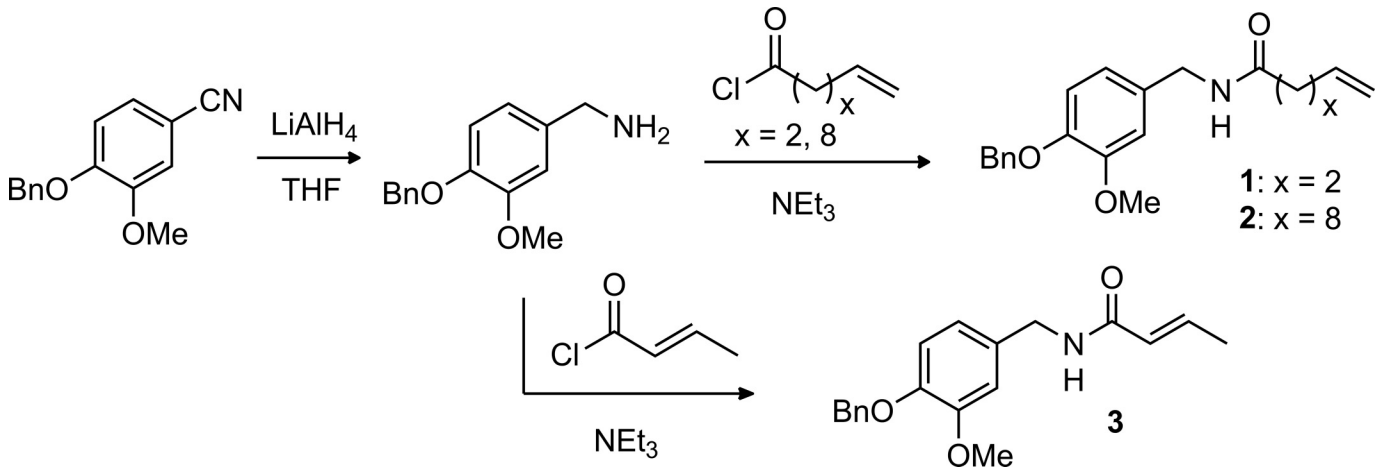
General biological testing

Antibiotic susceptibility tests were performed using BBL Mueller Hinton II cation adjusted broth (Becton Dickinson, Mississauga, ON) for *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa*, Bacto Brain Heart Infusion broth (Becton Dickinson, Mississauga, ON) for *Enterococcus faecium* and vancomycin-resistant *Enterococcus faecium* (VRE), Difco Sabouraud Dextrose broth (Becton Dickinson, Mississauga, ON) for *Candida albicans*, and Difco Yeast Mold broth (Becton Dickinson, Mississauga, ON) for *Saccharomyces cerevisiae* in nontissue culture treated 96-well microtitre plates. Optical densities (OD) were measured using a Molecular Devices Emax microplate reader with a 600 nm filter.

Antibiotic susceptibility screening assay

Antibacterial activity against *S. aureus* (ATCC 29213), MRSA (ATCC 33591), *E. faecium* (ATCC 35667), VRE (ATCC 51559), and *P. aeruginosa* (ATCC 10145) and antifungal activity against *C. albicans* (ATCC 14053) and *S. cerevisiae* (ATCC 9763) were evaluated using a microbroth dilution antibiotic susceptibility assay modified from McCulloch et al.[9] Stock solutions of the test compounds (10 mmol/L) were prepared with sterile-filtered DMSO, stored at 4 °C, and used within 1 week of preparation. Immediately prior to use, stock solutions (40 μL) were diluted with the appropriate nutrient broth (960 μL), and the resulting test solutions (100 μL)

Scheme 1. Synthesis of unsaturated amides **1–3**.



were transferred to nonperipheral wells of a 96-well microtitre plate in triplicate. Wells were then inoculated with suspensions of the appropriate microbial strain (100 μL) of cell density 5 × 10^5 colony-forming units (CFU)/mL. To reduce evaporation from the plates, sterile water (200 μL) was added to perimeter wells. Positive controls consisted of an appropriate antibiotic tested in triplicate (0.625 μg/mL, erythromycin for *S. aureus*; 5 μg/mL, vancomycin for MRSA; 0.625 μg/mL, tetracycline for *E. faecium* and VRE; 1.25 and 10 μg/mL, and *P. aeruginosa*, respectively; 2.5 μg/mL, nystatin for *C. albicans*; and 2.5 μg/mL, amphotericin B for *S. cerevisiae*) as 4% DMSO solutions in the appropriate nutrient broth inoculated with suspensions of the appropriate pathogen (100 μL). In addition to test and positive control wells, each plate contained three untreated controls [4% DMSO in the appropriate nutrient broth (100 μL) inoculated with suspensions of the appropriate pathogen (100 μL)] and three uninoculated blanks (200 μL of 2% DMSO in the appropriate nutrient broth). The optical densities of the wells were measured before and after a 24 h incubation period (37 °C), and the change in optical density (ΔOD) was calculated by subtracting the initial optical density from the final optical density of corresponding wells. The ΔOD values were corrected for background absorbance of the media by subtracting the mean ΔOD readings of the blanks from the mean ΔOD readings of the control and test compound wells. The percentage inhibition of bacterial or fungal growth was then defined as [1 – (mean test or positive control ΔOD/mean negative control ΔOD)] × 100.

Determination of minimum inhibitory concentration (MIC) and median lethal concentration (IC_{50})

MIC and IC_{50} values were determined for test compounds that exhibited >20% growth inhibition at a concentration of 200 μmol/L in the initial screening assays. Assays were performed as described previously on two-fold serial dilutions of active compounds in triplicate. All compounds were tested at a minimum of 7 and maximum of 17 concentrations obtained from two-fold dilution series (400–3.125 and 300–1.172 μmol/L) for median lethal concentrations against susceptible bacteria and fungi. The MIC of a compound was considered to be the lowest assay concentration at which it inhibited microbial growth by more than a mean value of 90%.[10] Absolute IC_{50} values were estimated by fitting a four parameter logistic curve to the microbial growth data (percent inhibition) using Prism 6.0 (GraphPad).[11]

Results and discussion

Chemistry

In this preliminary study, we have incorporated the boron group into the aliphatic group via the iridium-catalyzed hydroboration of unsaturated amides using pinacolborane. We therefore designed a small family of compounds of unsaturated amides with two short aliphatic chains and one longer lipophilic derivative to see if the corresponding borocapsaicinoid products would display any appreciable antimicrobial activity. Compounds **1–3** were prepared via initial reduction of the nitrile group of a protected 4-hydroxy-3-methoxybenzonitrile derivative using LAH,[12] which resulted in the corresponding benzylamine (Scheme 1). This amine could be isolated as the HCl salt in 81% as a white solid.[8] Further reaction of this amine with readily prepared acyl chlorides (derived from three commercially available unsaturated carboxylic acids) in the presence of base afforded the desired unsaturated capsaicinoids (**1–3**) in good yields (78%–94%). It is important to note at this point that both compounds **1** and **2** have a terminal alkene with varying chain lengths (**1** has a short chain and **2** contains a considerably longer lipophilic tail) and that compound **3** has an internal alkene.

The transition metal catalyzed hydroboration reaction is a powerful synthetic methodology for introducing boryl groups (BR_2) into unsaturated compounds.[13] In this study, we have found that $[IrCl(cod)]_2$/2 dppe (cod = 1,5-cyclooctadiene; dppe = 1,2-bis (diphenylphosphino)ethane) can be employed (6 mol%) to catalyze the addition of pinacolborane (HBpin: pin = $O_2C_2Me_4$) to **1** and **2** to give selective formation of the corresponding terminal hydroboration products **4** and **5**, respectively (Scheme 2). Compounds **4** and **5** have been characterized using multinuclear NMR and FTIR spectroscopy, melting point determination and elemental analysis. A broad peak at 33 ppm in the ^{11}B NMR spectra for both complexes confirms that the Bpin group has been incorporated into the capsaicinoid and the boron atom lies in a three coordinate environment.[14] Unfortunately, all attempts to affect the hydroboration of **3** to give a branched boron-containing product using $[IrCl(cod)]_2$/2 dppe and a number of other late metal catalysts proved unsuccessful, including the ones developed by Smith and Takacs for amide-direction hydroborations.[13d] It should also be noted that a number of catalyst systems have been developed that facilitate the isomerization/hydroboration of internal alkenes to give the corresponding terminal linear boronate esters,[13c] which can alternatively be prepared using the methodology developed herein using a terminal unsaturated acyl chloride.

Biological activity

With two representative boron-containing capsaicinoid derivatives in hand, one with a short aliphatic tail and the other with a longer lipophilic chain, we decided to conduct preliminary antimicrobial tests on these boron compounds, along with the unsaturated starting materials using capsaicin as a natural products

Scheme 2. The iridium-catalysed hydroboration of unsaturated amides **1** and **2** to give boronate ester derivatives **4** and **5**.

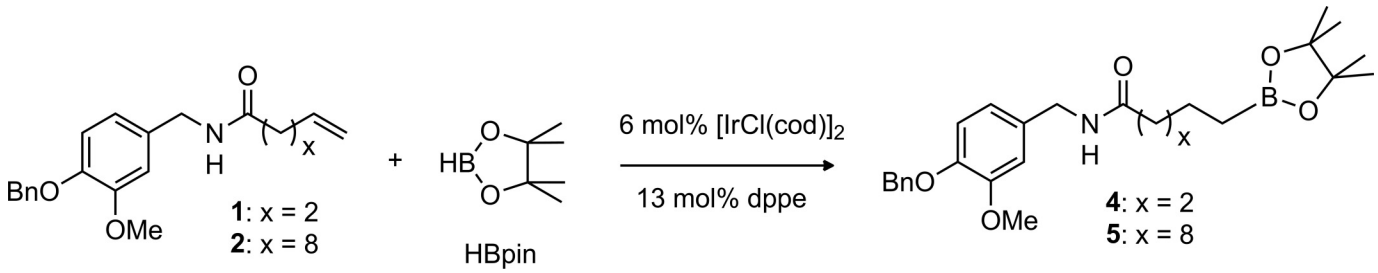


Table 1. Antibacterial activities (IC_{50} values in μmol/L) of compounds **1–5**, PBA, and capsaicin.

	E. faecium	VRE	*S. aureus*	MRSA
Compound	IC_{50} (95% CI)	IC_{50} (95% CI)	IC_{50} (95% CI)	IC_{50} (95% CI)
1	Inactive	Inactive	Inactive	Inactive
2	Inactive	Inactive	Inactive	Inactive
3	Inactive	Inactive	Inactive	Inactive
4	Inactive	Inactive	105.9 (80.7, 138.9)	107.1 (75.4, 152.2)
5	228.6 (215.5, 242.6)	276.7 (262.4, 291.8)	279.5 (248.1, 314.8)	244.7 (198.9, 301.1)
PBA	Inactive	Inactive	Inactive	169.6 (125.4, 229.5)
Capsaicin	Inactive	Inactive	Inactive	Inactive

Note: No compound inhibited the growth of any test organism by 90% or greater at the highest test concentration (400 μmol/L), and therefore, accurate MICs could not be determined. Less than 20% inhibition in initial screening (200 μmol/L) was considered inactive. IC_{50}, median lethal concentration; 95% CI, 95% confidence interval ($n = 3$); PBA, phenylboronic acid.

Table 2. Antifungal activities (IC_{50} and MIC values in μmol/L) of compounds **1–5**, PBA, and capsaicin.

	C. albicans	*S. cerevisiae*	
Compound	IC_{50} (95% CI)	IC_{50} (95% CI)	MIC
1	Inactive	Inactive	Inactive
2	Inactive	Inactive	Inactive
3	Inactive	Inactive	Inactive
4	Inactive	Inactive	Inactive
5	Inactive	150.6 (126.8, 178.9)	400
PBA	Inactive	Inactive	Inactive
Capsaicin	Inactive	Inactive	Inactive

Note: Less than 25% inhibition in initial screening (200 μmol/L) was considered to be inactive. MIC is defined as the lowest testing concentration that inhibited growth by a mean value of >90%. IC_{50}, median lethal concentration; 95% CI, 95% confidence interval ($n = 3$); PBA, phenylboronic acid.

control and phenylboronic acid (PBA). PBA was chosen as an organoboronic acid control in these antimicrobial studies. Antibacterial studies were conducted on Gram-positive *Enterococcus faecium*, Vancomycin-resistant *Enterococcus faecium* (VRE), *Staphylococcus aureus*, and Methicillin-resistant *Staphylococcus aureus* (MRSA) (Table 1). Not surprisingly, the unsaturated capsaicinoids (as well as capsaicin) did not show any appreciable activity against these bacteria.[15] We were pleased to observe, however, that the short-chain aliphatic derivative **4** showed activity against both *S. aureus* and MRSA, although no activity was observed against *E. faecium* and VRE. However, the more lipophilic boron-derivative **5** showed significant activity against all bacterial cell lines. These results are significant as Vancomycin is considered the last resort medication for the treatment of septicemia and several other infections caused by Gram-positive bacteria.[16,17] Encouraged by these results, we then examined all new compounds for potential antifungal activity against *Candida albicans* and *Saccharomyces cerevisiae*. Remarkably, only the long chain boron-containing compound **5** showed any activity against one of these fungi, *S. cerevisiae* (Table 2). These results are quite promising and suggest that both the boron group and the increased length of the aliphatic chain are necessary for pronounced bioactive efficacy in these compounds.

Conclusions

In this preliminary study, we have prepared two new boron-capsaicin derivatives containing either a short or long chain aliphatic tail group using an iridium-catalyzed hydroboration reaction with pinacolborane. The boronate ester groups reside on the terminal position of the tail groups, and it appears, in these initial studies, that it is necessary for the bioactivity of these compounds. Indeed, both compounds showed considerable activity against two Gram-positive bacteria, including Vancomycin-resistant *Enterococcus*. Vancomycin is considered the last resort medication for the treatment of septicemia and new antibacterial agents that can treat sepsis are of paramount importance. The more lipophilic boron compound with the longer aliphatic chain also showed antifungal activity against *S. cerevisiae*. Encouraged by these initial results, we are now developing a more exhaustive family of aliphatic compounds loosely based on the capsaicin scaffold that incorporate boron groups within the molecule, the results of which will be reported in due course.

Supplementary data

Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjc-2018-0193.

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References

(1) (a) Russo, A.; Bronte, G.; Fulfaro, F.; Cicero, G.; Adamo, V.; Gebbia, N.; Rizzo, S. *Curr. Cancer Drug Targets* **2010**, *10*, 55. doi:10.2174/156800910790980250; (b) Smoum, R.; Rubinstein, A.; Dembitsky, V. M.; Srebnik, M. *Chem. Rev.* **2012**, *112*, 4156. doi:10.1021/cr608202m; (c) Ban, H. S.; Nakamura, H. *Chem. Rec.* **2015**, *15*, 616. doi:10.1002/tcr.201402100; (d) Andrés, P.; Ballano, G.; Calaza, M. I.; Cativiela, C. *Chem. Soc. Rev.* **2016**, *45*, 2291. doi:10.1039/

C5CS00886G; (e) Yang, F.; Zhu, M.; Zhang, J.; Zhou, H. *Med. Chem. Commun.* **2018**, *9*, 201. doi:10.1039/C7MD00552K.

(2) (a) Dewar, M. J. S.; Dougherty, R. C. *J. Am. Chem. Soc.* **1962**, *84*, 2648. doi:10.1021/ja00872a040; (b) Dewar, M. J. S.; Dougherty, R. C. *J. Am. Chem. Soc.* **1964**, *86*, 433. doi:10.1021/ja01057a028; (c) Gronowitz, S.; Dalgren, T.; Namvedt, J.; Roos, C.; Sjoberg, B.; Forsgren, U. *Acta Pharm. Suec.* **1971**, *8*, 377; (d) Svensson, C. *Acta Crystallogr., Sect. B: Struct. Sci., Cryst. Eng. Mater.* **1976**, *32*, 3341. doi:10.1107/S0567740876010297; (e) Grassberger, M. A.; Turnowsky, F.; Hildebrandt, J. *J. Med. Chem.* **1984**, *27*, 947. doi:10.1021/jm00374a003; (f) Robinson, P. D.; Groziak, M. P.; Yi, L. *Acta Crystallogr., Sect. C: Struct. Chem.* **1996**, *52*, 2826. doi:10.1107/S010827019600697X; (g) Baldock, C.; Rafferty, J. B.; Sedelnikova, S. E.; Baker, P. J.; Stuitje, A. R.; Slabas, A. R.; Hawkes, T. R.; Rice, D. W. *Science* **1996**, *274*, 2107. doi:10.1126/science.274.5295.2107; (h) Groziak, M. P.; Chen, L.; Yi, L.; Robinson, P. D. *J. Am. Chem. Soc.* **1997**, *119*, 7817. doi:10.1021/ja963784i; (i) Davis, M. C.; Franzblau, S. G.; Martin, A. R. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 843. doi:10.1016/S0960-894X(98)00126-7; (j) Robinson, P. D.; Groziak, M. P.; Chen, L. *Acta Crystallogr., Sect. C: Struct. Chem.* **1998**, *54*, 71. doi:10.1107/S0108270197012432; (k) Baldock, C.; de Boer, G.-J.; Rafferty, J. B.; Stuitje, A. R.; Rice, D. W. *Biochem. Pharmacol.* **1998**, *55*, 1541. doi:10.1016/S0006-2952(97)00684-9; (l) Robinson, P. D.; Groziak, M. P. *Acta Crystallogr., Sect. C: Struct. Chem.* **1999**, *55*, 1701. doi:10.1107/S0108270199007520; (m) Lee, G. T.; Prasad, K.; Repič, O. *Tetrahedron Lett.* **2002**, *43*, 3255. doi:10.1016/S0040-4039(02)00365-9; (n) Groziak, M. P.; Robinson, P. D. *Collect. Czech. Chem. Commun.* **2002**, *67*, 1084. doi:10.1135/cccc20021084; (o) Hicks, J. W.; Kyle, C. B.; Vogels, C. M.; Wheaton, S. L.; Baerlocher, F. J.; Decken, A.; Westcott, S. A. *Chem. Biodivers.* **2008**, *5*, 2415. doi:10.1002/cbdv.200890206; (p) Kanichar, D.; Roppiyakuda, L.; Kosmowska, E.; Faust, M. A.; Tran, K. P.; Chow, F.; Buglo, E.; Groziak, M. P.; Sarina, E. A.; Olmstead, M. M.; Silva, I.; Xu, H. H. *Chem. Biodivers.* **2014**, *11*, 1381. doi:10.1002/cbdv.201400007; (q) Groziak, M. P.; Ganguly, A. D.; Robinson, P. D. *J. Am. Chem. Soc.* **1994**, *116*, 7597. doi:10.1021/ja00096a017; (r) Jordan, C. A.; Sandoval, B. A.; Serobyan, M. V.; Gilling, D. H.; Groziak, M. P.; Xu, H. H.; Vey, J. L. *Acta Crystallogr., Sect. F: Struct. Biol. Commun.* **2015**, *71*, 1521. doi:10.1107/S2053230X15022098; (s) Sarina, E. A.; Olmstead, M. M.; Kanichar, D.; Groziak, M. P. *Acta Crystallogr., Sect. C: Struct. Chem.* **2015**, *71*, 1085. doi:10.1107/S2053229615021841; (t) Yang, J.; Johnson, B. J.; Letourneau, A. A.; Vogels, C. M.; Decken, A.; Baerlocher, F. J.; Westcott, S. A. *Aust. J. Chem.* **2015**, *68*, 366. doi:10.1071/CH14534; (u) Dilek, O.; Lei, Z.; Mukherjee, K.; Bane, S. *Chem. Commun.* **2015**, *51*, 16992. doi:10.1039/C5CC07453C; (v) Stojanović, M.; Baranac-Stojanović, M. *J. Org. Chem.* **2016**, *81*, 197. doi:10.1021/acs.joc.5b02499; (w) Ward, M. S.; Silva, I.; Martinez, W.; Jefferson, J.; Rahman, S.; Garcia, J. M.; Kanichar, D.; Roppiyakuda, L.; Kosmowska, E.; Faust, M. A.; Tran, K. P.; Chow, F.; Buglo, E.; Zhou, F.; Groziak, M. P.; Xu, H. H. *Bioorg. Med. Chem.* **2016**, *24*, 3267. doi:10.1016/j.bmc.2016.05.058; (x) Gillingham, D. *Org. Biomol. Chem.* **2016**, *14*, 7606. doi:10.1039/C6OB01193D; (y) Stress, C. J.; Schmidt, P. J.; Gillingham, D. G. *Org. Biomol. Chem.* **2016**, *14*, 5529. doi:10.1039/C6OB00168H; (z) Reddy, G. N.; Giri, S. *RSC Adv.* **2016**, *6*, 47145. doi:10.1039/C6RA08625J; (aa) Bandyopadhyay, A.; Cambray, S.; Gao, J. *J. Am. Chem. Soc.* **2017**, *139*, 871. doi:10.1021/jacs.6b11115; (bb) Satta, Y.; Nishiyabu, R.; James, T. D.; Kubo, Y. *Tetrahedron* **2017**, *73*, 2053. doi:10.1016/j.tet.2017.02.050; (cc) Nourie, H. C.; Buglo, E.; Groziak, M. P.; Sarina, E. A.; Olmstead, M. M. *J. Heterocycl. Chem.* **2017**, *54*, 749. doi:10.1002/jhet.2608.

(3) (a) Baker, S. J.; Zhang, Y.-K.; Akama, T.; Lau, A.; Zhou, H.; Hernandez, V.; Mao, W.; Alley, M. R. K.; Sanders, V.; Plattner, J. J. *J. Med. Chem.* **2006**, *49*, 4447. doi:10.1021/jm0603724; (b) Das, B. C.; Thapa, P.; Karki, R.; Schinke, C.; Das, S.; Kambhampati, S.; Banerjee, S. K.; van Veldhuizen, P.; Verma, A.; Weiss, L. M.; Evans, T. *Future Med. Chem.* **2013**, *5*, 653. doi:10.4155/fmc.13.38; (c) Sharma, N.; Sharma, D. *J. Pharmacol. Pharmacother.* **2015**, *6*, 236. doi:10.4103/0976-500X.171870; (d) Jinna, S.; Finch, J. *Drug Des. Dev. Ther.* **2015**, *9*, 6185.

(4) (a) Webb, M. I.; Halcovitch, N. R.; Bowes, E. G.; Lee, G. M.; Geier, M. J.; Vogels, C. M.; O'Neill, T.; Li, H.; Flewelling, A.; Decken, A.; Gray, C. A.; Westcott, S. A. *J. Heterocycl. Chem.* **2014**, *51*, 157. doi:10.1002/jhet.1997; (b) Hébert, M. J. G.; Flewelling, A. J.; Clark, T. N.; Levesque, N. A.; Jean-François, J.; Surette, M. E.; Gray, C. A.; Vogels, C. M.; Touaibia, M.; Westcott, S. A. *Int. J. Med. Chem.* **2015**, *2015*, 418362. doi:10.1155/2015/418362; (c) Cormier, K.; Curry, R. D.; Betsch, M. P.; Goguen, J. A.; Vogels, C. M.; Decken, A.; Turcotte, S.; Westcott, S. A. *J. Heterocycl. Chem.* **2016**, *53*, 1807. doi:10.1002/jhet.2490; (d) St-Coeur, P.-D.; Kinley, S.; Vogels, C. M.; Decken, A.; Morin, P. J.; Westcott, S. A. *Can. J. Chem.* **2017**, *95*, 207. doi:10.1139/cjc-2016-0570; (e) Zhu, D.; Hunter, C. D.; Baird, S. R.; Davis, B. R.; Bos, A.; Geier, S. J.; Vogels, C. M.; Decken, A.; Gray, C. A.; Westcott, S. A. *Heteroat. Chem.* **2017**, *28*, e21045. doi:10.1002/hc.21405.

(5) (a) Fernandes, E. S.; Cerqueira, A. R. A.; Soares, A. G.; Costa, S. K. P. In *Drug Discovery from Mother Nature: Advances in Experimental Medicine and Biology*; Gupta, S. C., Prasad, S., Aggarwal, B. B., Eds.; Springer: Cham, Switzerland, 2016; p. 91. doi:10.1007/978-3-319-41342-6_5; (b) Chen, L.; Huang, Z.; Du, Y.; Fu, M.; Han, H.; Wang, Y.; Dong, Z. *J. Alzheimer's Dis.* **2017**, *59*, 683. doi:10.3233/JAD-170337; (c) Serafini, M.; Griglio, A.; Aprile, S.; Seiti, F.; Travelli, C.; Pattarino, F.; Grosa, G.; Sorba, G.; Genazzani, A. A.; Gonzalez-Rodriguez, S.; Butron, L.; Devesa, I.; Fernandez-Carvajal, A.; Pirali, T.; Ferrer-Montiel, A. *J. Med. Chem.* **2018**, *61*, 4436. doi:10.1021/acs.jmedchem.8b00109.

(6) Herde, J. L.; Lambert, J. C.; Senoff, C. V. *Inorg. Synth.* **1974**, *15*, 18.

(7) Liu, F.; Chen, X.; Allali-Hassani, A.; Quinn, A. M.; Wasney, G. A.; Dong, A.; Barsyte, D.; Kozieradzki, I.; Senisterra, G.; Chau, I.; Siarheyeva, A.; Kireev, D. B.; Jadhav, A.; Herold, J. M.; Frye, S. V.; Arrowsmith, C. H.; Brown, P. J.; Simeonov, A.; Vedadi, M.; Jin, J. *J. Med. Chem.* **2009**, *52*, 7950. doi:10.1021/jm901543m.

(8) Wang, E.-C.; Lin, G.-J.; Wang, L. F.; Huang, K.-S.; Wang, J.-P.; Huang, K.-K.; Kuo, Y. H. *Chin. Pharm. J.* **1999**, *51*, 319.

(9) McCulloch, M. W. B.; Berrue, F.; Haltli, B.; Kerr, R. G. *J. Nat. Prod.* **2011**, *74*, 2250. doi:10.1021/np2006555.

(10) Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004. PMID:9145860.

(11) Sebaugh, J. L. *Pharm. Stat.* **2011**, *10*, 128. doi:10.1002/pst.426.

(12) Liu, F.; Chen, X.; Allali-Hassani, A.; Quinn, A. M.; Wigle, T. J.; Wasney, G. A.; Dong, A.; Senisterra, G.; Chau, I.; Siarheyeva, A.; Norris, J. L.; Kireev, D. B.; Jadhav, A.; Herold, J. M.; Janzen, W. P.; Arrowsmith, C. H.; Frye, S. V.; Brown, P. J.; Simeonov, A.; Vedadi, M.; Jin, J. *J. Med. Chem.* **2010**, *53*, 5844. doi:10.1021/jm100478y.

(13) (a) Crudden, C. M.; Edwards, D. *Eur. J. Org. Chem.* **2003**, *2003*, 4695. doi:10.1002/ejoc.200300433; (b) Vogels, C. M.; Westcott, S. A. *Curr. Org. Chem.* **2005**, *9*, 687. doi:10.2174/1385272053765060; (c) Lata, C. J.; Crudden, C. M. *J. Am. Chem. Soc.* **2010**, *132*, 131. doi:10.1021/ja904142m; (d) Smith, S. M.; Takacs, J. M. *J. Am. Chem. Soc.* **2010**, *132*, 1740. doi:10.1021/ja908257x; (e) Fernández, E.; Guiry, P. J.; Connole, K. P. T.; Brown, J. M. *J. Org. Chem.* **2014**, *79*, 5391. doi:10.1021/jo500512s; (f) Fujihara, T.; Semba, K.; Terao, J.; Tsuji, Y. *Catal. Sci. Technol.* **2014**, *4*, 1699. doi:10.1039/c4cy00070f; (g) Chong, C. C.; Kinjo, R. *ACS Catal.* **2015**, *5*, 3238. doi:10.1021/acscatal.5b00428.

(14) Nöth, H.; Wrackmeyer, B. *Nuclear Magnetic Resonance Spectroscopy of Boron Compounds*; Springer-Verlag: Berlin, 1978. doi:10.1007/978-3-642-66757-2.

(15) Patterson, A. E.; Flewelling, A. J.; Clark, T. N.; Geier, S. J.; Vogels, C. M.; Masuda, J. D.; Gray, C. A.; Westcott, S. A. *Can. J. Chem.* **2015**, *93*, 1305. doi:10.1139/cjc-2015-0400.

(16) Rincón, S.; Panesso, D.; Díaz, L.; Carvajal, L. P.; Reyes, J.; Munita, J. M.; Arias, C. *Biomédica* **2014**, *34*, 191. doi:10.7705/biomedica.v34i0.2210.

(17) Uttley, A. H.; Collins, C. H.; Naidoo, J.; George, R. C. *Lancet* **1988**, *1*, 57. PMID:2891921.