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Atty. Dkt. No. 045471-0750

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Yong-Kang ZHANG, et al.

Title: BORON-CONTAINING SMALL MOLECULES FOR USE IN THE TOPICAL TREATMENT OF ONYCHOMYCOSIS AND CUTANEOUS FUNGAL INFECTIONS

Appl. No.: Unknown

Filing Date: Herewith

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Art Unit: Unknown

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Sir:

Transmitted herewith for filing under 37 C.F.R. § 1.53(c) is the provisional patent application of:

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[X] Applicant claims small entity status under 37 CFR 1.27(c)(1).

016.352550.1

Enclosed are:

[X] Description (35 pages).

[ ] Informal drawings (0 sheets, Figures 0).

[ ] Assignment of the invention.

[ ] Assignment Recordation Cover Sheet.

[ ] Small Entity statement(s).

[X] Application Data Sheet (37 CFR 1.76).

The filing fee is calculated below:

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				Rate		
Basic Fee				$200.00		$200.00
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[X] A check in the amount of $100.00 to cover the filing fee is enclosed.

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Respectfully submitted,

Date February 16, 2005

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U.S. PATENT APPLICATION

FOR

BORON-CONTAINING SMALL MOLECULES FOR USE IN THE TOPICAL TREATMENT OF ONYCHOMYCOSIS AND CUTANEOUS FUNGAL INFECTIONS

Inventors: YONG-KANG ZHANG
STEPHEN J. BAKER

BORON-CONTAINING SMALL MOLECULES FOR USE IN THE TOPICAL TREATMENT OF ONYCHOMYCOSIS AND CUTANEOUS FUNGAL INFECTIONS

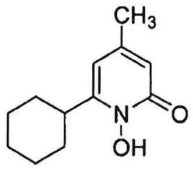
Field of the Invention

[0001] This invention relates to compounds useful for treating fungal infections, more specifically topical treatment of onychomycosis and/or cutaneous fungal infections. This invention is directed to compounds that are active against fungi and have properties that allow the compound, when placed in contact with a patient, to reach the particular part of nail or skin infected by the fungus. In particular the present compounds have physiochemical properties that facilitate penetration of the nail plate.

State of the Art

[0002] Onychomycosis is a fungal infection of the toe and finger nails. The causative pathogens in this infection include the dermatophytes, *Trichophyton*, *Microsporum* and *Epidermophyton* species and yeast-like fungi including *Candida* species.

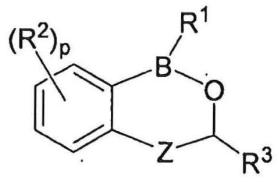
[0003] In the USA, onychomycosis is currently managed by only one FDA approved topical treatment, PENLAC®, which contains the active ingredient ciclopirox:



[0004] PENLAC® is known to have very poor efficacy rates: it is only effective in 8% of all onychomycosis cases treated using this topical treatment. Thus, there remains a need for new antifungal agents that can applied safely to toe- and finger nails. The present invention meets these and other needs.

SUMMARY OF THE INVENTION

[0005] The compounds useful for treating fungal infections, more specifically topical treatment of cutaneous fungal infections and/or onychomycosis, have the structure given by Formula I below:



I

wherein

Z is selected from a bond, $-CH_2$, and $-CHR^4$;

R^3 is selected from hydrogen and methyl; alternatively, R^3 and R^4 and the atoms bonded thereto join together to form a substituted or unsubstituted aryl;

R^1 is selected from OH, substituted or unsubstituted C_{1-4}alkyl, substituted or unsubstituted C_{3-7}cycloalkyl, substituted or unsubstituted C_{2-4}alkenyl, substituted or unsubstituted C_{2-4}alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl; and

p is 0, 1 or 2;

R^2 is selected from halo, OH, CN, substituted or unsubstituted C_{1-4}alkyl, substituted or unsubstituted C_{3-7}cycloalkyl, substituted or unsubstituted C_{2-4}alkenyl, substituted or unsubstituted C_{2-4}alkynyl, acyl, aminoacyl, acylamino, carboxyl, carboxyl ester, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted alkoxy, substituted or unsubstituted aryloxy, substituted or unsubstituted heteroaryloxy, and NR^aR^b, wherein R^a and R^b are independently selected from hydrogen, substituted or unsubstituted C_{1-4}alkyl, substituted or unsubstituted C_{3-7}cycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl;

alternatively, when p is 2 and the two R^2 groups are bonded to adjacent carbon atoms, then these two R^2 groups and the atoms bonded thereto may join together to form a substituted or unsubstituted aryl.

In a preferred embodiment, the physicochemical properties of the molecule of Formula I, described by quantities predictive for migration of the compound through the nail plate, including, but not limited to, molecular weight, logP, solubility in water and activity in the presence of keratin, and the like, are effective to provide substantial penetration of the nail plate.

[0006] Compounds with a molecular weight of less than 200 Da penetrate the nail plate in a manner superior to the commercially available treatment for onychomycosis (See Examples). In one embodiment of the present invention the antifungal compounds have a molecular weight of less than 170 Da. In another embodiment of this invention, the compounds have a molecular weight of from about 145 to about 170 Da. In yet another embodiment the molecular weight is either 151.93 or 168.39 Da.

[0007] In one embodiment of the present invention the antifungal compounds have a LogP value of less than about 2.5. In another embodiment, the antifungal compounds have a LogP value of from about 1.0 to about 2.0. In yet another embodiment, the antifungal compounds have a LogP value of 1.9 or 2.3.

[0008] Also contemplated by the present invention are compounds with a LogP value greater then 2.5, with a molecular weight less than 200 Da, that are still able to penetrate the nail plate.

[0009] In one embodiment of the present invention the antifungal compounds have a water solubility greater than about 0.1 mg/mL in octanol saturated water.

[0010] In one embodiment of the present invention, the minimum inhibitory concentration, MIC, against *Trichophyton rubrum* of the antifungal compounds of the present invention did not change in the presence of about 5% keratin. In one embodiment, the MIC of the compound exhibits less than about an 8 fold decrease in activity in the presence of of about 5% keratin.

[0011] In one embodiment, Z is a bond.

[0012] In another embodiment Z is CH_2. In yet another embodiment Z is $-CHR^4$, and R^3 and R^4 and the atoms bonded thereto join together to form a phenyl group.

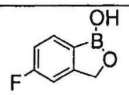
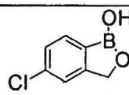
[0013] In one embodiment R^1 is OH.

[0014] In one embodiment *p* is 1 and R^2 is selected from fluoro, chloro, methyl, cyano, methoxy, hydroxymethyl, and p-cyanophenyloxy.

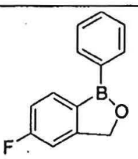
[0015] In one embodiment *p* is 2 and each R^2 is independently selected from fluoro, chloro, methyl, cyano, methoxy, hydroxymethyl, and p-cyanophenyl.

[0016] In one embodiment *p* is 2 and the two R^2 groups, which are on adjacent carbon atoms, join to form a phenyl group.

[0017] Some examples of molecules with optimal physicochemical properties are given in the table below.

Structure:	 (compound **1**)	 (compound **2**)
Formula:	$C_7H_6BFO_2$	$C_7H_6BClO_2$
Molecular weight (Da):	151.93	168.39
Plasma protein binding (%):	66	83
LogP:	1.9	2.3
Water solubility (μg/mL):	>100	>100

[0018] Compound 3 below is an example of a compound similar in molecular weight to ciclopirox, and like ciclopirox, does not penetrate the nail plate.

Structure:	 (compound **3**)
Formula:	$C_{13}H_{10}BFO$
Molecular weight (Da):	212.03
Plasma protein binding (%):	100
LogP:	not determined
Water solubility (μg/mL):	not determined

[0019] This invention is also directed to pharmaceutical compositions comprising a pharmaceutically acceptable diluent and a therapeutically effective amount of a compound described herein or mixtures of one or more of such compounds.

[0020] In another embodiment, the present invention is directed to topical formulations comprising a pharmaceutically acceptable topical carrier and at least one compound of Formula I as described above.

[0021] In a preferred embodiment the topical formulations the compound of Formula I described structurally above has a total molecular weight of less than 200 Da, has a logP of less than 2.5, and a minimum inhibitory concentration against *Trichophyton rubrum* that is substantially unchanged in the presence of 5% keratin.

[0022] This invention is still further directed to methods for treating a viral infection mediated at least in part by dermatophytes, *Trichophyton*, *Microsporum* or *Epidermophyton* species, or a yeast-like fungi including *Candida* species, in mammals, which methods comprise administering to a mammal, that has been diagnosed with said viral infection or is at risk of developing said viral infection, a pharmaceutical composition comprising a pharmaceutically acceptable diluent and a therapeutically effective amount of a compound described herein or mixtures of one or more of such compounds. In one embodiment the infection is onychomycosis. Penetration of the nail by the active ingredient may be effected by the polarity of the formulation. However, the polarity of the formulation is not expected have as much influence on nail penetration as some of the other factors, such as the molecular weight or the LogP of the active ingredient. The presence of penetration enhancing agents in the formulation are likely to increase penetration of the active agent when compared to similar formulations containing no penetration enhancing agent.

[0023] Compounds contemplated by the present invention may have broad spectrum antifungal activity and as such may be candidates for use against other cutaneous fungal infections.

Utility

[0024] It is believed that poor penetration of the active agent through the nail plate and/or excessive binding to keratin, (the major protein in nails and hair) are the reasons

for the poor efficacy of PENLAC® and other topical treatments (that have failed in clinical trials). In mild cases of onychomycosis, the pathogenic fungi reside in the nail plate only. In moderate to severe cases the pathogenic fungi establish a presence in the nail plate and in the nail bed. If the infection is cleared from the nail plate but not from the nail bed, the fungal pathogen can re-infect the nail plate. Therefore, to effectively treat onychomycosis, the infection must be eliminated from the nail plate and the nail bed. To do this, the active agent must penetrate and disseminate substantially throughout the nail plate and nail bed.

[0025] It is believed that in order for an active agent to be effective once disseminated throughout the infected area, it must be bioavailable to the fungal pathogen and cannot be so tightly and/or preferentially bound to keratin that the drug is rendered inactive.

[0026] An understanding of the morphology of the nail plate suggests certain physicochemical properties of an active agent that would facilitate penetration of the nail plate. The desired physicochemical properties are described throughout. The tested compounds of the present invention are able to penetrate the nail plate and were also active against *Trichophyton rubrum* and *mentagrophytes* and other species. In addition, the tested compounds are also active against *Trichophyton rubrum* in the presence of 5% keratin powder. Finally, penetration of the nail plate by the compounds of this invention is weakly, if at all, influenced by the polarity of the formulation within which the active compound is dispersed.

Administration and Pharmaceutical Composition

[0027] In general, the compounds prepared by the methods, and from the intermediates, described herein will be administered in a therapeutically effective amount by any of the accepted modes of administration for agents that serve similar utilities. The actual amount of the compound of this invention, i.e., the active ingredient, will depend upon numerous factors such as the severity of the disease to be treated, the age and relative health of the subject, the potency of the compound used, the route and form of administration, and other factors. The drug can be administered more than once a day, for example, about 4 times a day, and preferably once or twice a day.

[0028] The pharmaceutical compositions of the present invention are comprised of in general, a compound of the present invention in combination with at least one pharmaceutically acceptable diluent or excipient. Acceptable excipients are non-toxic, aid administration, and do not adversely affect the therapeutic benefit of the active compound. Such excipient may be any solid, liquid, semi-solid or, in the case of an aerosol composition, gaseous excipient that is generally available to one of skill in the art.

[0029] Solid pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk and the like. Liquid and semisolid excipients may be selected from glycerol, propylene glycol, water, ethanol and various oils, including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, etc. Preferred liquid carriers, particularly for injectable solutions, include water, saline, aqueous dextrose, and glycols.

[0030] The amount of the compound in a topical formulation can vary within the full range employed by those skilled in the art. Typically, the topical formulation will contain, on a weight percent (wt%) basis, from about 0.01-99.99 wt% of a compound of the present invention based on the total formulation, with the balance being one or more suitable pharmaceutical excipients. Preferably, the compound is present at a level of about 1-50 wt%, more preferably about 1 to about 25%, even more preferably about 1 to about 10%, still more preferably about 5 to about 10%.

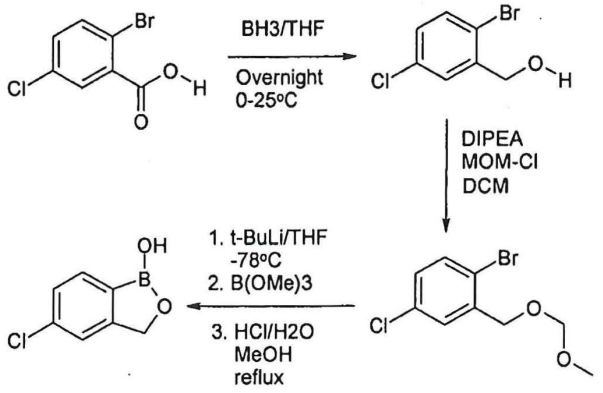
[0031] The topical formulations may be administered 1 to 4 times per day. More preferably the formulation is applied to the patient 1 to 2 times per day.

EXAMPLES

[0032] Proton NMR are recorded on Varian AS 300 spectrometer and chemical shifts are reported as δ (ppm) down field from tetramethylsilane. Mass spectra are determined on Micromass Quattro II.

Example 1

5-Chloro-1,3-Dihydro-1-Hydroxy-2,1-Benzoxaborole



[0033] (a) *2-bromo-5-chlorobenzyl alcohol.* To a solution of 2-bromo-5-chlorobenzoic acid (5.49 g, 23.3 mmol) in anhydrous THF (70 ml) under nitrogen was added dropwise a BH_3 THF solution (1.0 M, 55 mL, 55 mmol) at 0°C and the reaction mixture was stirred overnight at room temperature. Then the mixture was cooled again with ice bath and MeOH (20 mL) was added dropwise to decompose excess BH_3. The resulting mixture was stirred until no bubble was released and then 10%NaOH (10 mL) was added. The mixture was concentrated and the residue was mixed with water (200 mL) and extracted with EtOAc. The residue from rotary evaporation was purified by flash column chromatography over silica gel eluted with a mixed solvents of hexane and EtOAc (5:1, v/v) to give 4.58 g of 2-bromo-5-chlorobenzyl alcohol as a white solid (yield 88.8%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.57 (d, J = 8.7 Hz, 1H), 7.50-7.49 (m, 1H), 7.28-7.24 (m, 1H), 5.59 (t, J = 6.0 Hz, 1H) and 4.46 (d, J = 6.0 Hz, 2H) ppm.

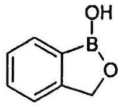
[0034] (b) *2-bromo-5-chloro-1-(methoxymethoxymethyl)benzene.* The obtained 2-bromo-5-chlorobenzyl alcohol was dissolved in CH_2Cl_2 (150 mL) and cooled to 0°C with ice bath. To this solution under nitrogen were added in sequence di-isopropyl ethyl amine (5.4 mL, 31.02 mmol, 1.5 eq) and chloromethyl methyl ether (2 mL, 25.85 mmol, 1.25 eq). The reaction mixture was stirred overnight at room temperature and washed with $NaHCO_3$-saturated water and then NaCl-saturated water. The residue after rotary evaporation was

purified by flash column chromatography over silica gel eluted with a mixed solvents of hexane and EtOAc (5:1, v/v) to give 4.67 g of 2-bromo-5-chloro-1-(methoxymethoxymethyl)benzene as a colorless liquid (yield 85.0%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.63 (d, J = 8.7 Hz, 1H), 7.50 (dd, J = 2.4 & 0.6 Hz, 1H), 7.32 (dd, J = 8.4 & 2.4 Hz, 1H), 4.71 (s, 2H), 4.53 (s, 2H) and 3.30 (s, 3H) ppm.

[0035] (c) *5-Chloro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole*. To a solution of 2-bromo-5-chloro-1-(methoxymethoxymethyl)benzene (4.6 g, 17.3 mmol) in anhydrous THF (80 mL) at -78°C under nitrogen was added dropwise *tert*-BuLi (1.7 M in n-pentane, 11.7 mL, 1.15 eq) and the solution became brown colored. Then, $B(OMe)_3$ (1.93 mL, 17.3 mmol) was injected in one portion and the cooling bath was removed. The mixture was warmed gradually with stirring for 30 min and then stirred with a water bath for 2 h. After addition of 6N HCl (6 mL), the mixture was stirred overnight at room temperature and about 50% hydrolysis has happened as shown by TLC analysis. The solution was rotary evaporated and the residue was dissolved in MeOH (50 mL) and 6N HCl (4 mL). The solution was refluxed for 1 h and the hydrolysis was completed as indicated by TLC analysis. Rotary evaporation gave a residue which was dissolved in EtOAc, washed with water, dried and then evaporated. The crude product was purified by flash column chromatography over silica gel eluted with mixed solvents of hexane and EtOAc (2:1, v/v) to provide a solid with 80% purity. The solid was further purified by washing with hexane to afford 1.22 g of the desired title compound as a white solid (yield 41.9%). M.p. 142-150°C. MS (ESI): m/z = 169 (M+1, positive) and 167 (M-1, negative). HPLC (220 nm): 99% purity. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.30 (s, 1H), 7.71 (d, J = 7.8 Hz, 1H), 7.49 (s, 1H), 7.38 (d, J = 7.8 Hz, 1H) and 4.96 (s, 2H) ppm.

Example 2

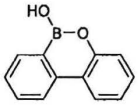
1,3-Dihydro-1-Hydroxy-2,1-Benzoxaborole



[0036] The title compound was prepared by using 2-bromobenzyl alcohol as a starting material and following the procedures as described in Example 1b and 1c. The product of the title compound was obtained as a white solid. M.p. 83-86°C. MS (ESI): m/z = 135 (M+1, positive) and 133 (M-1, negative). HPLC (220 nm): 95.4% purity. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.14 (s, 1H), 7.71 (d, J = 7.2 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.38 (d, J = 7.5 Hz, 1H), 7.32 (t, J = 7.1 Hz, 1H) and 4.97 (s, 2H) ppm.

Example 3

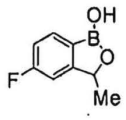
6-Hydroxy-6H-Dibenz[c,e][1,2]oxaborin



[0037] To a three-necked flask with hexane (250 mL) under nitrogen were added in sequence BCl_3 (1.0 M, 45 mL, 45 mmol) and 2-phenylphenol solid (5 g, 29.3 mmol) at room temperature. After the solid was dissolved, $AlCl_3$ (0.2 g, 5 mol%) was added in one portion. The mixture was refluxed for 5 h and then cooled to 0°C. Ethanol (30 mL, 0.51 mol) were slowly added and the mixture was stirred for a while. Water was added and the mixture was extracted with EtOAc. Evaporation gave the residue that was purified by flash column chromatography over silica gel eluted with mixed solvents of hexane and EtOAc (4:1, v/v) to provide 3.13 g of the desired title compound as a cream solid (yield 54.5%). M.p. 203-206°C. MS (ESI): m/z = 197 (M+1, positive) and 195 (M-1, negative). HPLC (220 nm): 98.9% purity. ^{1}H NMR (300 MHz, DMSO-d_6+D2O): δ 9.42 (s, trace), 8.27-8.21 (m, 2H), 8.05 (dd, J = 7.2 & 0.9 Hz, 1H), 7.71 (td, J_t = 7.8 Hz, J_d = 1.8 Hz, 1H), 7.46 (td, J_t = 7.4 Hz, J_d = 0.9 Hz, 1H), 7.41-7.35 (m, 1H) and 7.25-7.17 (m, 2H) ppm.

Example 4

5-Fluoro-1-hydroxy-3-methyl-2,1-benzoxaborolane



[0038] To a solution of 2-bromo-5-fluoro-[1-(methoxymethoxy)ethyl]benzene (1.32 g, 5.00 mmol) in dry tetrahydrofuran (10 mL) were added *sec*-butyl lithium (1.4 mol/L in cyclohexane; 5.0 mL) and trimethyl borate (1.1 mL, 10 mmol) at –78°C under nitrogen atmosphere, and the mixture was stirred for 1h while allowing to warm to room temperature. The reaction was quenched with 2 mol/L hydrochloric acid, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure. To a solution of the residue in tetrahydrofuran (15 mL) was added 6 mol/L hydrochloric acid (3 mL), and the mixture was stirred at room temperature for overnight. Water was added and the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure. Recrystallization from pentane gave the target compound (473 mg, 58%) as a white powder.

[0039] ^{1}H-NMR (300.058 MHz, DMSO-d_6) δ ppm 1.37 (d, J = 6.4 Hz, 3H), 5.17 (q, J = 6.4 Hz, 1H), 7.14 (m, 1H), 7.25 (dd, J = 9.7, 2.3 Hz, 1H), 7.70 (dd, J = 8.2, 5.9 Hz, 1H), 9.14 (s, 1H).

Example 5

2-Bromo-5-fluoro-[1-(methoxymethoxy)ethyl]benzene

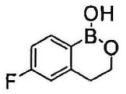
[0040] To a solution of 2-bromo-5-fluorobenzaldehyde (4.23 g, 20.0 mmol) in tetrahydrofuran (30 mL) was added methylmagnesium bromide (1.4 mol/L in tetrahydrofuran; 18 mL, 25 mmol) at – 78 °C under nitrogen atmosphere, and the mixture was stirred for 2 h while allowing to warm to room temperature. The reaction was quenched with 2 mol/L hydrochloric acid, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure. To a solution of the residue (4.62 g) in

dichloromethane (100 mL) were added diisopropylethylamine (5.2 mL, 30 mmol) and chloromethyl methyl ether (2.0 mL, 26 mmol) at 0 °C, and the reaction mixture was stirred at room temperature fro overnight. Water was added, and the mixture was extracted with chloroform. The organic layer was washed with brine and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (15:1 hexane/ethyl acetate) to give 2-bromo-5-fluoro-[1-(methoxymethoxy)ethyl]benzene (4.97 g, 2 steps 94%).

[0041] ^{1}H-NMR (300.058 MHz, $CDCl_3$) δ ppm 1.43 (d, J = 6.5 Hz, 3H), 3.38 (s, 3H), 4.55 (d,J = 6.5 Hz, 1H), 4.63 (d,J = 6.5 Hz, 1H), 5.07 (q, J = 6.5 Hz, 1H), 6.85 (m, 1H), 7.25 (dd, J = 9.7, 2.6 Hz, 1H), 7.46 (dd, J = 8.8, 5.3 Hz, 1H).

Example 6

5-Fluoro-1-hydroxy-1,2,3,4-tetrahydro-2,1-benzoxaborine



[0042] A mixture of -bromo-5-fluorobenzaldehyde (4.23 g, 20.0 mmol), (methoxymethyl)triphenylphosphonium chloride (8.49 g, 24. 0 mmol), and potassium *tert*-butoxide (2.83 g, 24.0 mol) in *N,N*-dimethylformamide (50 mL) was stirred at room temperature for overnight. The reaction was quenched with 6 mol/L hydrochloric acid, and the mixture was extracted with ethyl acetate. The organic layer was washed with water (x 2) and brine, and dried on anhydrous sodium sulfate. The solvent was removed under reduced. To the residue were added tetrahydrofuran (60 mL) and 6 mol/L hydrochloric acid, and the mixture was heated at reflux for 8 h. Water was added, and the mixture was extracted with ether. The organic layer was washed with brine and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure to afford 2-bromo-5-fluorophenylacetaldehyde (3.60 g, 83%).

[0043] To a solution of 2-bromo-5-fluorophenylacetaldehyde (3.60 g, 16.6 mmol) in methanol (40 mL) was added sodium borohydride (640 mg, 16.6 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. Water was added, and the mixture

was extracted with ethyl acetate. The organic layer was washed with brine and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure. To the residue were added dichloromethane (50 mL), diisopropylethylamine (3.5 mL, 20 mmol) and chloromethyl methyl ether (1.5 mL, 20 mmol) at 0 °C, and the reaction mixture was stirred at room temperature fro overnight. Water was added, and the mixture was extracted with chloroform. The organic layer was washed with brine and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (15:1 hexane/ethyl acetate) to give 2-bromo-5-fluorophenyl-*O*-methoxymethylethanol (2.99 g, 2 steps 68%).

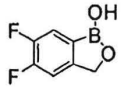
[0044] ^{1}H-NMR (300.058 MHz, $CDCl_3$) δ ppm 3.04 (t, J = 6.7 Hz, 2H), 3.31 (s, 3H), 3.77 (t, J = 6.7 Hz, 2H), 4.62 (s, 2H), 6.82 (td, J = 8.2, 3.2 Hz, 1H), 7.04 (dd, J = 9.4, 2.9 Hz, 1H), 7.48 (dd, J = 8.8, 5.3 Hz, 1H).

[0045] The above compound was converted into the cyclic boronic ester by following the general procedure (A). Silica gel column chromatography (2:1 hexane/ethyl acetate) followed by trituration with pentane gave the target compound as a white powder.

[0046] ^{1}H-NMR (300.058 MHz, DMSO-d_6) δ ppm 2.86 (t, J = 5.9 Hz, 2H), 4.04 (t, J = 5.9 Hz, 2H), 7.0-7.1 (m, 2H), 7.69 (dd, J = 8.2, 7.2 Hz, 1H), 8.47 (s, 1H).

Example 7

5,6-Difluoro-1-hydroxy-2,1-benzoxaborolane



[0047] To a solution of 2-bromo-4,5-difluoro-*O*-methoxymethylbenzylalcohol (2.97 g; 11.1 mmol) and triisopropoxyborane (2.8 mL, 12 mmol) in tetrahydrofuran (30 mL) was added *n*-butyllithium (1.6 mol/L in hexane; 7.5 mL, 12 mmol) over 30 min at – 78°C under nitrogen atmosphere, and the mixture was stirred for 1h while allowing to warm to room temperature. The reaction was quenched with 2 mol/L hydrochloric acid, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried

on anhydrous sodium sulfate. The solvent was removed under reduced pressure. To a solution of the residue in tetrahydrofuran (25 mL) was added 6 mol/L hydrochloric acid (5 mL), and the mixture was stirred at room temperature for overnight. Water was added and the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure. Recrystallization from ethyl acetate/diisopropyl ether gave the target compound (1.14 g, 60%) as a white powder.

[0048] ^{1}H-NMR (300.058 MHz, DMSO-d_6) δ ppm 4.94 (s, 2H), 7.50 (dd, J = 10.7, 6.8 Hz, 1H), 7.62 (dd, J = 9.7, 8.2 Hz, 1H), 9.34 (s, 1H).

Example 8

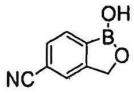
2-Bromo-4,5-difluoro-*O*-(methoxymethyl)benzylalcohol

[0049] To a solution of 2-bromo-4,5-difuluorobenzoic acid (6.11 g, 25.0 mmol) in tetrahydrofuran (40 mL) was added borane-tetrahydrofuran (1 mol/L solution in tetrahydrofuran; 50 mL, 50 mmol) at 0 °C, and the mixture was stirred at room temperature for overnight. The mixture was cooled to 0 °C, then water was added dropwise, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure. To the residue were added dichloromethane (100 mL), diisopropylethylamine (6.1 mL, 35 mmol) and chloromethyl methyl ether (2.3 mL, 30 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for overnight. Water was added, and the mixture was extracted with chloroform. The organic layer was washed with brine and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (9:1 hexane/ethyl acetate) to give 2-bromo-4,5-difluoro-*O*-methoxymethylbenzylalcohol (5.69 g, 2 steps 85%).

[0050] ^{1}H-NMR (300.058 MHz, $CDCl_3$) δ ppm 3.42 (s, 3H), 4.57 (d, J = 1.2 Hz, 2H), 4.76 (s, 2H), 7.3-7.5 (m, 2H).

Example 9

5-Cyano-1-hydroxy-2,1-benzoxaborolane



[0051] To a solution of 4-bromo-3-methylbenzonitrile (10 .0 g, 49.5 mmol) in carbon tetrachloride (200 mL) were added *N*-bromosuccinimide (8.81 g, 49.5 mmol) and *N,N*-azoisobutylonitrile (414 mg, 5 mol%), and the mixture was heated at reflux for 3 h. Water was added, and the mixture was extracted with chloroform. The organic layer was washed with brine and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure. To the residue were added dimethylformamide (150 mL) and sodium acetate (20.5 g, 250 mmol), and the mixture was stirred at 80 °C for overnight. Water was added, and the mixture was extracted with ether. The organic layer was washed with water and brine, and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure. To the residue was added methanol (150 mL) and 1 mol/L sodium hydroxide (50 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated to about a third of volume under reduced pressure. Water and hydrochloric acid were added, and the mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (3:1 hexane/ethyl acetate) followed by trituration with dichloromethane to give 2-bromo-5-cyanobenzylalcohol (4.63 g, overall 44%).

[0052] ^{1}H-NMR (300.058 MHz, DMSO-d_6) δ ppm 4.51 (d, J = 5.9 hz, 2H), 5.67 (t, J = 5.6 Hz, 1H), 7.67 (dd, J = 8.2, 2.0 Hz, 1H), 7.80 (s, J = 8.2 Hz, 1H), 7.83 (d, J = 2.0 Hz, 1H).

[0053] To a solution of 2-bromo-5-cyanobenzylalcohol (4.59 g, 21.7 mmol) in dichloromethane (80 mL) were added diisopropylethylamine (5.6 mL, 32 mmol) and chloromethyl methyl ether (2.3 mL, 30 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for overnight. Water was added, and the mixture was extracted with chloroform. The organic layer was washed with brine and dried on anhydrous sodium

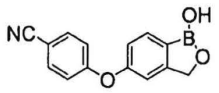
sulfate. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (6:1 hexane/ethyl acetate) to give 2-bromo-5-cyano-*O*-(methoxymethyl)-benzylalcohol (4.08 g, 71%).

[0054] ^{1}H-NMR (300.058 MHz, $CDCl_3$) δ ppm 3.43 (s, 3H), 4.65 (s, 2H), 4.80 (s, 2H), 7.43 (dd, J = 8.2, 4.1 Hz, 1H), 7.66 (d, J = 8.2 Hz, 1H), 7.82 (d, J = 4.1 Hz, 1H). The above compound was converted into the cyclic boronic ester by following the general procedure (B). Recrystallization from ethyl acetate/ether gave the target compound as a white powder.

[0055] ^{1}H-NMR (300.058 MHz, DMSO-d_6) δ ppm 5.03 (s, 2H), 7.76 (d, J = 8.2 Hz, 1H), 7.89 (d, J = 8.2 Hz, 1H), 7.90 (s, 1H), 9.53 (s, 1H).

Example 10

5-(4-Cyanophenoxy)-1-hydroxy-2,1-benzoxaborolane



[0056] A mixture of 4-bromo-3-methylphenol (5.00 g, 26.2 mmol), 4-fluorobenzonitrile (3.20 g, 26.2 mmol) , and potassium carbonate (3.98 g, 28.8 mmol) in dimethylformamide (100 mL) was stirred at 100 °C fro overnight. Water was added, and the mixture was extracted with ether. The organic layer was washed with water and brine, and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (9:1 hexane/ethyl acetate) followed by trituration with pentane to afford 4-(4-bromo-3-methylphenoxy)benzonitrile (6.20 g, 82%).

[0057] ^{1}H-NMR (300.058 MHz, $CDCl_3$) δ ppm 2.39 (s, 3H), 6.77 (dd, J = 8.8, 2.9 Hz, 1H), 6.95 (d, J = 2.9 Hz, 1H), 7.00 (d, J = 8.5 Hz, 2H), 7.54 (d, J = 8.8 Hz, 1H), 7.61 (d, J = 8.5 Hz, 2H).

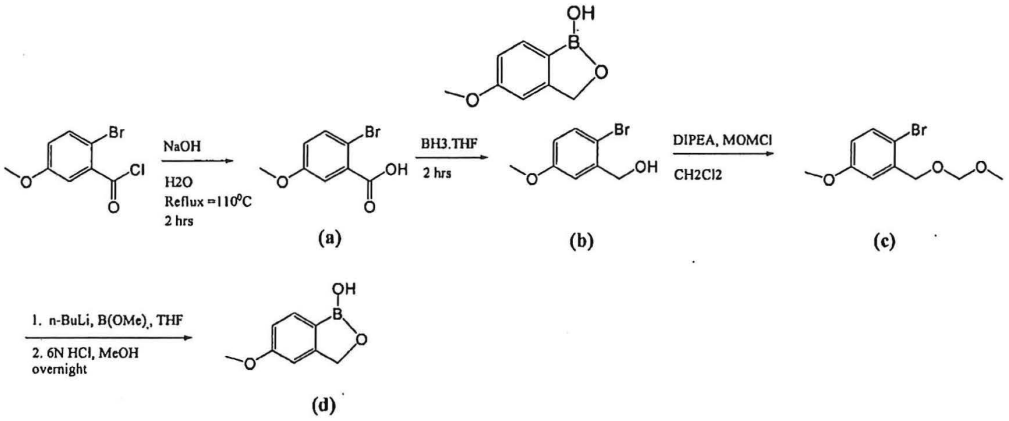
[0058] The above compound was converted into 2-bromo-5-(4-cyanophenoxy)-*O*-(methoxymethyl)benzylalcohol in a similar manner to Example (AN2727), which was further converted into the the cyclic boronic ester by following the

general procedure (B). Silica gel column chromatography (1:4 hexane/ethyl acetate) gave the target compound as a white powder.

[0059] ^{1}H-NMR (300.058 MHz, DMSO-d_6) δ ppm 4.95 (s, 2H), 7.08 (dd, J = 7.9, 2.1 Hz, 1H), 7.14 (d, J = 8.8 Hz, 1H), 7.15 (d, J = 2.1 hz, 1H), 7.78 (d, J = 7.9 Hz, 1H), 7.85 (d, J = 9.1Hz, 2H), 9.22 (s, 1H).

Example 11

1,3-dihydro-1-hydroxy-5-methoxy-2,1-benzoxaborole



[0060] ***(a) 2-bromo-5-methoxy-benzoic acid.*** To a solution of 2-bromo-5-methoxy-benzoyl chloride(6g, 24 mmol) in 36 mL 1N NaOH was stirred under reflux T=110^{0}C in oil bath for 2 hours. Remove the oil bath and let the mixture cool down to room temperature. Adjust the pH of the reaction mixture to pH 7. Water was added and the reaction mixture was extracted 3 times with EtOAc. The combined EtOAc extract was dried over magnesium sulfate. Solvent was removed on a rotary evaporator to yield 5.42 g (98% yield) of white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.38(s, 1H), 7.57(d, J = 9 Hz, 1H), 7.24(d, J = 3.3 Hz, 1H), 7.01(dd, J_1 = 3.3 Hz, J_2= 3Hz, 1H), 3.77(s, 3H).

[0061] ***(b) 2-bromo-5-methoxy-benzoic alcohol.*** 2-bromo-5-methoxy-benzoic acid (5.42g , 23.5 mmol) was placed into a 250 mL reaction flask, followed by 80mL of THF, and the flask was immersed in an ice bath and cooled to 0^0 C. Then 47 mL(47 mmol)of borane-THF was slowly added over a period of 15 min and the solution was

vigorously stirred for half an hr. The ice bath was then removed and the reaction mixture was continued to stir at room temperature for another 1.5 hrs. Then the reaction mixture was cooled down again in ice bath. Excess hydride was carefully destroyed with 15 mL of water and the aqueous phase was saturated with 4-5 g of potassium carbonate. The THF layer was separated and the aqueous phase was extracted 3 times with EtOAc. The combined organic extracts were dried over magnesium sulfate. Solvent was removed on a rotary evaporator to yield 3.57 g of white solid(70% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.42(d, J = 8.7 Hz, 1H), 7.09(d, J = 2.4 Hz, 1H), 6.77 (dd, J_1 = 3 Hz, J_2= 3Hz, 1H), 5.43(t, J = 5.7 Hz, 1H), 4.44(d, J = 5.1 Hz, 2H), 3.76(s, 3H)

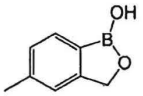
[0062] ***(c) 2-bromo-5-methoxy-1-(methoxymethoxymethyl)benzene.*** 2-bromo5-methoxy-benzoic alcohol (3.57g, 16.43 mmol) was dissolved in CH_2Cl_2, 65 mL under nitrogen and cooled to 0^0C in an ice bath. Then 4.3 mL (24.6 mmol) of diisopropyl ethyl amine and 1.5 mL(32.86 mmol) were added. The reaction mixture was stirred overnight at room temperature. The solution was washed with $NaHCO_3$ solution and brine, and dried over magnesium sulfate. Solvent was removed on a rotary evaporator. The crude product was purified by flash chromatography on silica gel, eluted with a mixed solvents of EtOAc/Hex(1:1) to give 2.21g (52% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.48(dd, J_1 = 1.2 Hz, J_2= 1.2 Hz, 1H), 7.05(d, J = 2.7 Hz, 1H), 6.83(dd, J_1 = 3 Hz, J_2= 3Hz, 1H), 4.69(d, J = 1.2 Hz, 2H), 4.5(s, 2H), 3.74(d, J = 1.5 Hz, 3H), 3.32(d, J = 2.1Hz, 3H)

[0063] (d) ***1,3-dihydro-1-hydroxy-5-methoxy-2,1-benzoxaborole.*** To a solution of 2-bromo-5-methoxy-1-(methoxymethoxymethyl)benzene (645 mg, 2.5mmol) was dissolved in 8 mL THF under nitrogen and cooled to -78 ^{0}C was added n-BuLi(1.6 M in hexane, 1.85 mL, 3 mmol) dropwise. Then, $B(OMe)_3$ (0.31 mL, 2.7mmol) was added and the reaction mixture was continued to stir for another 15 min. Then, the dry ice bath was removed. The mixture was allowed to warm up to room temperature and stirred for another 2h. 2.5 mL 6N HCl was added and the mixture was stirred for 15 minutes. Two layers formed in the reaction mixture. THF solvent was removed by rotary evaporator. 15 mL MeOH was added to dissolve the residue and then, another 2.5 mL 6N HCl was added. The mixture was stirred overnight. The solvent was removed and the residue was dissolved in EtOAc, washed with water, dried over $MgSO_4$. The crude product was purified by flash

chromatography on silica gel, eluted with a mixed solvents of EtOAc/Hex(1:10) and crystallized in hexane to afford 156 mg(38% yield) as a white crystal. M.P. 102-104 ^{0}C. MS ESI:m/z = 165.3 (M+1) and 162.9 (M-1). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.95 (s, 1H), 7.60 (d, J = 8.1 Hz, 1 H), 6.94 (s, 1H), 6.88 (d, J = 8.1 Hz, 1H), 4.91 (s, 2H), 3.77 (s,3 H).

Example 12

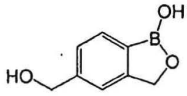
5-methyl-1,3-dihydro-1-hydroxy-2,1-Benzoxaborole



[0064] Same procedure as 1b, 1c and 1d starting with 2-bromo-5-methyl benzoic acid. The product is clear crystal. M.P. 124-128 ^{0}C. MS ESI:m/z = 148.9 (M+1) and 146.9 (M-1). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.05 (s, 1H), 7.58 (d, J = 7.2 Hz, 1H), 7.18 (s, 1H), 7.13 (d, J = 7.2 Hz, 2H), 4.91 (s, 2H), 2.33 (s, 3H).

Example 13

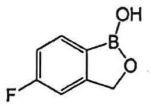
1,3-dihydro-1-hydroxy-5-hydroxymethyl-2,1-benzoxaborole.



[0065] Same procedure as 1b, 1c and 1d starting with 4-bromoisophtalic acid. The product is clear crystal. M.P. 124-128 ^{0}C. MS ESI:m/z = 148.9 (M+1) and 146.9 (M-1). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.05 (s, 1H), 7.58 (d, J = 7.2 Hz, 1H), 7.18 (s, 1H), 7.13 (d, J = 7.2 Hz, 2H), 4.91 (s, 2H), 2.33 (s, 3H).

Example 14

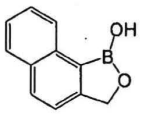
5-fluoro-1,3-dihydro-1-hydroxy-2,1-Benzoxaborole



[0066] The product is white powder. M.P. 110-114 °C. MS ESI:m/z = 150.9 (M-1). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.20 (s, 1H), 7.73 (dd, J_1 = 6 Hz, J_2= 6Hz, 1H), 7.21 (m, 1H), 7.14 (m, 1H), 4.95 (s, 2H).

Example 15

1,3-dihydro-2-oxa-1-cylopenta[ά]naphthalene



[0067] Same procedure as 1b, 1c and 1d starting with 1-bromo-2-napthaldehyde. The product is clear crystal. M.P. 139-143°C. MS ESI:m/z = 184.9 (M+1). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.21 (s, 1H), 8.28 (dd, J_1 = 6.9 Hz, J_2= 0.6Hz, 1H), 7.99(d, J = 8.1 Hz, 1H), 7.95 (d, J = 7.5 Hz, 1H), 7.59-7.47 (m, 3H), 5.09 (s, 2H).

Example 16

Formulations

[0068] Compounds of the present invention can be administered to a patient using a therapeutically effective amount of a compound of Formula I in anyone of the following three lacquer formulations and one solvent formulation. Solvent has good ease of use. Lacquer has good durability. However, these compounds could be applied using a spray formulation, paint-on lacquer, drops, or other.

1. 20%propylene glycol, 70% ethanol 10% API
2. 70% ethanol, 20% poly(vinyl methyl ether-alt-maleic acid monobutyl ester), 10% API
3. 56% ethanol, 14% water, 15% poly(2-hydroxyethyl methacrylate), 5% dibutyl sebacate, 10% API

4. 55% ethanol, 15% ethyl acetate, 15% poly(vinyl acetate), 5% dibutyl sebacate, 10% API

Example A
Protocol for Anacor Antifungal MIC Testing

Instrumentation

- Biomek Automated Plate Reader (Beckman)
- Novaspec II Spectrophotometer (Pharmacia)
- O_2 Incubator (Sheldon)
- Sonicator (Branson)
- Autoclave (Amsco)

Reagents and Materials

- Sabouraud Dextrose Agar (BBL)
- RPMI 1640 (Gibco)
- MOPS (Sigma)
- $NaHCO_3$ (J.T. Baker)
- NaOH (BDH)
- HCl (Fisher)
- NaCl (Fisher)
- DMSO (Sigma)
- dH_2O (Zenon)
- Fluconazole
- Amphotericin B (Sigma)
- Itraconazole

Preparation

Media is prepared 1-2 weeks before the start of the experiment. Media is stored in the cold room (4°C) prior to use.

- Sabouraud Dextrose Agar Plates:
    1. Add 65g of powdered of Sabouraud Dextrose Agar media into 1L of dH_2O with gentle stirring
    2. Autoclave at 121°C and 22 psi for 15 minutes
    3. Allow the media to cool to ~50°C
    4. Pour media into 100x15 mm sterile petri dishes with 20 ml aliquots

- RPMI 1640 + MOPS Broth:
    1. Add 1 packet of powdered RPMI media to 1L of dH_2O (15°C-30°C) with gentle stirring
    2. Add 2g of $NaHCO_3$
    3. Add 34.5 g of MOPS

4. Adjust the pH to 7.0 using NaOH or HCl
5. Sterilize with membrane filtration (0.22 micron cellulose acetate filter)

- Sterile Saline (0.9%)
  1. Dissolve 9g of NaCl to 1L of dH_2O
  2. Autoclave at 121°C and 22 psi for 15 minutes

- Sterile dH_2O
  1. Autoclave dH_2O at 121°C and 22 psi for 15 minutes

Procedure

1. The 10 organism panel is plated from stock vials stored at -80°C (suspended in broth with 20% glycerol) and incubated at 37°C for 24 hours. The organisms are then sub-cultured and incubated at 37°C for 24 hours. These will be used to prepare fresh inoculums for Step 6.

2. Approximately 2.5 mg of the compounds to be tested are weighed into 2 ml cryovials. Fluconazole, Amphotericin B and Itraconazole are tested as reference compounds.

3. 5 mg/ml stock solutions are made by adding DMSO to the samples accordingly. Compounds insoluble with vortexing only are sonicated.

4. 256 µg/ml working solutions are made by using the 5 mg/ml stock solutions and adding sterile distilled water accordingly.

5. 96-well plates are used for MIC determination. Each of the 8 rows can be used to test a different compound. Compounds are loaded into the first column and two-fold dilutions of are made from column 1 to 10. Column 11 is a growth control (no compound) and column 12 is a blank control (no compound or organism). Manual addition of broth and compounds is performed as follows:

   - 100 µl of RPMI + MOPS broth is added to columns 1-11
   - 200 µl of RPMI + MOPS broth is added to column 12
   - 100 µl of compounds at the 256 µg/ml working solution are added to column 1 (one compound per row)
   - Two-fold serial dilutions are done from column 1 to 10
   - Column 11 serves as the growth control (media + organism only)

6. The sub-cultured organisms are used to prepare fresh inoculums for testing on the 96-well plates. Each 96-well plate will test a different organism.

   - Colonies from the sub-cultured organisms (Step 1) are used to prepare inoculums with sterile saline. The target is adjusted to 70-75% transmittance at 530 nm wavelength using a Novospec II spectrophotometer.
   - A 1/1000 dilution is made into RPMI + MOPS broth

- 100 µl of this broth with organism is added to columns 1-11 (column 12 serves as the blank control)

7. The completed 96-well plates are incubated at 37°C for 24 hours. The 96 well plates are then read for absorbance at 650 nm wavelength using a Biomek Automated Plate Reader.

Calculations

The absorbance readings from the Biomek Automated Plate Reader are used to determine the percent inhibition for each test well. The formula used is as follows:

$$\% \text{ Inhibition} = [\ 1 - (ABS_{test} - ABS_{blank}) / (ABS_{mean\ growth} - ABS_{blank})\ ] \times 100\%$$

ABS_{test}: Absorbance of the test well
ABS_{blank}: Absorbance of the blank well in the same row as the test well (column 12)
$ABS_{mean\ growth}$: Mean absorbance of the growth control wells (column 11)

The minimum inhibitory concentration (MIC) is found at the lowest concentration of compound where percent inhibition is greater than or equal to 80%.

Example B
Keratin Assay

1. Weigh out 1.0 to 1.5 mg of test compound. Make a stock solution of 6.4mg/mL.
   $\frac{\text{Wt. of compound (mg)} \times 1000}{6.4\ \text{mg/mL}} = \mu L$ DMSO required
2. Make 1:2 serial dilutions of stock solution in DMSO to obtain stock solutions of 3.2 mg/mL down to 0.1 mg/mL. (Do same day as assay)
3. Prepare sterile 13mm x100mm tubes by adding 50mg of keratin in each. Need 7 bes for each compound tested plus tubes for positive and negative controls. (Prepare in advance)
4. Add 0.490 mL of RPMI+MOPS to each tube. To negative controls, add 1.0 mL PMI+MOPS and to positive controls, add 0.5 mL of same.
5. Add 10 µL of each compound dilution to tubes. (e.g. 10 µL of 6.4mg/mL solution to first tube of seven, 10 µL of 3.2mg/mL solution to second tube, etc.)
6. From a 2 week old culture of *Trichophyton rubrum,* take enough microconidia and add to 3 ml. of sterile water to read between 70 and 75% T at 530nm. (Thoroughly

mix the microconidia to get an even suspension.) Make a 1/1000 dilution of this suspension in RPMI+MOPS.

7. Add 0.5 mL of final microconidia suspension to each tube except the negative controls. Total volume in each tube is 1.0 mL. The concentration of compounds range from 64μg/mL down to 1.0μg/mL.

8. Incubate the tubes for 1 week at 30°C. Read for visual growth. MIC's are defined as the lowest drug concentration that inhibits invisible growth.

Example C

The Solubility, Stability and Log P Determination of compounds of the present invention by LC/MS/MS

Reagents and Standards used in the following methods

Ethanol:	200 proof ACS Grade (EM Science)
Octanol:	octyl alcohol (EM Science)
Acetonitrile:	HPLC Grade (Burdick & Jackson)
Ammonium Acetate:	lot 3272X49621 (Mallinckrodt)
COMPOUND (1):	lot A032-103
Compound (2):	lot A032-116
PNP:	lot OGN01 (TCI)
Water:	Deionized water (from Millipore systems, Billerica, MA)

Method for determining Solubility

[0069] N-Octanol and water were mutually pre-saturated by vigorously stirring a mixture of both solvents for up to 12 hours and the mixture was allowed to separate. Solubility in each solvent was determined by adding 10 μL of 20, 40 200, 1000 and 5000 μg/mL of Compound (1)and Compound (2) in DMSO to the pre-saturated n-octanol or water. After the sample was vortexed for 10s, the sample was centrifuged for 10 min 3000 rpm. A visual inspection was made to determine if the sample was clear or if a pellet had formed on the bottom of the tube.

Method for determining Log P

[0070] Compound (1) and Compound (2) (10 µL of 5000 µg/mL) at 2X the final concentration was added to 0.5 mL pre-saturated n-octanol and mixed. An equal volume (0.5 mL) of pre-saturated water was added, vortex mixed and then mixed on a rotating shaker for one hour and 24 hours in triplicates at ca. 25 °C. The organic and aqueous layers were separated by centrifugation for 5 min at ca. 2000 rpm. Twenty five µL of the octanol (top) layer were removed and placed in a pre-labeled tube. Twenty five µL of the aqueous layer (bottom) were removed, taking care to avoid octanol contamination, and placed in a pre-labeled tube. The samples were stored at *ca.* -20 °C prior to analysis.

Method for determining Stability at Room Temperature

[0071] Compound (1) and Compound (2) (10 µL of 5000 µg/mL) was added both to 0.5 mL n-octanol and 0.5 mL water in triplicates. Samples were mixed. At 0 hour and 24 hours samples were stored at *ca.* -20 °C. Twenty five µL of sample was used for analysis.

Method for Performing Calculations

[0072] A 1/concentration weighted linear regression was used for the quantification of Compound (1) and Compound (2). All integrations were performed with peak areas using Analyst version 1.3, Applied Biosystems. For Compound (1) and Compound (2) peak area ratios analyte to internal standard PNP were used for all quantification.

[0073] The partition coefficient (P) was calculated according to the equation detailed below:

$$P = [\text{Sample concentration}]_{\text{octanol}} / [\text{Sample concentration}]_{\text{water}}$$

$$\text{Log } P = \log 10(\text{partition coefficient})$$

Analytical Methods

[0074] The extraction and LC/MS/MS procedure used for the analysis of Compound (1) and Compound (2) is provided below.

Solutions

[0075] A calibrator was prepared using solutions of Compound (1) (5 mg/mL in DMSO), Compound (2), (5 mg/mL in DMSO), and p-Nitrophenol (PNP), (1 mg/mL in DMSO), ethanol.

[0076] The working internal standard was 60 mL of Acetonitrile add 6 µL of PNP (1000 µg/mL).

Extraction Procedure Compound (1) AND Compound (2)

[0077] For the octanol sample, 25 µL of ethanol, 25 µL of water and 300 µL of acetonitrile containing the internal standard was added. For the water sample, 25 µL of ethanol, 25 µL of octanol and 300 µL of acetonitrile containing the internal standard was added. For the calibrators 25 µL of octanol, 25 µL of water and 300 µL of acetonitrile containing the internal standard was added. The sample was vortexed for 10 seconds. Two hundred µL of the organic layer were transferred into a clean deactivated auto-sampler vial.

LC/MS/MS Conditions Compound (1) AND Compound (2)

[0078] The following conditions and solutions were used in the LC/MS/MS procedures:

Column:	Zorbax Eclipse SCB-C18 5 µm 2.1x50mm (Agilent)
Mobile Phase:	A: 5 mM Ammonium Acetate filtered (Mallinckrodt) B: Acetonitrile HPLC Grade filtered (Burdick & Jackson)
Injection Vol.:	10 µL
Needle Wash:	Water and Acetonitrile
Column Temp:	Ambient
HPLC Pumps:	LC10-AD (Shimadzu)
Autosampler:	HTS PAL Leap (CTC Analytics)
Gradient:	

Time (min)	%A	%B	Flow (µL/min)
0	95	5	300
1.0	95	5	300
1.1	5	95	300
2.0	5	95	300
2.1	95	5	300
3.5	95	5	300

Mass spectrometer:	API3000 (MDS Sciex)
MS Source:	APCI-Negative

Temperature: 400 °C

Compound:	Q1 Mass	Q3 Mass	DP	FP	CE	CXP
Compound (1)	150.84	42.65	-51	-100	-38	-5
Compound (2)	166.87	42.85	-81	-340	-40	-5
PNP	137.91	107.75	-46	-270	-24	-17

Dwell Time: 250 msec
Entrance Potential: -10

Results

[0079] As shown in the table below the solubility of Compound (1) and Compound (2) in both octanol and water is very good over the concentration range tested.

Solubility of Compound (1) and Compound (2) in water and octanol

Targeted Conc (µg/mL)	Water Visual	Octanol Visual
0.400	Clear	Clear
0.800	Clear	Clear
4.00	Clear	Clear
20.0	Clear	Clear
100	Clear	Clear

[0080] The tables below show the results of the log P determination after 1h and 24h for Compound (1) and Compound (2), respectively. The mean log P after 1h was 1.97 (n=3). After 24h the concentrations in both the octanol and water layer remained the same. The mean log P after 24h was 1.93 (n=3). For Compound (2) the mean log P after 1h was 2.34 (n=3). After 24h the mean log P was 2.48 (n=3).

Log P of Compound (1)

Sample	Conc. in Water (µg/mL)	Conc. in Octanol (µg/mL)	Log P
1h-1	1.26	108	1.93
1h-2	1.21	103	1.93
1h-3	1.05	115	2.04
24h-1	1.27	104	1.91
24h-2	1.17	109	1.97
24h-3	1.28	99.0	1.89

Log P of Compound (2)

Sample	Conc. in Water (µg/mL)	Conc. in Octanol (µg/mL)	Log P
1h-1	0.580	94.5	2.21
1h-2	0.439	112	2.41
1h-3	0.455	112	2.39
24h-1	0.384	113	2.47
24h-2	0.400	122	2.48
24h-3	0.325	101	2.49

[0081] In parallel a stability study for Compound (1) and Compound (2) was initiated at room temperature over 24h without continuous mixing. The tables below show that Compound (1) in pure water and octanol is stable over 24h. The found concentration for Compound (2) in water was only 51.4 µg/mL at 0 h, compared to 100 µg/mL theoretical. A possible explanation is that Compound (2) has reached its solubility limit in water, although visually this was not observed.

Water and Octanol stability for Compound (1) and Compound (2) are stable at room temperature after 24h.

Sample	Mean (µg/mL)	SD	Percent Remaining 24h versus 0h
Water-0h	82.5	3.72	115
Water-15h	95.0	21.4	
Octanol-0h	115	3.06	93
Octanol-15h	107	6.11	

Water and Octanol stability for Compound (2) at room temperature after 24h

Sample	Mean (µg/mL)	SD	Percent Remaining 24h versus 0h
Water-0h	51.4	0.3	108
Water-15h	55.6	10.0	
Octanol-0h	106	6.65	107
Octanol-15h	113	7.77	

Example D

Determination of Penetration into Human Nail.

[0082] This is a single dose 72 hour dosing period.

Species:	Healthy human cadaver finger nail plates.
Source:	UCSF Anatomy Laboratory.
Site of Sampling:	Larger size finger nails (finger nails number 1, 2, 3, and 4, at least 1.3 cm diameter).
Storage:	Samples are gently cleaned by saline solution. They are sealed in a plastic container and refrigerated at 2 - 8 °C until use.
Acceptance Criteria:	Following visual inspection, non-damaged nails with dimensions of 1.3 cm or larger will be accepted for inclusion into the study.
Rejection Criteria:	Nail will not be used if damaged or has abnormal length and shape, surface texture and color or has been contacted with chemicals (such as formalin).

EXPERIMENTAL DESIGN

[0083] Permeation cells were assembled according to standard operating procedure.

Cell:	Teflon one-chamber diffusion cell.
Receptor Fluid:	A small cotton ball wetted with 0.1 ml normal saline.
Incubation Temp. and Humidity:	Room temperature (20 – 30°C), 30 - 45% RH.
Surface Area:	One (1) cm in diameter, 0.785 cm^2.
Formulation:	To be determined.
Dose Volume and Frequency:	Ten (10) $\mu l/cm^2$/dose, once (9 – 10 AM).
Dose Contact Time and Surface Washing:	Dose contact time is 72 hrs. Surface washing is with ethanol, soap and water.

Sampling Sources:
1. Surface washes
2. Cotton ball (supporting bed)
3. Nail content

STUDY DESIGN

[0084] Randomly pick nail samples (human finger nail).

Replicates	Chemical / Formulation									
	A	B	C	D	E	F	G	H	I	J
1	X	X	X	X	X	X	X	X	X	X
2	X	X	X	X	X	X	X	X	X	X
3	X	X	X	X	X	X	X	X	X	X

Treatments : Single Dose Screening Human Fingernail

A comparison of 10 formulation with 3 replicates dose application for 72 hours (n = 30 fingernails).

Day 1 •Single dose application test articles

Day 3 •Remove receptor cotton ball 72 hours post dosing

•Wash nail surfaces with ethanol followed by soap and water wash.

•Retail all cotton balls and washes for assay.

•Remove inner nail area as powder for assay

•Remove dorsal dosing area for assay.

•Retain remainder of nail for assay.

Dosing procedure: Chemicals in formulations, 10 % w/v, dosed at 10 µl once only.

Surface Washing and Final Cell Washing:

Ten minutes prior to the morning application (including the first application), the dosed area of the nail plate is washed with cotton tips in a cycle as follows:

a tip wetted with ethanol, then

a tip wetted with ethanol, then

a tip wetted with 50% skin cleansing liquid IVORY soap, then

a tip wetted with distilled water, then

a tip wetted with distilled water, then

a final dry tip.

Collect all tips into a single vial.

Apply test material in quantities identified in "XI." above.

After a waiting period of 30 minutes, record appearance of the nail.

When the whole incubation period finished, after removal of the nail plate and nail bed, the cell is washed as the same as the surface washing procedure.

SAMPLE COLLECTION, ANALYSIS AND STORAGE

[0085] Supporting Bed Samples: The cotton ball placed in the chamber is transferred into a scintillation vial and the chamber is washed with cotton tips (the procedure is the same as the dosed area nail washing). The washing samples are put into a separate vial to the cotton ball.

[0086] Nail Surface Wash Samples: The washing samples from each time point from each nail are pooled and collected by breaking off the cotton tip into scintillation glass vials.

[0087] Nail Sample Collection: Inner (ventral layer) - intermediate center of the nail is drilled to be powder, then collected into a scintillation vial. The top (dorsal layer) - intermediate center is cut and placed into the second vial. The remainder is placed into the third vial. Nail samples are weighed before and after sampling and vials are also weighed before and after being placed samples.

[0088] The quantity of Compounds (1) and (2) in the cotton ball, nail powder and "top of the nail" samples were measured using LC/MS/MS as described below. P-Nitrophenol (PNP) was used as an internal standard (IS). The analyte and internal standard were extracted from the cotton ball with acetonitrile. The nail powder and "top of the nail" were first solubilized by 1N sodium hydroxide. The solution was then neutralized and extracted with methylenechloride before analysis. The samples were transferred to glass

autosampler vials and injected onto an LC/MS/MS system, where they were analyzed using a Zorbax Eclipse XDB-C18 (2x10 mm) column and a Sciex API 3000 Mass Spectrometer.

Reagents and Solutions

Acetonitrile:	HPLC Grade (Burdick & Jackson)
Ammonium Acetate:	lot 3272X49621 (Mallinckrodt)
Normal Saline:	0.85 % (w/v) sodium chloride (Mallinckrodt)
Methylene Chloride:	HPLC Grade (Mallinckrodt)
Sodium Hydroxide:	1N (JT Baker)
Hydrochloric Acid:	6N (VWR)
Trisbuffer pH 7.4	0.1M (Sigma)
Compound 1:	lot A032-103, 25.6 mg/mL in DMSO
Compound 2:	lot A032-116, 25.6 mg/mL in DMSO
PNP:	lot OGN01, 1.0 mg/mL in DMSO (TCI)
Water:	Deionized water (from Millipore systems, Billerica, MA)
Spin-X centrifuge Tubes	0.22 µM nylon (Costar)

Method for Cotton Ball Analysis

[0089] Calibrator Preparation for cotton ball

Calibrator ID	Source Solution ID	Stock Conc. (µg/mL)	Vol. of stock added (µl)	Vol. of blank saline added (µl)	Final Conc. (µg/mL)	Absolute Amount (µg)
K	Stock	25600	100	900	2560	256
J	K	2560	500	500	1280	128
I	J	1280	500	500	640	64
H	I	640	500	500	320	32
G	H	320	500	500	160	16
F	G	160	500	500	80	8
E	F	80	500	500	40	4
D	E	40	500	500	20	2
C	D	20	500	500	10	1
B	C	10	500	500	5	0.5
A	B	5	500	500	2.5	0.25
BL	-	0	0	500	0	0

Working Internal Standard:

0.1 µg/mL PNP in Acetonitrile

To 60 mL of Acetonitrile add 6 µl of PNP (1 mg/mL)

Extraction Procedure:

- Aliquot 100 µl of standard or blank onto cotton ball into a glass scintillation vial
- Add additional 100 µl of blank saline to cotton ball
- Add 300 µL of internal standard to each sample
- Vortex for 5 seconds
- Transfer to x-spin centrifuge filter tubes
- Centrifuge the tubes for 10 min at 1600 rpm
- Transfer 200 µL of the organic layer into a clean deactivated autosampler vial

Method for Nail Powder and Top of the Nail Analysis

Calibrator ID	Source Solution ID	Stock Conc. (µg/mL)	Vol. of stock added (µl)	Vol. of DMSO (µl)	Final Conc. (µg/mL)	Absolute Amount (µg)
K	Stock	25600	-	0	25600	256
J	K	25600	100	100	12800	128
I	J	12800	100	100	6400	64
H	I	6400	100	100	3200	32
G	H	3200	100	100	1600	16
F	G	1600	100	100	800	8
E	F	800	100	100	400	4
D	E	400	100	100	200	2
C	D	200	100	100	100	1
B	C	100	100	100	50	0.5
A	B	50	100	100	25	0.25
BL	-	0	0	100	0	0

Working Internal Standard:

To 60 mL of methylenechloride add 6 µl of PNP (40 µg/mL stock)

Extraction Procedure:

- Aliquot 10 µl of standard or blank onto nail powder into a glass tube.
- Add 2 mL 1N NaOH, incubate at 45C overnight in waterbath
- Cool tubes in freezer for 10 min
- Add about 332 µl 6N HCl and 1 mL Trisbuffer 0.1M pH 7.4 to bring pH around 6.0
- Vortex for 10s
- Extract with 5 mL methylenechloride containing internal standard
- Invert tubes for 10 min
- Centrifuge the tubes for 10 min at approximately 3000 rpm
- Transfer organic layer (bottom) to glass tubes
- Evaporate under nitrogen in Zymark Turbovap LV evaporator till dryness at 40C
- Reconstitute in 200 µl of acetonitrile into a clean deactivated autosampler vial

LC/MS/MS Conditions

Column:	Zorbax Eclipse XDB-C18 5 µm 2.1x50mm (Agilent)
Mobile Phase:	A: 5 mM Ammonium Acetate filtered (Mallinckrodt) B: Acetonitrile HPLC Grade filtered (Burdick & Jackson)
Injection Vol.:	10 µL
Needle Wash:	Water and Acetonitrile
Column Temp:	Ambient
HPLC Pumps:	LC10-AD (Shimadzu)
Autosampler:	HTS PAL Leap (CTC Analytics)
Gradient:	

Time (min)	%A	%B	Flow (µL/min)
0	95	5	300
1.0	95	5	300
1.1	5	95	300
2.0	5	95	300
2.1	95	5	300
3.5	95	5	300

Mass spectrometer: API3000 (MDS Sciex)
MS Source: APCI-Negative
Temperature: 400 C

Compound:	Q1 Mass	Q3 Mass	DP	FP	CE	CXP
Compound 1:	150.84	42.65	-51	-100	-38	-5
Compound 2:	166.87	42.85	-81	-340	-40	-5
PNP	137.91	107.75	-46	-270	-24	-17

Dwell Time: 250 msec
Entrance Potential: -10

Data Processing

[0090] Data and chromatographic peaks were processed using Analyst 1.3 with peak smoothing setting 1. A standard curve was determined from peak area ratio vs. concentration using quadratic regression with 1/x weighting.

Application Data Sheet

Application Information

Application Type::	Provisional
Subject Matter::	Utility
Suggested classification::	
Suggested Group Art Unit::	
CD-ROM or CD-R?::	None
Computer Readable Form (CRF)?::	No
Title::	BORON-CONTAINING SMALL MOLECULES FOR USE IN THE TOPICAL...
Attorney Docket Number::	045471-0750
Request for Early Publication?::	No
Request for Non-Publication?::	No
Suggested Drawing Figure::	0
Total Drawing Sheets::	0
Small Entity?::	Yes
Petition included?::	No
Secrecy Order in Parent Appl.?::	No

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Representative Information

Representative Customer Number::	38706	

Domestic Priority Information

Application::	**Continuity Type::**	**Parent Application::**	**Parent Filing Date::**

Foreign Priority Information

Country::	**Application number::**	**Filing Date::**	**Priority Claimed::**

Assignee Information

Assignee Name::