Discovery of a Novel Broad-spectrum Antifungal Agent, Derived from Albaconazole

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Experimental Procedures

Chemistry

General methods

Melting points were determined using an Electrothermal IA9300 digital melting point apparatus and reported uncorrected. 1H and 13C NMR spectra were recorded on a Bruker Bruker Avance 400 spectrometer (400 MHz). Chemical shifts are expressed as δ values (ppm) relative to tetramethylsilane as internal standard (in NMR description, s = singlet, d = doublet, t = triplet, q = quadruplet, sext = sextuplet, m = multiplet and b = broad). Coupling constants *J* are given in Hertz. IR spectra were obtained in KBr pellets using a Perkin-Elmer Paragon FTIR 1000 PC spectrometer. Only the most significant absorption bands have been reported. Mass spectra analysis was performed by the Mass Spectrometry Laboratory of the University of Rouen. Mass spectra (EI) were recorded with a Waters LCP 1er XR spectrometer. The optical rotations were recorded with a polarimeter Schmidt-Haensch Polartronic NH8. All reactions were monitored by thin-layer chromatography (TLC) using 0.2 mm-thick silica gel plates 60F-254 (5735 Merck). Column chromatography was carried out using silica gel 60 (70-230 Mesh, ASTM, Merck). Chemicals and solvents used were commercially available. Optically oxirane (2*R*,3*S*) **15** and albaconazole were synthesized according to protocols described in Ref. 17 and 3, respectively (for compound **15**: $[\alpha]^{20}_D$ = -9.0 (c = 1.0 in MeOH) (lit: $[\alpha]^{20}_D$ = -8.3), for albaconazole: $[\alpha]^{20}_D$ = -8.0 (c = 1.0 in $CHCl_3$) (lit: $[\alpha]^{20}_D$ = -8.3)). Focused microwave irradiations were carried out with a CEM Discover^{TM} focused microwave reactor (300W, 2455 MHz, monomode system).

7-bromoquinazolin-4(3H)-one **(2)**

4-Bromo-2-nitrobenzoic acid (1g, 4.06 mmol, **1**), formamide (6.44 mL, 162.14 mmol) and indium chloride (900 mg, 4.06 mmol) were introduced under argon into a 50 mL round-bottomed flask equipped with a condenser and a magnetic stirring bar. The flask was placed

in the microwave cavity and exposed to microwave irradiation under argon for 40 min at 150°C using irradiation power of 80 W. After cooling, mixture was diluted with water and product was extracted with ethyl acetate. Organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified on silica gel column chromatography (dichloromethane/ethanol, 99:1) and compound **2** was obtained in a 81% yield as a white solid. Mp 259-260 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.69 (dd, 1H, ^3J = 8.4 Hz, ^4J = 1.6 Hz), 7.88 (d, 1H, ^4J = 1.6 Hz), 8.03 (d, 1H, ^3J = 8.4 Hz), 8,13 (s, 1H), 12.41 (bs, 1H); IR (KBr cm^{-1}): 858, 1239, 1609, 1668, 3050, 3466; HRMS calcd for $C_8H_6{}^{79}BrN_2O$ $[M+H]^+$ 224.9663 found 224.9665, for $C_8H_6{}^{81}BrN_2O$ $[M+H]^+$ 226.9643 found 226.9651.

7-bromo-6-nitroquinazolin-4(3H)-one **(3)**

To a stirred solution of **2** (1.47 g, 6.53 mmol) in concentrated sulfuric acid (23 mL) was added fuming nitric acid (0.552 mL, 13.06 mmol) and the solution was stirred at 100°C for 1 h. After cooling, mixture was poured into ice water and neutralized with ammonia. Product was extracted with ethyl acetate, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified on silica gel column chromatography (dichloromethane/ethanol, 99:1) and compound **3** was obtained in a 76% yield as a pale yellow solid. Mp 313-314 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.17 (s, 1H), 8.30 (s, 1H), 8.62 (s, 1H), 12.78 (bs, 1H); IR (KBr cm^{-1}): 810, 1249, 1332, 1518, 1605, 1656, 1695, 3082, 3504; HRMS calcd for $C_8H_3{}^{79}BrN_3O_3$ $[M-H]^-$ 267.9358 found 267.9355, for $C_8H_3{}^{81}BrN_3O_3$ $[M-H]^-$ 269.9337 found 269.9327.

3-benzyl-7-bromo-6-nitroquinazolin-4(3H)-one **(4)**

Compound **3** (1.335 g, 4.94 mmol), DMF (7.4 mL), sodium hydride (60% dispersion in mineral oil) (237 mg, 5.93 mmol) and benzyl bromide (0.588 mL, 4.94 mmol) were successively introduced into a 50 mL round-bottomed flask equipped with a condenser and a magnetic stirring bar. The flask was placed in the microwave cavity and exposed to microwave irradiation for 30 min at 80°C using irradiation power of 40 W. The solvent was removed under reduced pressure, and the residue was hydrolyzed with water and extracted with ethyl acetate. Organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified on silica gel column chromatography (dichloromethane) and compound **4** was obtained in a 84% yield as a white solid. Mp 169-170 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.25 (s, 2H), 7.33-7.43 (m, 5H), 8.25 (s, 1H), 8.70 (s, 1H), 8.83 (s, 1H); IR (KBr cm^{-1}): 813, 1216, 1350, 1532, 1602, 1679, 3082; HRMS calcd for $C_{15}H_{11}{}^{79}BrN_3O_3$ $[M+H]^+$ 359.9984 found 359.9983.

6-amino-3-benzyl-7-bromoquinazolin-4(3H)-one **(5)**

To a stirred solution of **4** (200 mg, 0.55 mmol) in ethanol (1.5 mL) and acetic acid (1.5 mL) was added iron powder (124 mg, 13.06 mmol) and the solution was stirred under reflux for 1 h. After cooling, mixture was poured into ice water and neutralized with NaOH (5M). Product was extracted with ethyl acetate, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified on silica gel column chromatography (dichloromethane/ethanol, 99.5:0.5) and compound **5** was obtained in a 93% yield as a white solid. Mp 166-167 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.18 (s, 2H), 5.91 (bs, 2H), 7.32-7.39 (m, 5H), 7.49 (s, 1H),

7.81 (s, 1H), 8,31 (s, 1H); IR (KBr cm^{-1}): 836, 1213, 1483, 1595, 1621, 1669, 3020, 3332, 3453; HRMS calcd for $C_{15}H_{13}{}^{79}BrN_3O$ $[M+H]^+$ 330.0242 found 330.0239 (93%), for $C_{15}H_{13}{}^{81}BrN_3O$ $[M+H]^+$ 332.0222 found 332.0222.

3-benzyl-7-bromo-6-[(4-chloro-5H-1,2,3-dithiazol-5-ylidene)amino]quinazolin-4(3H)-one **(6)**
To a stirred solution of **5** (2.035 g, 6.16 mmol) in anhydrous dichloromethane (39 mL) was added under argon Appel's salt (1.542 g, 7.40 mmol) and the solution was stirred at room temperature for 2 h. Pyridine (1.097 mL, 13.56 mmol) was added and the solution was stirred at room temperature for 1 h. Solvent were removed under reduced pressure and the residue was purified on silica gel column chromatography (dichloromethane). Compound **6** was obtained in a 58% yield as a yellow solid. Mp 214-215 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.23 (s, 2H), 7.34-7.43 (m, 5H), 8.03 (s, 1H), 8.19 (s, 1H), 8.65 (s, 1H); IR (KBr cm^{-1}): 868, 1366, 1451, 1586, 1677; HRMS calcd for $C_{17}H_{11}{}^{79}Br^{35}ClN_4OS_2$ $[M+H]^+$ 464.9246 found 464.9254.

7-bromo-6-[(4-chloro-5H-1,2,3-dithiazol-5-ylidene)amino]quinazolin-4(3H)-one **(7)**
To a stirred solution of aluminium chloride (2.64 g, 19.80 mmol) in toluene (33 mL) was added under argon **6** (1.64 g, 3.54 mmol) and the solution was stirred at 65°C for 1 h. Solvent was removed under reduced pressure and mixture was poured into ice water. Product was extracted with ethyl acetate and organic layers were washed with saturated sodium bicarbonate, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified on silica gel column chromatography (dichloromethane/ethanol, 98:2 and 96:4) and compound **7** was obtained in a 90% yield as an orange solid. Mp >350 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01 (s, 1H), 8.15 (s, 1H), 8.16 (d, 1H, ^3J = 3.5 Hz), 12.49 (bs, 1H); IR (KBr cm^{-1}): 861, 1273, 1447, 1596, 1671, 3030, 3451; HRMS calcd for $C_{10}H_5{}^{79}Br^{35}ClN_4OS_2$ $[M+H]^+$ 374.8777 found 374.8786.

8-oxo-7,8-dihydro-1,3-thiazolo[4,5-g]quinazoline-2-carbonitrile **(8)**
Compound **7** (600 mg, 1.60 mmol), pyridine (24 mL) and copper iodide (608 mg, 3.19 mmol) were successively introduced under argon into a 100 mL round-bottomed flask equipped with a condenser and a magnetic stirring bar. The flask was placed in the microwave cavity and exposed to microwave irradiation under argon for 15 min at 115°C using irradiation power of 60 W. After cooling, the mixture was dissolved in ethyl acetate, and washed with saturated sodium thiosulfate solution. Organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified on silica gel column chromatography (dichloromethane/ethanol, 98:2 and 95:5) and compound **8** was obtained in a 86% yield as a light brown solid. Mp >350 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.24 (d, 1H, ^3J = 3.2 Hz), 8.69 (s, 1H), 8.91 (s, 1H), 12.52 (bs, 1H); IR (KBr cm^{-1}): 1278, 1468, 1619, 1676, 2227, 3054, 3468; HRMS calcd for $C_{10}H_3N_4OS$ $[M-H]^-$ 227.0028 found 227.0039.

1,3-thiazolo[4,5-g]quinazolin-8(7H)-one **(9)**
Compound **8** (300 mg, 1.32 mmol) and HBr (48%) (33 mL) were introduced into a 100 mL round-bottomed flask equipped with a condenser and a magnetic stirring bar. The flask was placed in the microwave cavity and exposed to microwave irradiation for 30 min at 115°C

using irradiation power of 60 W. The solution was neutralized with NaOH (5M) and product was extracted with ethyl acetate. Organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified on silica gel column chromatography (dichloromethane/ethanol, 95:5) and compound **9** was obtained in a 93% yield as a white solid. Mp >350 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.16 (d, 1H, ^3J = 3.5 Hz), 8.56 (s, 1H), 8.77 (s, 1H), 9.58 (s, 1H), 12.32 (bs, 1H); IR (KBr cm^{-1}): 845, 1278, 1445, 1615, 1670, 3038, 3455; HRMS calcd for $C_9H_6N_3OS$ $[M+H]^+$ 204.0232 found 204.0220.

7-[(1R,2R)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]thiazolo[4,5-g]quinazolin-8(7H)-one **(I)**

To a stirred solution of compound **15** (111 mg, 0.44 mmol) in *N*-methyl-2-pyrrolidone (1 mL) was added compound **9** (90 mg, 0.44 mmol) and potassium carbonate (92 mg, 0.65 mmol). The mixture was stirred at 80°C for 3 days. After cooling, mixture was diluted with water and product was extracted with ethyl acetate. Organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified on silica gel column chromatography (dichloromethane/ethanol, 99:1) and compound **I** was obtained in a 47% yield as a white solid. Mp 140-141 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.27 (d, 3H, ^3J = 7.2 Hz), 4.27 (d, 1H, ^2J = 14.4 Hz), 4.92 (d, 1H, ^2J = 14.4 Hz), 5.93 (q, 1H, ^3J = 7.2 Hz), 6.43 (s, 1H), 7.01 (ddd, 1H, $^3J_{H\text{-}F}$ = $^3J_{H\text{-}H}$ = 8.4 Hz, $^4J_{H\text{-}H}$ = 2.2 Hz), 7.25-7.38 (m, 2H), 7.60 (s, 1H), 8.24 (s, 1H), 8.48 (s, 1H), 8.63 (s, 1H), 8.88 (s, 1H), 9.60 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 15.4, 51.6, 54.9, 77.6, 104.4, 111.3, 120.3, 120.7, 121.0, 124.2, 129.9, 140.7, 144.1, 144.9, 145.9, 150.6, 151.9, 158.9, 159.3, 161.5, 162,4; IR (KBr cm^{-1}): 848, 1140, 1259, 1442, 1501, 1609, 1674, 3418; HRMS calcd for $C_{21}H_{17}F_2N_6O_2S$ $[M+H]^+$ 455.1102 found 455.1101; $[\alpha]^{20}_D$ = -10.0 (c = 0.1 in $CHCl_3$).

8-[(1R,2R)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl]thiazolo[5,4-f]quinazolin-9(8H)-one **(II)**

Using the synthetic procedure used for compound **I** starting from **15** (149 mg, 0.59 mmol) and compound **14** (120 mg, 0.59 mmol) to obtain compound **II** in a 45% yield as a white solid. Mp 238-229 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.31 (d, 3H, ^3J = 6.8 Hz), 4.27 (d, 1H, ^2J = 14.1 Hz), 4.92 (d, 1H, ^2J = 14.1 Hz), 6.00 (q, 1H, ^3J = 6.8 Hz), 6.46 (s, 1H), 7.02 (ddd, 1H, $^3J_{H\text{-}F}$ = $^3J_{H\text{-}H}$ = 8.4 Hz, $^4J_{H\text{-}H}$ = 2.4 Hz), 7.35-7.38 (m, 2H), 7.61 (s, 1H), 7.96 (d, 1H, ^3J = 8.8 Hz), 8.23 (s, 1H), 8.60 (d, 1H, ^3J = 8.8 Hz), 8.63 (s, 1H), 9.64 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 15.5, 52.2, 54.8, 77.6, 104.4, 111.3, 115.9, 124.0, 126.0, 129.4, 129.9, 130.6, 144.9, 146.3, 146.4, 150.5, 152.2, 158.8, 159.4, 160.2, 162.4; IR (KBr cm^{-1}): 857, 1158, 1273, 1456, 1504, 1587, 1657, 3450; HRMS calcd for $C_{21}H_{17}F_2N_6O_2S$ $[M+H]^+$ 455.1102 found 455.1089; $[\alpha]^{20}_D$ = -30.0 (c = 0.1 in $CHCl_3$).

In vitro antifungal activity

Candida spp.

Candida spp. suspensions were prepared in RPMI 1640 medium (Sigma, Saint Quentin Fallavier, France) supplemented with 0.165 M morpholinopropanesulphonic acid (MOPS,

Sigma), 2% glucose and antibiotics and adjusted to give a final concentration of 10^3 cells mL^{-1}. A 96-well microplate (Nunc, Dutscher SA, France) was seeded with 100 µL of *Candida* suspension. Molecules were first dissolved in dimethylsulfoxide and then diluted in culture medium. Each concentration of molecule (100 µL) to be tested was added and plates were incubated at 37 °C for 24 h. The cellular viability was evaluated spectrofluorometrically with an excitation at 550 nm and an emission at 590 nm after a 4 h incubation with 10 µL of resazurin (Interchim, Montluçon, France). The minimal inhibitory concentration (MIC) is the concentration that inhibited 50% of the cell growth. MICs were determined by linear regression analysis.

Aspergillus fumigatus

Conidia of *A. fumigatus* were microscopically counted and diluted in RPMI 1640 medium (Sigma) supplemented with 0.165 M morpholinopropanesulphonic acid (MOPS, Sigma), 2% glucose and antibiotics. One hundred microliters of a 10^4 cells mL^{-1} suspension were inoculated in a 96-well microplate (Nunc). Drugs were prepared as described for *Candida spp.* evaluations and 100 µL of the drug dilutions were added to the cell suspension. After an incubation time of 48 h at 37 °C, the cellular viability was evaluated as in *Candida* assay after an incubation time of 20h. Activity of the studied and reference molecules was expressed as the MIC. The MIC is the concentration that inhibited 80% of the cell growth. MICs were determined by linear regression analysis.

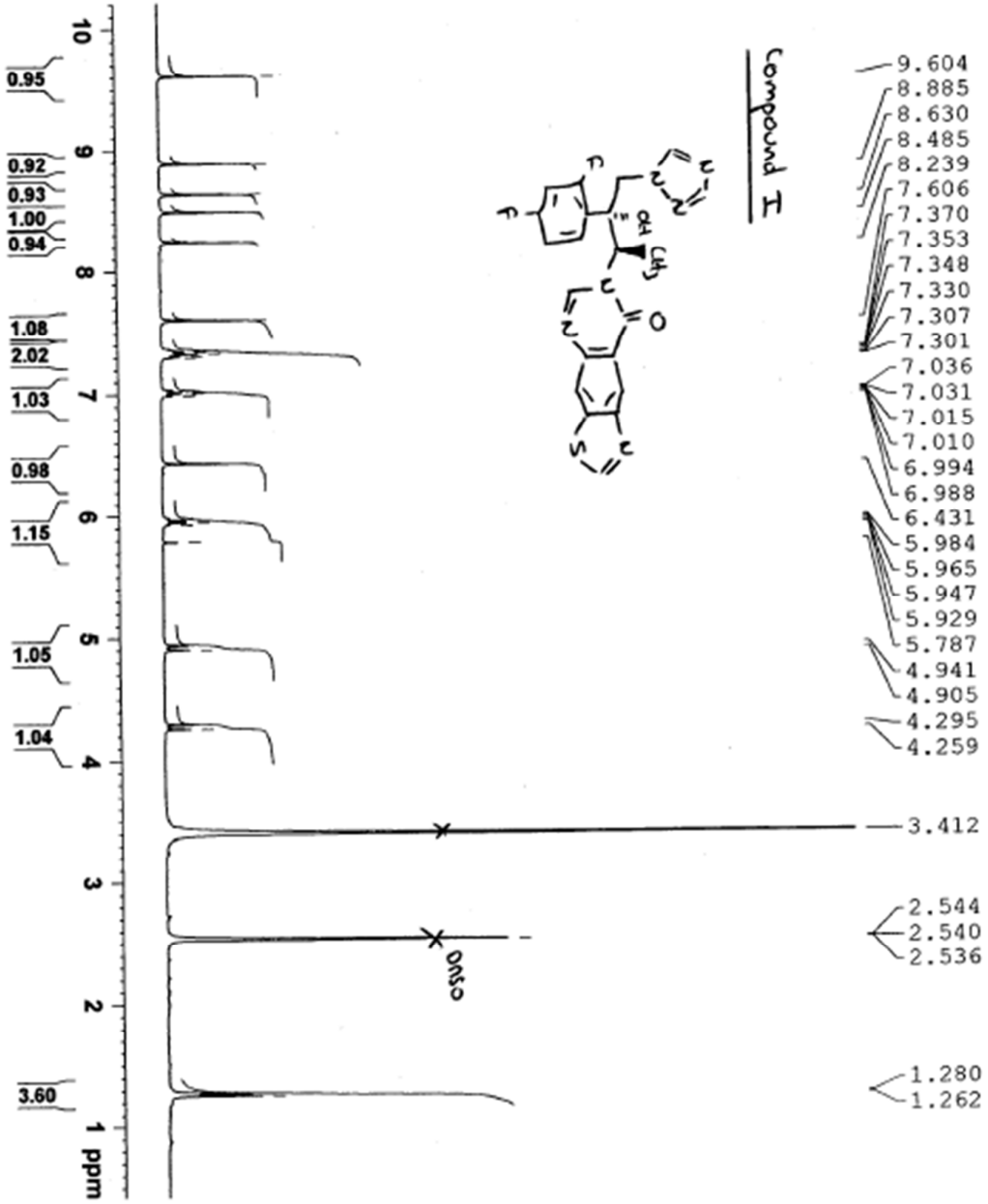
Other filamentous fungi

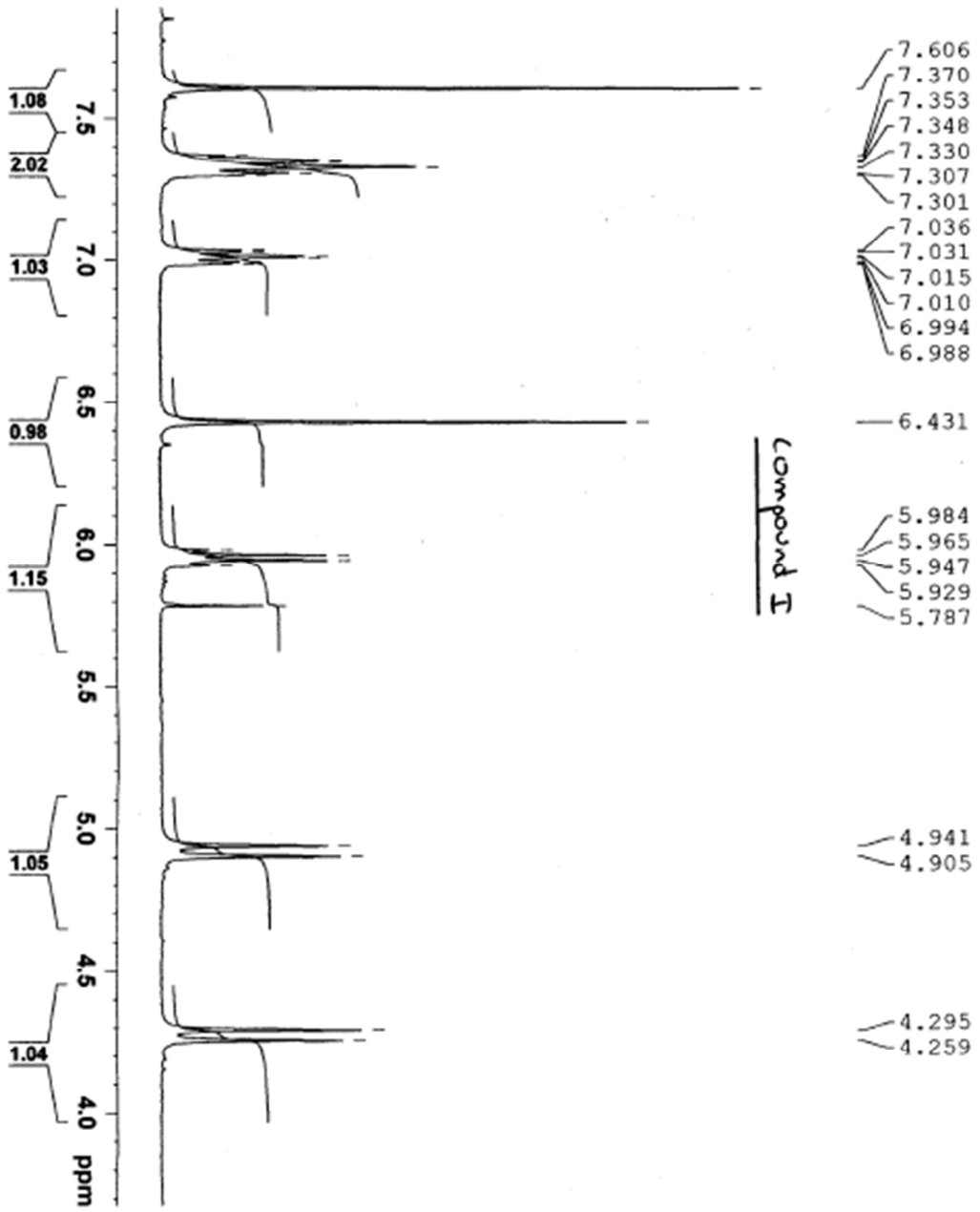
The broth microdilution test was done in accordance with the CLSI guidelines for filamentous fungi (CLSI document M38-A) using RPMI 1640 medium (Sigma) buffered to pH 7.0 with MOPS (Sigma). Drugs were diluted in DMSO and further in culture medium. Final drug range was 0.032–16 mg/L. Stock inoculum suspensions were prepared from 7-day-old cultures grown on potato dextrose agar following the CLSI guidelines (document M38-A). Stock suspensions contained conidia or sporangiospores. The diluted (2×) inoculum sizes ranged from 0.9×10^4 to 4.7×10^4 cfu/mL. Drug-free and cell-free controls were included. The microdilution plates were incubated at 35°C and read after 48 h except for the Zygomycetes, which were read at 24 h. The MIC endpoints were read visually with the aid of a reading mirror and expressed as the lowest drug concentration that prevented 100% growth.

Sterol analysis

After an overnight culture of *Candida albicans* CAAL93 in Sabouraud dextrose broth with two concentrations of compound **I**, 4.54 and 22.72 $ng.mL^{-1}$. Cells were then harvested and resuspended in 3 ml of 60% (wt/v) KOH and saponified by heating at 80°C for 2 h. Non saponifiable lipids (sterols) were extracted from the saponified mixture, twice, with 2 ml of n-hexane pooled, and dried under nitrogen. The sterols were suspended in 100 µl of bis(trimethylsilyl) trifluoride for 30 min for silylation. The silyled sterols were analyzed by gas chromatography-mass spectrometry (Agilent Technologies). The sterol identification was done in reference to the relative retention times and mass spectra previously reported.

^{1}H and ^{13}C NMR spectra for compound I

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4.905
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Compound I
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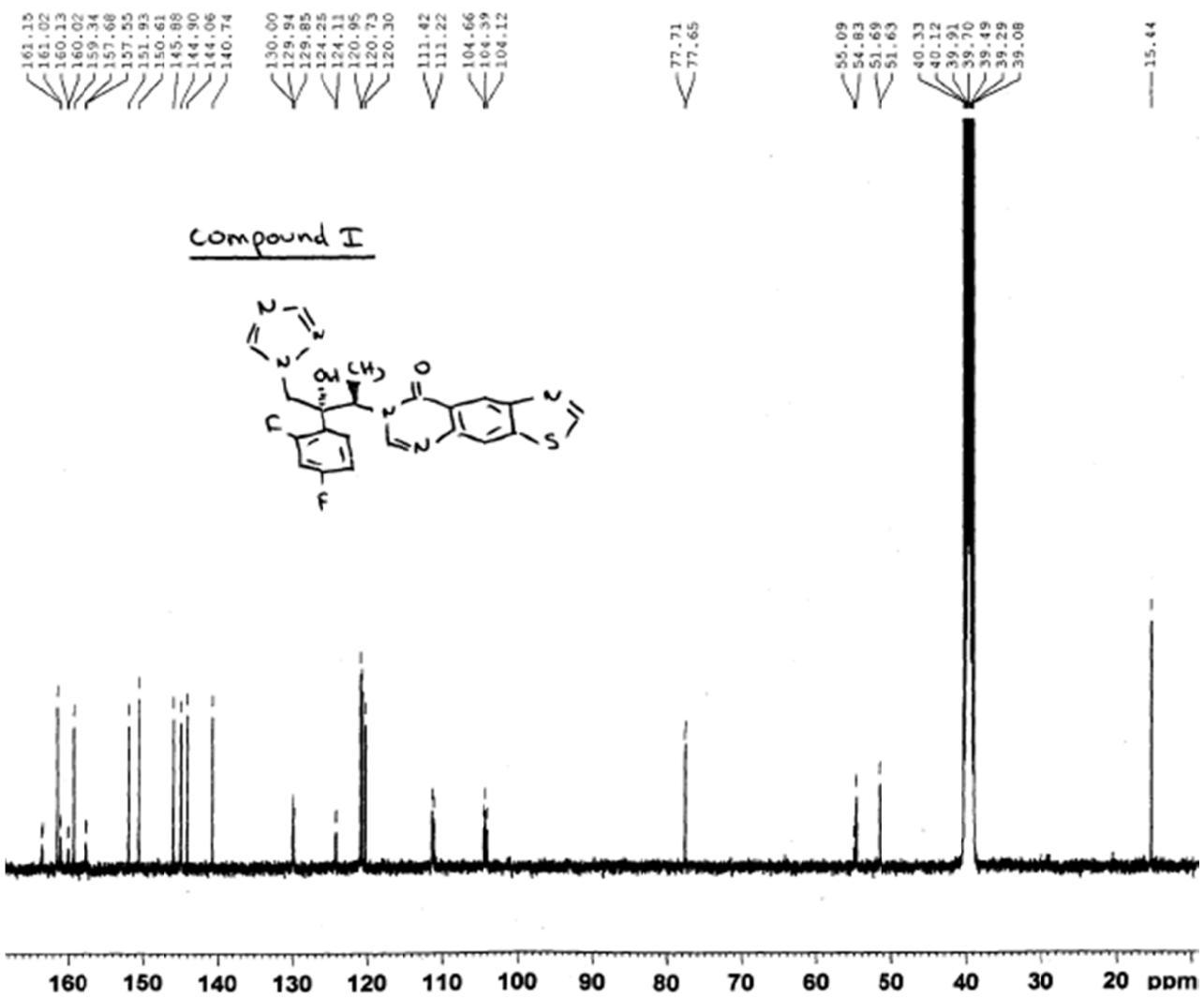
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Compound I
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