Supporting Information for:

Impact of Absolute Stereochemistry on the Antiangiogenic and Antifungal Activities of Itraconazole

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Chemistry. Reactions were carried out in oven dried glassware. All reagents were purchased from commercial sources and were used without further purification unless noted. Unless stated otherwise, all reactions were carried out under a positive pressure of argon and were monitored by Merck precoated silica gel 60F-254 plates and were visualized using 254 nm UV light. Column chromatography was performed on silica gel (200–400 mesh, Merck). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). NMR data were collected on a Varian Unity-400 (400 MHz ^{1}H, 100 MHz ^{13}C) machine in the Department of Pharmacology and Molecular Sciences, the Johns Hopkins University. ^{1}H NMR spectra were obtained in deuteriochloroform ($CDCl_3$) with either tetramethylsilane (TMS, δ = 0.00 for ^{1}H) or chloroform ($CHCl_3$, δ = 7.27 for ^{1}H) as an internal reference. ^{13}C NMR spectra were proton decoupled and were in $CDCl_3$ with either TMS (δ = 0.0 for ^{13}C) or $CHCl_3$ (δ = 77.0 for ^{13}C) as an internal reference. Chemical shifts are reported in ppm (δ). Data are presented in the form: chemical shift (multiplicity, coupling constants, and integration). ^{1}H data are reported as though they were first order. The errors between the coupling constants for two coupled protons were less than 0.5 Hz, and the average number was reported. The low-resolution mass spectra were obtained on a Voyager DE-STR, MALDI-TOF instrument at the AB Mass Spectrometry/Proteomics Facility at the Johns Hopkins University. The MALDI-samples were prepared by mixing droplets of the sample solutions in chloroform or methanol and 2,5-dihydroxybenzoic acid solution in acetone, where the latter served as the matrix. The high-resolution mass spectra were acquired on an ABI Voyager-DE STR instrument in the Department of Chemistry at the Texas A&M University. The ionization method is MALDI by using THAP (2,4,6-trihydroxyacetophenone) as the matrix. Optical rotations were measured on a Jasco P-1010 polarimeter at the sodium D line (589 nm) in the Department of Chemistry, the Johns Hopkins University. Optical rotations were measured at 22 ± 2 °C. Optical rotations are in units of deg•mL$(dm \cdot g)^{-1}$.

***Trans*-(*2R,4S*)-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-4-tosyloxymethyl-1,3-dioxolane (11c)** or ***trans*-(*2S,4R*)-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-4-tosyloxymethyl-1,3-dioxolane (11d)**

This compound was synthesized by following the literature-reported procedure.[1] After the salt formation with toluenesulfonic acid, the majority of tosylate salts of **11c** or **11d** were remained in the ethyl acetate solution, which was neutralized by washing with a saturated aqueous K_2CO_3 solution. The aqueous layer was then extracted with CH_2Cl_2 several times. The combined organic solution was dried (Na_2SO_4), filtered, and concentrated to yield the crude product, which was purified by column chromatography (50:1→5:1 CH_2Cl_2-acetone) to afford **11c** or **11d** as a yellowish oil: R_f 0.33 (5:1, CH_2Cl_2-acetone); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.11 (s, 1H), 7.85 (s, 1H), 7.60 (d, J = 8.1 Hz, 2H), 7.49 – 7.22 (m, 4H), 7.09 (dd, J = 8.5, 1.4 Hz, 1H), 4.73 – 4.60 (m, 2H), 4.14 – 3.98 (m, 1H), 3.94 – 3.80 (m, 3H), 3.59 (t, J = 7.8 Hz, 1H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 151.69, 145.53, 144.96, 136.14, 134.54, 133.00, 132.32, 131.33, 130.15, 129.40, 128.03, 127.38, 108.28, 75.01, 67.69, 67.07, 54.37, 21.92. MALDI-MS: 484.1 ($M+H^+$), 506.1 ($M+Na^+$).

Typical procedure for the preparation of *trans*-itraconazole 1e–1h

To a solution of **8a** or **8b** (22.6 mg, 0.057 mmol) in DMSO was added NaH (10.2 mg of a 60% dispersion in mineral oil, 0.26 mmol). After the mixture was stirred at 50 °C under argon for 1 h, a solution of **11c** or **11d** (30.0 mg, 0.062 mmol) in DMSO was added dropwise. After the addition, the temperature was increased to 90 °C, and the solution was stirred under argon for another 3 h. The reaction was then quenched by the addition of a 50% aqueous NaCl solution, and the resulting mixture was extracted with CH_2Cl_2. The organic fractions were dried (Na_2SO_4), filtered, and concentrated under vacuum to yield the crude product, which was purified by column chromatography (1:1 hexanes–EtOAc → neat EtOAc) to afford *trans*-itraconazole (27.9 mg, 69%) as a yellowish oil, which could be further purified by a second column (neat CH_2Cl_2 → 50:1 CH_2Cl_2–CH_3OH) if necessary: R_f 0.56 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.23 (br s, 1H), 7.93 (br s, 1H), 7.65 – 7.55 (m, 2H), 7.49 – 7.38 (m, 3H),

7.19 (dd, J = 8.5, 2.1 Hz, 1H), 7.13 – 6.85 (m, 4H), 6.68 (t, J = 15.2 Hz, 2H), 4.76 (q, J = 14.6 Hz, 2H), 4.36 – 4.15 (m, 2H), 4.00 (dd, J = 8.2, 6.4 Hz, 1H), 3.89 (dd, J = 10.0, 4.5 Hz, 1H), 3.80 (dd, J = 13.9, 6.6 Hz, 2H), 3.20 – 3.57 (m, 8H), 1.94 – 1.77 (m, 1H), 1.78 – 1.64 (m, 1H), 1.39 (d, J = 6.7 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 152.22, 136.03, 135.23, 134.09, 133.21, 131.37, 129.69, 127.30, 123.79, 119.12, 117.12, 115.48, 108.05, 76.25, 67.79, 67.60, 54.66, 52.90, 51.30, 49.12, 28.66, 19.50, 11.03. MALDI-MS: 705.3 ($M+H^+$), 727.3 ($M+Na^+$).

Biological methods

Materials and Strains

Pooled HUVEC and EGM-2 bullet kits were purchased from Lonza. Powdered RPMI 1640 media with L-glutamine and without sodium bicarbonate was purchased from Gibco. Amino acids, uracil, and agar were purchased from Sigma and morpholinepropanesulfonic acid (MOPS) was purchased from Fisher. Dimethyl Sulfoxide (DMSO) was purchased from J.T. Baker. *C. glabrata* strains BG1[2] and BG2[3], *C. neoformans* H99[4], *S. cerevisiae* BY4741 (MATa, *ura3, his3, leu2, met15*), and *C. albicans* (ATCC 10261) were used.

Cell Culture

HUVEC between passage 3 and 8 were grown in EGM-2 bullet kit media at 37°C in a humidified environment with 5% CO_2 present. Fungi were maintained on YES (5 g/L yeast extract, 30 g/L glucose) agar (2%) plates supplemented with 225 mg/L adenine, histidine, leucine, uracil, and lysine at 30°C.

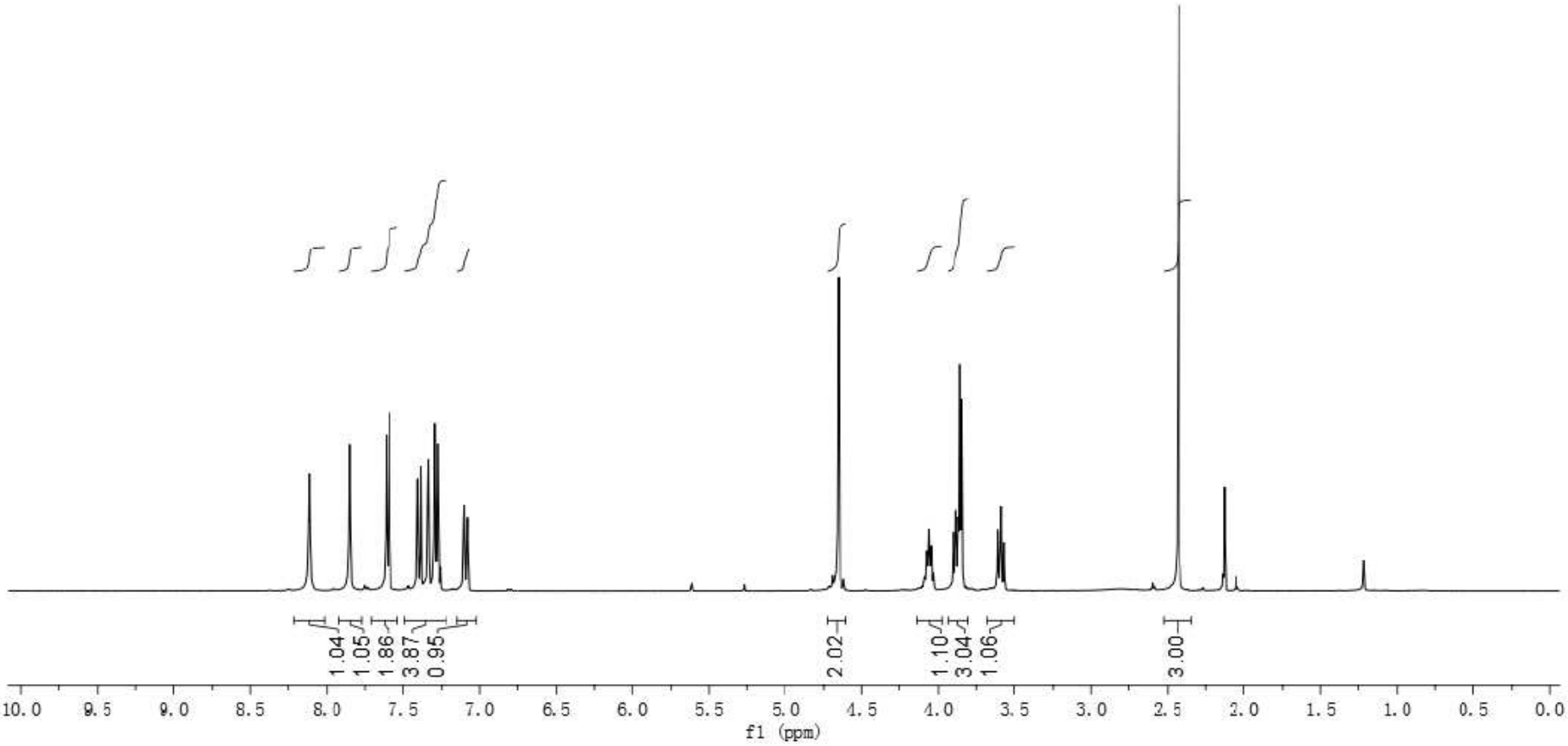
HUVEC proliferation assay

HUVEC were plated in a 96-well plate at a density of 2000 cells/well 199 μL media. After recovering overnight, cells were incubated for 24 hours with drugs added from 200X DMSO stocks. Next, cells were incubated with 0.9 μCi of [^{3}H]-thymidine for 6 h, washed once with PBS, trypsinized, and transferred to filtermats (Wallac) using a Mach III M Harvester 96 (Tomtec). The filtermats were then dried overnight. Using a 1450 Microbeta apparatus (Wallac), the amount of [^{3}H] at each position on the filtermat was determined by scintillation counting. Counts from vehicle only treated controls were used to normalize for maximum proliferation. Experiments were conducted in triplicate with multiple technical replicates for each data point. A four parameter logistic regression was used to determine IC_{50} values (GraphPad Prism [v4.03]).

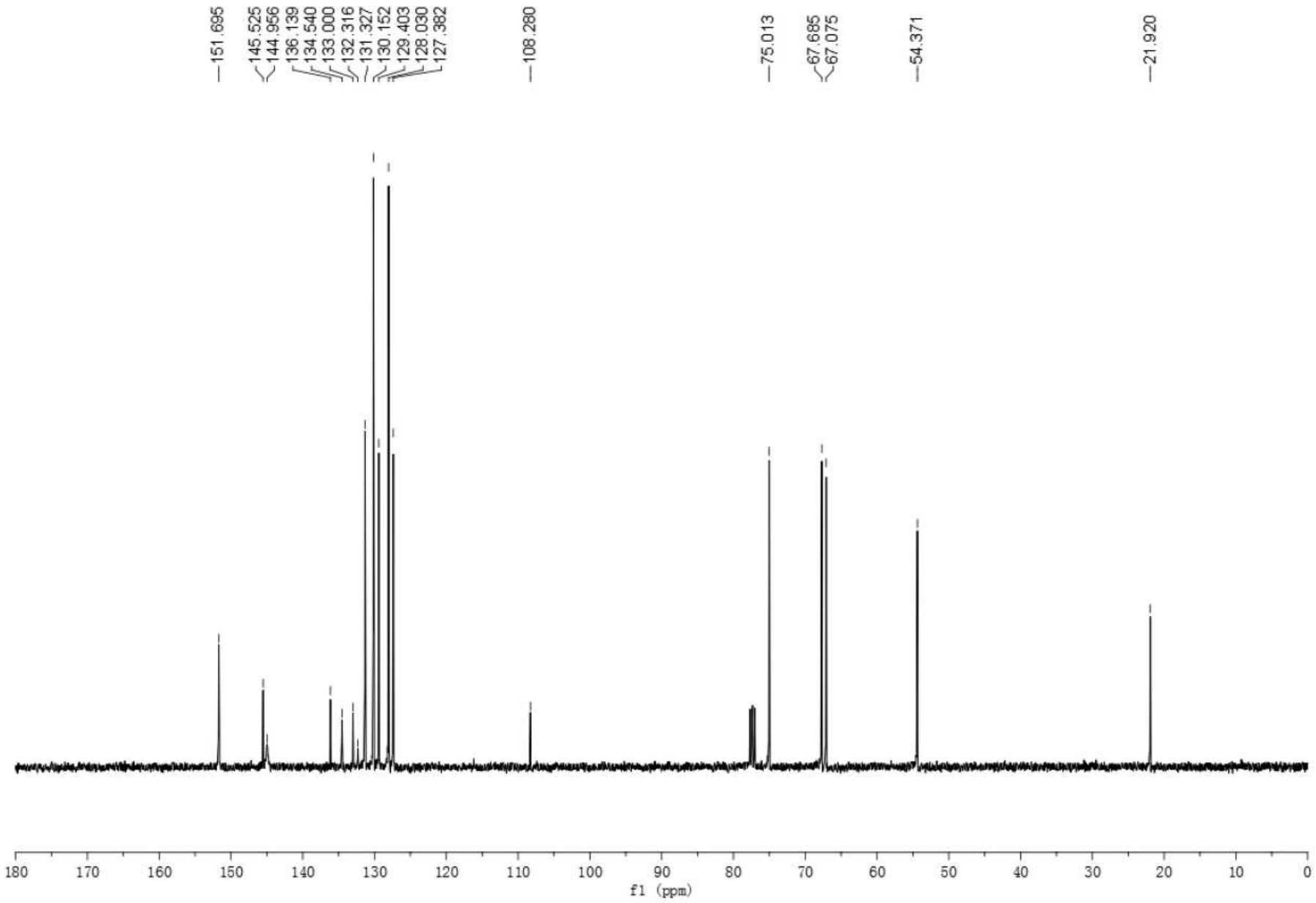
Fungal Susceptibility Assays

Fungi were grown overnight in 5 mL YES supplemented 225 mg/L adenine, histidine, leucine, uracil, and lysine (supplemented YES) at 30°C with shaking. Cultures were diluted to 0.01 OD_{600} in RPMI (with L-glutamine, without sodium bicarbonate) which was supplemented with 2% glucose, 225 mg/L adenine, histidine, methionine, leucine, uracil, and lysine and buffered with 165 mM MOPS to 7.0 (complete RPMI). This solution was then further diluted 190-fold in complete RPMI in 96-well plates containing test compounds in a final volume of 150 µL. When practical, the intermediate RPMI dilution was bypassed and the overnight cultures were directly diluted to the appropriate OD_{600} in the RPMI solution that was added to the 96-well plates. Two-fold serial dilutions of the compounds were tested starting at 4 ug/mL with each well containing 0.125% vehicle (DMSO). The plates were incubated in a humidified environment at 30°C with shaking for 30 h except for experiments with *C. neoformans* which was incubated for 54 h. Abs_{600} was measured using a plate reader (Bio-Tek Synergy HT) after vigorous shaking for 45 seconds to resuspend the cells. The background absorbance from non-inoculated wells was subtracted and the absorbance of test wells was divided by that of wells treated with vehicle alone. The percent of remaining growth for each drug concentration was averaged over 2-3 independent experiments, each with two technical replicates, and the minimum concentration giving 80% inhibition (MIC_{80}) was determined.

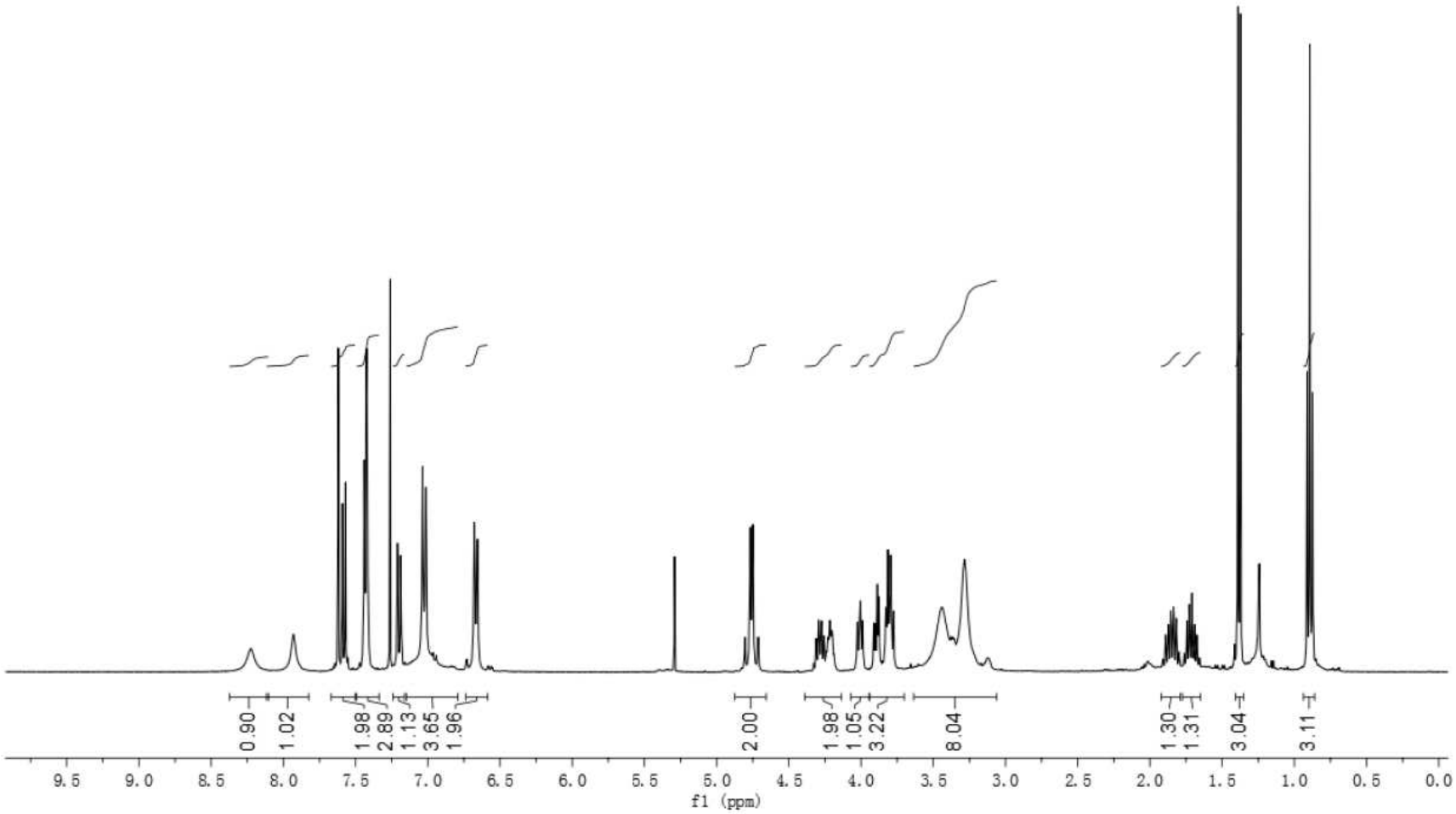
^{1}H NMR spectrum of **11c** or **11d**



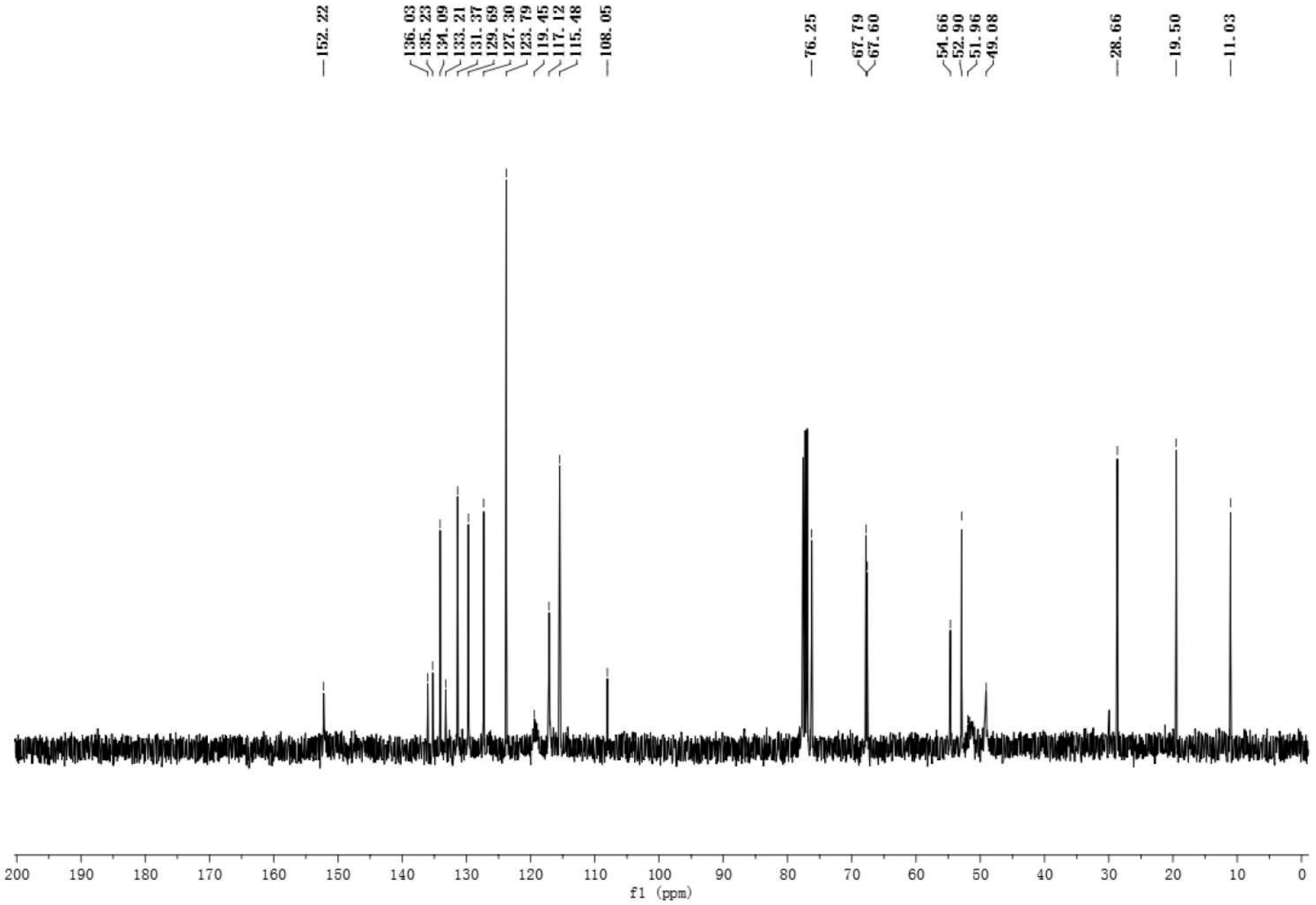
^{13}C NMR spectrum of **11c** or **11d**



^{1}H NMR spectrum of **1e**, **1f**, **1g**, or **1h**



^{1}H NMR spectrum of **1e**, **1f**, **1g**, or **1h**



High-resolution mass spectrometry data

Compounds	Calculated ($M+H^+$) ($C_{35}H_{39}Cl_2N_8O_4$)	Found
1a	705.2471	705.2458
1b		705.2455
1c		705.2476
1d		705.2458
1e		705.2455
1f		705.2459
1g		705.2474
1h		705.2456

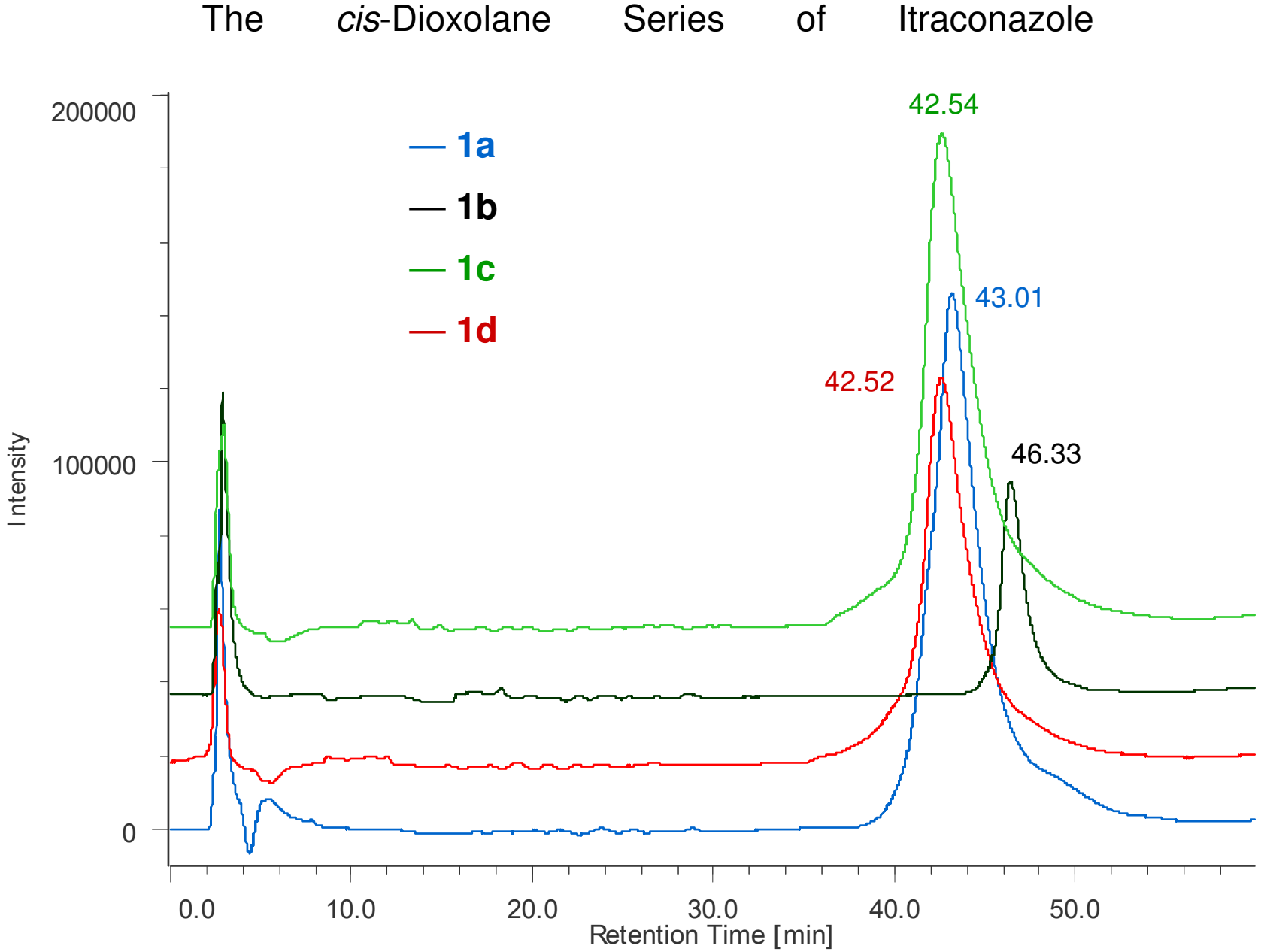
HPLC Analysis of itraconazole diastereomers 1a–1h

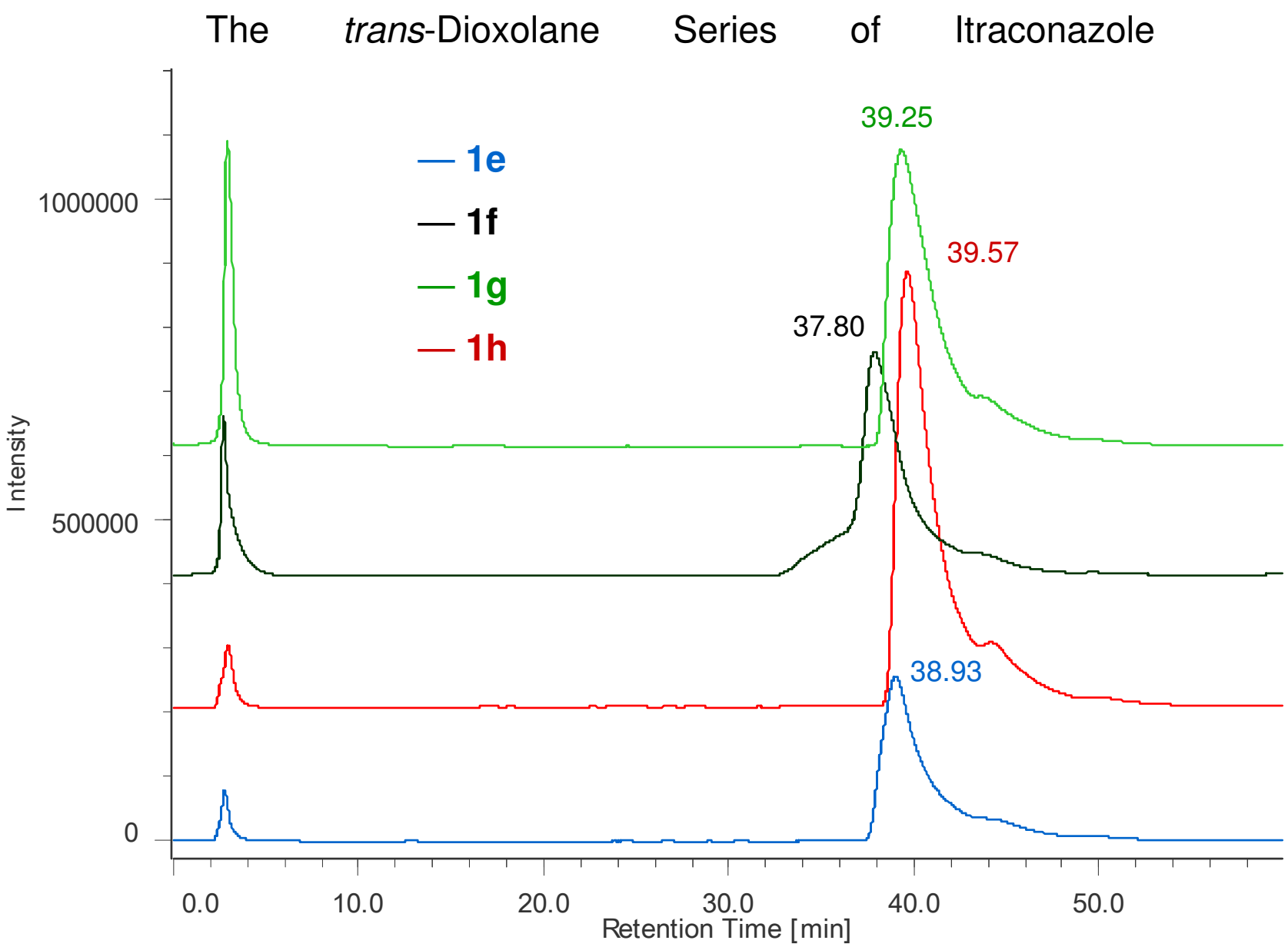
HPLC: JASCO PU-2089S Plus quaternary pump system

Column: CHIRALPAK® AS-RH (2.1x150 mm), 5 μm

Detector: MD-2010 Plus PDA

Conditions: Itraconazole diastereomers were analyzed on HLPC according to the conditions described by Kunze et al.[5] Briefly, 10 μL sample of each itraconazole diastereomer as ~1 mM solution in chloform was injected into a 7725i Rheodyne injection module. A mixture of acetonitrile and 5 mM aqueous ammonium acetate was used as the mobile phase with the flow rate of 0.2 mL/min. Initially, mobile phase composition was 70% 5 mM aqueous ammonium acetate and 30% acetonitrile, but the gradient was ramped up to 70% acetonitrile and 30% 5 mM aqueous ammonium acetate over 46 min starting at the 4th min. In the next 5 min, eluent was changed to 20% 5 mM aqueous and 80% acetonitrile to wash off the column, and then it was switched to the original composition to get ready for the next injection cycle.


The *cis*-Dioxolane Series of Itraconazole
200000
100000
0
Intensity
1a
1b
1c
1d
42.54
43.01
42.52
46.33
0.0
10.0
20.0
30.0
40.0
50.0
Retention Time [min]


The *trans*-Dioxolane Series of Itraconazole
1e
1f
1g
1h
39.25
39.57
37.80
38.93
Intensity
1000000
500000
0
0.0
10.0
20.0
30.0
40.0
50.0
Retention Time [min]

References

1. Tanoury, G. J.; Hett, R.; Wilkinson, H. S.; Wald S. A.; Senanayake, C. H. Total synthesis of (*2R,4S,2'S,3'R*)-hydroxyitraconazole: implementations of a recycle protocol and a mild and safe phase-transfer reagent for preparation of the key chiral units. *Tetrahedron: Asymmetry* **2003,** *14*, 3487-3493.
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