

Supporting information

High-throughput screening-based identification and structure-activity relationship-characterization defined (S)-2-(1-aminoisobutyl)-1-(3-chlorobenzyl) benzimidazole as a highly antimycotic agent, non-toxic to cell lines

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General synthesis and analysis procedures

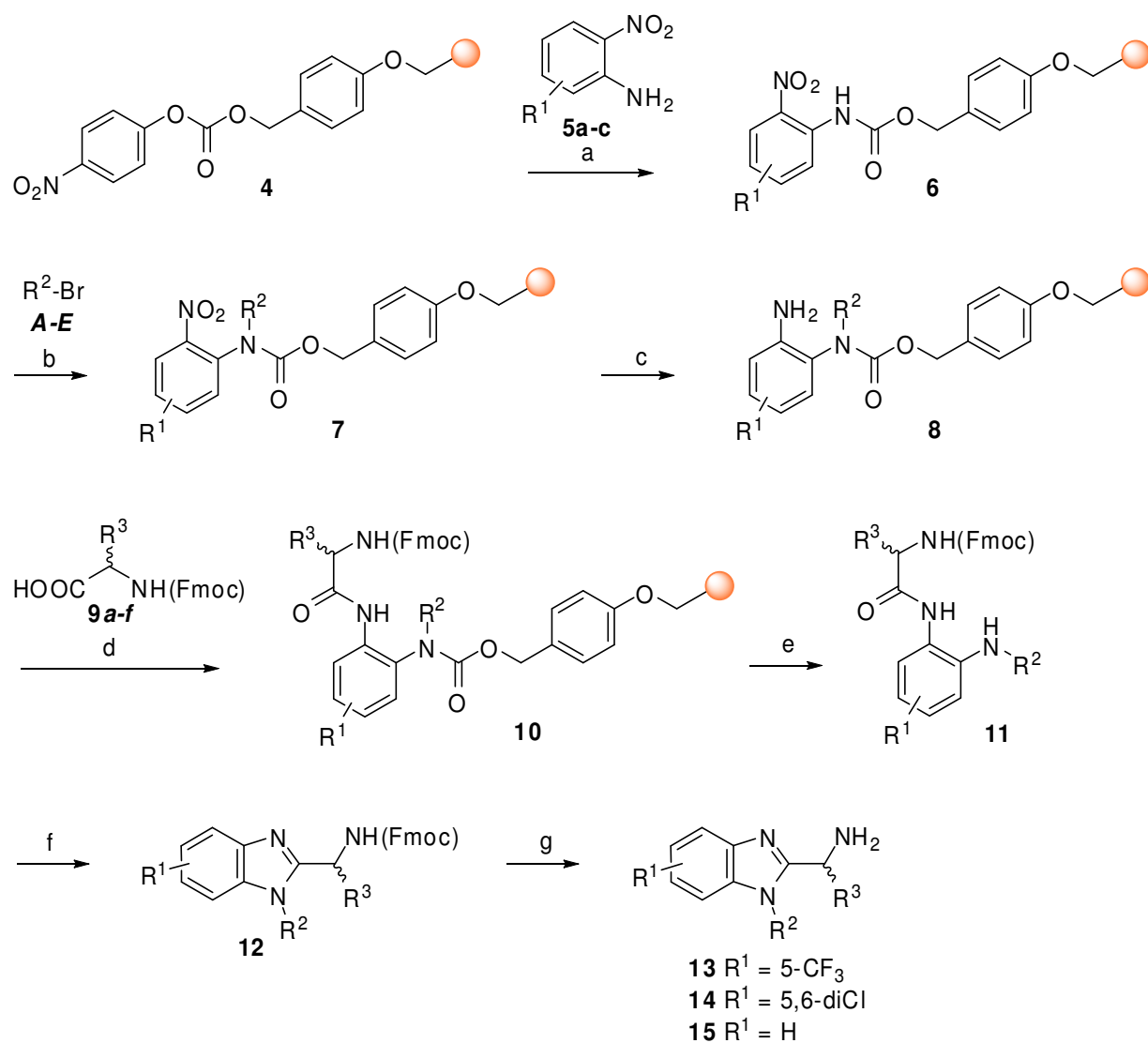
All reagents were purchased from Fluka and Sigma and used as received. Solid phase synthesis steps were performed on polystyrene resin (1 % DVB, 100-200 mesh) from Rapp Polymere, Tübingen, Germany. Reactions were carried out in commercially available dry solvents unless otherwise stated. Preparative flash chromatography was performed with MN Kieselgel 60 silica gel (Macherey-Nagel, 0.04-0.063 mm).

^1H and ^{13}C NMR spectra were recorded with a Bruker Avance 400 MHz spectrometer. ^1H and ^{13}C chemical shifts are given in ppm relative to the solvent signal as internal standard. Assignments were based on homonuclear decoupling experiments and homo- and heteronuclear correlation. Onbead FT-ATR-IR measurements were performed on a Bruker Vector 22 containing a Harrick split-pea ATR unit. HPLC-MS analytics were performed on a Waters 2795 Alliance HT HPLC system equipped with a RP-18 column (GromSil 80, ODS-7 pH, 4 μm , 40x2 mm) connected to a Waters Micromass ZQ quadrupole mass spectrometer.

Solid phase synthesis of benzimidazol-2-yl-alkylamines - exemplary procedures (Scheme S1):

Multistep syntheses of the compound collections were carried out by methods of parallel solid phase organic synthesis starting from *p*-nitrophenyl carbonate Wang resin **4**.¹ In the presence of NaH as a base, **4** was reacted with substituted nitroanilines **5a-c** to the corresponding 2-nitrophenylcarbamates **6**.² Alkylation of the carbamate with appropriate halides using lithium *tert*-butoxide as a base yielded alkyl-(2-nitrophenyl)carbamate Wang resin **7** in quantitative yields. The nitro group was then reduced employing a 1M solution of stannous chloride dihydrate in dry DMF to afford polymer bound carbamoyl anilines **8**. Further acylation of the weakly nucleophilic aniline **8** could be achieved most efficiently by PyBrOP mediated activation of *N*-Fmoc- α -amino acids **9** in the presence of DIEA in NMP.³ The complete conversion of the anilines was determined colorimetrically by treatment of the resin **10** with the sensitive chloranil test.⁴ Acidic cleavage (25 % TFA in DCM) and evaporation of the reagents provided *N*-Fmoc- α -amino acid anilides **11** in excellent yields. The *N*- α -protected benzimidazole-2-yl-alkylcarbamates **12** were obtained by refluxing the precursor molecules in

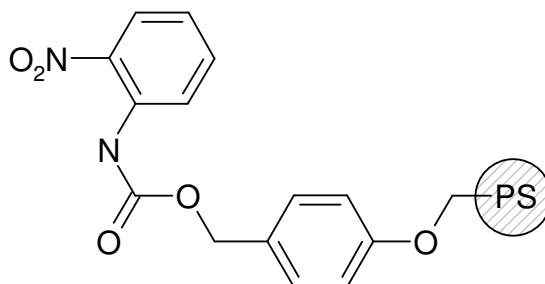
glacial acetic acid for 16 h.⁵ Deprotection of the primary amino group (5 % piperidine/DCM) afforded the required stereochemically pure (*S*)-2-aminoalkyl benzimidazoles **13-15**.



Scheme S1.^a

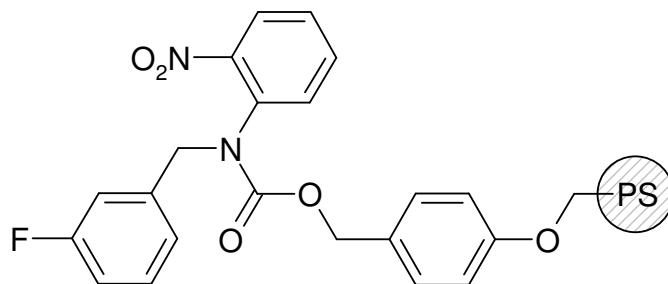
^aReagents and conditions: (a) 3 eq NaH, DMF, 2.5 eq nitroaniline **5a-c**, 12 h; (b) 5 eq alkyl bromide **A-E**, 8 eq LiOtBu, THF/DMSO (1:1, v/v), 12 h; (c) 1M SnCl₂ in DMF, 12 h; (d) 2.5 eq Fmoc-amino acid **9a-f**, 5 eq DIEA, 2.5 eq PyBrOP, NMP, 12 h; (e) 25% TFA/DCM, 1 h; (f) HOAc, refl., 12 h; (g) 5% piperidine, DCM, 2 h.

2-Nitrophenylcarbamate Wang resin (**6**)



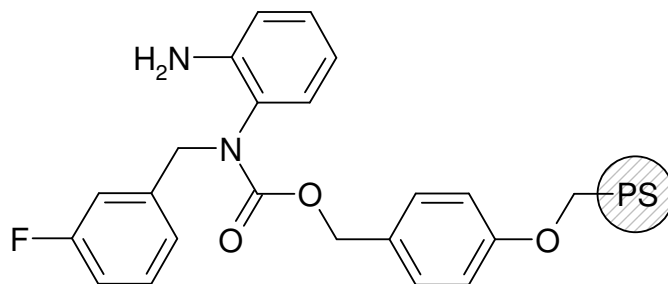
o-Nitroaniline (552 mg, 4.0 mmol) in dry DMF (3 ml) was added to a suspension of p-nitrophenyl carbonate Wang resin **4** (2.0 g, 0.8 mmol⁻¹) in dry DMF (20 ml). Sodium hydride (193 mg of a 60 % dispersion in mineral oil, 4.8 mmol) was carefully added and the suspension was agitated for 12 h. The resin **6** was collected by filtration, washed with DMF, MeOH, DCM and Et₂O and dried under high vacuum. FT-ATR-IR: ν [cm⁻¹] = 3053, 3022, 2918, 2849, 1740, 1610, 1583, 1504, 1425, 1338, 1234, 1196, 1036, 820, 743, 698.

3-Fluorobenzyl(2-nitrophenyl)carbamate Wang resin (7)



3-Fluorobenzylbromide (981 μl , 8.0 mmol) was added to a suspension of 2-nitrophenylcarbamate Wang resin **6** (2.0 g, 0.8 mmol g^{-1}) in a mixture of dry THF and DMSO (30 ml, 1:1 v/v). Lithium *tert.*-butoxide (1.025 g, 12.8 mmol) was added and the suspension was agitated for 12 h. The resin was collected by filtration, washed with DMF, MeOH, DCM and Et₂O and dried under high vacuum. After cleavage of the intermediate compound from a small amount (5 mg) of the resin **7** (25 % TFA/DCM) the HPLC-MS (214 nm) indicated a clean and quantitative alkylation.

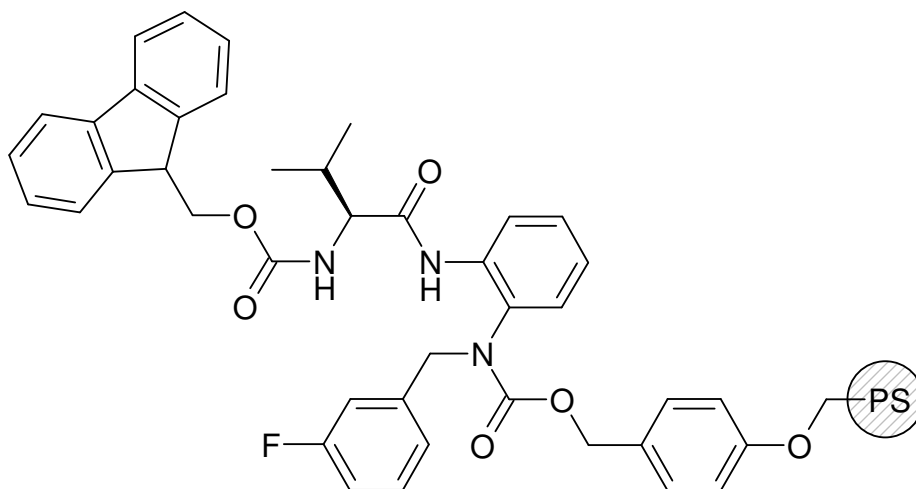
3-Fluorobenzyl(2-aminophenyl)carbamate Wang resin (**8**)



3-Fluorobenzyl(2-nitrophenyl)carbamate Wang resin **7** (2.15 g, 0.73 mmol g⁻¹) was added to a 1M solution of stannous chloride dihydrate in dry DMF (30 ml) and the suspension was agitated for 12 h. The resin was collected by filtration, washed with DMF, MeOH, DCM and Et₂O and dried under high vacuum. After cleavage of the intermediate compound from a small amount (5 mg) of the resin **8** (25 % TFA/DCM) the HPLC-MS (214 nm) indicated a clean and quantitative reduction.

FT-ATR-IR: ν [cm⁻¹] = 3580 (b), 3057, 3024, 2920, 2852, 1699, 1614, 1508, 1491, 1450, 1396, 1292, 1242, 1173, 1146, 1113, 1016, 822, 746, 698.

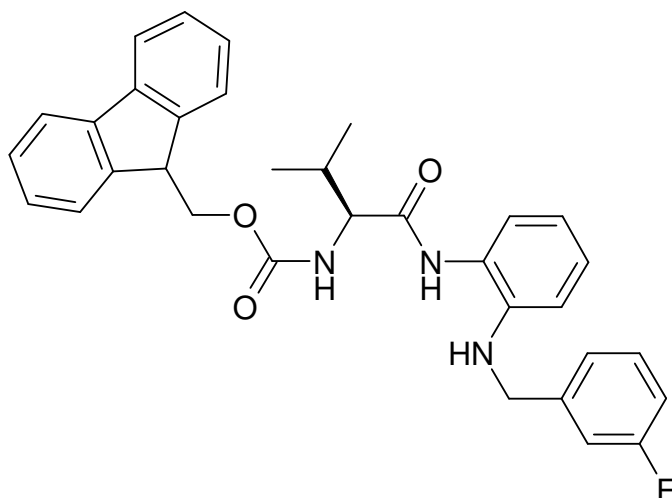
3-Fluorobenzyl[2-(Fmoc-L-valylamino)phenyl]carbamate Wang resin (**10**)



Fmoc-L-valine (1.27 g, 3.75 mmol), N,N'-diisopropylethylamine (1.31 ml, 7.5 mmol) and bromo-tris-pyrrolidino phosphonium hexafluorophosphate PyBrOP (1.75 g, 3.75 mmol) were dissolved in dry N-methyl pyrrolidone (10 ml) and added to a suspension of 3-fluorobenzyl(2-aminophenyl)carbamate Wang resin **8** (2.00 g, 0.75 mmol^g⁻¹) in dry N-methyl pyrrolidone (15 ml). The suspension was agitated for 12 h. The resin was collected by filtration, washed with DMF, MeOH, DCM and Et₂O and dried under high vacuum. Upon treatment with the chloranil test reagents the resin **10** (in contrast to a non-acylated control sample) did not show the typical blue-stained resin beads indicating the complete conversion of the aniline.

FT-ATR-IR: ν [cm⁻¹] = 3057, 3024, 2922, 2847, 1689, 1614, 1541, 1506, 1491, 1452, 1396, 1292, 1223, 1175, 1111, 1018, 820, 743, 698.

***N*-{2-[(3-Fluorobenzyl)amino]phenyl}-*N*-Fmoc-L-valinamide (**11**)**

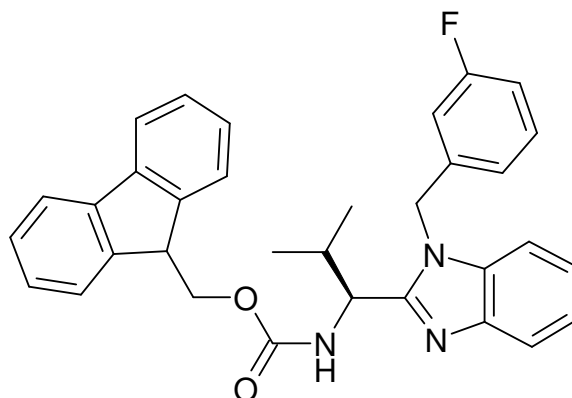


3-Fluorobenzyl[2-(Fmoc-L-valylamino)phenyl]carbamate Wang resin **10** (2.35 g, 0.6 mmol g⁻¹), was suspended in a mixture of 25 % trifluoroacetic acid in DCM (25 ml). The suspension was agitated for 1 h and then filtered off. For complete product release, the resin was washed with DCM and methanol (5 ml). The filtrates were collected, solvents were removed by evaporation and the crude product **11** was lyophilized from *tert*.-butylalcohol/water (4:1 v/v) to yield a pale yellow solid.

Yield: 698 mg (92 %), C₃₃H₃₂FN₃O₃, M = 537.64 g/mol.

Purity: 95 % (HPLC-ESI-MS, 214 nm), [M+H]⁺ = 538 m/z.

(S)-2-[1-(N-Fmoc-amino)isobutyl]-1-(3-fluorobenzyl)benzimidazole (12)



N-{2-[(3-Fluorobenzyl)amino]phenyl}-N-Fmoc-L-valinamide **11** (506 mg, 0.94 mmol) was dissolved in glacial acetic acid (15 ml) and refluxed for 12 h at 80 °C. Solvents were removed by evaporation and the crude product was lyophilized from *tert.*-butylalcohol/water (4:1 v/v) to yield a dark oil. For analytics, the product **12** was purified by flash chromatography (DCM/MeOH) to yield a colorless solid.

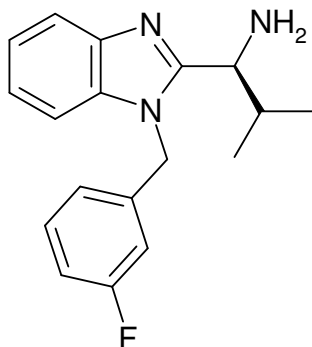
Yield: 337 mg (69 %), C₃₃H₃₀FN₃O₂, M = 519.62 g/mol.

Purity: 94 % (HPLC-ESI-MS, 214 nm), [M+H]⁺ = 520 m/z.

¹H-NMR (400 MHz, CDCl₃): δ = 7.79 (d, 1H, Ar-*H*, ³J = 7.3 Hz), 7.72 (d, 2H, Ar-*H*, ³J = 7.3 Hz), 7.56-7.49 (m, 2H, Ar-*H*), 7.39-7.17 (m, 8H, Ar-*H*), 6.98-6.80 (m, 3H, Ar-*H*), 5.58 (m, 2H, Ar-CH₂), 4.74 (dd, 1H, CH-NH, ³J = 9.4 Hz), 4.37-4.19 (m, 2H, O-CH₂), 4.10 (m, 1H, CH-CH₂O), 2.42 (m, 1H, CH(CH₃)₂), 1.04 (d, 3H, CH(CH₃), ³J = 6.6 Hz), 0.67 (d, 3H, CH(CH₃), ³J = 6.6 Hz).

¹³C-NMR (100 MHz, CDCl₃): δ = 164.3, 156.4, 154.9, 143.7, 141.2, 138.4, 138.3, 134.5, 130.6, 130.5, 127.6, 127.0, 125.1, 123.5, 123.1, 122.2, 119.9, 119.2, 115.2, 115.0, 113.8, 113.6, 110.2, 67.1, 53.2, 47.0, 46.7, 32.7, 19.6, 18.7.

(S)-2-(1-Aminoisobutyl)-1-(3-fluorobenzyl)benzimidazole (15Ea)



(S)-2-[1-(*N*-Fmoc-amino)isobutyl]-1-(3-fluorobenzyl)benzimidazole **12** (330 mg, 0.64 mmol) was dissolved in DCM (10 ml). Piperidine (500 μ l) was added and the reaction mixture was stirred for 2 h at r.t. Solvents were removed by evaporation and the crude product was purified by flash chromatography (DCM/MeOH) to yield the pure compound **15Ea** as viscous oil.

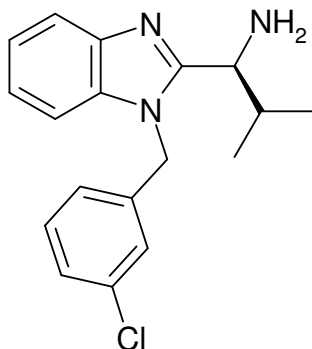
Yield: 141 mg (74 %), C₁₈H₂₀FN₃, M = 297.38 g/mol.

Purity: 99 % (HPLC-ESI-MS, 214 nm), [M+H]⁺ = 298.7 m/z.

¹H-NMR (400 MHz, CDCl₃): δ = 7.87 (d, 1H, Ar-*H*, ³J = 7.6 Hz), 7.61-7.49 (m, 3H, Ar-*H*), 7.35-7.29 (m, 1H, Ar-*H*), 7.05-7.01 (m, 2H, Ar-*H*), 6.89 (d, 1H, Ar-*H*, ³J = 7.6 Hz), 5.70 (dd, 2H, Ar-CH₂, ²J = 17.0 Hz), 4.74 (d, 1H, CH-NH₂, ³J = 8.7 Hz), 2.59 (m, 1H, CH(CH₃)₂), 1.16 (d, 3H, CH(CH₃), ³J = 6.1 Hz), 0.49 (d, 3H, CH(CH₃), ³J = 6.3 Hz).

¹³C-NMR (100 MHz, CDCl₃): δ = 164.3, 161.9, 152.4, 148.8, 135.6, 135.5, 132.3, 132.3, 131.3, 127.4, 127.1, 127.0, 122.8, 116.5, 116.4, 116.2, 114.2, 114.0, 112.3, 48.6, 32.3, 18.8, 18.6.

(S)-2-(1-Aminoisobutyl)-1-(3-chlorobenzyl)benzimidazole (15Da)



(S)-2-(1-aminoisobutyl)-1-(3-chlorobenzyl)benzimidazole **15Da** was prepared analogously as compound **15Ea**.

Yield: 121.8 mg (89 %), C₁₈H₂₀ClN₃, M = 313.83 g/mol.

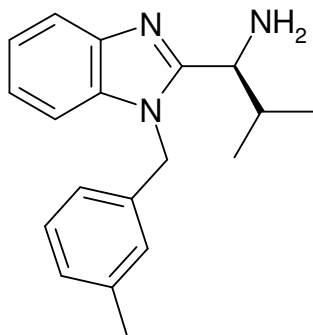
Purity: 98 % (HPLC-ESI-MS, 214 nm), [M+H]⁺ = 314.6 m/z.

¹H-NMR (400 MHz, CDCl₃): δ = 7.88 (d, 1H, Ar-H, ³J = 7.6 Hz), 7.62-7.49 (m, 3H, Ar-H), 7.33-7.26 (m, 2H, Ar-H), 7.18-7.13 (m, 2H, Ar-H), 5.66 (dd, 2H, Ar-CH₂, ²J = 17.0 Hz), 4.75 (d, 1H, CH-NH₂, ³J = 8.7 Hz), 2.60 (m, 1H, CH(CH₃)₂), 1.17 (d, 3H, CH(CH₃), ³J = 6.1 Hz), 0.49 (d, 3H, CH(CH₃), ³J = 6.3 Hz).

¹³C-NMR (100 MHz, CDCl₃): δ = 167.9, 148.7, 135.5, 135.1, 132.1, 130.8, 129.5, 127.4, 127.1, 125.3, 124.7, 116.3, 112.4, 70.8, 51.7, 48.7, 32.3, 18.9, 18.7.

The integrity of the stereocenter was confirmed by derivatization of **1a** = **15Da** and **1b** to the respective Mosher's amides with (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) and determination of the optical purities by NMR-spectroscopy of the resulting diastereomers indicating optical purities of **2a** and **2b** > 98.0 %.⁶

(S)-2-(1-Aminoisobutyl)-1-(3-methylbenzyl)benzimidazole (15Ca)



(S)-2-(1-aminoisobutyl)-1-(3-methylbenzyl)benzimidazole was prepared analogously as compound **15Ea**.

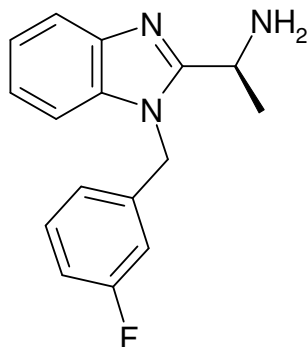
Yield: 95.7 mg (81 %), C₁₉H₂₃N₃, M = 293.42 g/mol.

Purity: 95 % (HPLC-ESI-MS, 214 nm), [M+H]⁺ = 294.7 m/z.

¹H-NMR (400 MHz, CDCl₃): δ = 7.77 (d, 1H, Ar-*H*, ³J = 7.8 Hz), 7.27-7.14 (m, 4H, Ar-*H*), 7.06 (d, 1H, Ar-*H*, ³J = 7.4 Hz), 6.88 (s, 1H, Ar-*H*), 6.81 (d, 1H, Ar-*H*, ³J = 7.6 Hz), 5.39 (s, 2H, Ar-CH₂), 3.80 (d, 1H, CH-NH₂, ³J = 7.1 Hz), 2.27 (s, 3H, Ar-CH₃), 2.15 (m, 1H, CH(CH₃)₂), 1.01 (d, 3H, CH(CH₃), ³J = 6.9 Hz), 0.81 (d, 3H, CH(CH₃), ³J = 6.6 Hz).

¹³C-NMR (100 MHz, CDCl₃): δ = 157.8, 142.3, 138.7, 136.1, 135.4, 128.9, 128.6, 126.8, 123.1, 122.5, 122.1, 119.5, 109.9, 54.8, 46.9, 33.9, 21.4, 20.2, 17.9.

(S)-2-(1-Aminoethyl)-1-(3-fluorobenzyl)benzimidazole (15Ef)



(S)-2-(1-aminoethyl)-1-(3-fluorobenzyl)benzimidazole was prepared analogously as compound **15Ea**.

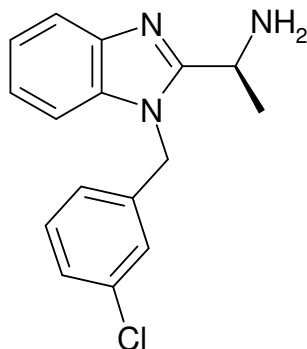
Yield: 141 mg (74 %), C₁₆H₁₆FN₃, M = 269.32 g/mol.

Purity: 99 % (HPLC-ESI-MS, 214 nm), [M+H]⁺ = 270.6 m/z.

¹H-NMR (400 MHz, CDCl₃): δ = 7.83 (d, 1H, Ar-*H*, ³J = 7.9 Hz), 7.34-7.23 (m, 4H, Ar-*H*), 7.01 (m, 1H, Ar-*H*), 6.88 (d, 1H, Ar-*H*, ³J = 7.6 Hz), 6.80 (d, 1H, Ar-*H*, ³J = 9.2 Hz), 5.52 (dd, 2H, Ar-CH₂, ²J = 17.0 Hz), 4.30 (q, 1H, CH-NH₂, ³J = 6.6 Hz), 1.59 (d, 3H, CH(CH₃), ³J = 6.6 Hz).

¹³C-NMR (100 MHz, CDCl₃): δ = 164.4, 161.9, 158.3, 142.2, 138.8, 138.7, 135.5, 130.7, 130.6, 122.9, 122.4, 121.7, 121.6, 119.7, 115.0, 114.8, 113.3, 113.1, 109.6, 46.4, 44.7, 23.6.

(S)-2-(1-Aminoethyl)-1-(3-chlorobenzyl)benzimidazole (15Df)



(S)-2-(1-aminoethyl)-1-(3-chlorobenzyl)benzimidazole was prepared analogously as compound **15Ea**.

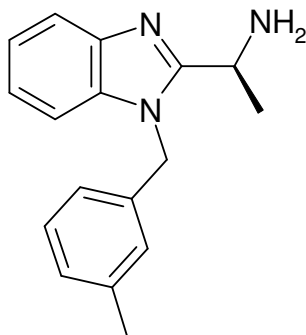
Yield: 77.6 mg (82 %), C₁₆H₁₆ClN₃, M = 285.76 g/mol.

Purity: 98 % (HPLC-ESI-MS, 214 nm), [M+H]⁺ = 286.6 m/z.

¹H-NMR (400 MHz, CDCl₃): δ = 7.96 (d, 1H, Ar-*H*, ³J = 7.6 Hz), 7.45-7.34 (m, 6H, Ar-*H*), 7.05 (d, 2H, Ar-*H*, ³J = 7.6 Hz), 5.61 (dd, 2H, Ar-CH₂, ²J = 17.0 Hz), 4.46 (q, 1H, CH-NH₂, ³J = 6.6 Hz), 1.71 (d, 3H, CH(CH₃), ³J = 6.6 Hz).

¹³C-NMR (100 MHz, CDCl₃): δ = 157.9, 142.1, 138.2, 135.4, 135.0, 130.3, 128.2, 126.3, 124.2, 122.9, 122.4, 119.7, 109.6, 46.4, 44.6, 23.5.

(S)-2-(1-Aminoethyl)-1-(3-methylbenzyl)benzimidazole (15Cf)



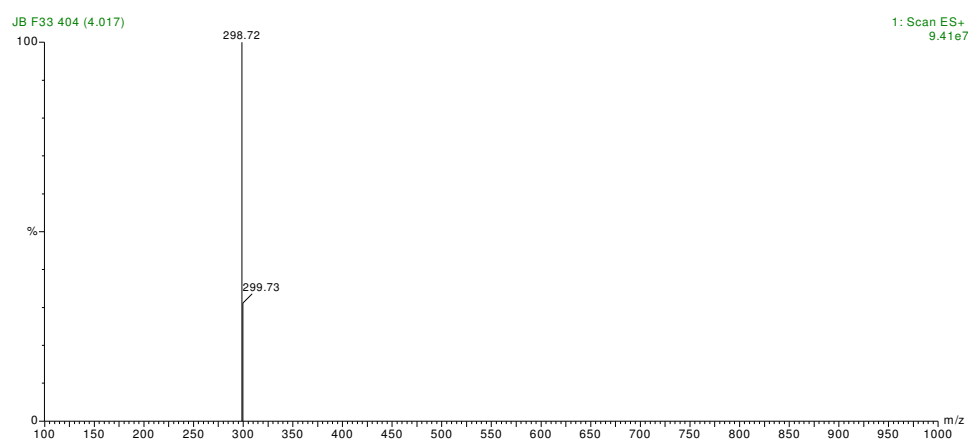
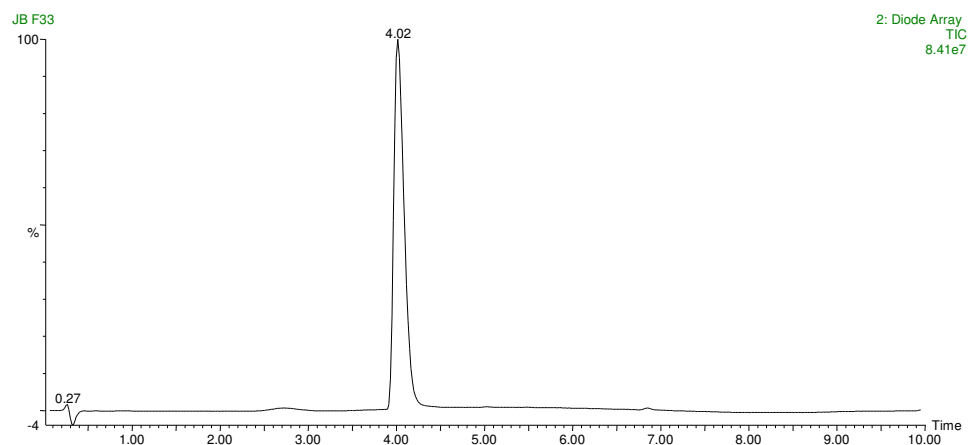
(S)-2-(1-aminoethyl)-1-(3-methylbenzyl)benzimidazole was prepared analogously as compound **15Ea**.

Yield: 95.7 mg (81 %), C₁₇H₁₉N₃, M = 265.36 g/mol.

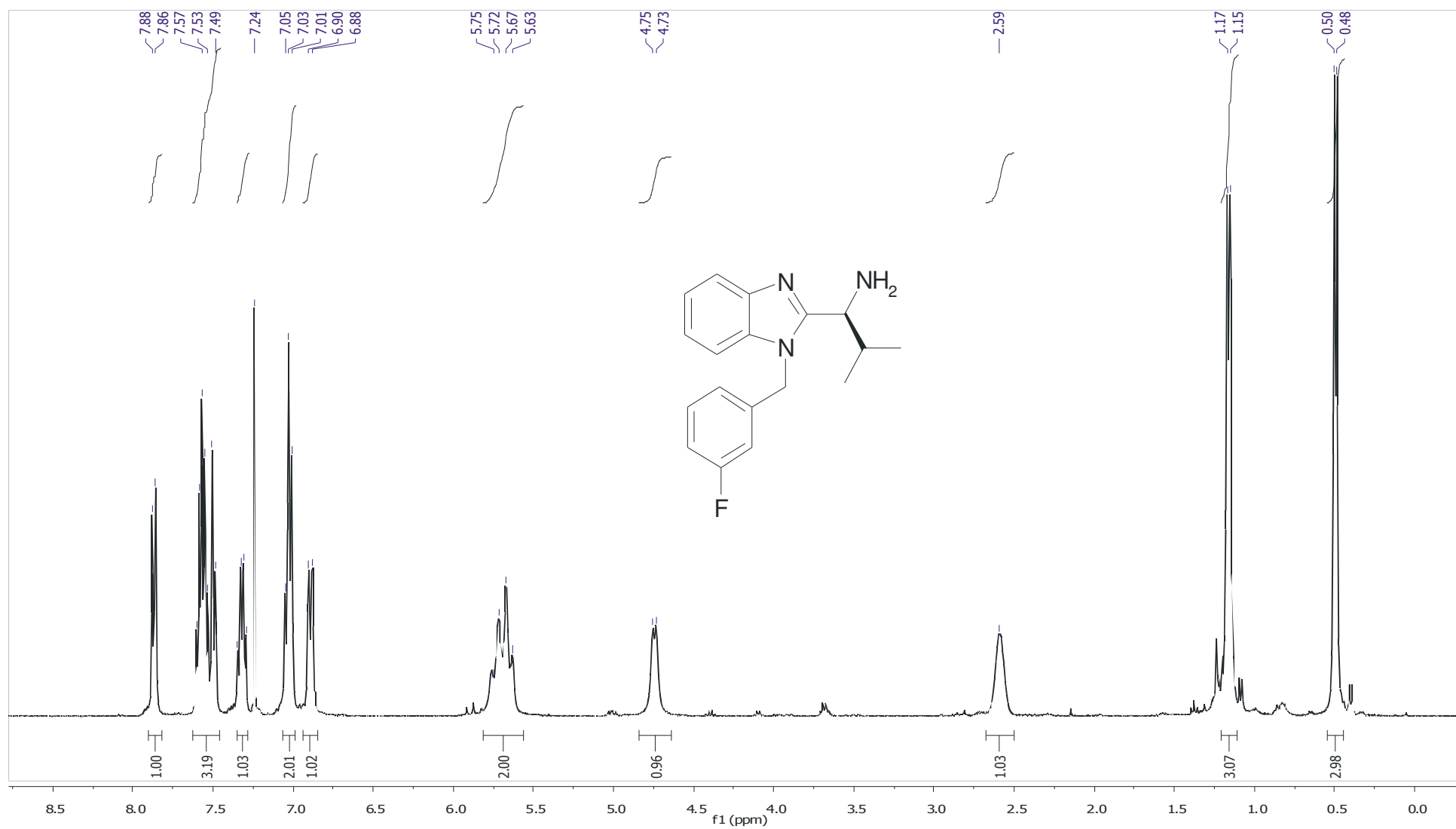
Purity: 95 % (HPLC-ESI-MS, 214 nm), [M+H]⁺ = 266.7 m/z.

¹H-NMR (400 MHz, CDCl₃): δ = 7.76 (d, 1H, Ar-H, ³J = 7.6 Hz), 7.24-7.13 (m, 4H, Ar-H), 7.06 (d, 1H, Ar-H, ³J = 7.4 Hz), 6.87 (s, 1H, Ar-H), 6.78 (d, 1H, Ar-H, ³J = 7.6 Hz), 5.37 (s, 2H, Ar-CH₂), 4.55 (d, 1H, CH-NH₂, ³J = 6.6 Hz), 2.25 (s, 3H, Ar-CH₃), 1.51 (d, 3H, CH(CH₃), ³J = 6.6 Hz).

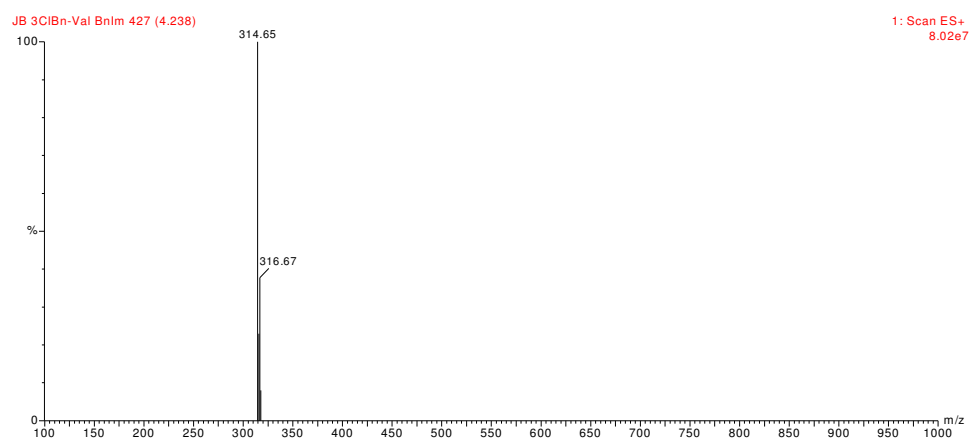
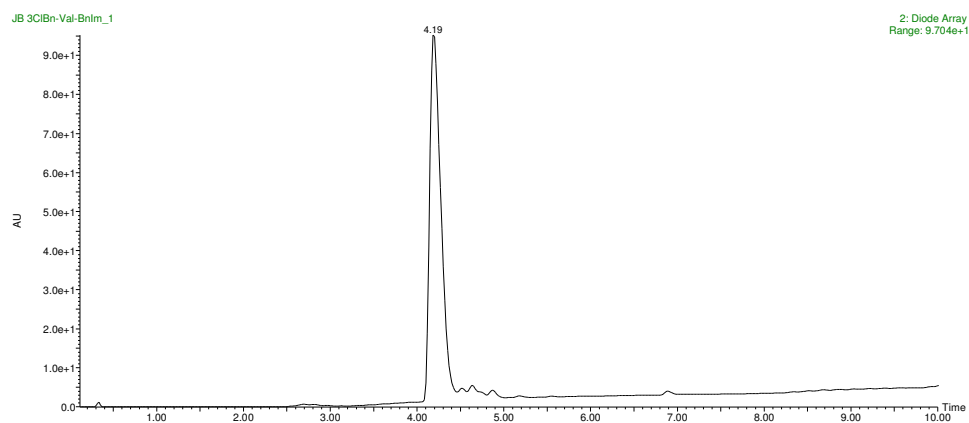
¹³C-NMR (100 MHz, CDCl₃): δ = 163.0, 149.0, 139.5, 133.4, 133.1, 132.7, 129.9, 129.3, 127.8, 126.7, 124.0, 116.4, 112.3, 51.5, 49.0, 21.2, 18.7.



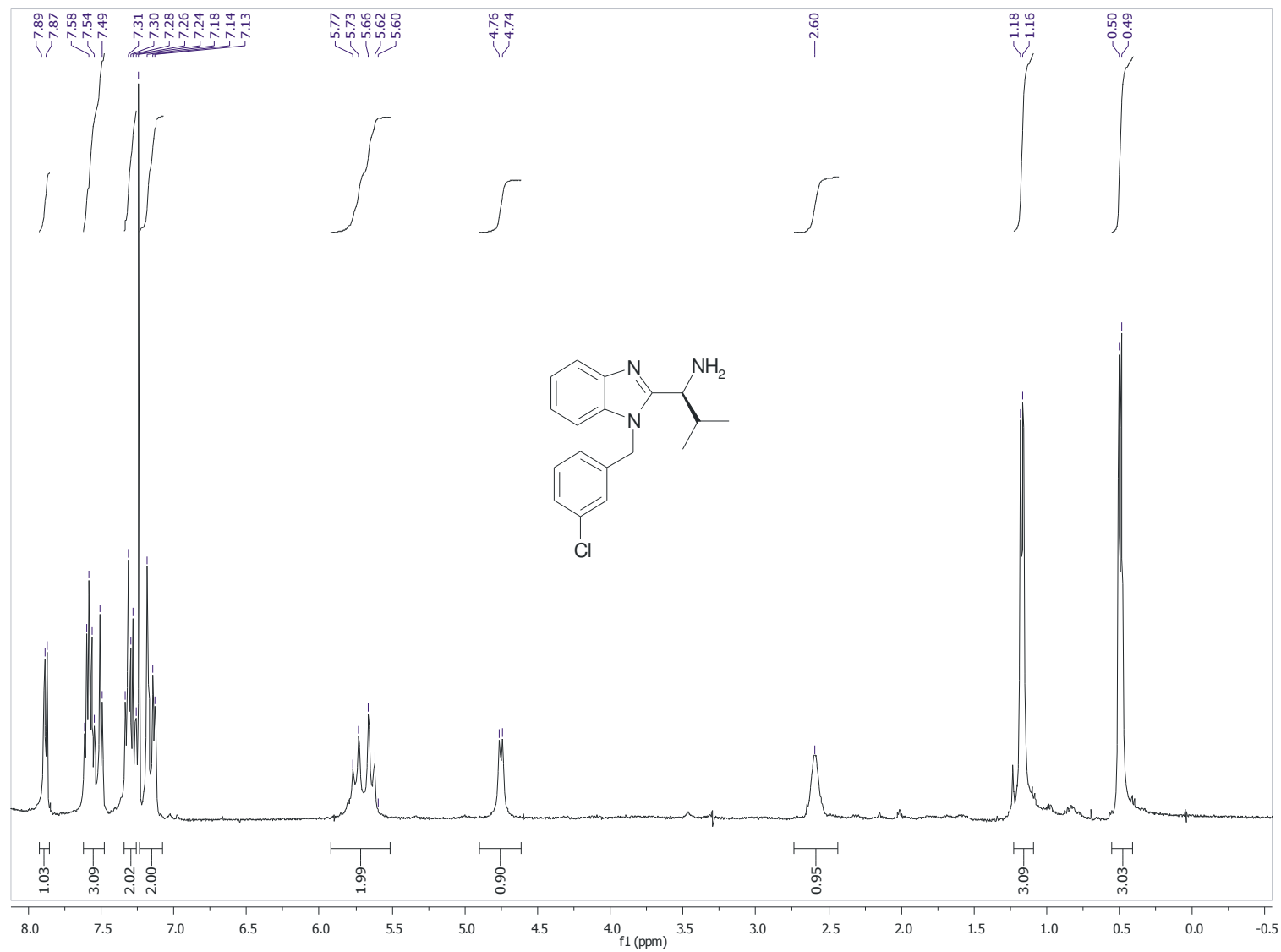
Scheme S2. HPLC-MS analysis (214 nm, 10 V) - chromatogram and corresponding ESI-MS spectrum of compound **15Ea**.



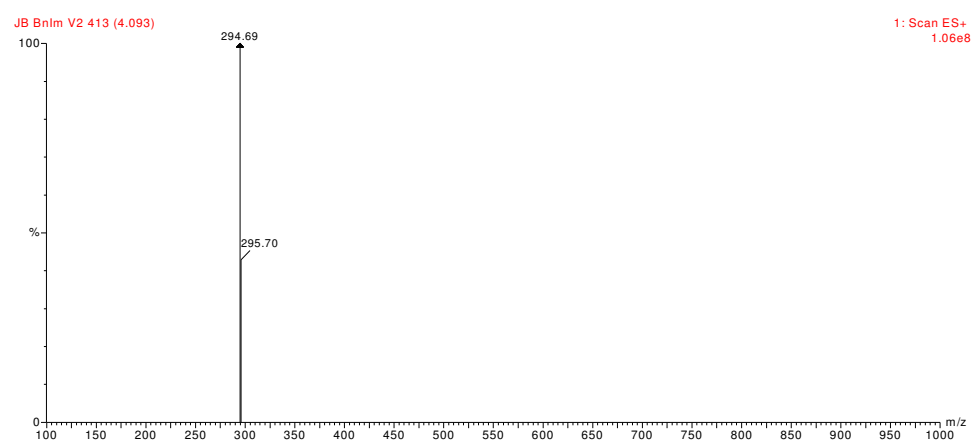
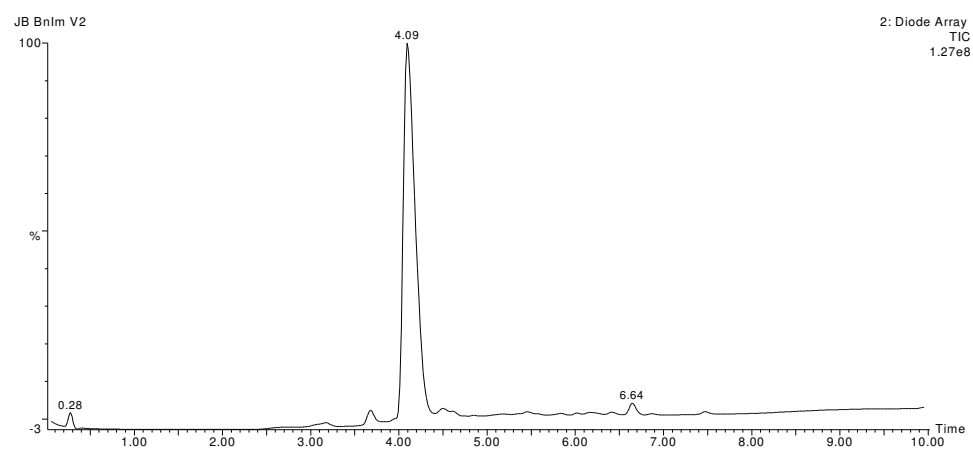
Scheme S3. ¹H-NMR spectrum (400 MHz) of compound **15Ea**.



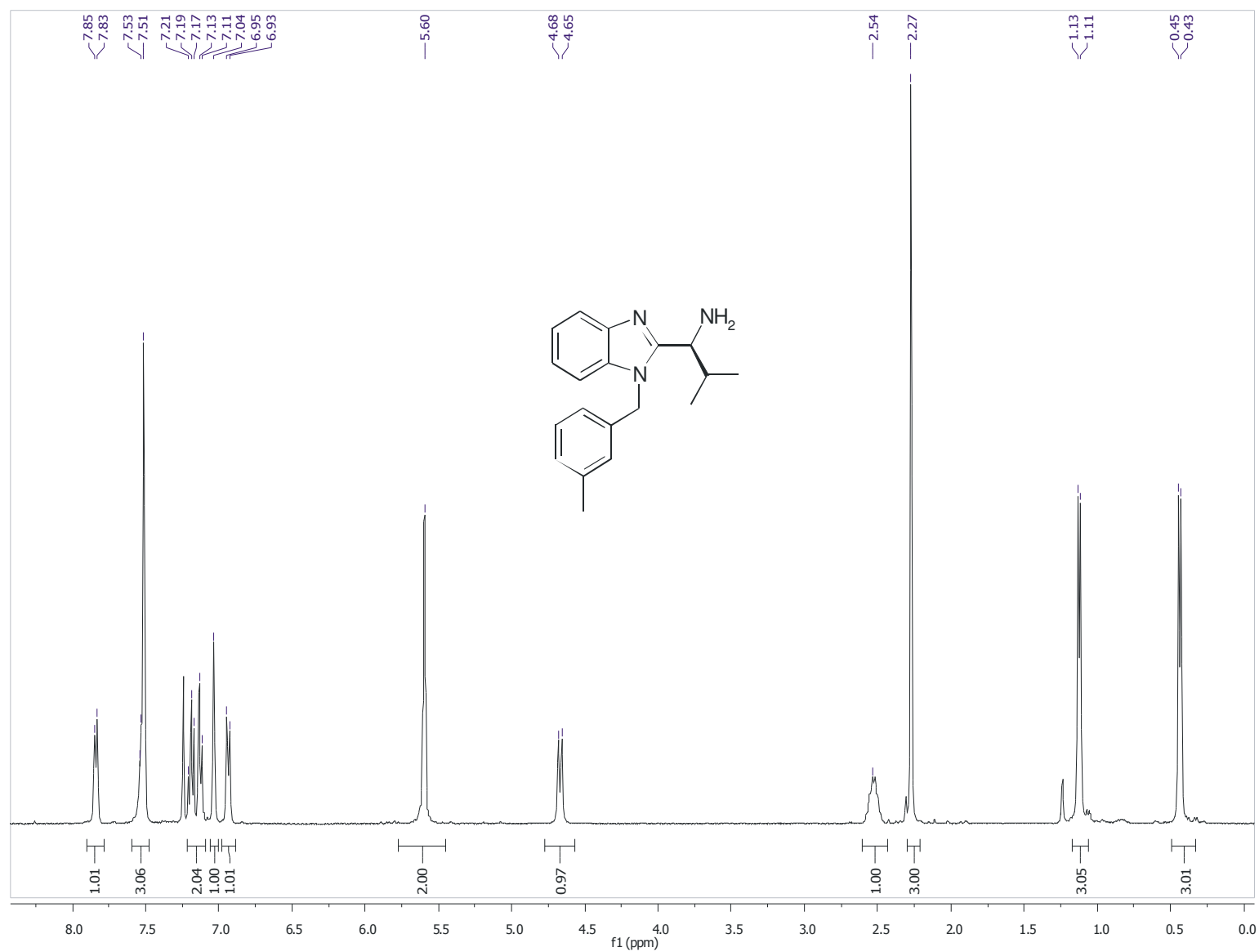
Scheme S4. HPLC-MS analysis (214 nm, 10 V) - chromatogram and corresponding ESI-MS spectrum of compound **15Da**.



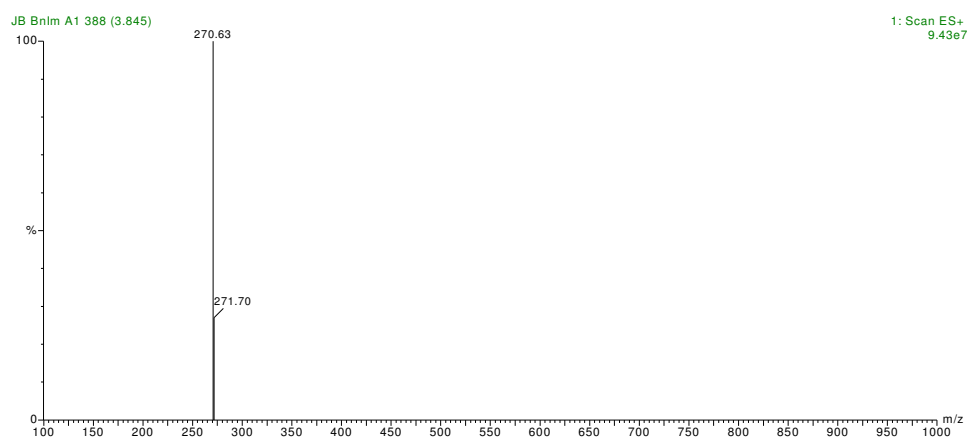
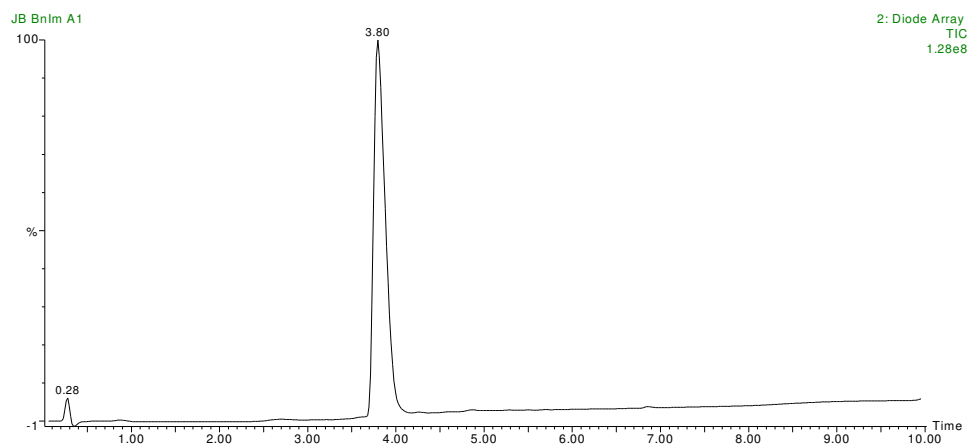
Scheme S5. ¹H-NMR spectrum (400 MHz) of compound **15Da**.



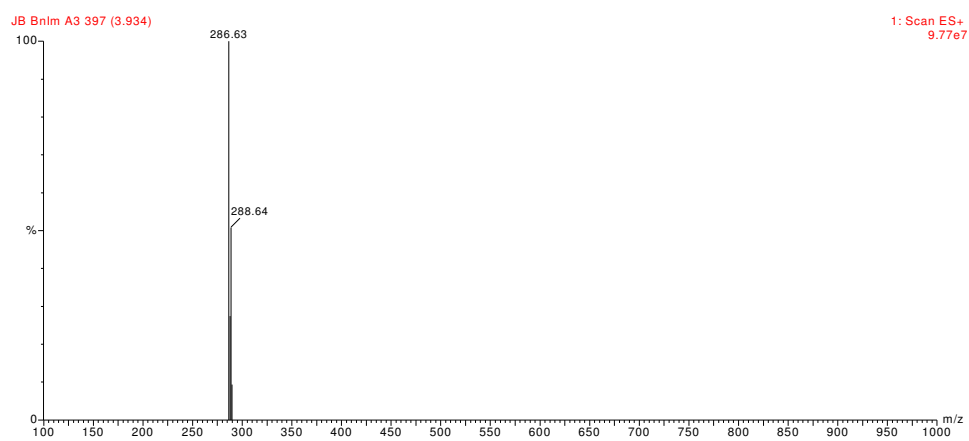
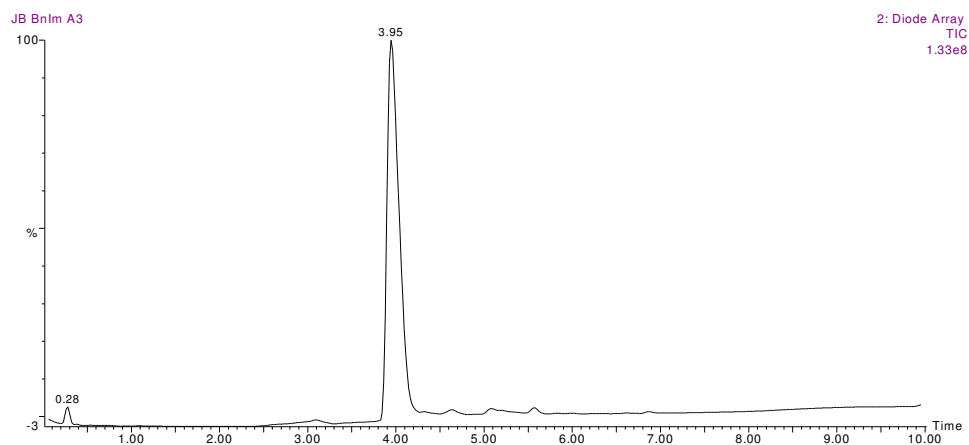
Scheme S6. HPLC-MS analysis (214 nm, 10 V) - chromatogram and corresponding ESI-MS spectrum of compound **15Ca**.



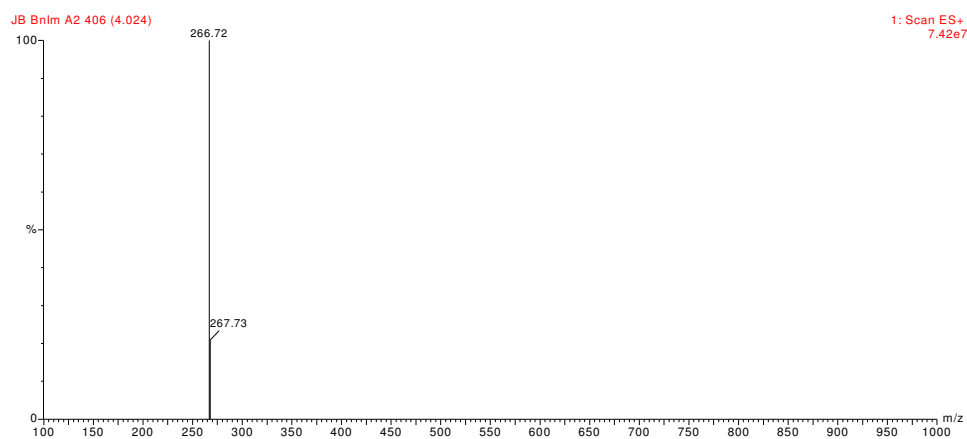
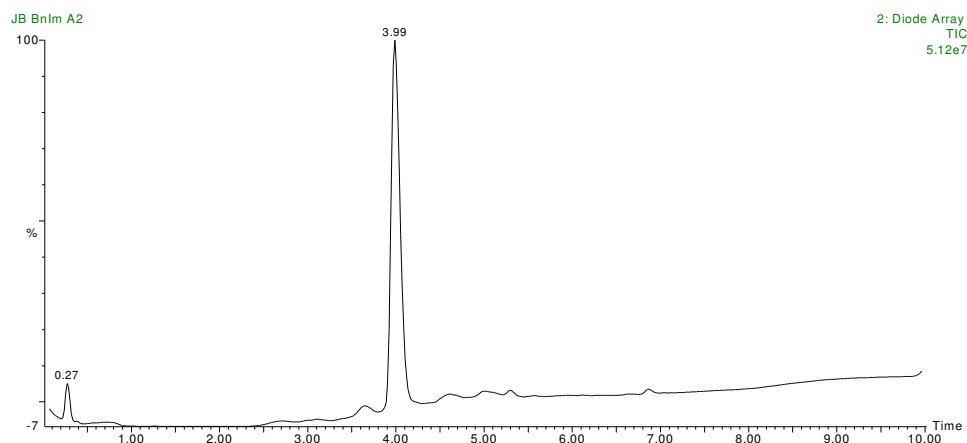
Scheme S7. ¹H-NMR spectrum (400 MHz) of compound **15Ca**.



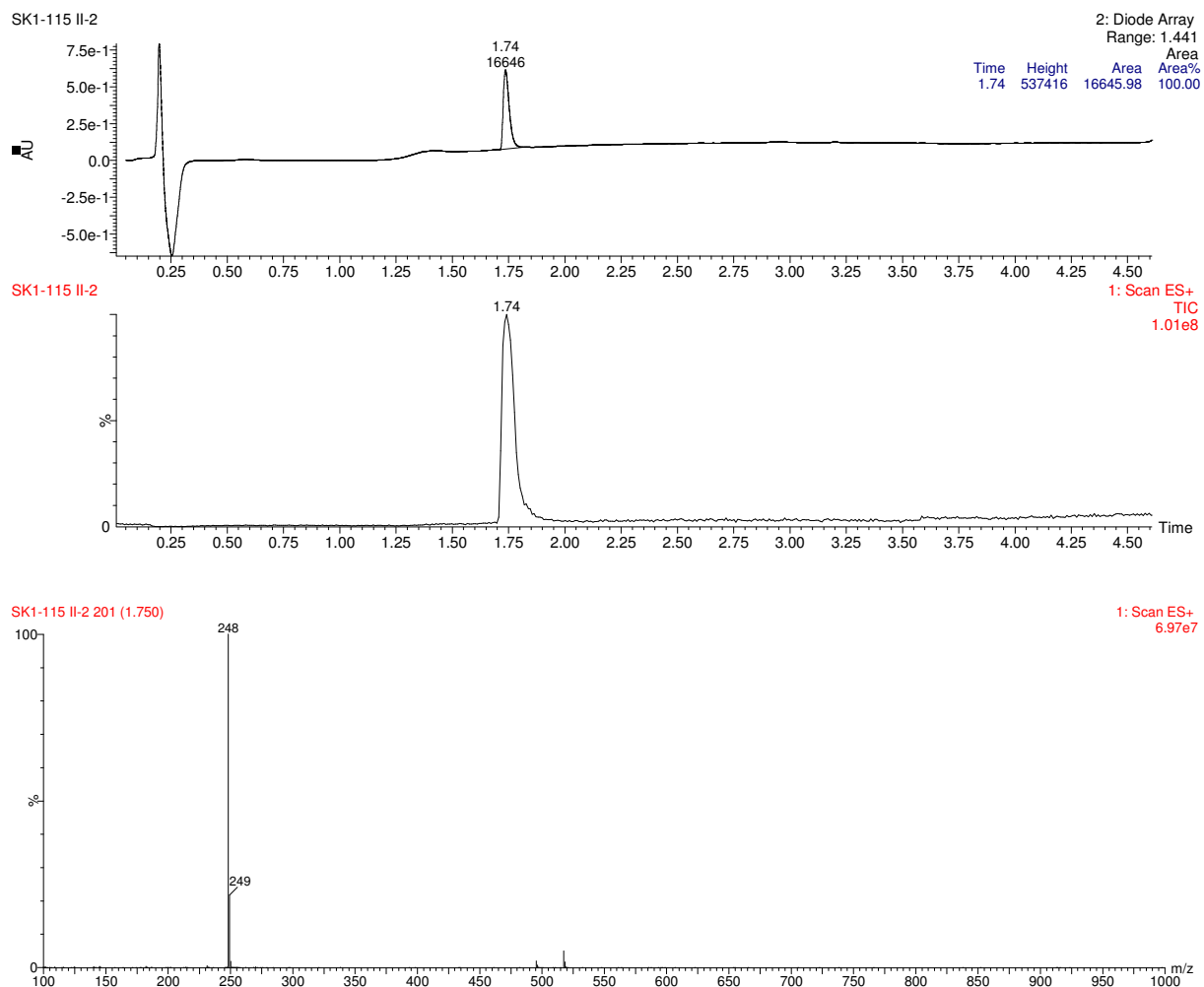
Scheme S8. HPLC-MS analysis (214 nm, 10 V) - chromatogram and corresponding ESI-MS spectrum of compound **15Ef**.



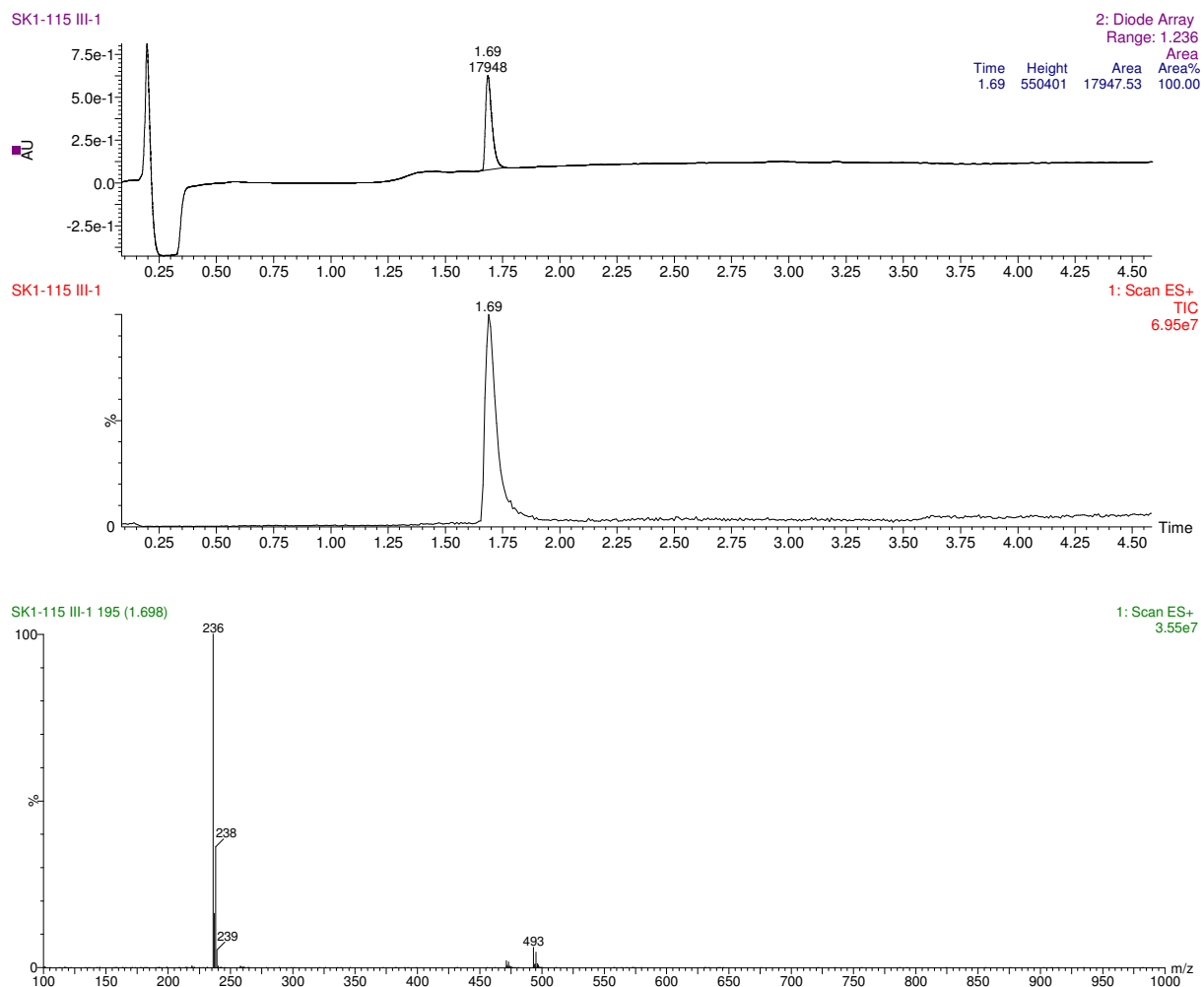
Scheme S9. HPLC-MS analysis (214 nm, 10 V) - chromatogram and corresponding ESI-MS spectrum of compound **15Df**.



Scheme S10. HPLC-MS analysis (214 nm, 10 V) - chromatogram and corresponding ESI-MS spectrum of compound **15Cf**.



Scheme S11. HPLC-MS analysis (214 nm, 10 V) – chromatogram, total ion current and corresponding ESI-MS spectrum of compound **22Ea**.

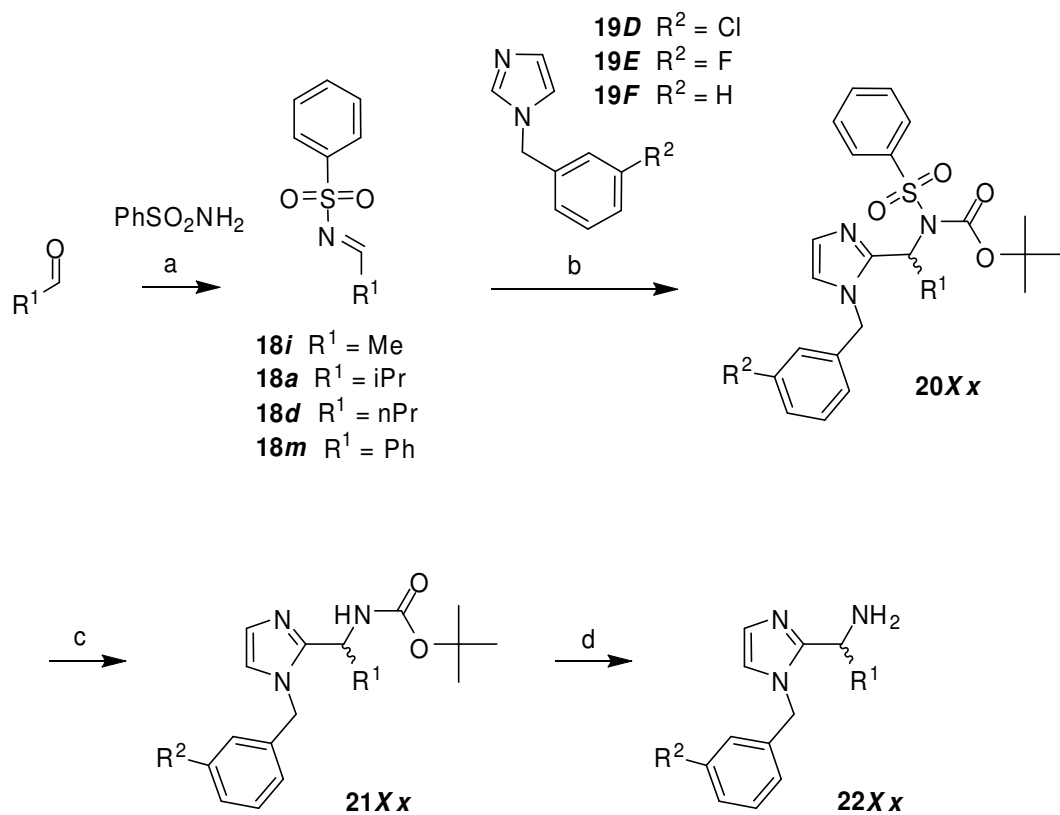


Scheme S12. HPLC-MS analysis (214 nm, 10 V) – chromatogram, total ion current and corresponding ESI-MS spectrum of compound **22Df**.

Synthesis of 2-(1-aminoalkyl)-1-benzyl imidazoles - exemplary procedures (Scheme S13):

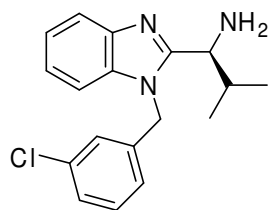
No obvious interaction sites are present in the modified part of the 2-aminoalkyl benzimidazole series, yet the smaller 2-(1-aminoalkyl)-1-benzyl imidazole scaffold carries an aromatic ring as hydrophobic contact with lipophilicity comparable to the related benzimidazoles. The parallel synthesis of a selection of such scaffolds (Scheme S13) started from *N*-sulfonyl aldimines **18x** which were available from the respective aldehydes by condensation of aldehydes and benzenesulfonamide in formic acid.⁷ The *N*-alkylidene- and arylidene-benzenesulfonamides **18i**, **18a**, **18d**, **18m** were then reacted with *N*-benzylimidazoles **19D-F** to yield the *N*-Boc-*N*-sulfonamide protected 1-(benzyl-imidazol-2-yl)-2-alkyl(phenylsulfonyl)carbamates **20Xx**.⁸ In this step, the presence of di-*tert.*-butyl dicarbonate (Boc₂O) is crucial for the generation of a 1,3-imidazolium ylide intermediate which was found to readily undergo addition to *N*-sulfonyl imines under mild heating.⁷ The racemic sulfonylcarbamates **20Xx** were then deprotected by treatment with magnesium under ultrasound sonication to yield imidazole-2-yl-alkylcarbamates **21Xx**.⁹ After TFA-mediated cleavage of the carbamate the 2-aminoalkyl imidazoles **22Xx** were isolated in acceptable yields and excellent purities.

Thus, the collection of 2-aminoalkyl imidazoles **22Xx** is designed to be concordant with the corresponding (*S*)-2-aminoalkyl benzimidazoles **15Xx** allowing the direct comparison of antifungal activities (Figure S1). Together with the activity data from the 5-CF₃ substitution (**13**) and 5,6-diCl substitution (**14**) of the benzimidazole core **15** this result indicates that the activity is based on the completely unsubstituted benzimidazole moiety as the pharmacophore. Benzoanellation is a structural prerequisite for the antifungal activity of the class of (*S*)-2-aminoalkyl benzimidazoles **15Xx**.



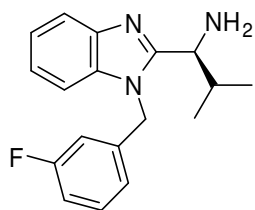
Scheme S13.^a

^aReagents and conditions: (a) 1 eq benzenesulfonamide, 1.1 eq sodium benzenesulfinate, HCHO/H₂O (1:1, v/v), 12 h; (b) 1 eq **19**, 2 eq Boc₂O, DCE, 1 h 60 °C, 12 h r.t.; (c) ~3 eq Mg⁽⁰⁾, HCl_(g), MeOH, ultrasonic sound, 2 h; (d) 25% TFA/DCM, 6 h.



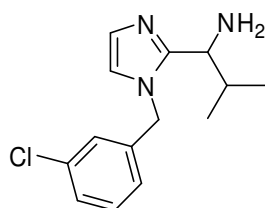
15Da

$IC_{50} = 0.75 \mu M$ (*C. albicans*)



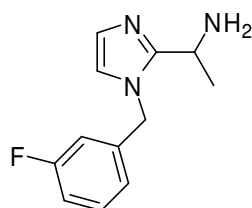
15Ea

$IC_{50} = 2.2 \mu M$ (*C. albicans*)



22Da

inactive

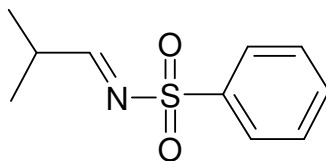


22Ei

inactive

Figure S1. Comparison of selected active (*S*)-2-aminoalkyl benzimidazoles **15Xx** and corresponding inactive 2-aminoalkyl imidazoles **22Xx**.

***N*-Isobutyridene-benzenesulfonamide (18a)**

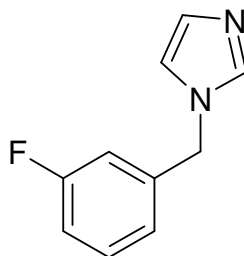


A solution of benzenesulfonamide (0.66 mmol, 104 mg), sodium benzenesulfinate (0.73 mmol, 120 mg) and isobutyric aldehyde (0.66 mmol, 60 μ l) in formic acid (1 ml) and water (1 ml) was stirred for 12 h at r.t. The reaction mixture was extracted with DCM (3 x 10 ml). The combined organic layers were washed with saturated aqueous NaHCO₃ solution, dried over sodium sulfate and the solvents were removed under reduced pressure to yield the desired sulfonylimine as a colorless solid which was used without further purification.

Yield: 105 mg (75 %), C₁₀H₁₃NO₂S, M = 211.3 g/mol.

Purity: 82 % (HPLC-ESI-MS, 214 nm), [M+H]⁺ = 212.3 m/z.

1-(3-Fluorobenzyl)-1H-imidazole (19E)

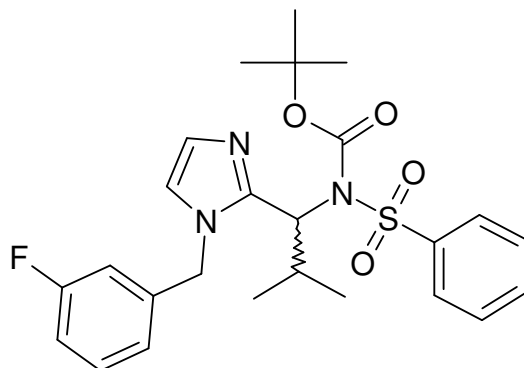


Imidazole (4.4 mmol, 300 mg) and sodium hydride (8.8 mmol, 211 mg) were dissolved in abs. THF (15 ml). 3-Fluorobenzyl bromide (4.4 mmol, 540 μ l) was slowly added and the mixture was stirred for 16 h. The reaction was quenched by slow addition of isopropyl alcohol and water and the aqueous phase was extracted with Et₂O (3 x 50 ml). The combined organic layers were washed with brine, dried over sodium sulfate and the solvent was removed completely under reduced pressure to yield a light brownish oil which was used without further purification.

Yield: 735 mg (95 %), C₁₀H₉FN₂, M = 176.2 g/mol.

Purity: 97 % (HPLC-ESI-MS, 214 nm), [M+H]⁺ = 177.2 m/z.

2-[1-(*N*-Boc-*N*-phenylsulfonyl)aminoisobutyl]-1-(3-fluorobenzyl)imidazole (20Ea**)**

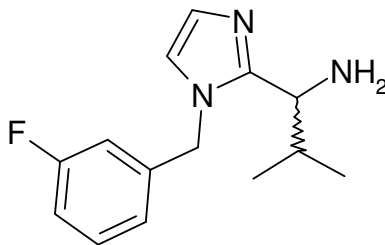


A solution of 1-(3-Fluoro-benzyl)-1H-imidazole **19E** (223 mg, 1.27 mmol), *N*-isobutylidene-benzenesulfonamide **18a** (1.27 mmol, 267 mg) and di-*tert*-butyl dicarbonate (2.53 mmol, 542 μ l) in dichloroethane (3 ml) was heated to 60°C for 1 h and allowed to stir at r.t. for 12 h. After completion of the reaction, the reaction mixture was carefully quenched by addition of water (20 ml) and the mixture was extracted three times with DCM (3 x 30 ml). The combined organic layers were dried over sodium sulfate and the solvents were removed under reduced pressure. The crude product was purified by gradient elution (hexane/diethylether) to yield a colorless solid.

Yield: 224 mg (36 %), C₂₅H₃₀FN₃O₄S, M = 487.6 g/mol.

Purity: 84% (HPLC-ESI-MS, 214 nm), [M+H]⁺ = 488.7 m/z.

2-(1-Aminoisobutyl)-1-(3-fluorobenzyl)imidazole (**22Ea**)



To a solution of 2-[1-(*N*-Boc-*N*-phenylsulfonyl)aminoisobutyl]-1-(3-fluorobenzyl)imidazole **20Ea** (181 mg, 0.37 mmol) in waterfree MeOH (5 ml) magnesium turnings (100 mg) were added. Gaseous hydrochloric acid (6 ml) was bubbled through the reaction mixture for the activation of Mg until the formation of hydrogen visibly occurred. The mixture was then treated with ultrasonic sound until all Mg was dissolved. Aqueous HCl (0.5 M, 10 ml) was added and the suspension was then extracted with DCM (3 x 50 ml). The combined organic layers were filtered through a pad of celite and the filtrate was concentrated *in vacuo* to yield the Boc-intermediate **21Ea** as a white solid. The crude Boc-protected amine **21Ea** was dissolved in DCM (3 ml), trifluoroacetic acid (1 ml) was added slowly and the mixture was stirred for 6 h at r.t. Upon completion of the reaction the solvents were removed under reduced pressure and the crude product was purified by flash chromatography (DCM/MeOH) to yield the pure compound **22Ea**.

Yield: 34 mg (37 %), C₁₄H₁₈FN₃, M = 247.3 g/mol.

Purity: 99 % (HPLC-ESI-MS, 214 nm), [M+H]⁺ = 248 m/z.

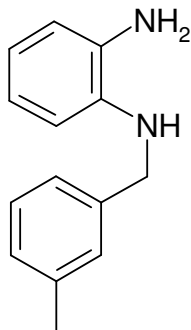
¹H-NMR (400 MHz, CDCl₃): δ = 7.59-7.48 (m, 3H, Ar-*H*), 7.32-7.29 (m, 1H, Ar-*H*), 7.13 (s, 1H, Ar-*H*), 6.85 (s, 1H, Ar-*H*), 5.25 (dd, 2H, Ar-CH₂, ²J = 15.6 Hz), 4.49 (d, 1H, CH-NH₂, ³J = 8.1 Hz), 2.33 (m, 1H, CH(CH₃)₂), 1.05 (d, 3H, CH(CH₃), ³J = 6.3 Hz), 0.63 (d, 3H, CH(CH₃), ³J = 6.3 Hz).

¹³C-NMR (100 MHz, CDCl₃): = 165.0, 162.4, 143.9, 138.3, 138.1, 130.6, 130.5, 124.3, 122.8, 118.1, 115.0, 114.7, 113.3, 113.0, 57.6, 51.9, 37.3, 19.5, 19.1.

Solution phase synthesis of benzimidazol-2-yl-alkylamines

- exemplary procedures:

N-(3-Methylbenzyl)benzene-1,2-diamine



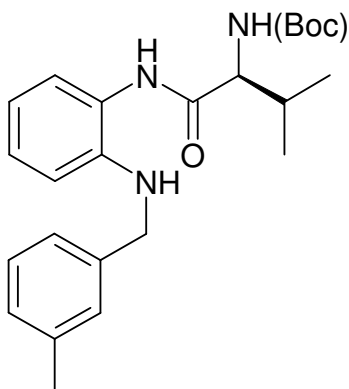
A solution of 1-fluoro-2-nitrobenzene (2.11 ml, 20 mmol) in 25 ml dry THF was added to a solution of 3-methylbenzylamine (2.50 ml, 20 mmol) in dry THF. After adding triethylamine (5.57 ml, 40 mmol) the reaction mixture was refluxed for 12 h. The cooled reaction mixture was evaporated and the crude product (HPLC: 82 %, 214 nm) was reduced without further purification. *N*-(3-Methylbenzyl)-2-nitroaniline was dissolved in 100 ml ethanol/water (1:1, v/v) and sodium dithionite (13.9 g, 80 mmol) was added. After stirring for 6 h at 70 °C the reaction mixture was extracted with dichloromethane (3 x 100 ml). The combined organic layers were washed with brine and water (each 100 ml), dried over sodium sulfate and concentrated *in vacuo*. The crude *N*-(3-methylbenzyl)-o-phenyldiamine was purified by column chromatography (ethyl acetate/hexane).

Yield: 3.10 g (73 %, for two steps), C₁₄H₁₆N₂, M = 212.3 g/mol.

TLC: R_f = 0.2 (DCM).

Purity (HPLC-MS, 214 nm): 99%.

***N*-{2-[(3-Methylbenzyl)amino]phenyl}-*N*-Boc-L-valinamide**

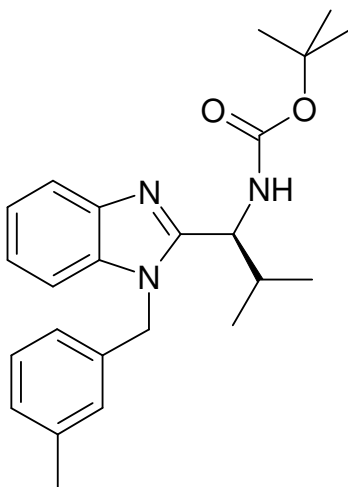


A solution of *N*-(3-methylbenzyl)-*o*-phenyldiamine (318 mg, 1.5 mmol) in 20 ml abs. DCM was prepared and *N*- α -Boc-L-valine (391 mg, 1.8 mmol), *N*-(3-dimethyl-aminopropyl)-*N*-ethylcarbodiimide hydrochloride (573 mg, 3 mmol) and a catalytic amount of 4-dimethylaminopyridine (10 mg) were added and stirred for 12 h at room temperature. The reaction mixture was washed with water (3 x 50 ml), dried over anhydrous sodium sulfate and evaporated to yield a pale yellow solid.

Yield: 570 mg (92.7 %), C₁₄H₁₆N₂, M = 411.6 g/mol.

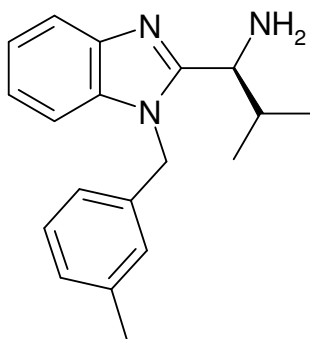
Purity (HPLC-MS, 214 nm): 98%.

(S)-2-[1-(*N*-Boc-amino)isobutyl]-1-(3-methylbenzyl)benzimidazole



Cyclization of *N*-{2-[(3-methylbenzyl)amino]phenyl}-*N*-Boc-L-valinamide was prepared analogously as described above in the solid-phase procedure.

(S)-2-(1-Aminoisobutyl)-1-(3-methylbenzyl)benzimidazole (15Ca)



The crude (S)-2-[1-(*N*-Boc-amino)isobutyl]-1-(3-methylbenzyl)benzimidazole (535 mg, 1.36 mmol) was suspended in 15 ml TFA/Wasser (2:1, v/v) and stirred at room temperature for 3 h. The reaction was quenched carefully with cold dilute sodium hydroxide (ca. pH 7.5) and extracted with DCM (3 x 100 ml). The combined organic layers were washed with brine (3 x 100 ml), dried over anhydrous sodium sulfate and evaporated to yield a pale brownish solid that could be further purified by crystallization from aqueous ethanol.

Yield: 379 mg (94.8 %), C₁₉H₂₃N₃, M = 293.42 g/mol.

Purity of the crude: (HPLC-MS, 214 nm): 81 %.

Purity after crystallization: (HPLC-MS, 214 nm): 97 %.

Screening assay. The screening assay¹⁰ was adapted for HTS as follows: 10,000 HeLa cells (human cervix carcinoma cells [ATCC, Cat. No. CCL-2]) were dispensed (50 μ l / 200 000/ml) per well of a 96 well plate in the presence or absence of 20 μ M compound (100 μ l 40 μ M), infected with *Candida albicans* (Sc5314) (CFU 5-500 (optimum 50, 50 μ l 1000 CFU/ml)) and incubated at 37 °C 5 % CO₂ in a total volume of 200 μ l media (RPMI 1640, 10 % FCS, 1 % glutamine) for 5 days . The IC₅₀ and CC₅₀ values were determined by analyzing the relative number and vitality of surviving human cell lines as measured by metabolization of fluorescein diacetate (FDA). Amphotericin B (Sigma-Aldrich A4888) was used as reference. A survival rate of higher than 40 % as measured by fluorescence intensity against a pathogen-only control was considered a hit and processed further. 100% equals the average fluorescent intensity of all control wells containing cells only.

Determination of MICs and comparison to commonly applied antimycotica. For the determination of MIC we applied the standard protocol EDef 7.1 *Antifungal Susceptibility Testing of Yeasts* according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) reference method.¹¹ We applied a test set for the determination of an average MIC value for each compound consisting of 16 clinical isolates of *C. albicans*, *C. glabrata* and *C. parapsilosis*. Furthermore, we used *C. albicans* DSMZ 11949, *C. glabrata* ATCC 90030, *C. parapsilosis* 22019, *C. parapsilosis* 90018 and *Issatchenkia orientalis* ATCC 6258 as reference control strains. Prior to the test, strains were grown at 25 °C for 24 h on solid sabouraud (Sab) plates supplemented with 40 μ g/ml gentamycin. Five individual colonies were suspended in sterile water, and the optical density of the suspension was adjusted to 0.5 McFarland. Working suspension was prepared by a 1 in 10 dilution of standardized suspension in sterile distilled water to yield $1-5 \times 10^5$ CFU/ml. 100 μ l of the final suspension were transferred into each of a 96 well microtiter plate preloaded with 100 μ l of a two-fold concentrated liquid RPMI medium containing the compound to be tested at a two-fold concentration as well. Column 1 containing sterile drug-free medium was inoculated with 100 μ l of cell suspension and used as a growth control for viability of fungal cells. Column 2 to column 12 contained an increasing amount of test compound in a 2-fold

titration scheme resulting in a range of concentration from 0.125 $\mu\text{g/ml}$ to 128 $\mu\text{g/ml}$ after addition of the cell suspension. Fluconazole was used as a control for antifungal activity of the test compounds. Microtiter plates were incubated at 35 °C and the growth of yeast cells was evaluated after 22 ± 2 h by measuring the optical density at 450 nm using a TECAN microtiter plate reader and analysed with Magellan software. MICs were defined as the lowest drug concentration giving rise to an inhibition of growth of more than 50 % of that of the drug free control. The average MIC of a compound for our set of test strains was calculated as the geometric mean of the MIC test results of our fourteen test strains.

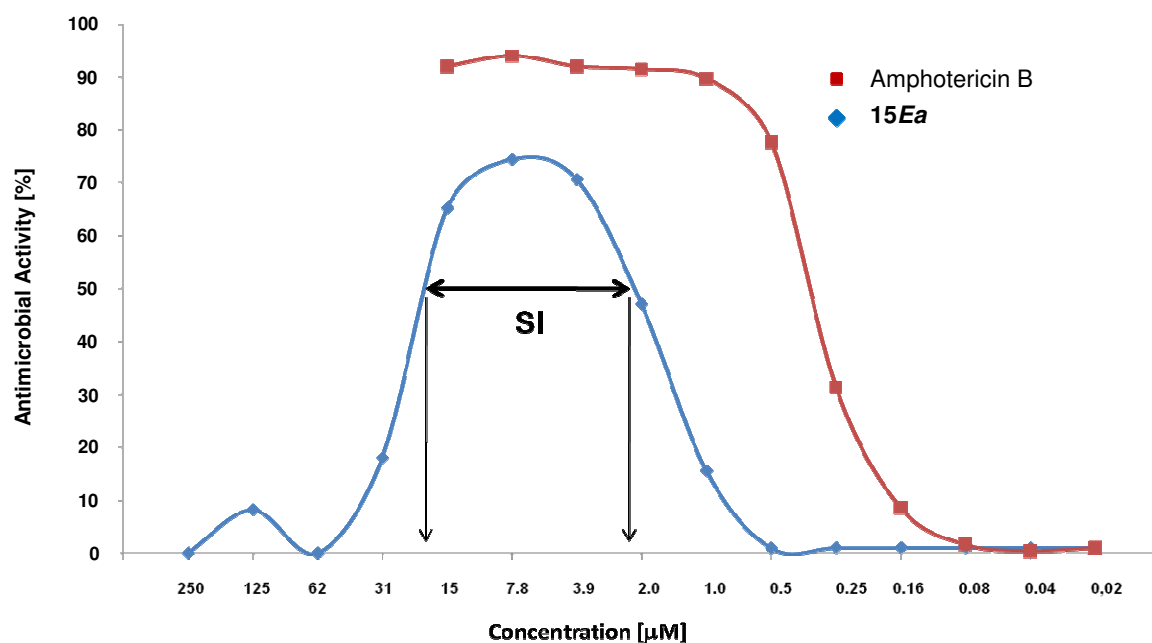
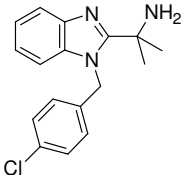
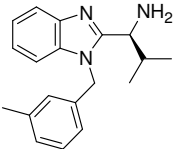
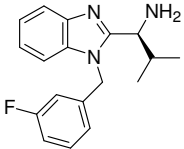
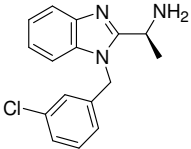


Figure S2. Exemplary determination of a dose response curve of a hit compound, **15Ea**. For **15Ea** $\text{IC}_{50} = 2.2 \mu\text{M}$ and $\text{CC}_{50} = 20.6 \mu\text{M}$ were determined, resulting in $\text{SI} = 9.4$.

Table S1. In vitro antifungal activities (IC₅₀) cytotoxicity (CC₅₀) and resulting selectivities (SI) of selected benzimidazoles **15Xx** against *Candida albicans*.

Compound	Structure	IC ₅₀	CC ₅₀	SI
1a = 15Da		0.75 μM	97.5 μM	130
1b		inactive	--	--
15Ec		30 μM	--	--
15Dc		15 μM	--	--
15Ac		7 μM	--	--
15Ab		4 μM	36 μM	9
15Cd		2 μM	30 μM	15
15Ad		2 μM	28 μM	14

15Ae		5 μ M	50 μ M	10
15Ca		3.3 μ M	10.6 μ M	3.2
15Ea		2.2 μ M	20.6 μ M	9.4
15Df		7.8 μ M	19.3 μ M	2.5

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