Supporting information for:

Photochemical Control of Protein Arginine Deiminase (PAD) Activity

Santanu Mondal[1,2], Sangram S. Parelkar[1,2], Mitesh Nagar[1,2], Paul R. Thompson[1,2*]

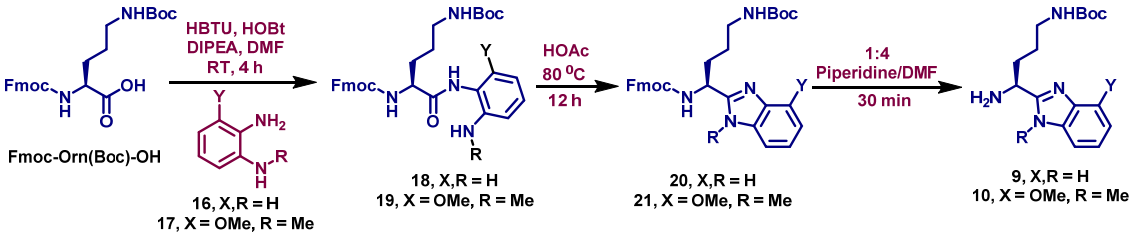
[1]Department of Biochemistry and Molecular Pharmacology, UMass Medical School, 364 Plantation Street, Worcester, MA 01605, USA

[2]Program in Chemical Biology, UMass Medical School, 364 Plantation Street, Worcester, MA, 01605, USA.

Running Title: Photochemical Control of PAD Activity

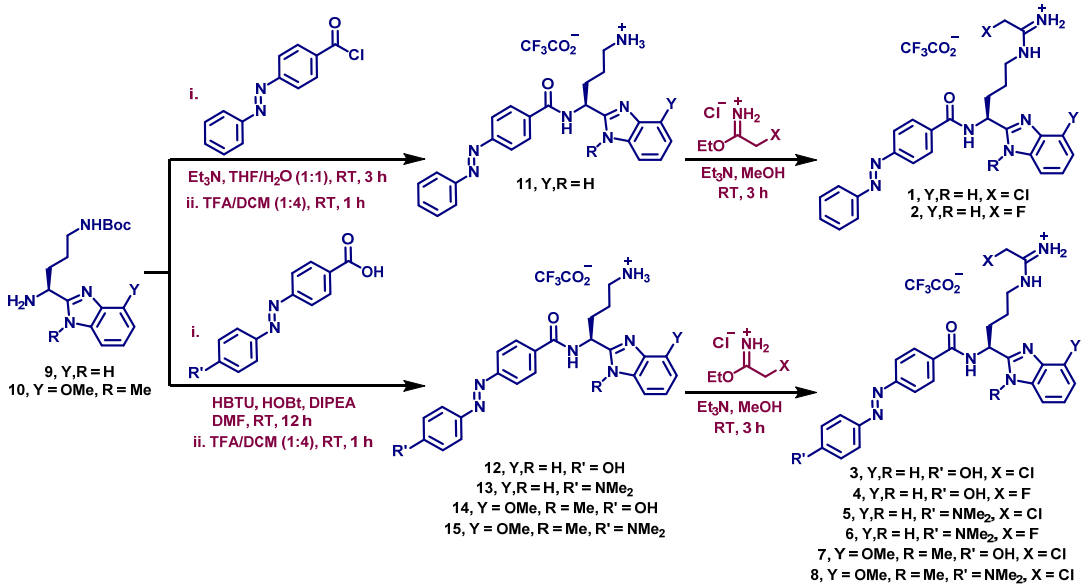
[*]Author to whom correspondence should be addressed: Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, LRB 826, 364 Plantation Street,
Worcester MA 01605 tel: 508-856-8492; fax: 508-856-6215; e-mail: paul.thompson@umassmed.edu.

Synthesis



General Procedure for the synthesis of 9 and 10

Compounds **9** and **10** were synthesized by following the reported procedure with minor modifications.[1,2] Briefly, Fmoc-Orn(Boc)-OH (1 g, 2.2 mmol) and 1,2-phenylenediamine (**16**) (238 mg, 2.2 mmol) or **17** (335 mg, 2.2 mmol) was dissolved in DMF and diisopropyl ethylamine (DIPEA) (1.2 mL, 6.6 mmol), HBTU (1.3 g, 3.3 mmol) and HOBt (297 mg, 2.2 mmol) were added sequentially to the solution. The reaction mixture was stirred for 12 h at 25 ^{0}C under nitrogen atmosphere and was poured into water to precipitate compound **18** or **19**, which was collected by vacuum filtration, washed with water and dried *in vacuo*. Crude compounds **18** or **19** were dissolved in glacial acetic acid (50 mL) and the mixture was refluxed for 12 h followed by cooling to room temperature and pouring into water. Excess acetic acid was neutralized with saturated sodium bicarbonate solution and the mixture was extracted with excess dichloromethane. The dichloromethane extract was then washed extensively with water, brine, dried over anhydrous sodium sulphate and concentrated *in vacuo* to afford compound **20** or **21**. The Fmoc-group in compounds **20** and **21** was removed by treating with 20% piperidine in dimethylformamide (v/v) for 30 min. The reaction mixture was then vigorously stirred with excess hexane. The hexane layer was decanted off and this procedure was repeated three times to afford **9** and **10** as gummy oils. These compounds were used in successive steps without further purification.



Synthesis of 11:

To a solution of **9** (300 mg, 1 mmol) in 1:1 THF/H_2O was added triethylamine (0.4 mL, 3 mmol) and 4-(phenylazo)benzoyl chloride (242 mg, 1 mmol) and the reaction mixture was stirred at room temperature for 3 h. The solution was evaporated *in vacuo* to remove excess THF and the mixture was extracted with dichloromethane twice. The combined organic extracts were washed with water, dried over anhydrous sodium sulphate, concentrated *in vacuo* to yield an orange solid. The crude product resulting from the coupling reaction between **9** and 4-(phenylazo)benzoyl chloride was purified by column chromatography using hexane/ethyl acetate as mobile phase. The orange solid was then dissolved in 1:4 trifluoroacetic acid/dichloromethane and the solution was stirred for 1 h at room temperature after which the residual trifluoroacetic acid/dichloromethane was evaporated *in vacuo* to afford **11** as a gummy red-coloured liquid. Compound **11** was used in successive steps without further purification.

General Procedure for the synthesis of 12-15

To a solution of **9** (1 mmol) or **10** (1 mmol) in DMF was added sodium p-(p-dimethylaminophenylazo)benzoate (1 mmol) or 4'-Hydroxyazobenzene-4-carboxylic Acid (1 mmol), DIPEA (3 mmol), HBTU (2 mmol), HOBt (2 mmol) and the reaction mixture was stirred at room temperature for 15 h. The mixture was poured into excess water and was extracted with excess dichloromethane three times. The combined organic extracts were washed with saturated lithium chloride, water, brine, dried over anhydrous sodium sulphate and concentrated *in vacuo*. The crude product resulting from the coupling reaction between **9** and **10** and the carboxylic acids was purified by column chromatography using hexane/ethyl acetate as the mobile phase. The purified product was then dissolved in 1:4 trifluoroacetic acid/dichloromethane and the solution was stirred for 1 h at room temperature after which the mixture was evaporated *in vacuo* to afford **12-15** as gummy red-coloured liquids. These compounds were used in successive steps without further purification.

General Procedure for the synthesis of 1-8 from 11-15

The trifluoroacetate salt of the amine (1 mmol) was dissolved in anhydrous methanol and was treated with triethylamine (4 mmol) and ethyl-2-choloroacetimidate hydrochloride (2 mmol) (for the chloroacetamidine warheads) or ethyl-2-fluoroacetimidate hydrochloride (2 mmol) (for the fluoroacetamidine warheads). The reaction mixture was stirred at room temperature for 3 h under nitrogen atmosphere. The solution was evaporated *in vacuo* to remove excess triethylamine and the resulting slurry was resuspended in methanol. The crude product was purified by reversed-phase HPLC using a pre-packed C18 column (Agilent, 21.2 × 250 mm, 10 μm) and water/acetonitrile gradient supplemented with 0.05% trifluoroacetic acid.

All the compounds used in this study were characterized by ^{1}H and ^{13}C NMR spectroscopy and ESI-Mass spectrometric techniques. These data are provided in Figure S11-S26.

Compound 1. ^{1}H NMR (CD_3OD) δ (ppm): 8.05 (d, J = 10 Hz, 2H), 7.93 (d, J = 8.6 Hz, 2H), 7.85-7.87 (m, 2H), 7.64-7.66 (m, 2H), 7.43-7.49 (m, 5H), 5.54-5.57 (m, 1H), 4.28 (s, 1H),

3.33-3.43 (m, 2H), 2.21-2.33 (m, 2H), 1.85-1.91 (m, 1H), 1.75-1.82 (m, 1H); ^{13}C NMR (CD_3OD) δ (ppm): 168.2, 163.4, 161.7, 161.4, 161.1, 160.9, 154.7, 153.7, 152.5, 134.6, 132.1, 131.7, 129.0, 128.6, 125.8, 122.7, 122.4, 113.8, 41.9, 38.7, 29.1, 23.6; ESI-MS (m/z) calculated for $C_{26}H_{27}Cl_1N_7O_1$ $[M + H]^+$: 488.196, found 488.2.

Compound 2. ^{1}H NMR (CD_3OD) δ (ppm): 8.05 (d, *J* = 10 Hz, 2H), 7.93 (d, *J* = 8.6 Hz, 2H), 7.85-7.87 (m, 2H), 7.65-7.67 (m, 2H), 7.44-7.48 (m, 5H), 5.54-5.57 (m, 1H), 5.22 (s, 1H), 5.13 (s, 1H), 3.36-3.45 (m, 2H), 2.22-2.32 (m, 2H), 1.86-1.94 (m, 1H), 1.73-1.82 (m, 1H); ^{13}C NMR (CD_3OD) δ (ppm): 168.2, 163.2, 163.0, 161.5, 161.2, 154.7, 153.8, 152.5, 134.7, 132.3, 129.0, 128.6, 125.6, 122.7, 122.4, 113.8, 78.3, 76.9, 41.4, 29.1, 23.7; ESI-MS (m/z) calculated for $C_{26}H_{27}F_1N_7O_1$ $[M + H]^+$: 472.2256, found 472.2.

Compound 3. ^{1}H NMR (CD_3OD) δ (ppm): 8.01 (d, *J* = 8.6 Hz, 2H), 7.84 (d, *J* = 8.6 Hz, 2H), 7.77 (d, *J* = 8.9 Hz, 2H), 7.65-7.67 (m, 2H), 7.43-7.45 (m, 2H), 6.84 (d, *J* = 9 Hz, 2H), 5.54-5.57 (m, 1H), 4.28 (s, 2H), 3.33-3.43 (m, 2H), 2.21-2.33 (m, 2H), 1.85-1.94 (m, 1H), 1.74-1.82 (m, 1H); ^{13}C NMR (CD_3OD) δ (ppm): 168.3, 163.4, 161.6, 161.5, 161.2, 155.1, 153.8, 146.2, 133.7, 132.5, 128.5, 125.6, 125.1, 122.0, 115.5, 113.8, 42.0, 38.7, 29.1, 23.6; ESI-MS (m/z) calculated for $C_{26}H_{27}Cl_1N_7O_2$ $[M + H]^+$: 504.1909, found 504.2.

Compound 4. ^{1}H NMR (CD_3OD) δ (ppm): 8.01 (d, *J* = 8.6 Hz, 2H), 7.84 (d, *J* = 8.5 Hz, 2H), 7.77 (d, *J* = 8.9 Hz, 2H), 7.65-7.67 (m, 2H), 7.44-7.46 (m, 2H), 6.84 (d, *J* = 8.9 Hz, 2H), 5.54-5.57 (m, 1H), 5.22 (s, 1H), 5.13 (s, 1H), 3.37-3.44 (m, 2H), 2.22-2.31 (m, 2H), 1.87-1.94 (m, 1H), 1.75-1.82 (m, 1H); ^{13}C NMR (CD_3OD) δ (ppm): 168.3, 163.2, 163.0, 161.6, 161.4, 161.1, 155.1, 153.8, 146.2, 133.7, 132.3, 128.5, 125.7, 125.1, 122.0, 115.5, 113.8, 78.3, 76.9, 41.4, 29.1, 23.7; ESI-MS (m/z) calculated for $C_{26}H_{27}F_1N_7O_2$ $[M + H]^+$: 488.2205, found 488.2.

Compound 5. ^{1}H NMR (CD_3OD) δ (ppm): 7.99 (d, *J* = 8.7 Hz, 2H), 7.76-7.79 (m, 4H), 7.67-7.70 (m, 2H), 7.48-7.51 (m, 2H), 6.76-6.79 (m, 2H), 5.55-5.58 (m, 1H), 4.28 (s, 2H), 3.33-3.43 (m, 2H), 3.04 (s, 6H), 2.26-2.30 (m, 2H), 1.86-1.95 (m, 1H), 1.73-1.82 (m, 1H); ^{13}C NMR (CD_3OD) δ (ppm): 168.4, 163.4, 160.9, 160.6, 155.2, 153.8, 153.6, 143.3, 132.5, 131.5, 128.5, 126.1, 125.4, 121.5, 113.7, 111.5, 41.9, 39.1, 38.7, 29.0, 23.6; ESI-MS (m/z) calculated for $C_{28}H_{32}Cl_1N_8O_1$ $[M + H]^+$: 531.2382, found 531.2.

Compound 6. ^{1}H NMR (CD_3OD) δ (ppm): 7.99 (d, *J* = 8.7 Hz, 2H), 7.77-7.79 (m, 4H), 7.68-7.70 (m, 2H), 7.48-7.50 (m, 2H), 6.77 (d, *J* = 9.3 Hz, 2H), 5.55-5.58 (m, 1H), 5.22 (s, 2H), 5.13 (s, 1H), 3.35-3.46 (m, 2H), 3.04 (s, 6H), 2.25-2.30 (m, 2H), 1.86-1.95 (m, 1H), 1.75-1.82 (m, 1H); ^{13}C NMR (CD_3OD) δ (ppm): 168.4, 163.2, 163.0, 160.8, 160.5, 155.2, 153.8, 153.6, 143.3, 132.5, 131.5, 128.5, 126.1, 125.5, 121.5, 113.7, 111.5, 78.3, 76.9, 41.4, 39.1, 28.9, 23.7; ESI-MS (m/z) calculated for $C_{28}H_{32}F_1N_8O_1$ $[M + H]^+$: 515.2678, found 515.4.

Compound 7. ^{1}H NMR (CD_3OD) δ (ppm): 7.95 (d, *J* = 8.6 Hz, 2H), 7.80 (d, *J* = 8.6 Hz, 2H), 7.76 (d, *J* = 8.9 Hz, 2H), 7.38 (t, *J* = 8.2 Hz, 1H), 7.24 (d, *J* = 8.3 Hz, 1H), 6.95 (d, *J* = 8.1 Hz, 1H), 6.82-6.85 (m, 2H), 5.56-5.59 (m, 1H), 4.28 (s, 2H), 3.98 (s, 3H), 3.94 (s, 3H), 3.34-3.38 (m, 2H), 2.18-2.31 (m, 2H), 1.87-1.92 (m, 1H), 1.69-1.77 (m, 1H); ^{13}C NMR (CD_3OD) δ (ppm): 168.1, 163.4, 161.6, 161.4, 161.1, 155.0, 152.1, 148.8, 146.1, 135.1, 133.6, 128.4,

126.4, 125.0, 122.0, 115.5, 105.5, 103.6, 55.2, 46.1, 42.1, 38.7, 30.5, 29.0, 23.6; ESI-MS (m/z) calculated for $C_{28}H_{31}Cl_1N_7O_3$ $[M + H]^+$: 548.2171, found 548.2.

Compound 8. ^{1}H NMR (CD_3OD) δ (ppm): 7.93 (d, *J* = 8.7 Hz, 2H), 7.75-7.77 (m, 4H), 7.43 (t, *J* = 8.3 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 1H), 7.00 (d, *J* = 8.1 Hz, 1H), 6.76 (d, *J* = 9.3 Hz, 2H), 5.56-5.59 (m, 1H), 4.28 (s, 2H), 4.02 (s, 3H), 3.95 (s, 3H), 3.33-3.40 (m, 2H), 3.03 (s, 6H), 2.17-2.34 (m, 2H), 1.87-1.94 (m, 1H), 1.69-1.77 (m, 1H); ^{13}C NMR (CD_3OD) δ (ppm): 168.3, 163.4, 160.7, 160.4, 155.2, 153.6, 152.1, 148.4, 143.3, 134.7, 132.4, 128.4, 126.9, 125.4, 121.5, 111.5, 106.1, 103.8, 55.3, 46.2, 42.0, 39.1, 38.7, 30.8, 28.8, 23.6; ESI-MS (m/z) calculated for $C_{30}H_{36}Cl_1N_8O_2$ $[M + H]^+$: 575.2644, found 575.2.

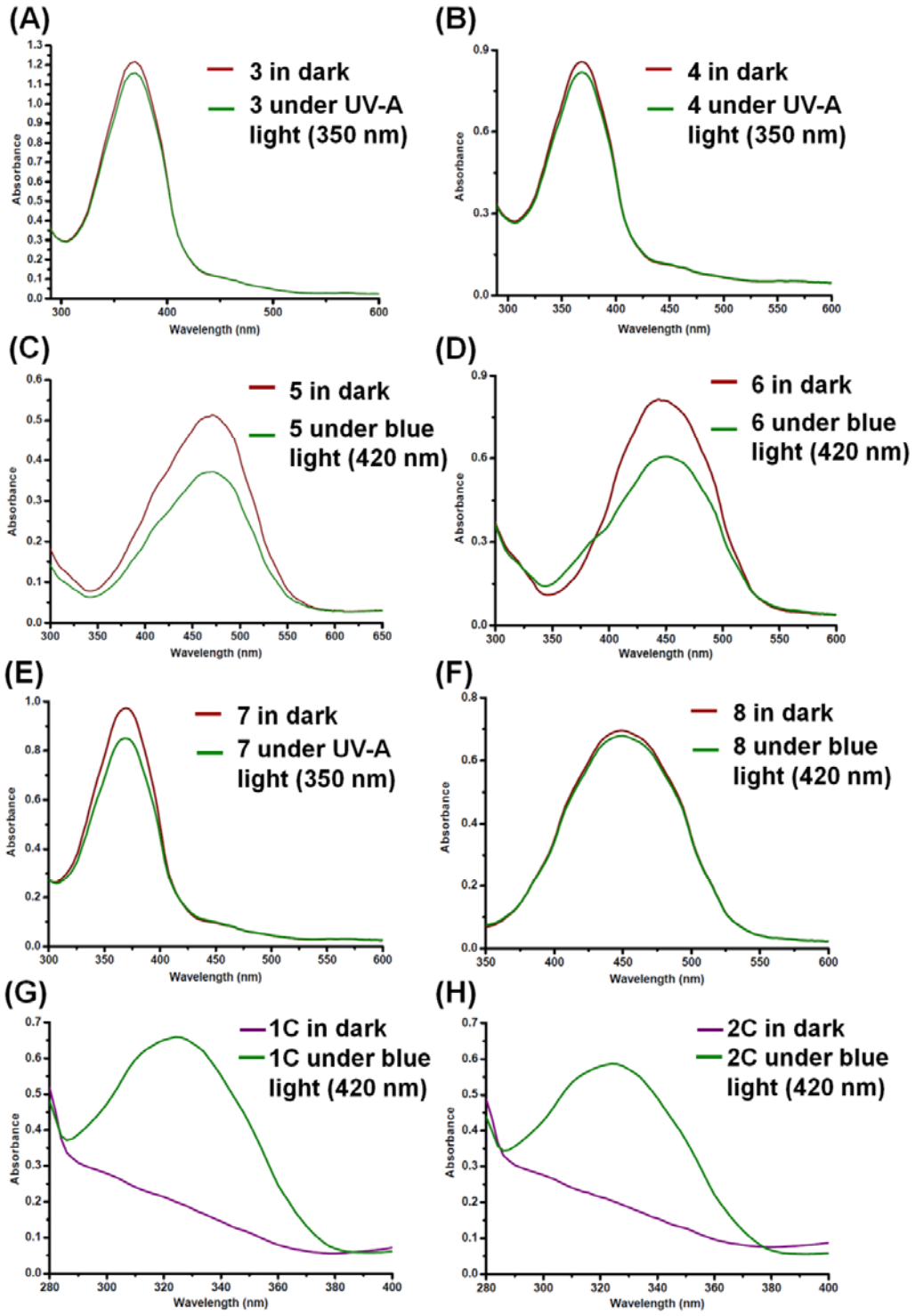


Figure S1. Photoisomerization of compounds **3**-**8** monitored by UV-visible spectroscopy (A-F). While compounds **5**-**7** exhibit significant changes in the UV-Vis spectra upon *trans* to *cis* isomerisation, compounds **3**, **4** and **8** show negligible changes, which is in agreement with the previously reported observations for hydroxy- and dimethylamino- substituted azobenzenes.[3,4] (G-H) The change in UV-vis spectra of compound **1** and **2** upon *cis* to *trans* isomerisation with blue light.

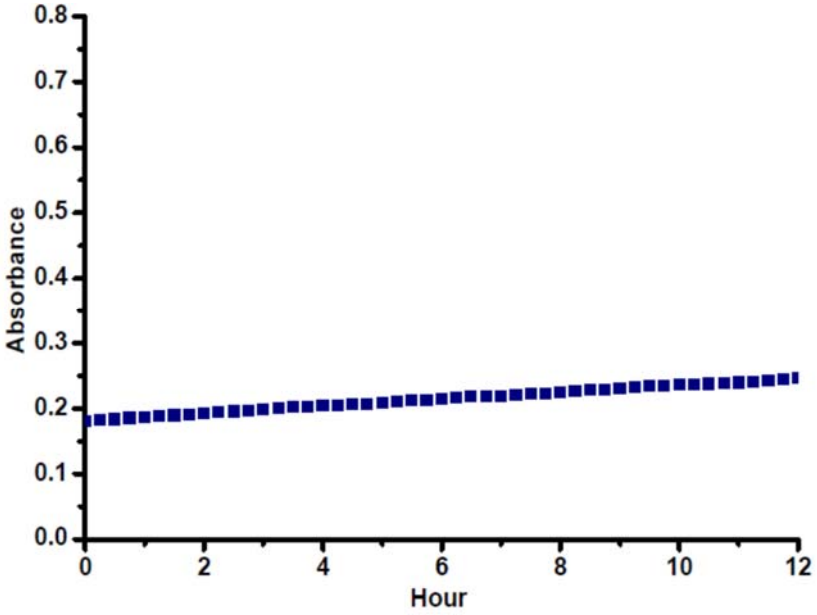


Figure S2. Thermal stability of **2C** in aqueous buffer at 37 ^{0}C.

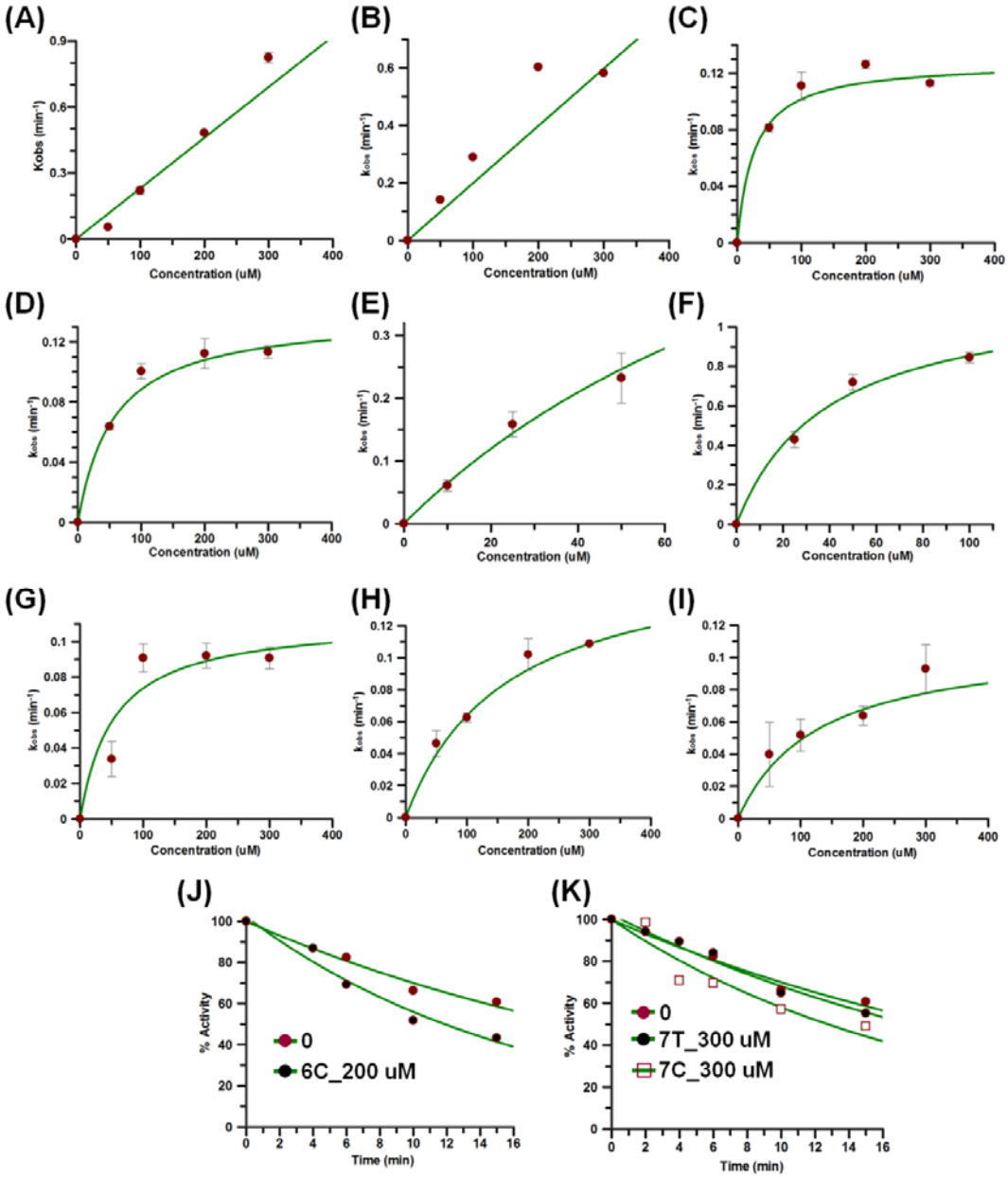


Figure S3. Inhibition of PAD1 by compounds **1**-**8**. **1T** (A), **1C** (B), **2T** (C), **2C** (D), **3T** (E), **3C** (F), **4T** (G), **4C** (H), **6T** (I), **6C** (J) and **7** (K). Due to poor inhibitory activity, a single k_{obs} value was determined for **6C**, **7T** and **7C**. For all other compounds, full k_{inact}/K_I profiles were obtained.

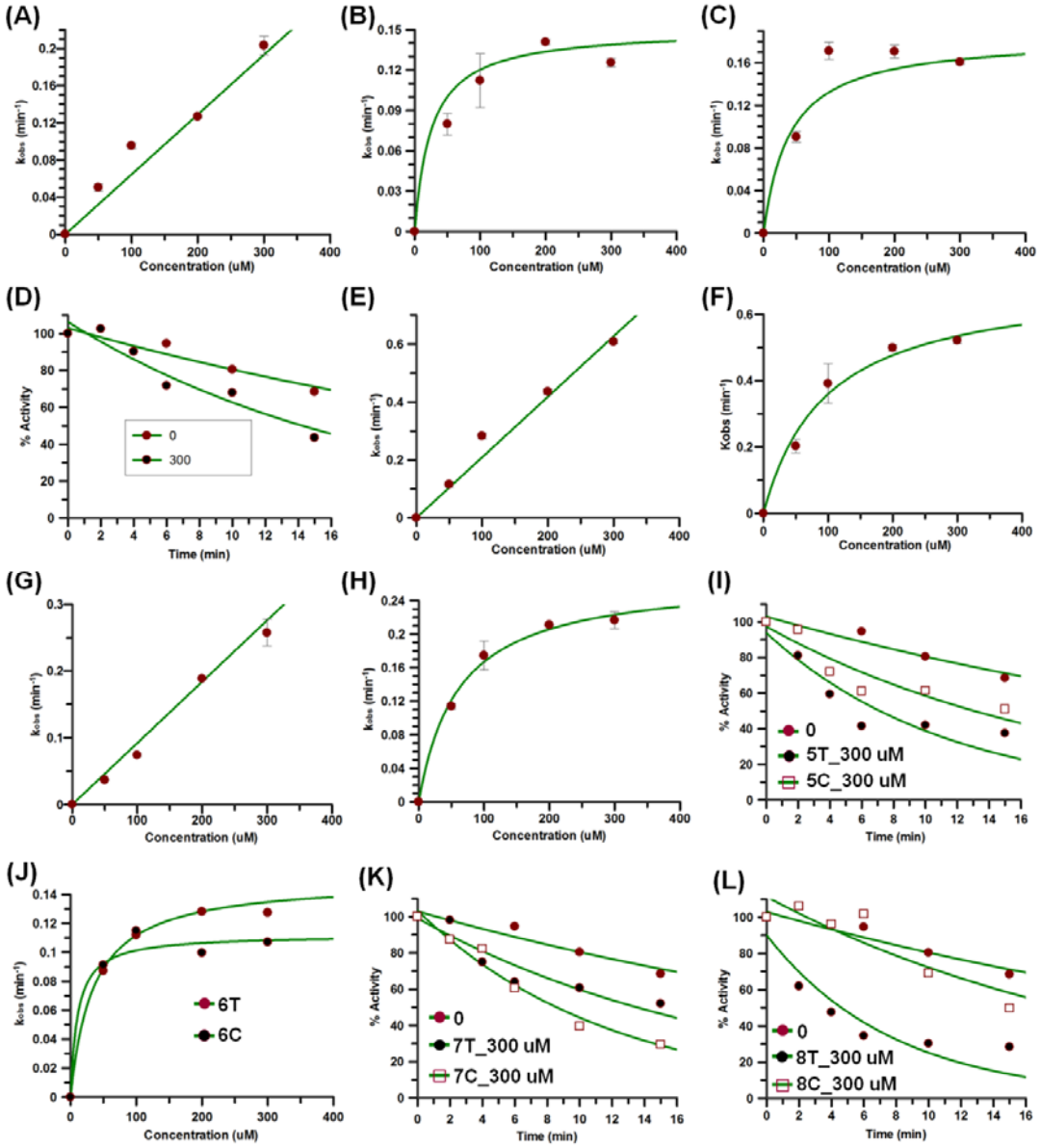


Figure S4. Inhibition of PAD2 by compounds **1**-**8**. **1T** (A), **1C** (B), **2T** (C), **2C** (D), **3T** (E), **3C** (F), **4T** (G), **4C** (H), **5** (I), **6** (J), **7** (K) and **8** (L). Due to poor inhibitory activity, a single k_{obs} value was determined for **5**, **7** and **8**. For all other compounds, full k_{inact}/K_I profiles were obtained.

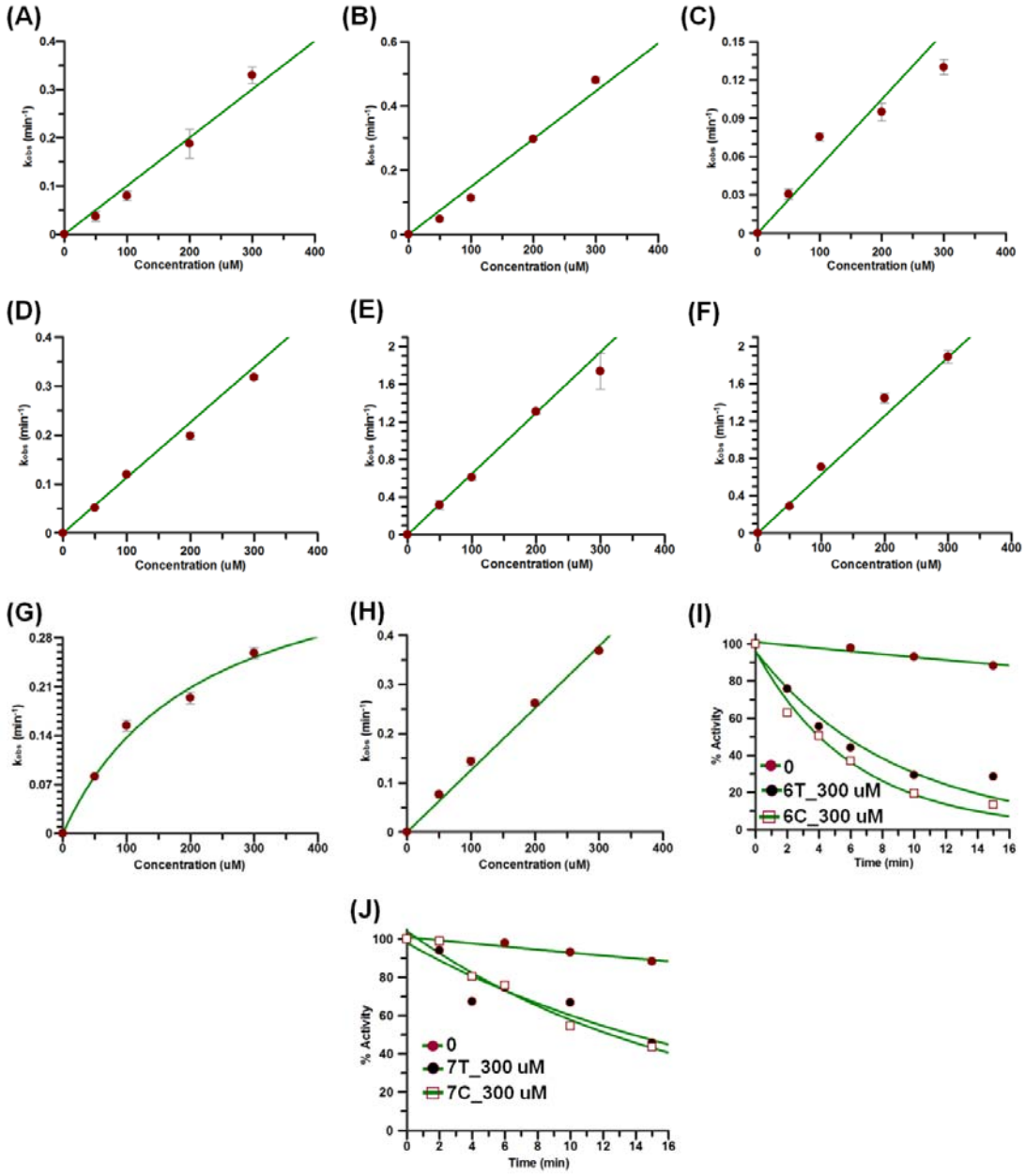


Figure S5. Inhibition of PAD3 by compounds **1**-**8**. **1T** (A), **1C** (B), **2T** (C), **2C** (D), **3T** (E), **3C** (F), **4T** (G), **4C** (H), **6** (I) and **7** (J). Due to poor inhibitory activity, a single k_{obs} value was determined for **6** and **7**. For all other compounds, full k_{inact}/K_I profiles were obtained.

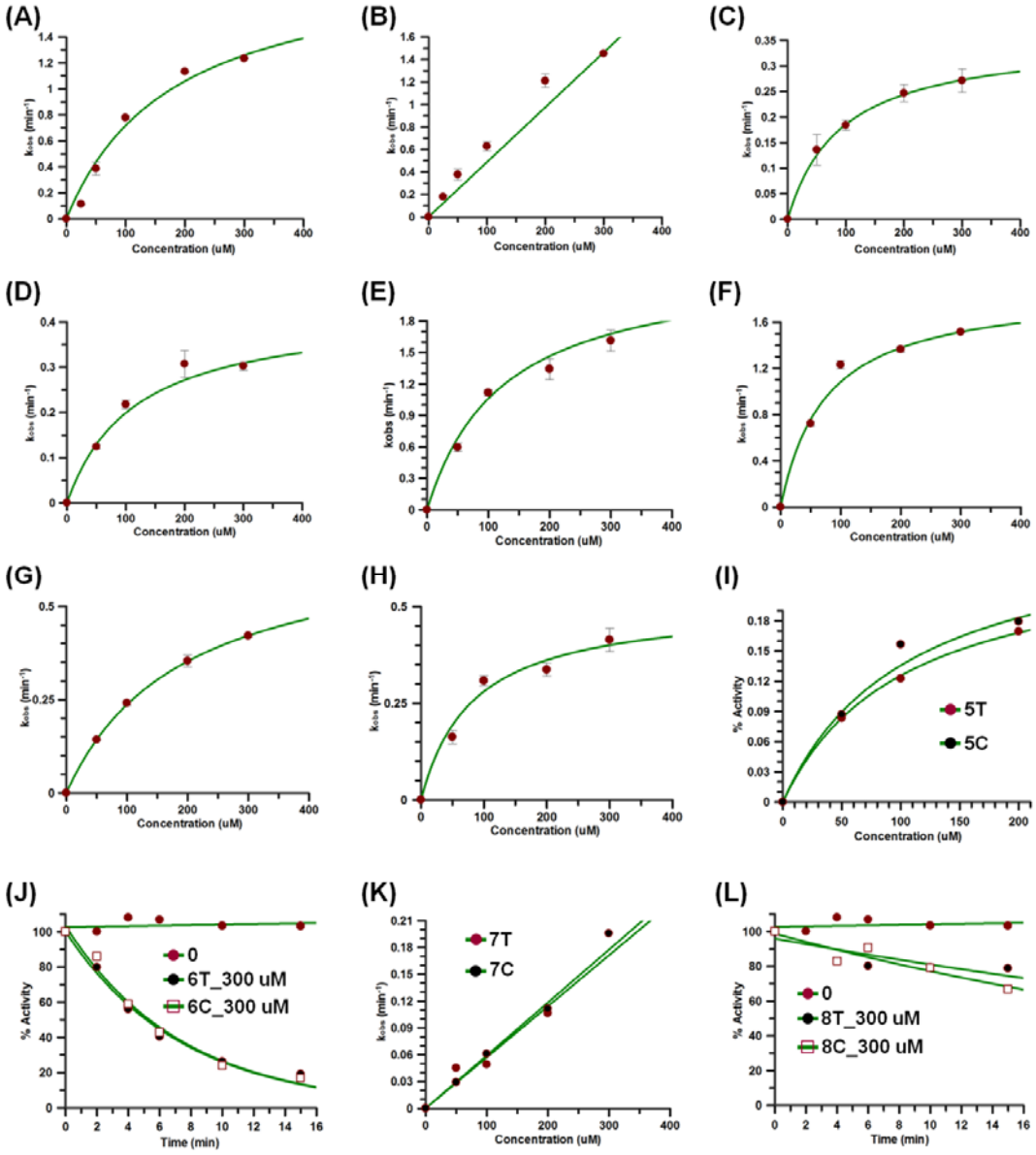


Figure S6. Inhibition of PAD4 by compounds **1**-**8**. **1T** (A), **1C** (B), **2T** (C), **2C** (D), **3T** (E), **3C** (F), **4T** (G), **4C** (H), **5** (I), **6** (J), **7** (K) and **8** (L). Due to poor inhibitory activity, a single k_{obs} value was determined for **6** and **7**. For all other compounds, full k_{inact}/K_I profiles were obtained.

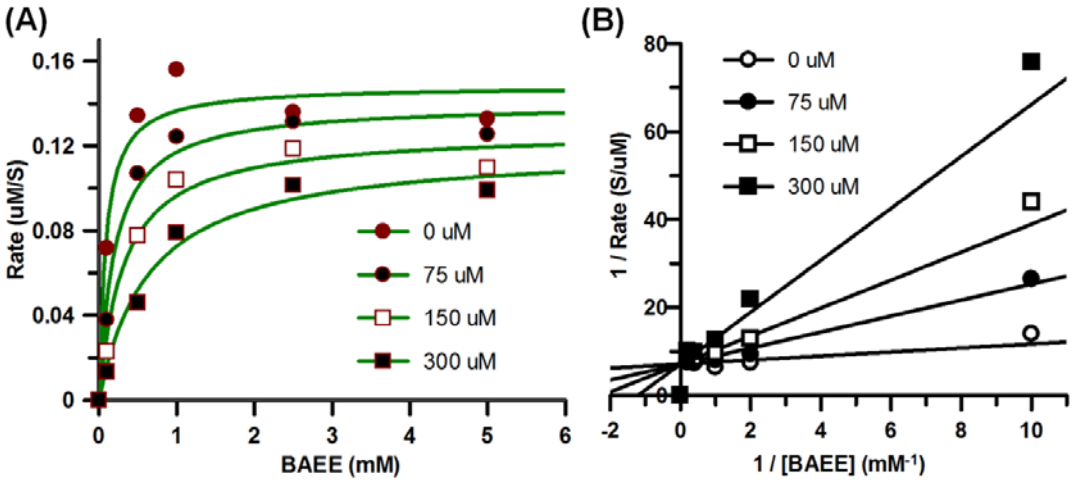


Figure S7. (A) Determination of K_m of BAEE for PAD2 in the presence of increasing concentrations of **2C**. (B) Lineweaver-Burk plot for the competitive inhibition of PAD2 by **2C**.

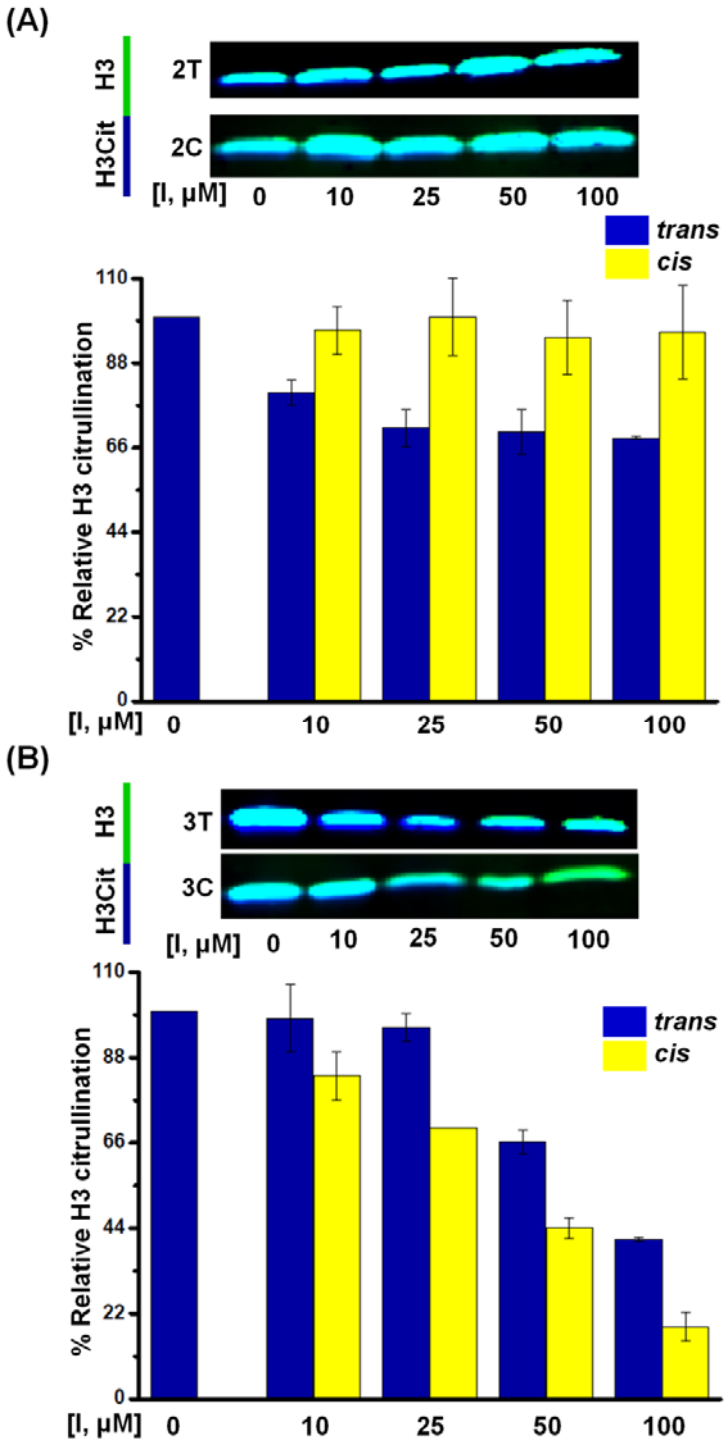


Figure S8. Inhibition of histone H3 citrullination in HEK293T/PAD2 cells by compounds **2** (A) and **3** (B). Inhibitor concentrations [I, μM] are given under each lane of the western-blot image. In each western-blot, citrullinated H3 (H3Cit) and H3 are shown in blue and green, respectively. Quantification of each band yielded the H3Cit/H3 ratio, from which the % relative H3 citrullination was calculated.

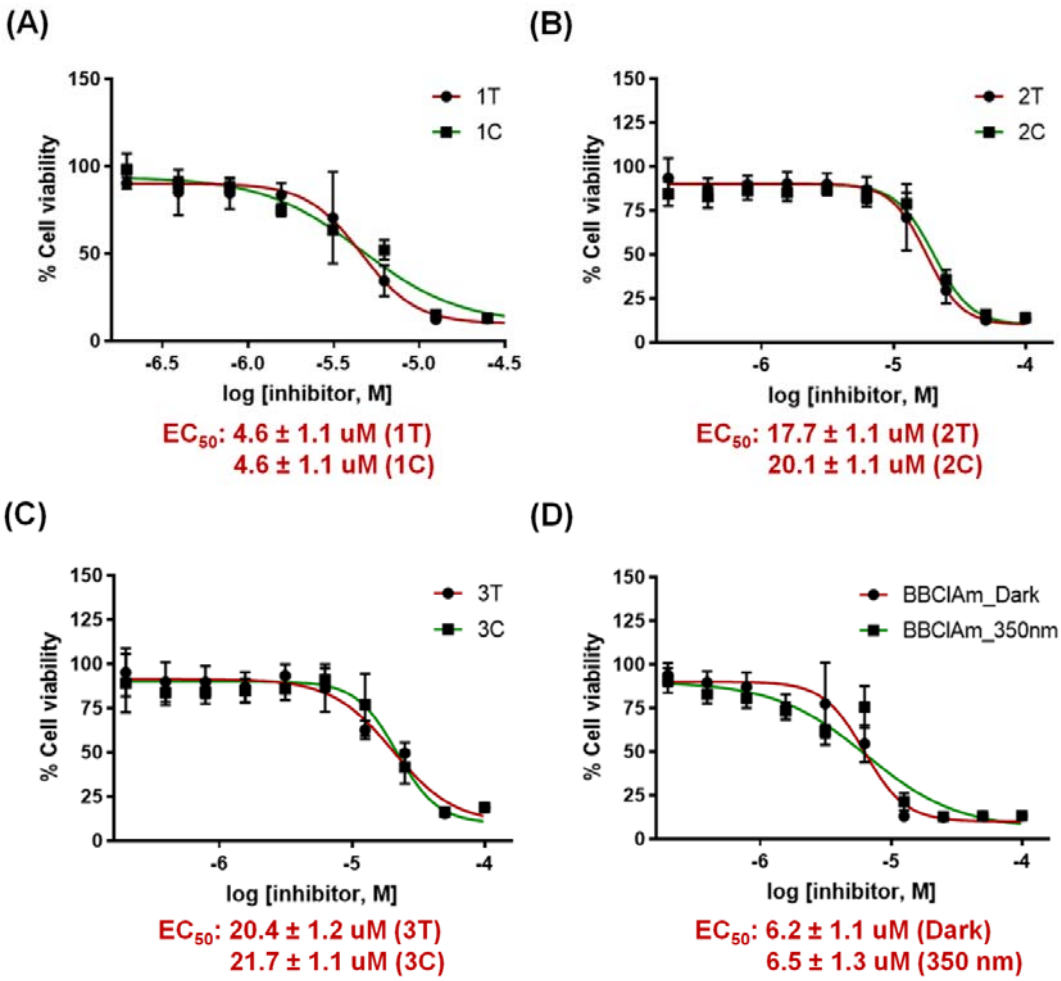


Figure S9. Cell viability experiments with compounds **1**-**3** and BB-Cl-Amidine. These experiments were carried out in HEK293T/PAD2 cells.

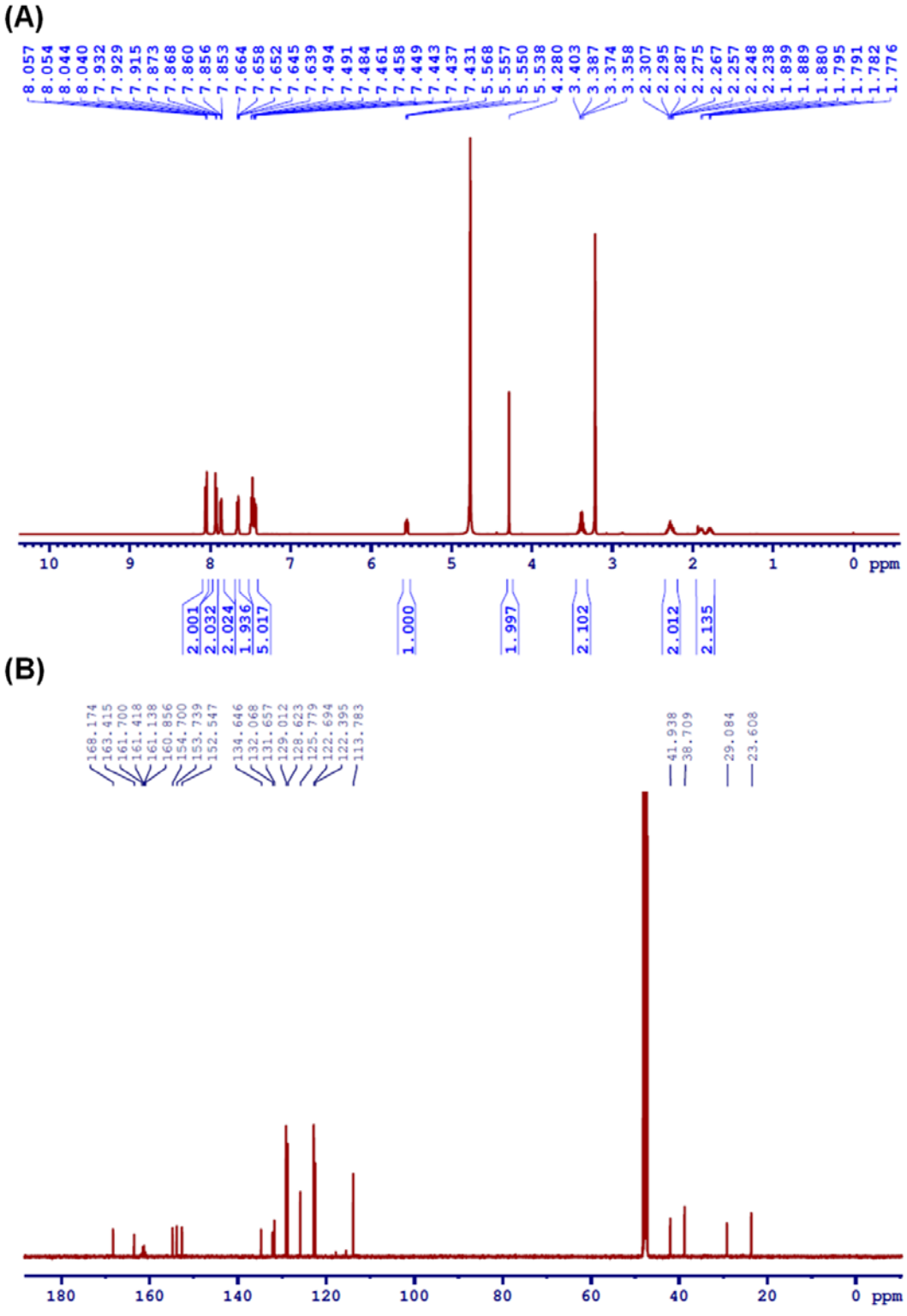


Figure S10. ^{1}H (A) and ^{13}C (B) NMR spectra of compound **1** in CD_3OD.

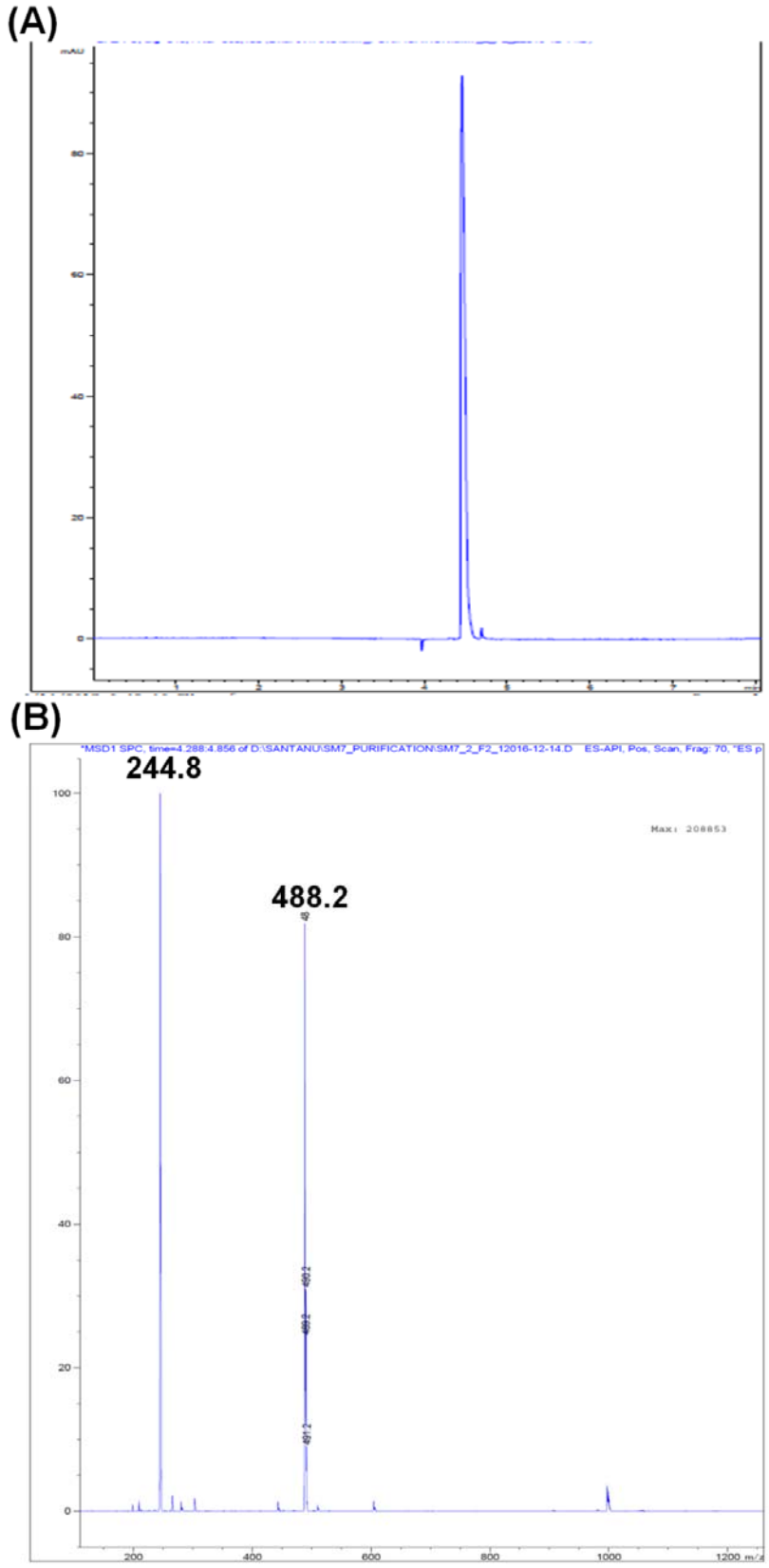


Figure S11. HPLC trace (A) and ESI-Mass spectra of compound **1**.

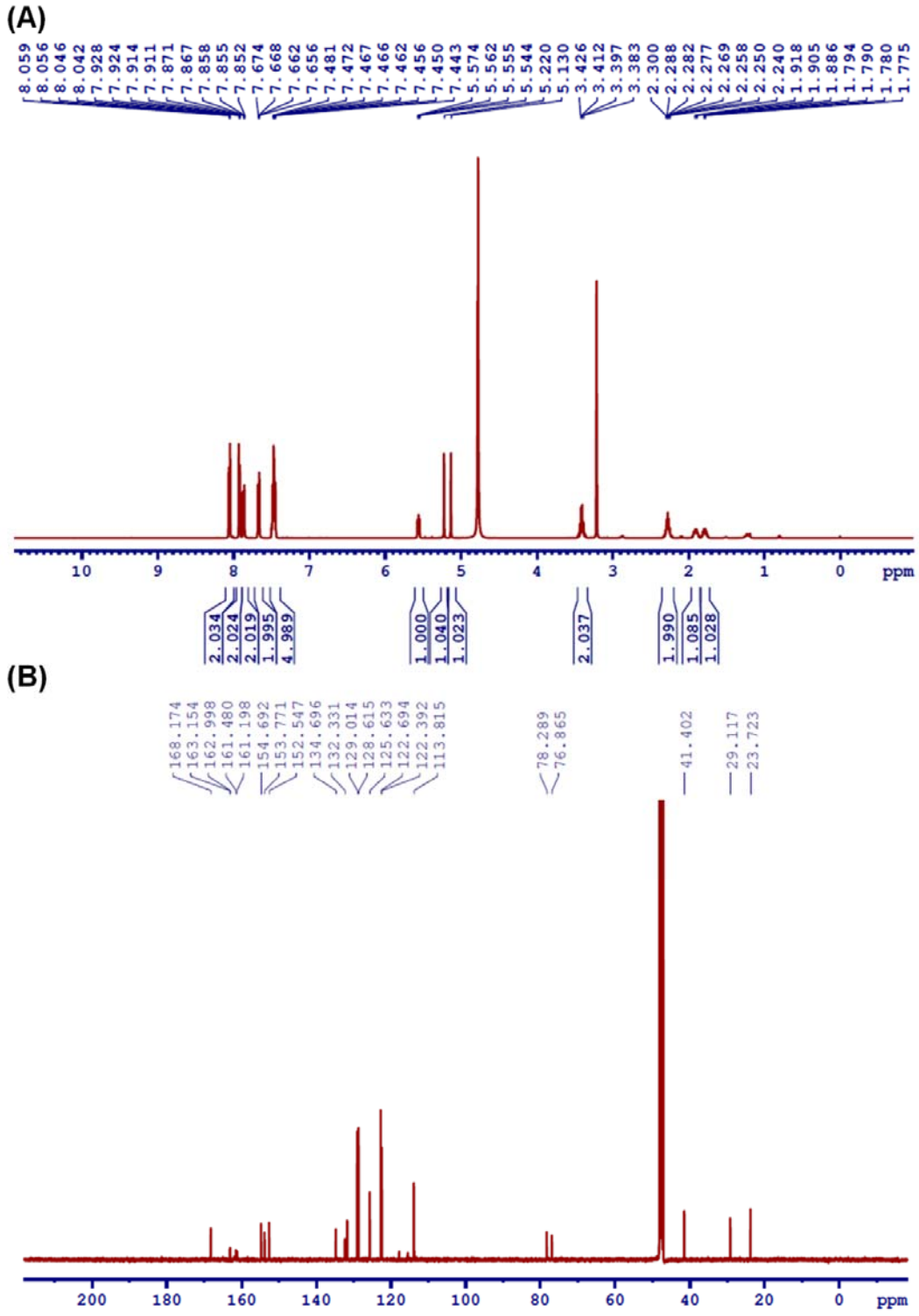


Figure S12. ^{1}H (A) and ^{13}C (B) NMR spectra of compound **2** in CD_3OD.

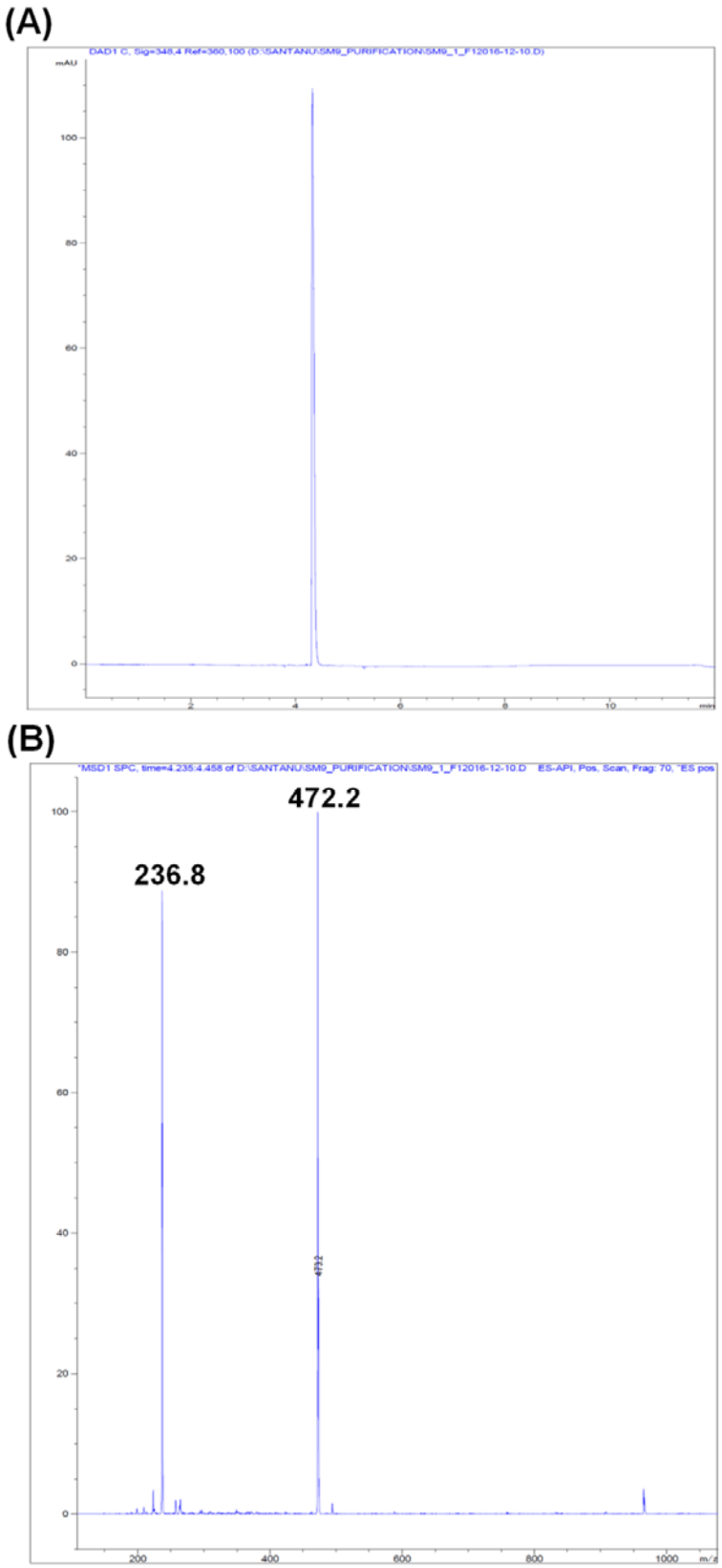


Figure S13. HPLC trace (A) and ESI-Mass spectra of compound **2**.

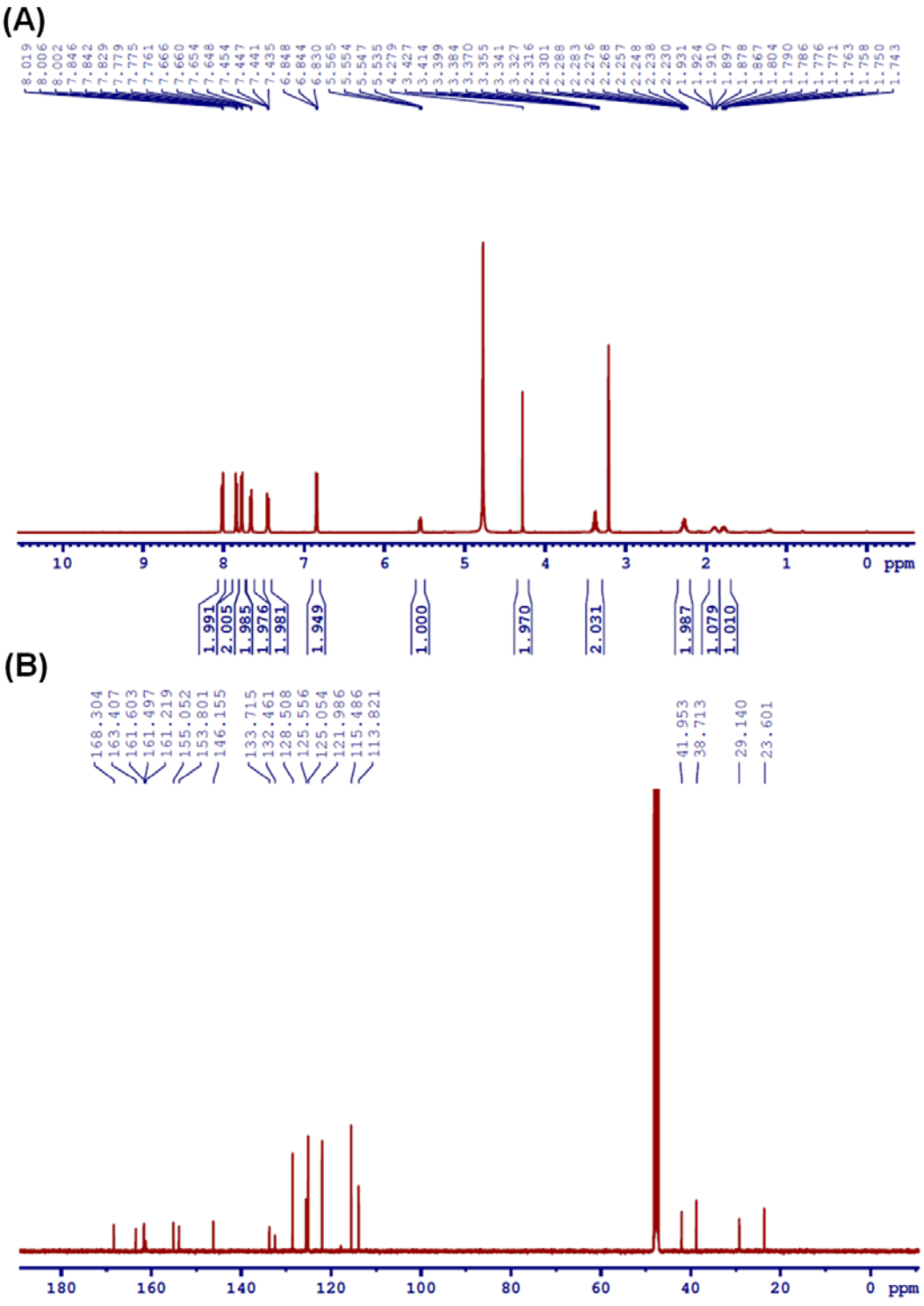


Figure S14. ^{1}H (A) and ^{13}C (B) NMR spectra of compound **3** in CD_3OD.

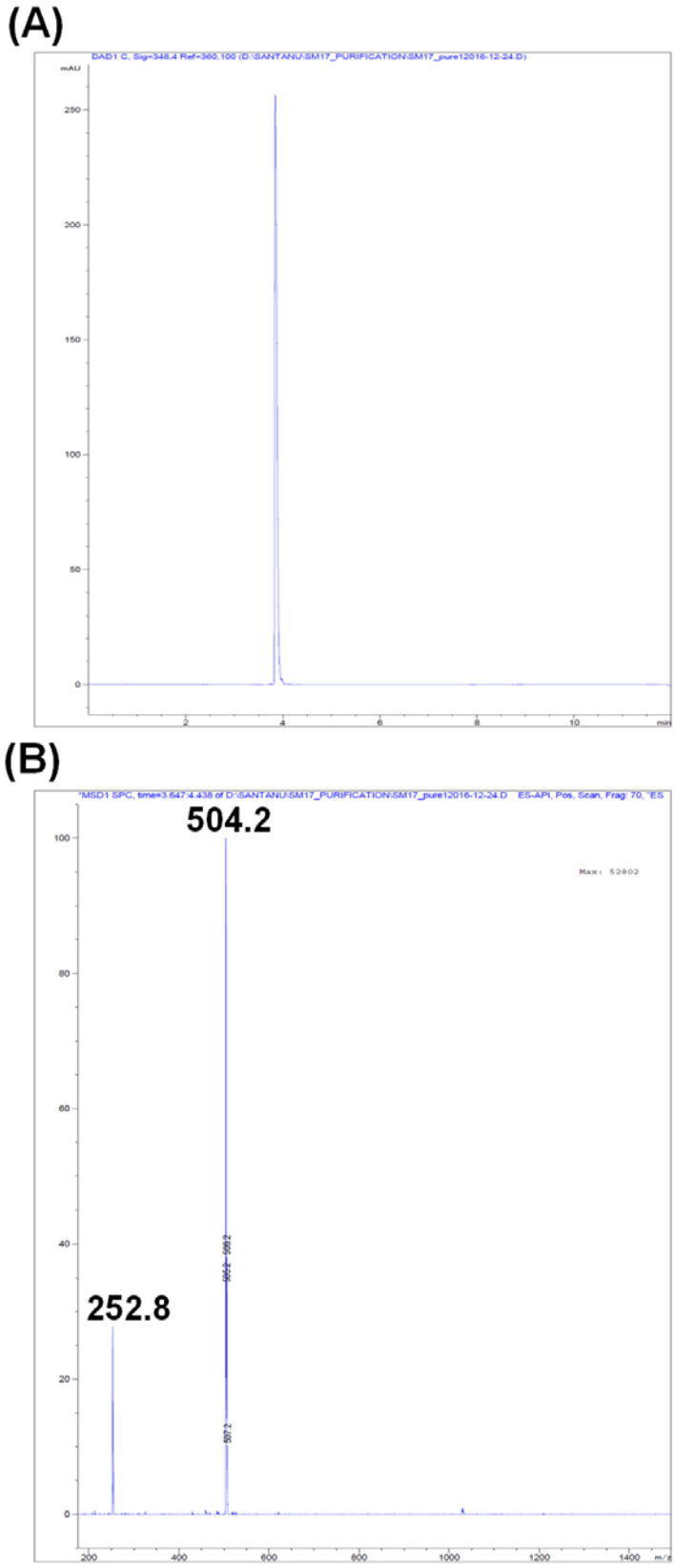


Figure S15. HPLC trace (A) and ESI-Mass spectra of compound **3**.

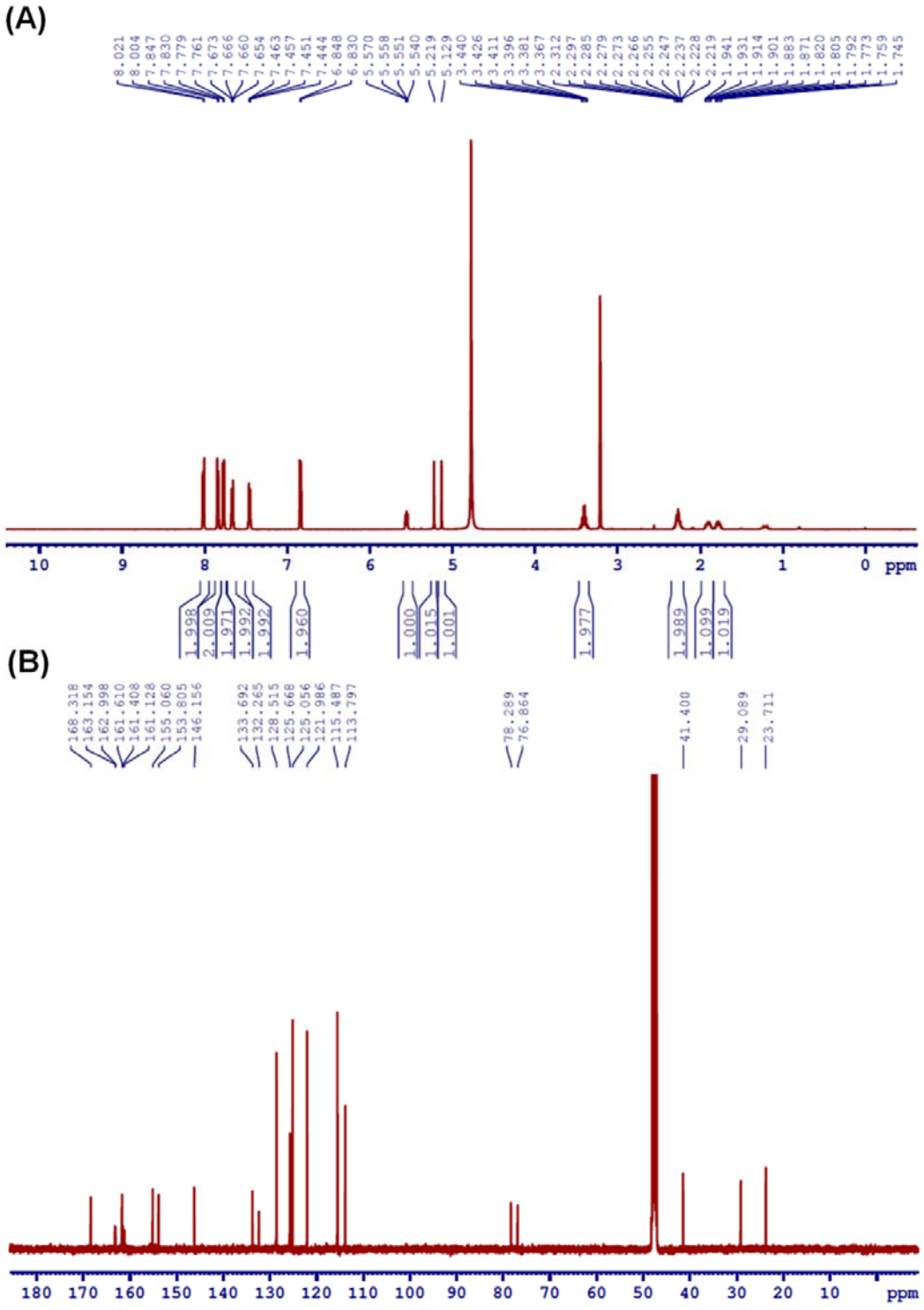


Figure S16. ^{1}H (A) and ^{13}C (B) NMR spectra of compound **4** in CD_3OD.

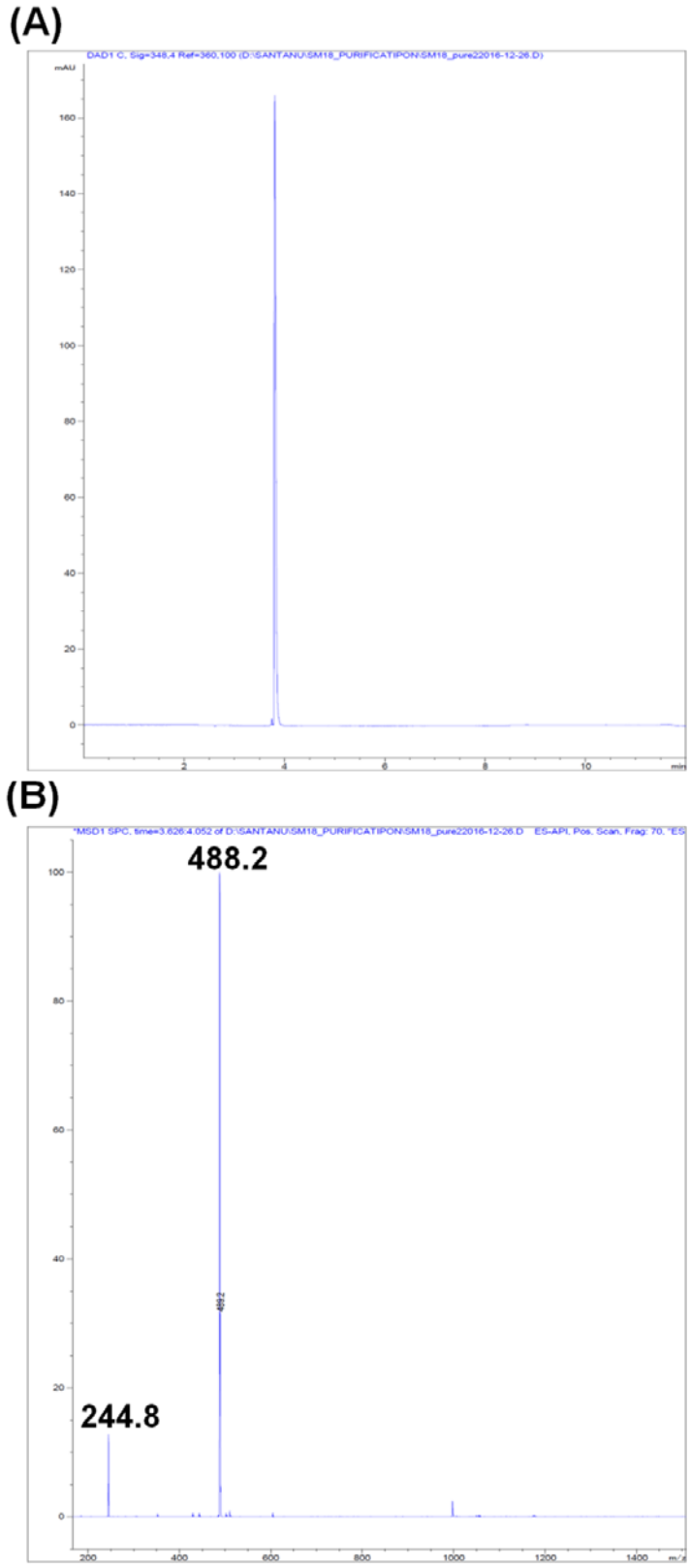


Figure S17. HPLC trace (A) and ESI-Mass spectra of compound **4**.

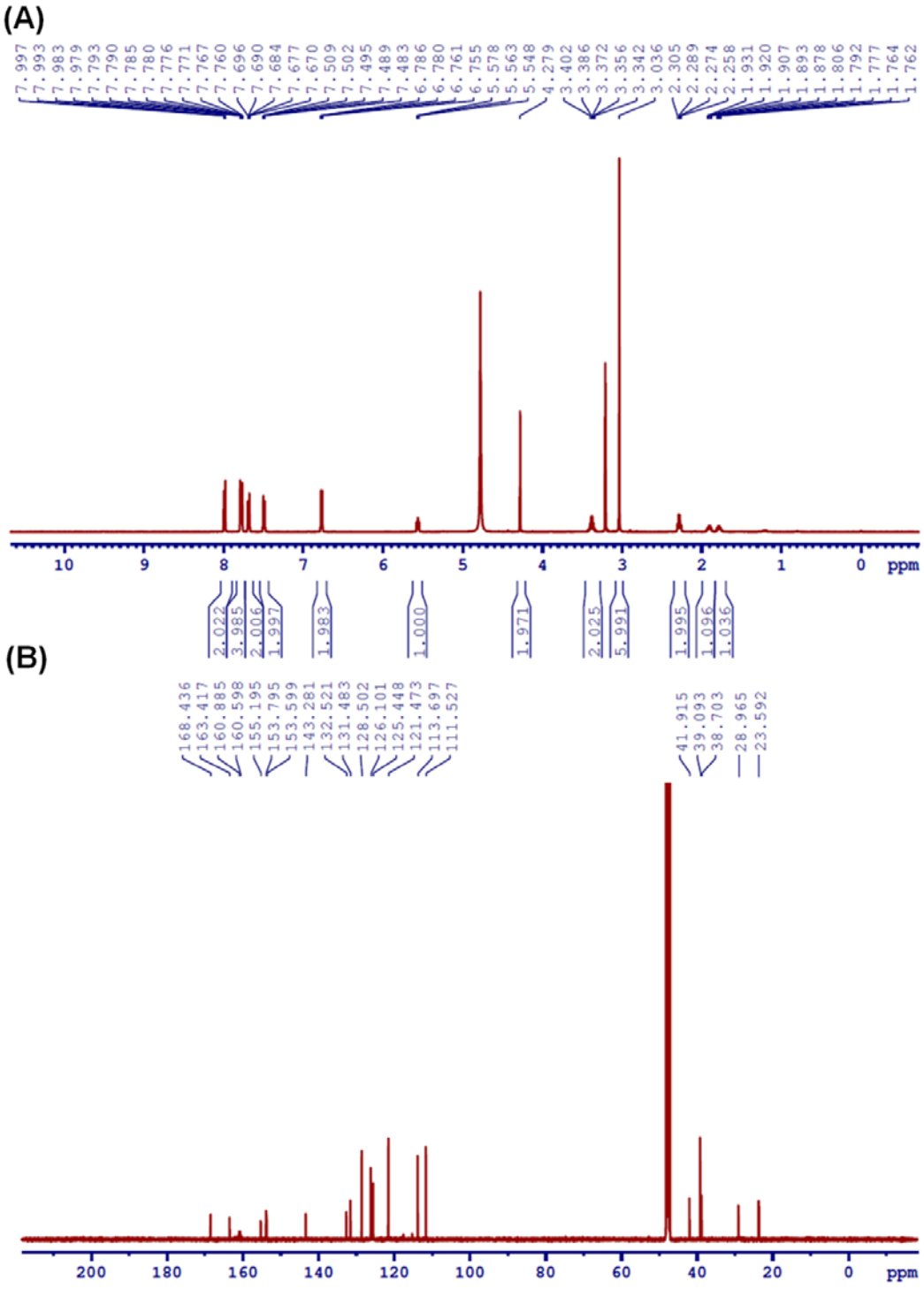


Figure S18. ^{1}H (A) and ^{13}C (B) NMR spectra of compound **5** in CD_3OD.

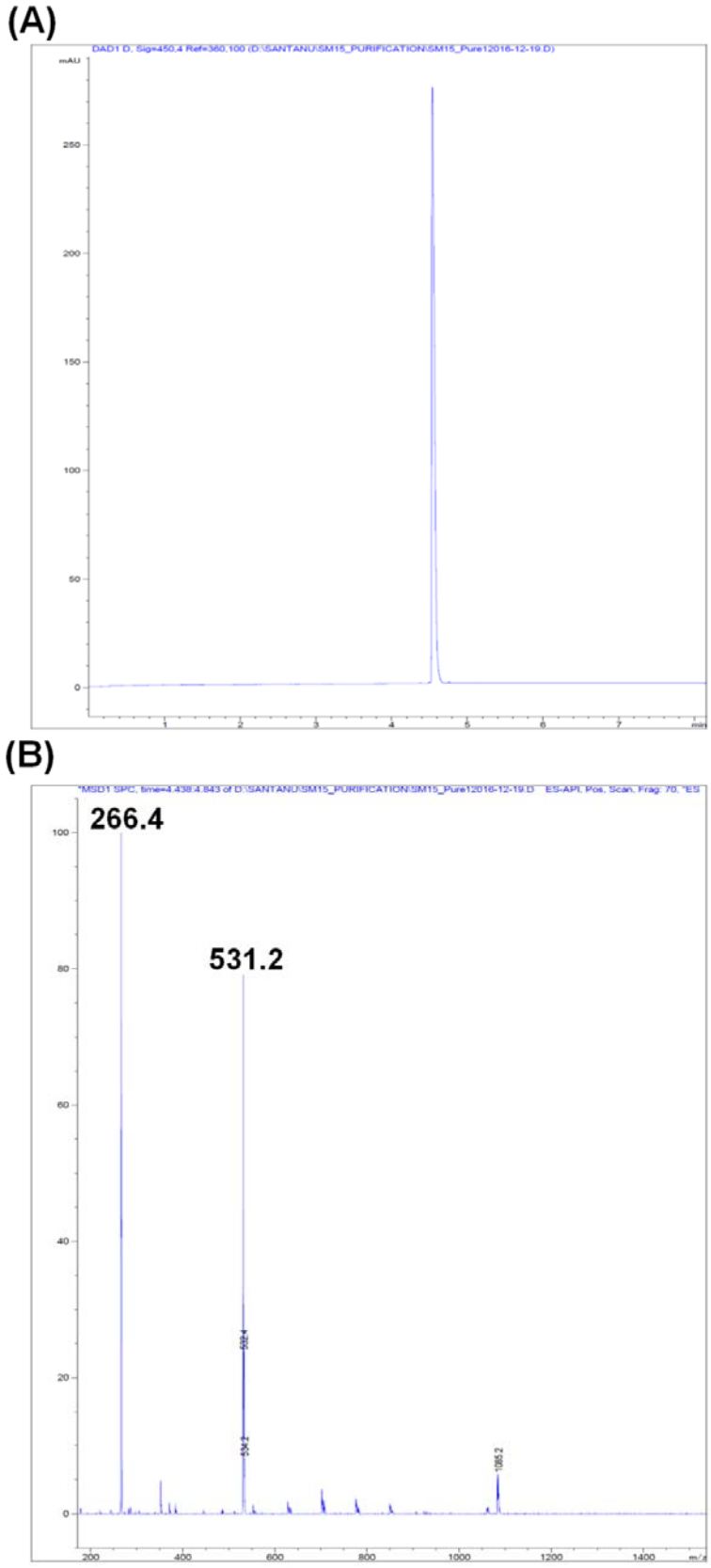


Figure S19. HPLC trace (A) and ESI-Mass spectra of compound **5**.

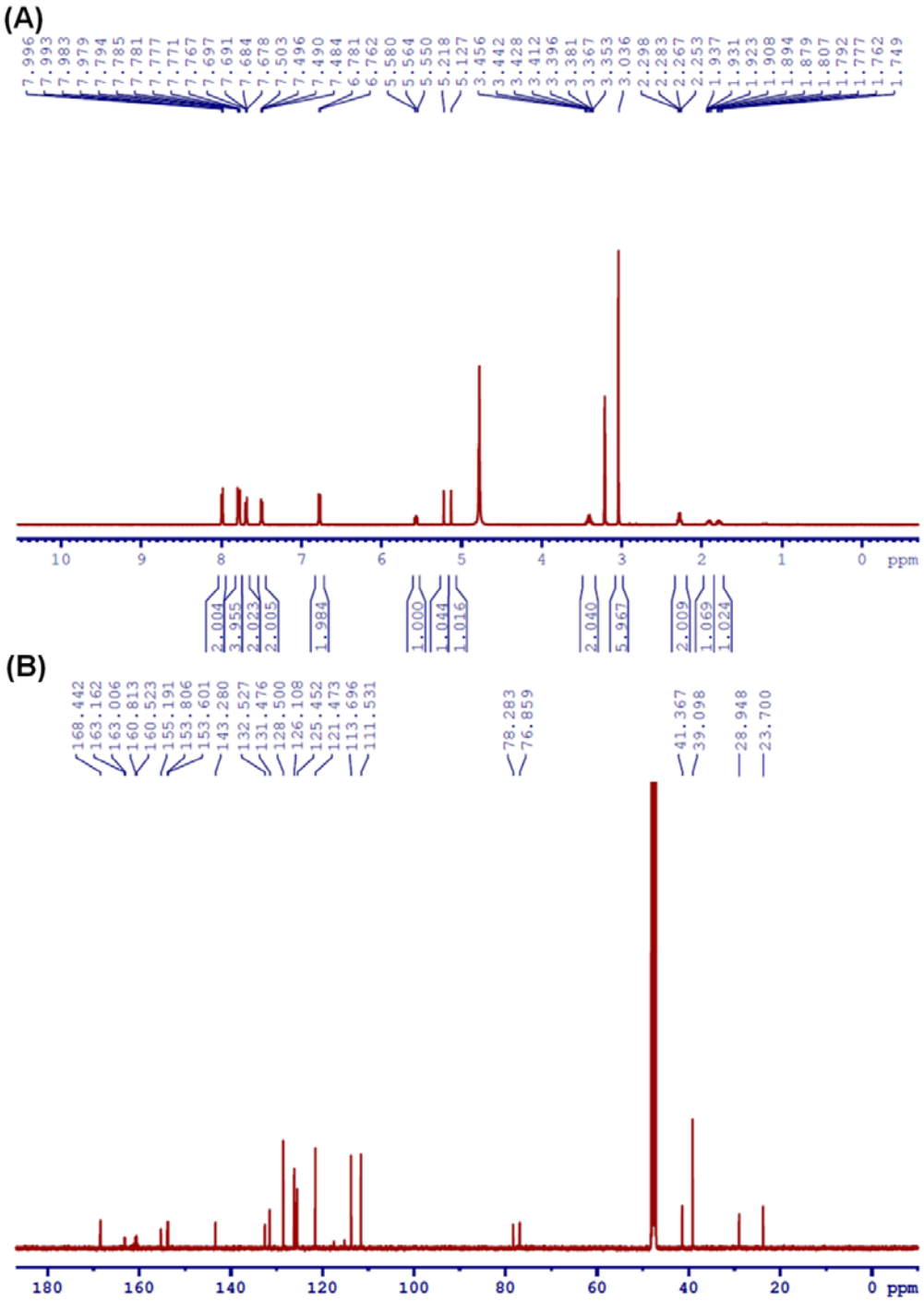


Figure S20. ^{1}H (A) and ^{13}C (B) NMR spectra of compound **6** in CD_3OD.

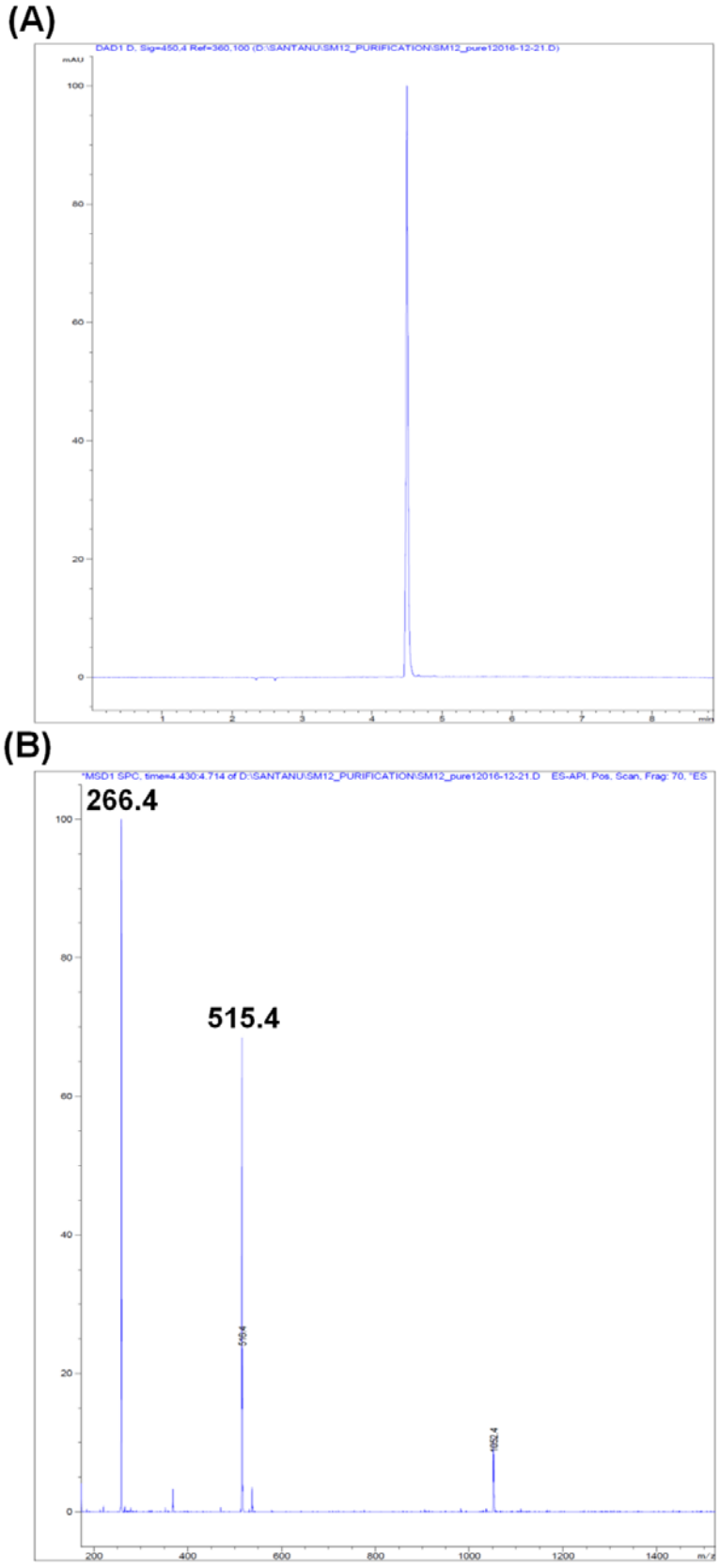


Figure S21. HPLC trace (A) and ESI-Mass spectra of compound **6**.

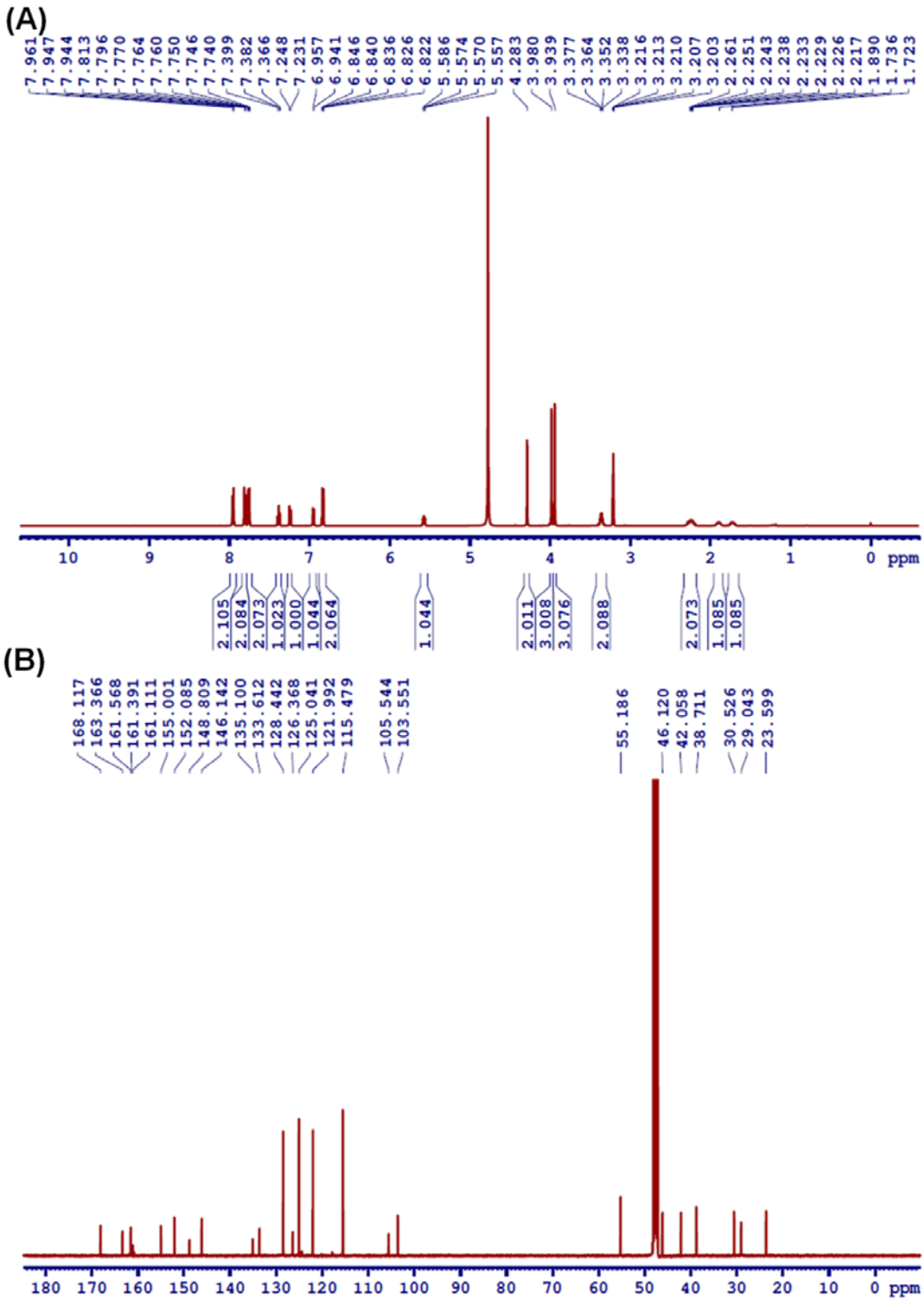


Figure S22. ^{1}H (A) and ^{13}C (B) NMR spectra of compound **7** in CD_3OD.

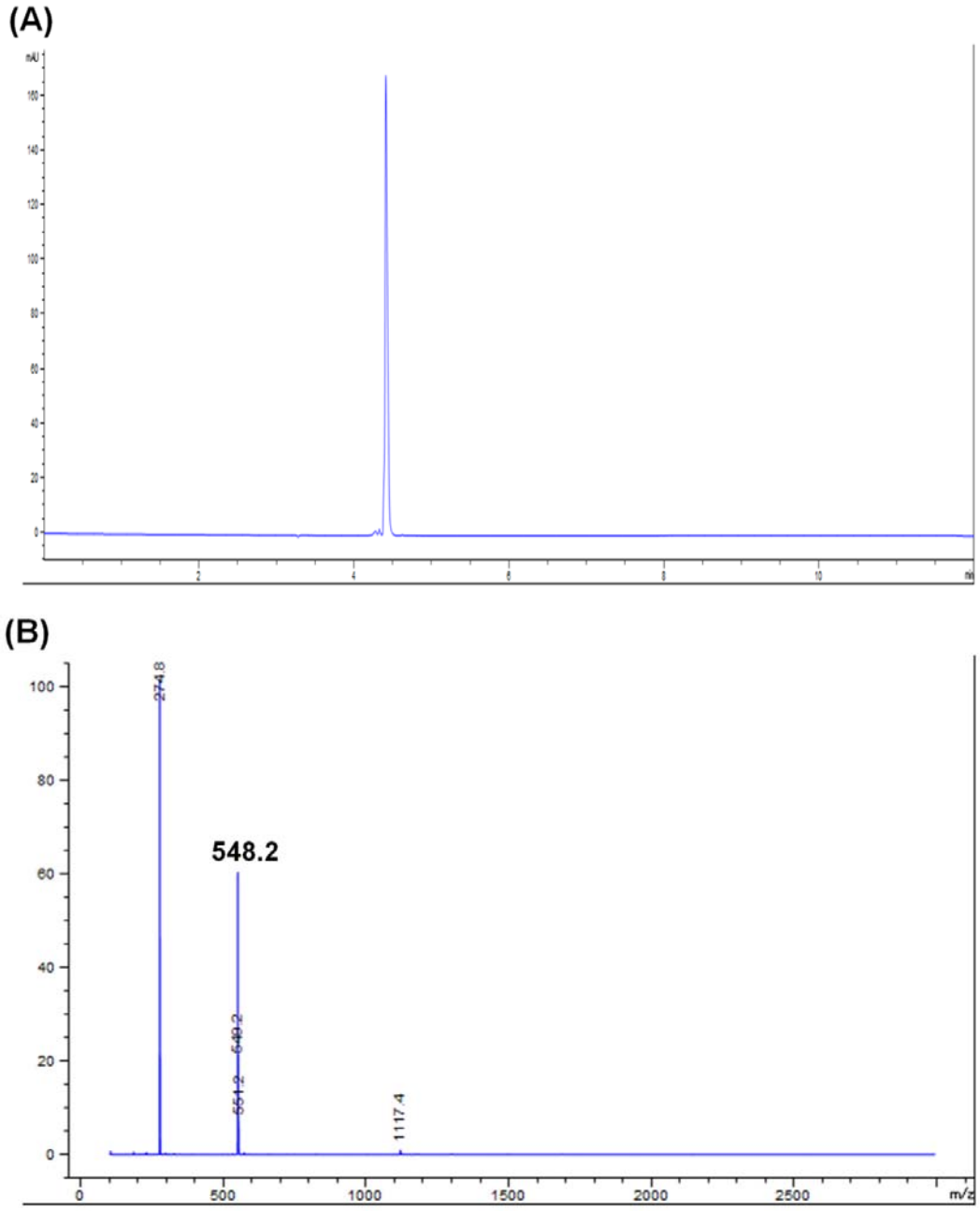


Figure S23. HPLC trace (A) and ESI-Mass spectra of compound **7**.

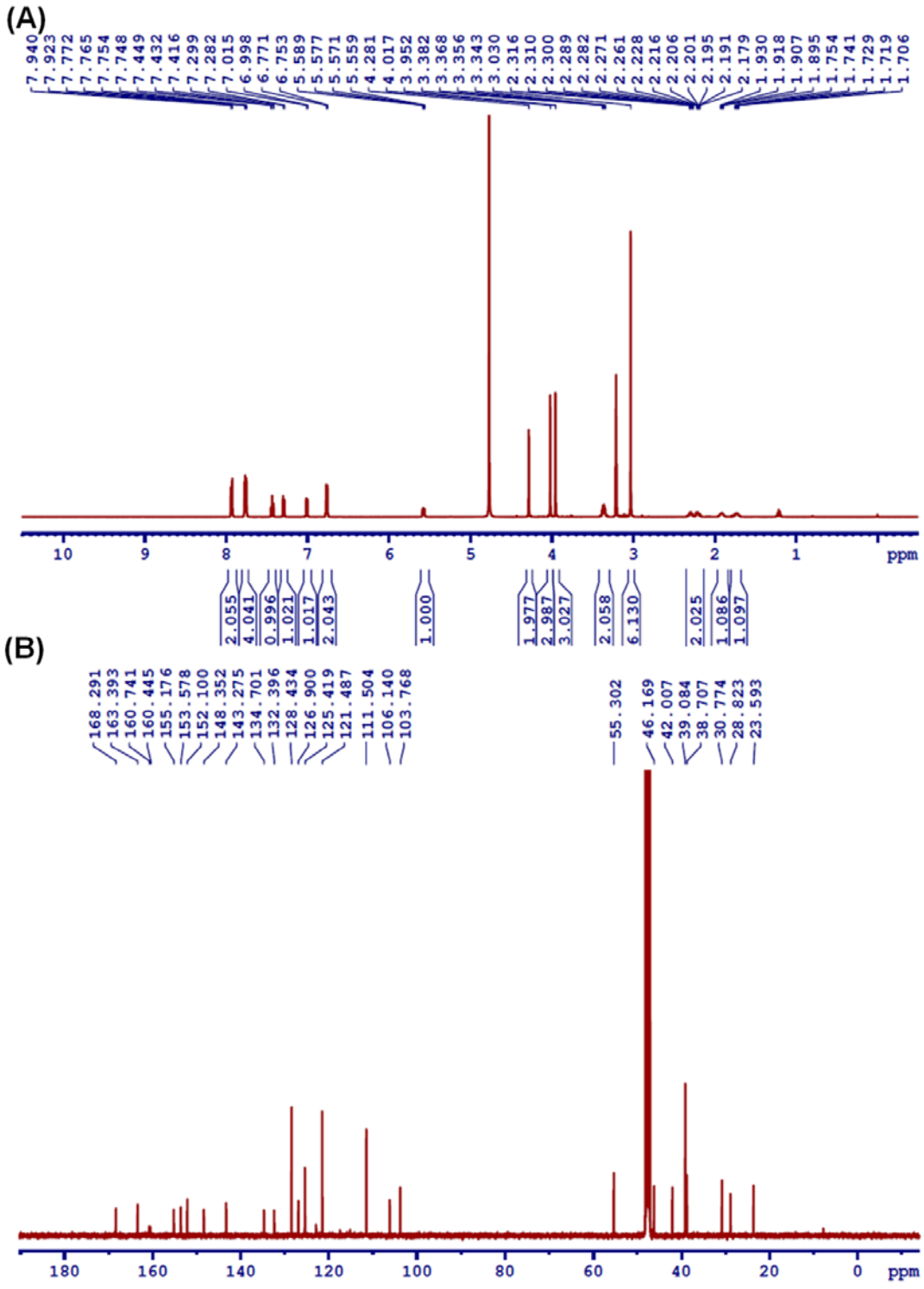


Figure S24. ^{1}H (A) and ^{13}C (B) NMR spectra of compound **8** in CD_3OD.

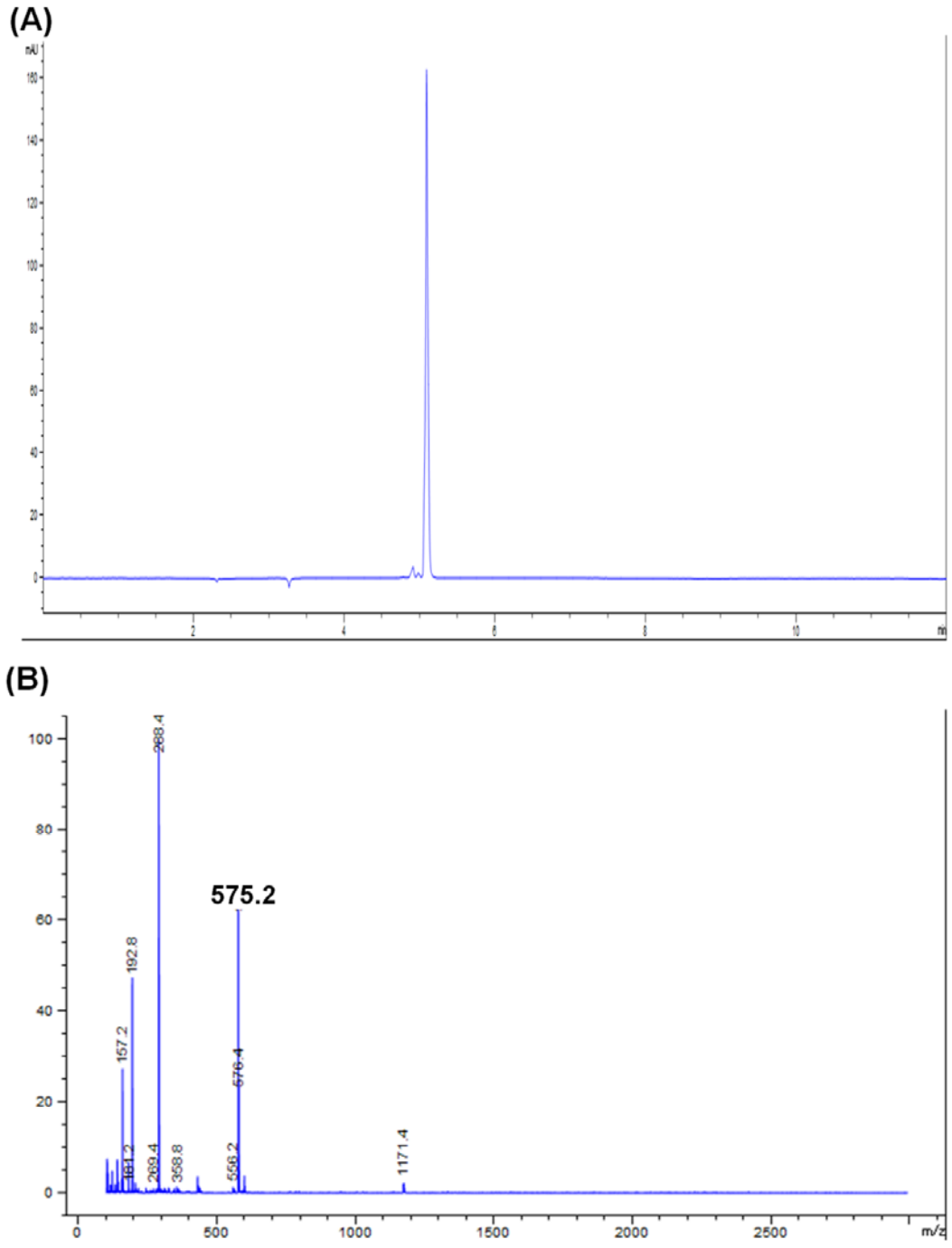


Figure S25. HPLC trace (A) and ESI-Mass spectra of compound **8**.

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