Bicyclic peptide-enhanced covalent inhibitor of SARS-CoV-2 3CL protease

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General synthesis methods

Unless otherwise specified, the materials and solvents were purchased from commercial sources and used without further purification. Chromatographic separations were performed by medium-pressure chromatography (Teledyne Isco 68-5230-066) unless otherwise specified. Silica gel (FCP 300–400 mesh) was used for column chromatography. Commercial plates (silica gel HSGF 254, 0.15–0.2 mm thickness) were used for analytical thin-layer chromatography to follow the progress of reactions. Unless otherwise specified, 1H and 13C nuclear magnetic resonance spectra (NMR) spectra were obtained on a Bruker 500 or 600 MHz NMR spectrometer. Chemical shifts (δ) for 1H or 13C were expressed in parts per million (ppm) using tetramethylsilane (TMS) as an internal reference, and the coupling constants (J) were indicated in Hz. The proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint), broad (br), or multiplet (m). Mass spectra (m/z) of chemical compounds were recorded on an Electrospray Ionization (ESI) mass spectrometer (Waters) and a HPLC SQ Detector

2 system (Waters). Reverse-phase HPLC (RP-HPLC) purifications were performed using a 1260 Infinity II HPLC equipped with a prep C18 column (5 μm, C18, 100Å, 21.2×50 mm preparative LC column; Agilent) eluted over a linear gradient from 95% solvent A (water and 0.1% trifluoroacetic acid) to 95% solvent B (acetonitrile and 0.1% trifluoroacetic acid). Unless otherwise noted, chemicals were purchased from Sigma Aldrich, J&K Chemical, or Sinopharm and were used without further purification. Protected Fmoc-amino acids and coupling reagents were purchased from Bidepharm and GL Biochem (Shanghai, China) Ltd. RinkAmide-MBHA resin (0.458mmol/g) was purchased from GL Biochem (Shanghai) Ltd (Lot. Nr. GLS220524-4910).

NMR spectra

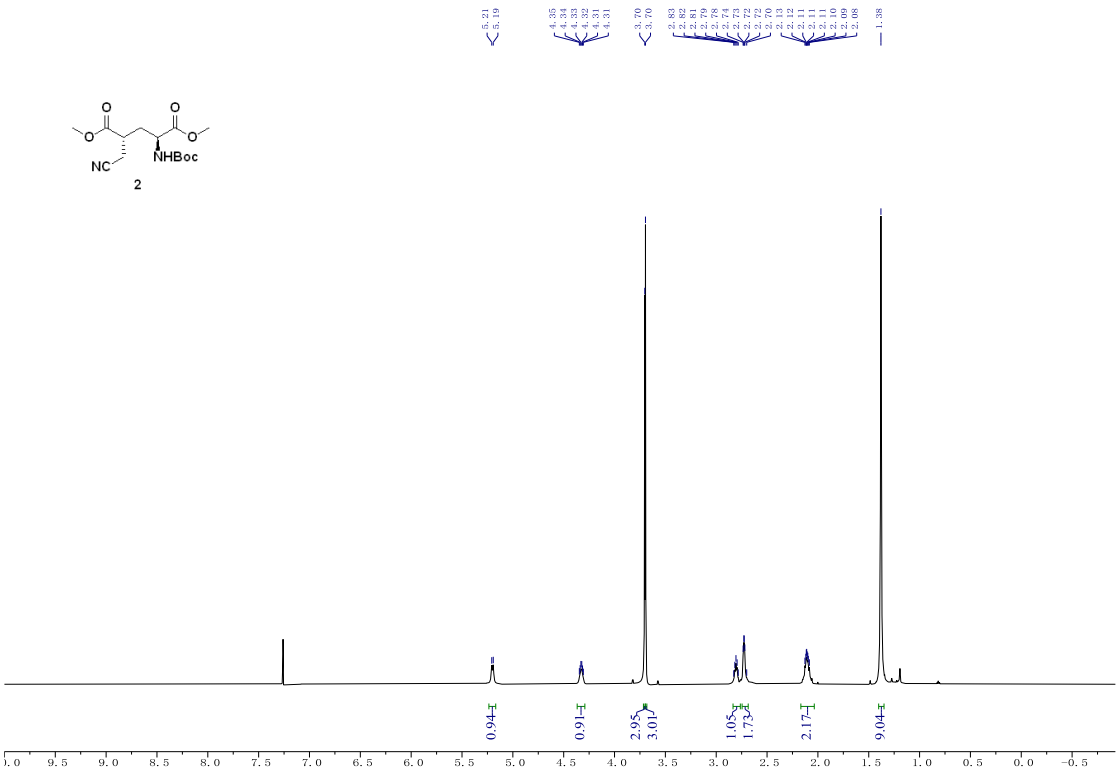

Figure S1. ^{1}H NMR spectrum (600 MHz) of compound **2** in $CDCl_3$.

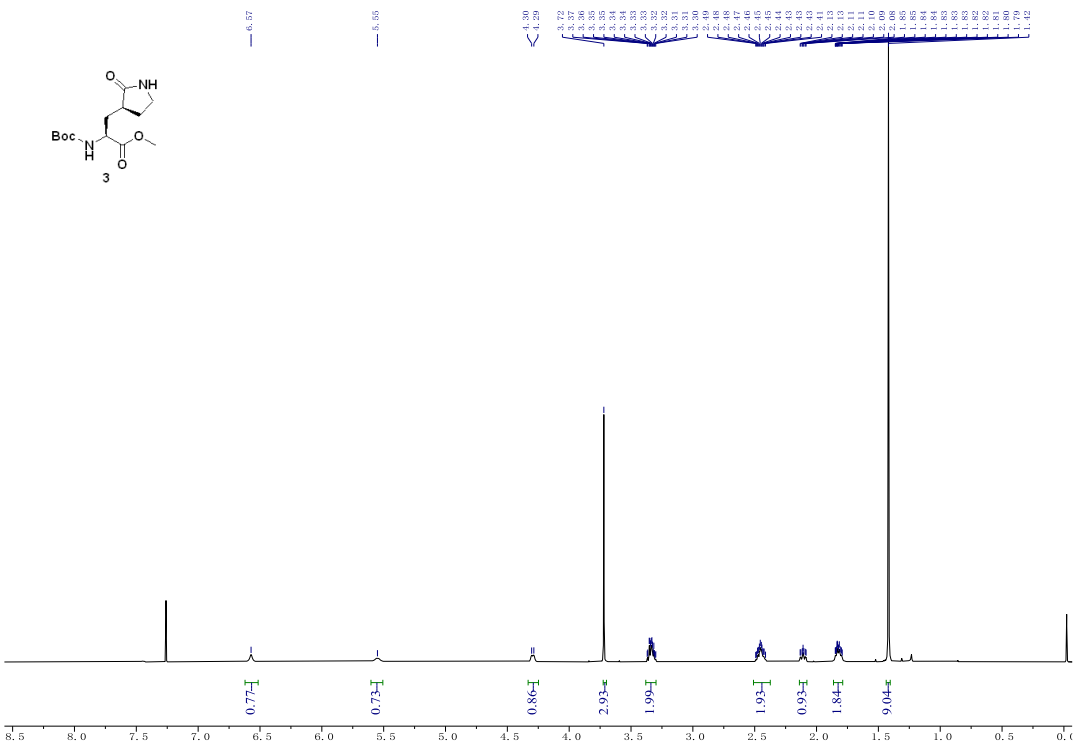

Figure S2. ^{1}H NMR spectrum (600 MHz) of compound **3** in $CDCl_3$.

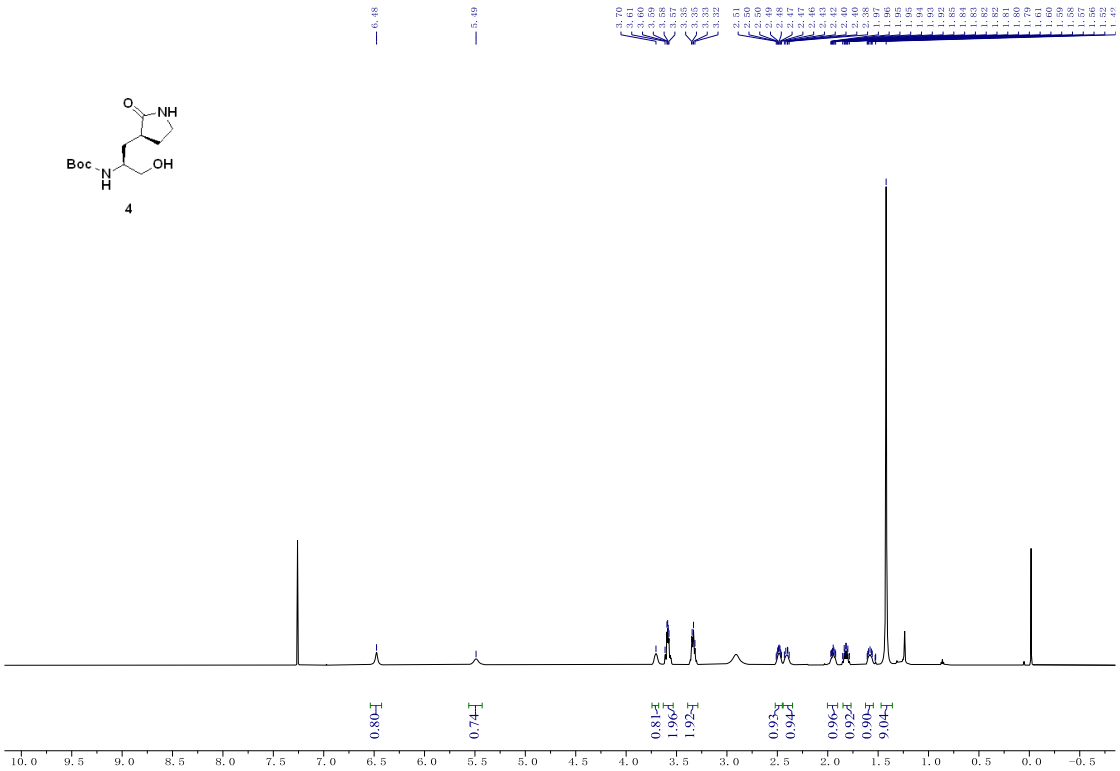

Figure S3. ^{1}H NMR spectrum (600 MHz) of compound **4** in $CDCl_3$.

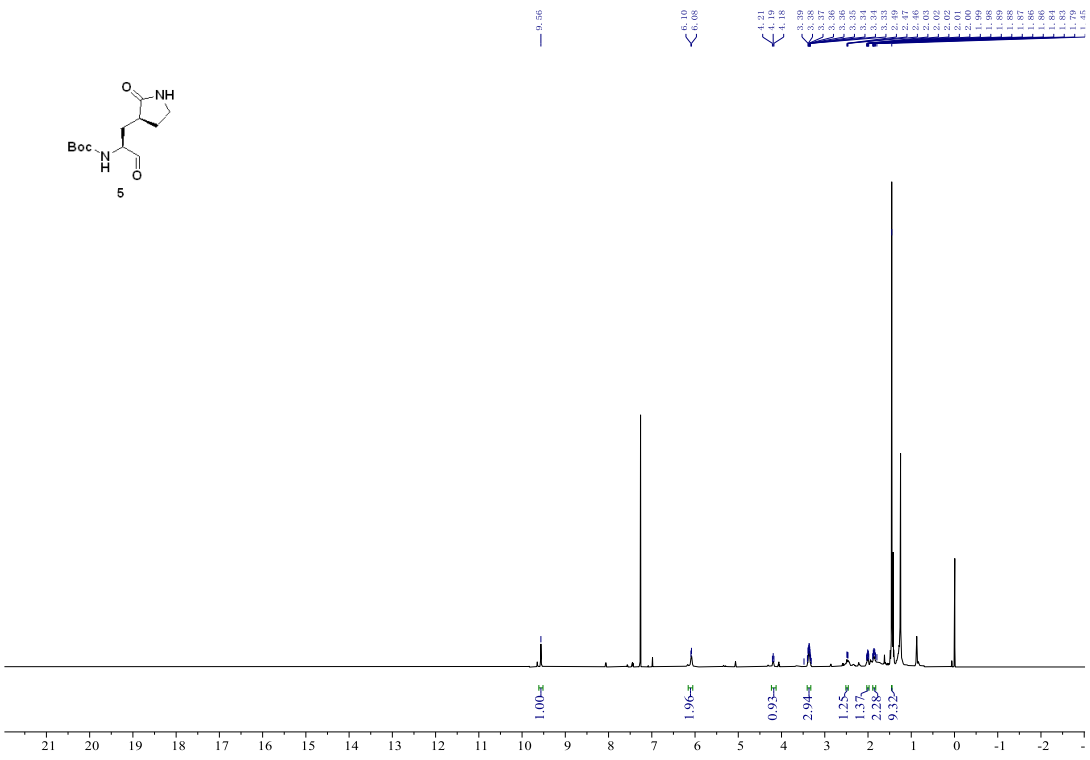

Figure S4. ^{1}H NMR spectrum (600 MHz) of compound **5** in $CDCl_3$.

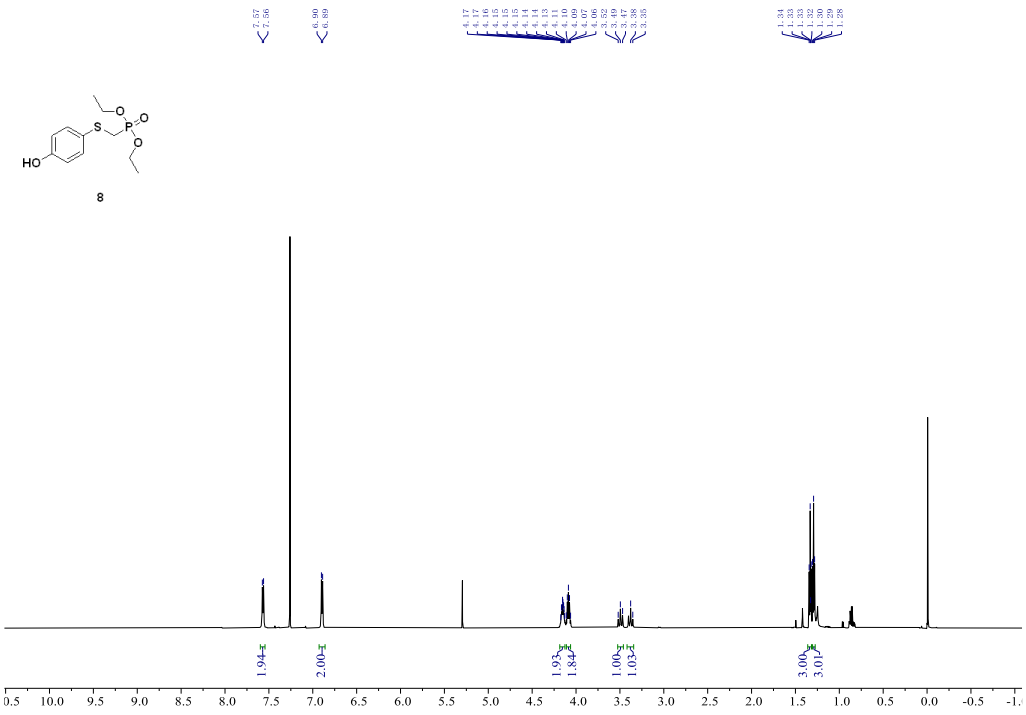

Figure S5. ^{1}H NMR spectrum (600 MHz) of compound **8** in $CDCl_3$.

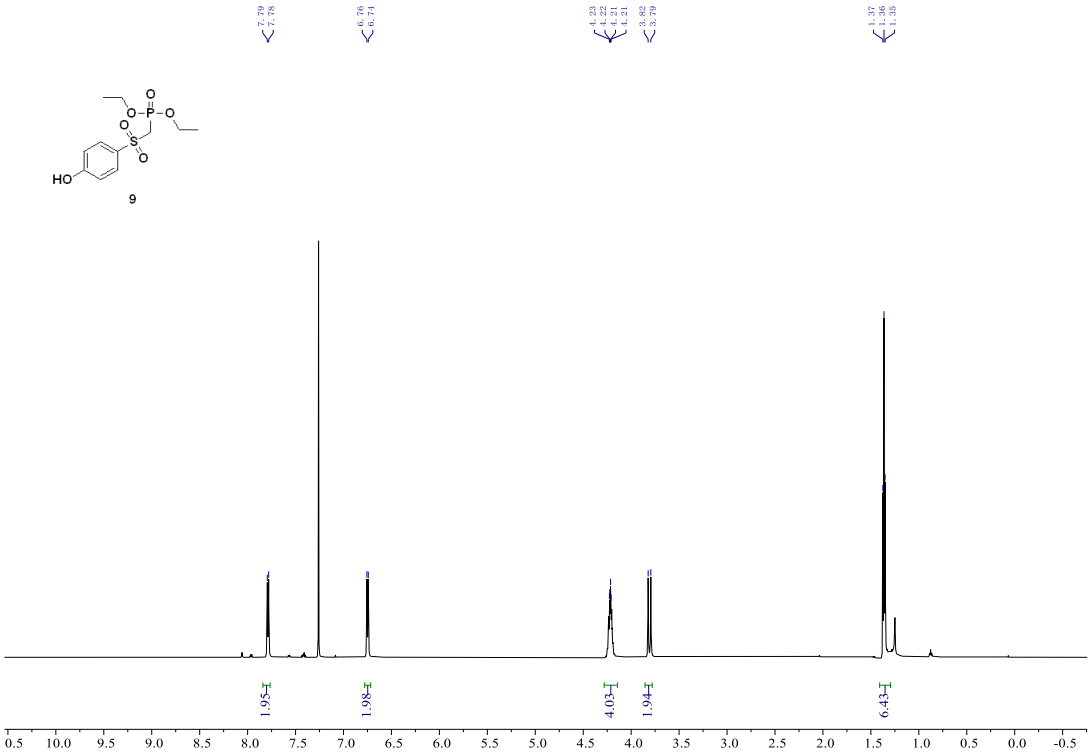

Figure S6. ^{1}H NMR spectrum (600 MHz) of compound **9** in $CDCl_3$.

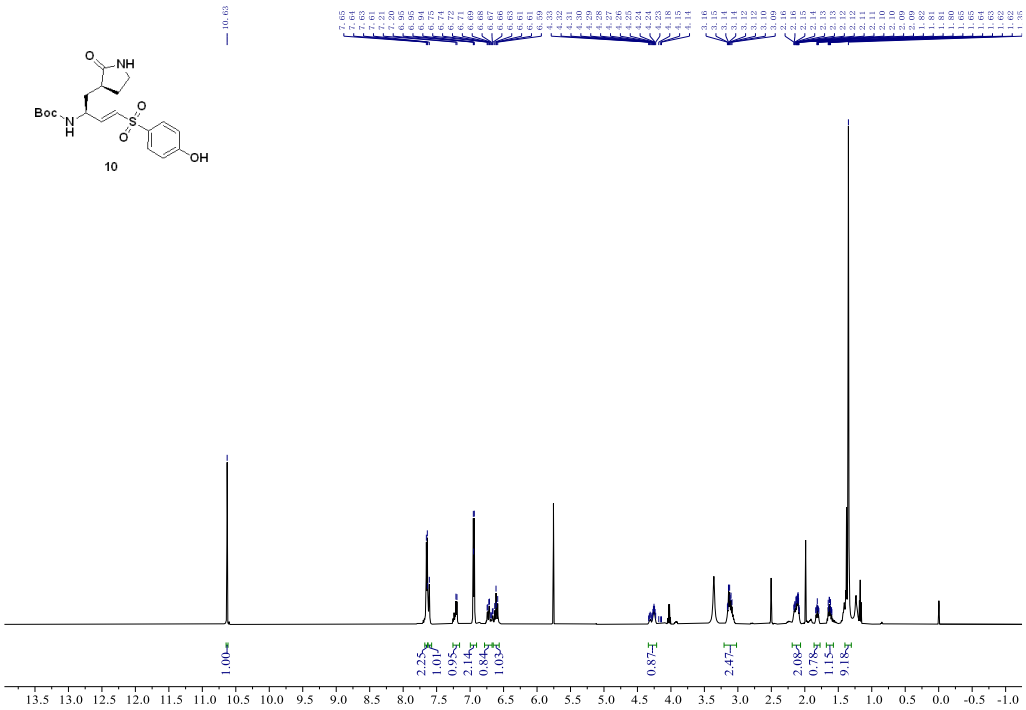

Figure S7. ^{1}H NMR spectrum (600 MHz) of compound **10** in DMSO-d_6.

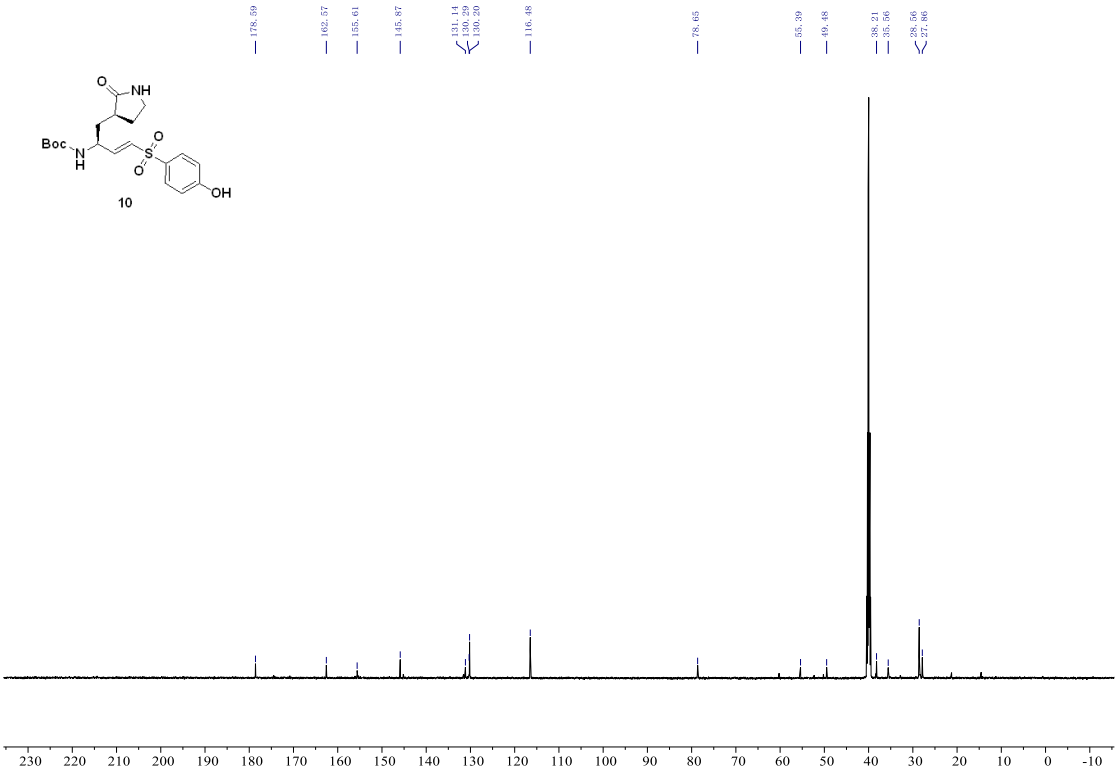

Figure S8. $^{13}C\{^{1}H\}$ NMR spectrum (151 MHz) of compound **10** in DMSO-d_6.

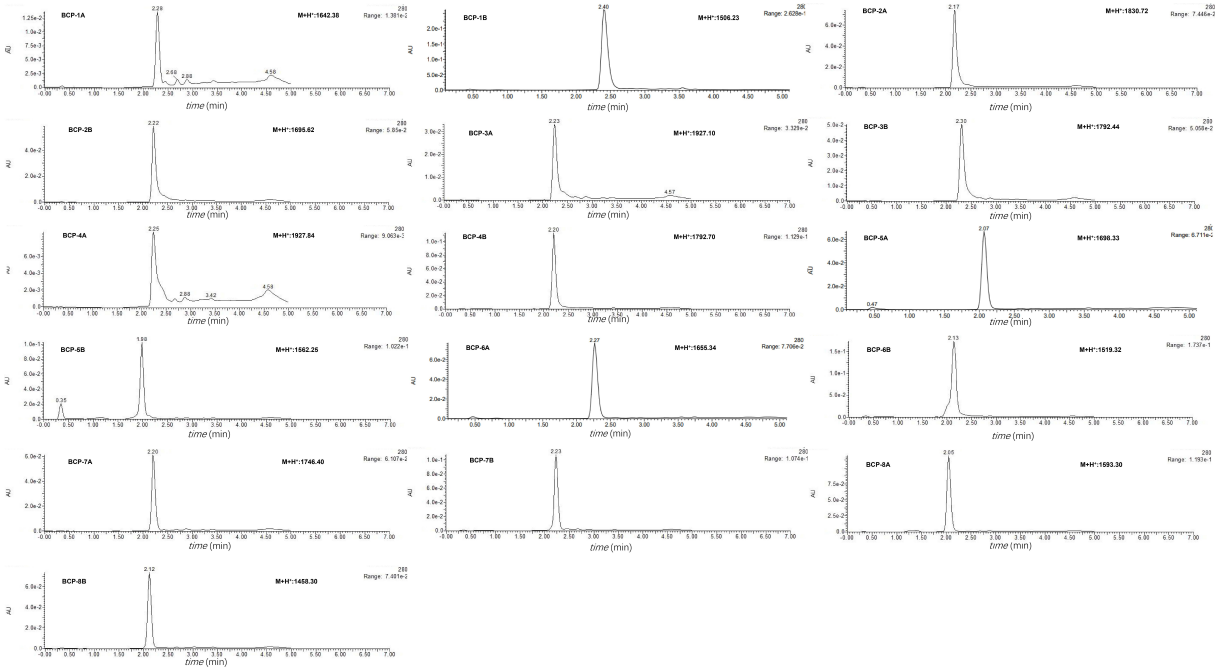

Figure S9. HPLC analysis of the vinyl sulfone bicyclic peptides.

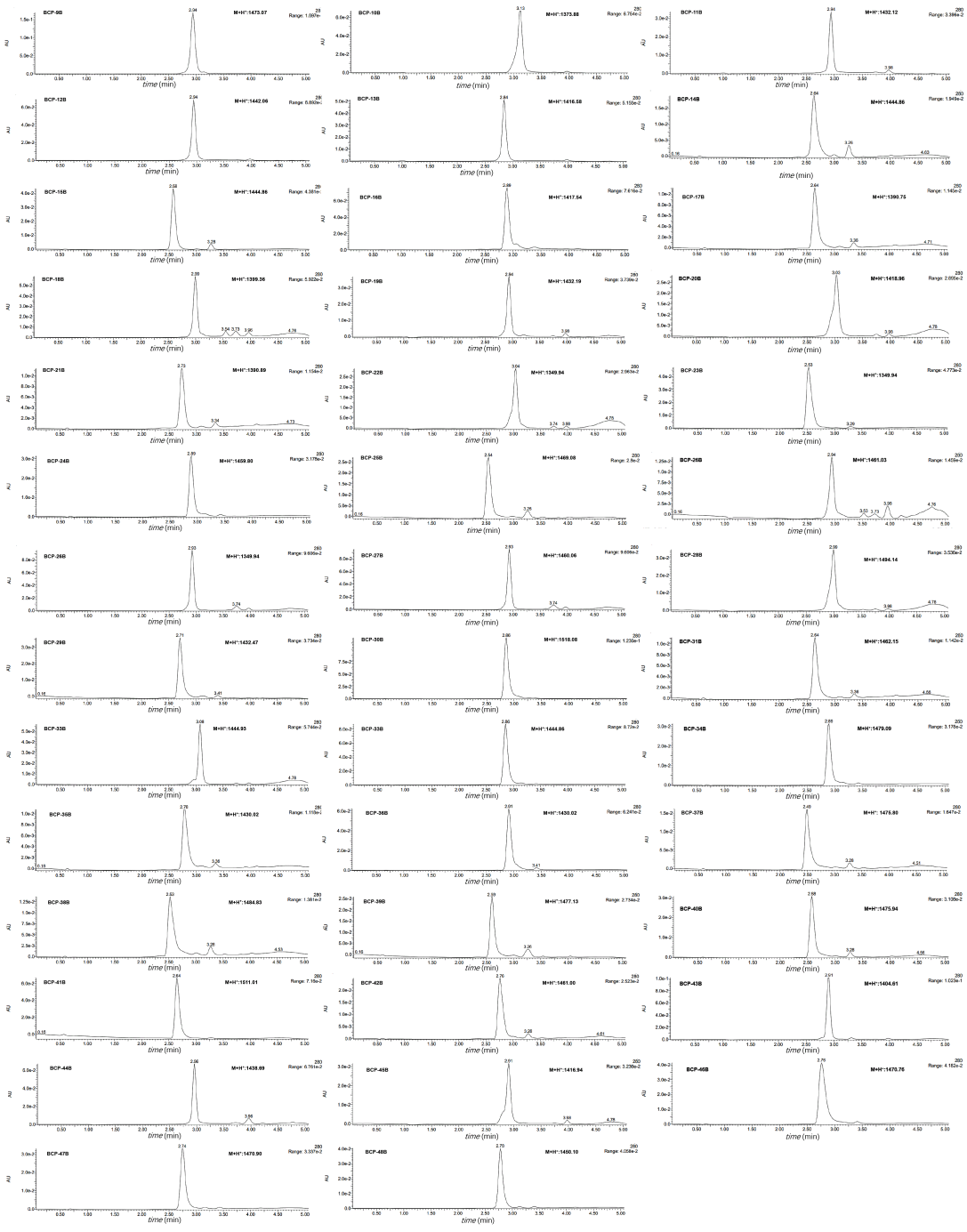

Figure S9 (Continued). HPLC analysis of the vinyl sulfone bicyclic peptides.

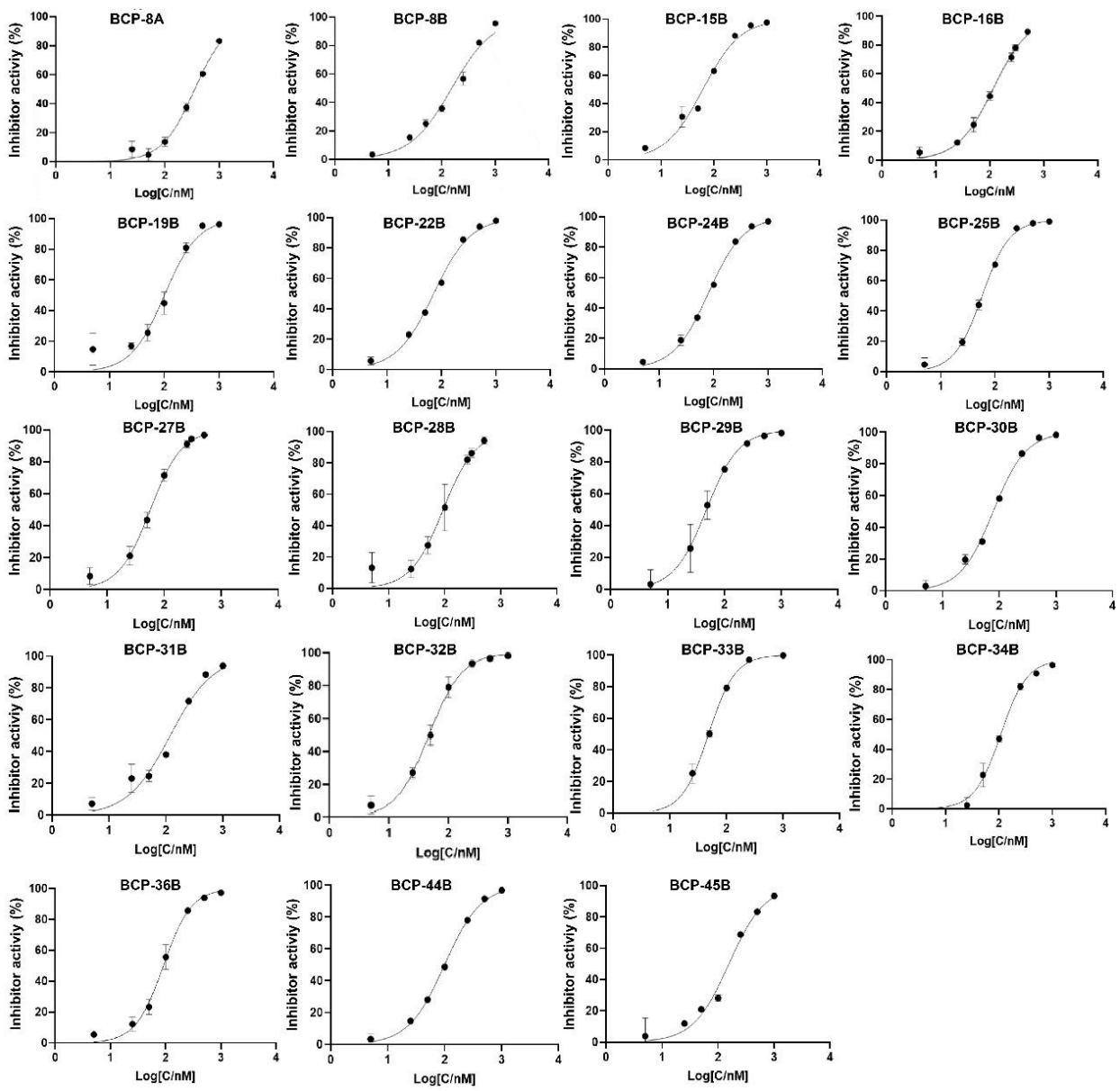

Figure S10. Dose response inhibition studies of the bicyclic peptides.