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Supporting Information

# Fragment-Based De Novo Design Reveals a Small-Molecule Inhibitor of *Helicobacter Pylori* HtrA**

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***Supplementary Information***

## Table of contents

## 1. Experimental section

### 1.1 Computational

#### 1.1.1 Similarity searching

For computational compound screening, we merged the compound collections from Asinex Ltd. (Gold, Platin, Synergy, v2010; Moscow, Russia), Specs (NP, SC, v2010, Delft, The Netherlands), Enamine (historical collection, collections 1-6, v2011; Monmouth Junction, NJ, USA), Chembridge (v2011; San Diego, CA, USA), the LOPAC1280 collection (Sigma-Aldrich, St. Louis, MO, USA), the Dictionary of Natural Products (DNP) (CRC Press, Boca Raton, FL, USA), and a collection of pharmaceutically active reference compounds (COBRA v10.0; inSili.com, Zürich, Switzerland). The resulting screening pool contained 3,255,508 unique compounds. Similarity searching and hit prioritization was performed with a KNIME workflow (knime tech, Zürich, Switzerland). All molecules were standardized using "wash", "protonate", and "add hydrogens" functions from MOE 2011.10 (Chemical Computing Group, Montreal, Canada). Single conformers were computed with Corina 3.4 (Molecular Networks, Erlangen, Germany). All compounds were encoded by substructure fingerprints (Morgan fingerprints with radius = 2; RDkit node obtained from http://www.rdkit.org), and the pharmacophore descriptors CATS[1] and LIQUID.[2] We selected the top-ranking 1000 compounds on the basis of lowest average ranks. The condensed compound library was further reduced to contain only compounds with clog*P* < 6 (MOE 2011.10 implementation). All molecules tested were ordered from the respective supplier. Molecular properties were computed with MOE 2011.10. For activity testing the stock solutions were prepared in DMSO (Sigma-Aldrich Chemie, Switzerland) in a concentration of 20 mM or 2 mM.

#### 1.1.2 *De novo* design

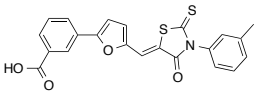
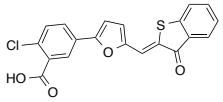
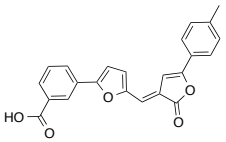
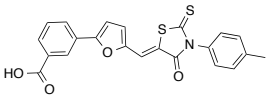
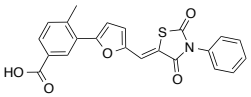
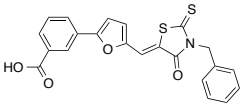
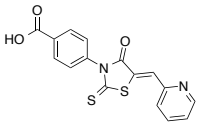
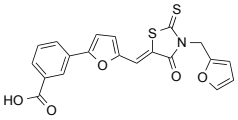
Compound **1a** was standardized using the "wash", "protonate", and "add hydrogens" functions from MOE 2011.10 (Chemical Computing Group, Montreal, Canada). The DOGS software was run with the options α = 0.1-0.9 in steps of 0.1 (favouring a balance of explorative and conservative designs), and 250 start fragments (broad sampling of chemical space). Similarity between the designs and the template was computed using the ISOAK graph kernel method on reduced graph representations.[3] The pooled designs were filtered according to predicted water solubility (logS > -6) (MOE 2011.10), and further processed with a 3D pharmacophore model generated with LigandScout (version 3.02, Inte:Ligand, Vienna, Austria). The pharmacophore model was built manually (see 1.1.3), based on the flexible alignment of eight HtrA inhibitors, and only the molecules matching all pharmacophoric features were considered further.

#### 1.1.3 Pharmacophore modelling

A pharmacophore model for HtrA inhibition was built with LigandScout (version 3.02, Inte:Ligand, Vienna, Austria) using default settings. A consensus model, based on flexible alignment of eight E-cadherin cleavage-inhibiting compounds (Table S1) was used to post-process the *de novo* designed structures. For conformer generation settings the "best settings" option was used, which creates a

maximum of 500 conformations based on the OMEGA conformer generator. For ligand clustering the “alignment score” was chosen and the pharmacophore model was generated with the “pharmacophore fit- and atom overlay” function. The top model (score 0.9230) was then transferred to the screening interface and applied to the set of unique DOGS compounds with clogS > -6. Sixty-five *de novo* generated compounds matched with the model in all query features. One compound from the top five ranked solutions was manually selected for synthesis and testing.

**Table S1.** Reference compounds for pharmacophore model.

| Molecular ID | Molecular structure |
|---|---|
| 1a |  |
| 1b |  |
| 1c |  |
| 1d |  |
| 1f |  |
| 1g |  |
| 1h |  |
| 1i |  |

## 1.2 Chemistry

### 1.2.1 General considerations

All starting materials and solvents were obtained from ABCR Chemicals, Aldrich, Fluka, Alfa Aesar or Acros, and were used without further purification.

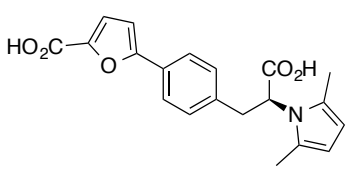
Microwave-assisted syntheses were conducted in a Biotage Initiator reactor. Flash chromatography was carried out on a Biotage IsoleraTM Spektra Systems with Biotage SNAP Ultra flash chromatography cartridges of 60 g ($C_{18}$). Melting points (mp) were recorded on a Büchi M560 apparatus and are uncorrected. Analytical LC-MS was carried out in a Hitachi LaChrom Ultra – Advion CMS system, equipped with a Nucleodur $C_{18}$ HTec column, under an appropriate gradient of acetonitrile : $H_2O$ (+ 0.1% formic acid in each solvent), and a total flow rate of 0.5 mL/min. The mass spectrometer was operated in positive-ion mode with ESI. Proton and carbon nuclear magnetic resonance spectra ($^1H$ and $^{13}C$ NMR, respectively) were recorded on Bruker Avance 400 or 500 spectrometers. Chemical shifts (δ) are reported in units of parts per million (ppm) downfield from SiMe4 (δ 0.0) and relative to the respective solvent's peak. Multiplicities are given as: s (singlet), d (doublet), dd (double of doublet), m (multiplet), br.s (broad singlet), t (triplet). $^1H$-$^1H$ Coupling constants (*J*) are reported in Hertz (Hz).

### 1.2.2 Organic syntheses

All compounds were obtained following Suzuki coupling and Paal-Knorr pyrrole syntheses.

Boronic acid (1 molar eq.) and bromofuran (1 molar eq.) were dissolved in a mixture of 1M $Na_2CO_3$ (2 mL) and acetonitrile (1 mL). $Pd(PPh_3)_2Cl_2$ (20 mol%) was added, followed by degasing under nitrogen. The vial was sealed and reacted for 30 minutes at 150°C under microwaves.[4] The solvent was evaporated under reduced pressure and the resulting crude product was purified by reverse phase flash chromatography (Eluent A: acetonitrile + 0.1% formic acid; Eluent B: $H_2O$ + 0.1% formic acid) with a 5-50% acetonitrile gradient run over 16 minutes. The solvent was evaporated affording the required intermediate. The intermediate compound (1 molar eq.) and hexane-2,5-dione (2 molar eq.) were dissolved in 300 $\mu$L AcOH and reacted at 170°C for 15 minutes under microwaves.[5] The solvent was evaporated under reduced pressure and the resulting crude product was purified by reverse phase flash chromatography (Eluent A: acetonitrile + 0.1% formic acid; Eluent B: $H_2O$ + 0.1% formic acid) with a 30-95% acetonitrile gradient run over 16 minutes. The solvent was evaporated affording title compounds.

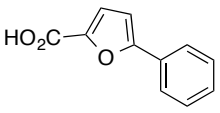
**(*S*)-5-(4-(2-carboxy-2-(2,5-dimethyl-1*H*-pyrrol-1-yl)ethyl)phenyl) furan-2-carboxylic acid (2)**



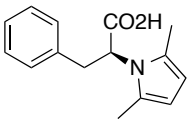

Reaction of 4-borono-L-phenylalanine (1 molar eq.) and 5-bromo-2-furoic acid (1 molar eq.) afforded the required intermediate (*S*)-5-(4-(2-amino-2-carboxyethyl)phenyl)furan-2-carboxylic acid (**S1**) as white powder, 64%; m.p. > 240ºC, LC-MS *m/z* 317.2 $(MH + CH_3CN)^+$, $R_t$ = 4.42 min. Reaction of the intermediate compound (1 molar eq.) and hexane-2,5-dione (2 molar eq.) afforded compound **2** as red powder, 100%, m.p. = 158°C; $R_t$ = 6.96 min (100%); $^1H$ NMR ($CD_3OD$,

400.13 MHz) δ 1.85 (6H, s, $2CH_3$), 3.12 (1H, dd, *J* = 14.0 and 11.2 Hz, $CH_2$), 3.41 (1H, dd, *J* = 13.6 and 4.0 Hz, $CH_2$), 4.82 (1H, m, CH), 5.52 (2H, s, Ar-H), 6.74 (1H, d, *J* = 3.6 Hz, Ar-H), 6.84 (2H, d, *J* = 8.4 Hz, Ar-H), 7.15 (1H, d, *J* = 3.6 Hz, Ar-H), 7.50 (2H, d, *J* = 8.0 Hz, Ar-H); $^{13}C$ NMR ($CD_3OD$, 100.61 Hz) δ 13.29, 38.39, 59.76, 107.33, 107.89, 120.54, 121.19, 125.62, 129.37, 131.14, 140.30, 145.23, 158.92, 162.01, 173.98. HRMS-ESI calc. ($C_{20}H_{19}NO_5+H^+$) = 353.1263 Da, found = 354.1337 Da.

**5-Phenylfuran-2-carboxylic acid (3)** Obtained from reaction of 5-bromo-2-furoic acid and phenylboronic acid as a white powder, 62%, m.p. = 142-144 ºC. $^1H$ NMR ($CD_3OD$, 400.13 MHz) δ 6.95 (1H, d, *J* = 3.6 Hz, Ar-H), 7.30 (1H, d, *J* = 3.6 Hz, Ar-H), 7.38 (1H, m, Ar-H), 7.47 (2H, m, Ar-H), 7.85 (2H, m, Ar-H). $^{13}C$ NMR ($CD_3OD$, 100.61 MHz) δ 108.12, 121.18, 125.72, 129.99, 130.02, 130.96, 145.26, 158.95, 161.90. HRMS-ESI calc. ($C_{11}H_8O_3+H^+$) = 189.0546 Da, found = 189.0550 Da.

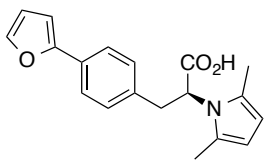



**(*S*)-2-(2,5-Dimethyl-1H-pyrrol-1-yl)-3-phenylpropanoic acid (4)**

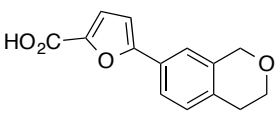



Obtained from the reaction of L-phenylalanine and hexane-2,5-dione as a brown gum, 34%. $^1H$ NMR ($CD_3OD$, 400.13 MHz) δ 1.82 (6H, br.s, $CH_3$), 3.07 (1H, dd, *J* = 14.0 and 11.2 Hz, $CH_2$), 3.38 (1H, dd, *J* = 14.0 and 4.0 Hz, $CH_2$), 4.76 (1H, m, CH), 5.50 (2H, s, Ar-H), 6.74-6.78 (2H, m, Ar-H), 7.02-7.03 (3H, m, Ar-H). $^{13}C$ NMR ($CD_3OD$, 100.61 MHz) δ 13.20, 38.60, 60.12, 107.06, 127.58, 127.79, 129.25, 130.43, 139.18, 174.16. HRMS-ESI calc. ($C_{15}H_{15}NO_2+H^+$) = 242.1187 Da, found = 242.1181 Da.

**(*S*)-2-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-3-(4-(furan-2-yl)phenyl)propanoic acid (5)** Reaction of 2-bromofuran and 4-borono-L-phenylalanine afforded the intermediate (*S*)-2-amino-3-(4-(furan-2-yl)phenyl)propanoic acid as a white powder, 70%, m.p. > 220 ºC. $^1H$ NMR (DMSO-$d_6$, 400.13 MHz) δ 2.86 (1H, m, $CH_2$), 3.15 (1H, m, $CH_2$), 3.5 (1H, m, CH), 6.59 (1H, d, *J* = 2.8 Hz, Ar-H), 6.91 (1H, d, *J* = 2.8 Hz, Ar-H), 7.32 (2H, d, *J* = 8.0 Hz, Ar-H), 7.62 (2H, d, *J* = 8.0 Hz, Ar-H), 7.73 (1H, s, Ar-H). Reaction of the intermediate with hexane-2,5-dione afforded **5** as red gum, 63%. $^1H$ NMR ($CD_3OD$, 400.13 MHz) δ 1.97 (6H, s, $2CH_3$), 3.21 (1H, dd, *J* = 14.0 and 11.2 Hz, $CH_2$), 3.50 (1H, dd, *J* = 13.6 and 4.0 Hz, $CH_2$), 4.91 (1H, m, CH), 5.64 (2H, s, Ar-H), 6.49 (1H, m, Ar-H), 6.68 (1H, d, *J* = 4.0 Hz, Ar-H), 6.90 (2H, d, *J* = 8.4 Hz, Ar-H), 7.48 (2H, d, *J* = 8.4 Hz, Ar-H), 7.52 (1H, d, *J* = 2.4 Hz, Ar-H); $^{13}C$ NMR ($CD_3OD$, 100.61 Hz) δ 13.26, 38.38, 60.01, 105.69, 112.64, 124.55, 124.60, 129.19, 130.74, 130.84, 138.42, 143.16, 155.23, 174.21. HRMS-ESI calc. ($C_{19}H_{19}NO_3+H^+$) = 310.1438 Da, found = 310.1431 Da.



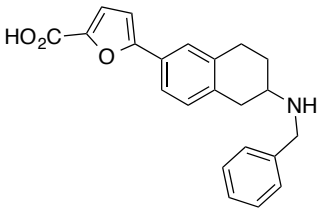

**5-(Isochroman-7-yl)furan-2-carboxylic acid (6)**




Obtained from reaction of 5-bromo-2-furoic acid and 7-bromoisochromane as a white solid, 45%, m.p. = 181-183 ºC. $^1H$ NMR ($CD_3OD$, 400.13 MHz) δ 2.76 (2H, t, *J* = 6.0 Hz, $CH_2$), 3.87 (2H, t, *J* = 5.6 Hz, $CH_2$), 4.68 (2H, s, $CH_2$), 6.77 (1H, d, *J* = 3.6 Hz, Ar-H), 7.11 (1H, d, *J* = 8.0 Hz, Ar-H), 7.16 (1H, d, *J* = 3.6 Hz, Ar-H),

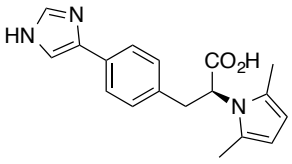
7.39 (1H, s, Ar-H), 7.51 (1H, dd, *J* = 8.0 and 1.6 Hz, Ar-H). $^{13}$C NMR ($CD_3OD$, 100.61 MHz) δ 29.23, 66.40, 68.83, 107.82, 121.23, 121.72, 123.96, 128.87, 130.58, 135.69, 136.90, 145.09, 158.96, 161.87. HRMS-ESI calc. ($C_{14}H_{12}O_4$+$H^+$) = 245.0808 Da, found = 245.0804 Da.

**5-(6-(Benzylamino)-5,6,7,8-tetrahydronaphthalen-2-yl)furan-2-carboxylic acid, formic acid salt (7)**



Reaction of 5-bromo-2-furoic acid and 6-bromo-2-tetralone afforded the intermediate 5-(6-oxo-5,6,7,8-tetrahydronaphthalen-2-yl)furan-2-carboxylic acid as a brown powder, 81%, m.p. 190-192 ºC. $^1$H NMR ($CD_3OD$, 400.13 MHz) δ 2.45 (2H, t, *J* = 6.8 Hz, $CH_2$), 3.05 (2H, t, *J* = 6.8 Hz, $CH_2$), 3.55 (2H, s, $CH_2$), 6.82 (1H, d, *J* = 3.6 Hz, Ar-H), 7.13 (1H, d, *J* = 8.0 Hz, Ar-H), 7.18 (1H, d, *J* = 3.6 Hz, Ar-H), 7.57 (1H, d, *J* = 8.0 Hz, Ar-H), 7.65 (1H, s, Ar-H). The intermediate (1 molar eq.) and benzylamine (1 molar eq.) were dissolved in dichloroethane (4 mL/mmol). $NaBH(OAc)_3$ (1.2 molar eq.) was added to the solution and the reaction stirred at room temperature until completion. The crude product was purified via preparative HPLC (Eluent A: acetonitrile + 0.1% formic acid; Eluent B: $H_2O$ + 0.1% formic acid) with a 5-50% acetonitrile gradient run over 16 minutes, to afford a brown oil, 64%. $^1$H NMR ($CD_3OD$, 400.13 MHz) δ 1.91 (1H, m, $CH_2$), 2.44 (1H, m, $CH_2$), 2.90-3.05 (3H, m, $CH_2$), 3.37 (1H, m, $CH_2$), 3.60 (1H, m, CH), 4.37 (2H, s, $CH_2$), 6.79 (1H, d, *J* = 3.6 Hz, Ar-H), 7.10 (1H, d, *J* = 3.6 Hz, Ar-H), 7.17 (1H, d, *J* = 8.0 Hz, Ar-H), 7.47-7.52 (3H, m, Ar-H), 7.57-7.60 (4H, m, Ar-H), 8.26 (1H, br.s, NH). $^{13}$C NMR ($CD_3OD$, 100.61 Hz) δ 27.01, 28.51, 31.99, 49.85, 55.79, 107.66, 118.47, 123.65, 125.57, 130.12, 130.36, 130.67, 130.74, 131.02, 132.90, 133.60, 136.65, 148.97, 156.95, 165.03, 166.48. HRMS-ESI calc. ($C_{22}H_{21}NO_3$+$H^+$) = 348.1594 Da, found = 348.1600 Da.

**(*S*)-3-(4-(1*H*-Imidazol-4-yl)phenyl)-2-(2,5-dimethyl-1H-pyrrol-1-yl)propanoic acid (8)**



Reaction of 4-borono-L-phenylalanine and 4-bromo-1*H*-imidazole afforded the intermediate (*S*)-3-(4-(1*H*-imidazol-4-yl)phenyl)-2-aminopropanoic acid as a white powder, 45%, m.p. > 250 ºC. $^1$H NMR ($D_2O$, 400.13 MHz) δ 3.02 (1H, dd, *J* = 14.2 and 8.0 Hz, $CH_2$), 3.17 (1H, dd, *J* = 14.2 and 5.2 Hz, $CH_2$), 3.90 (1H, d, *J* = 8.0 and 5.2 Hz, CH), 7.23 (2H, d, *J* = 8.0 Hz, Ar-H), 7.40 (1H, s, Ar-H), 7.57 (2H, d, *J* = 8.0 Hz, Ar-H), 7.78 (1H, s, Ar-H), 8.35 (1H, br.s, NH). Reaction of the intermediate with hexane-2,5-dione afforded **8** as brown oil, 36%. $^1$H NMR (DMSO-$d_6$, 400.13 MHz) δ 1.92 (6H, s, $CH_3$), 3.05 (1H, dd, *J* = 14.0 and 12 Hz, $CH_2$), 3.40 (1H, dd, *J* = 14.0 and 4.0 Hz, $CH_2$), 4.84 (1H, *J* = 12.0 and 4.0 Hz, CH), 5.52 (2H, s, Ar-H), 6.87 (2H, d, *J* = 8.4 Hz, Ar-H), 7.49 (1H, s, Ar-H), 7.53 (2H, d, *J* = 8.4 Hz, Ar-H), 7.67 (1H, s, Ar-H). $^{13}$C NMR (DMSO-$d_6$, 100.61 MHz) δ 12.95, 36.69, 58.74, 105.53, 123.81, 123.92, 127.45, 129.26, 131.83, 135.77, 135.86, 137.91, 172.07. HRMS-ESI calc. ($C_{18}H_{19}N_3O_2$+$H^+$) = 310.1550 Da, found = 310.1555 Da.

### 1.3 Biophysical methods

#### 1.3.1 Dynamic light scattering

Dynamic light scattering (90Plus Particle Size Analyzer, Brookhaven Instruments Corp., USA) was used to determine compound aggregation potential. The particle sizes were measured at 25 °C under default settings for water and the dust filter parameter was set to 50.

#### 1.3.2 Surface plasmon resonance

Binding experiments were performed on an SPR-2/SPR-4 instrument (Sierra Sensors GmbH, Hamburg, Germany) at 25 °C with a flow rate of 25 µL/mL. The measurements were performed on carboxymethyl dextran matrix high-density sensor chips (Affinity Sensor HC, Sierra Sensors, Hamburg, Germany) on which we immobilized wild type (*wt*) HtrA. Immobilization procedure (running buffer for all three steps: 10 mM HEPES, 150 mM NaCl, 0.005% Tween):

(i) amine coupling with an activation solution (200 mM *N*-ethyl-3-(3-dimethylaminopropyl)carbodiimide and 50 mM *N*-hydroxysuccinimide),

(ii) protein injection of 20 µg/mL *wt*HtrA in 10 mM HEPES (Fisher Scientific),

(iii) inactivation of excess coupling groups with 1 M ethanolamine-HCl (pH 8.5).

Samples were analyzed in running buffer containing 10 mM HEPES buffered saline, 150 mM NaCl, 0.005% Tween, 3% DMSO. For sample preparation, 20 mM compound stock solutions (100% DMSO) were sequentially diluted to the required test concentrations with DMSO-free running buffer to obtain 3% final DMSO content. Monitoring of the integrity of the enzyme was provided by regular injection of compound **1a** as reference.

#### 1.3.2 STD NMR

The NMR measurements were performed on a Bruker Avance III 500 MHz spectrometer (5 mm BBI probe and *z*-axis gradient) at 298 K. We prepared 20 mM stock solutions in DMSO-$d_6$ of compound **2** and β-methyl-D-glucoside. All samples were measured under conditions of 5 % DMSO-$d_6$, $D_2O$, 150 mM NaCl at pH 6.3 after 1 hour of pre-incubation. $^1$H STD-NMR experiments were performed under saturation at 0.8 ppm (*on*-resonance spectra), 40 ppm (*off*-resonance spectra, 50 dB, 190 Hz) and a total saturation time of 2 s (soft pulse delay of 100 µs). In experiment A we recorded a proton NMR of compound **2** (1 mM), in experiment B a proton NMR of HtrA (10 µM). STD experiment of a sample of compound **2** (1 mM) and HtrA (10 µM) led to the spectra C (*off*-resonance) and D (*on*-resonance), while the STD experiment with a sample of the non-binder β-methyl-D-glucoside (1 mM) and HtrA (10 µM) led to the spectra E (*off*-resonance) and F (*on*-resonance). The STD experiment with compound **2**, β-methyl-D-glucoside and HtrA (1 : 100 : 100; 10 µM : 10,000 µM : 10,000 µM) yielded the spectra G (*off*-resonance) and H (*on*-resonance).

## 1.4 Biology

### 1.4.1 Western blots

Purification of recombinant *H. pylori* HtrA and *in vitro* cleavage assays were performed like previously described.[6] In summary, the cleavage assay was executed by incubating 200 ng recombinant HtrA with 50 ng recombinant E-cadherin (R&D Systems, Germany) in 50 mM HEPES pH 7.5 for 16 hours at 37°C. Compounds were added at several concentrations as indicated. The resulting proteins were separated by SDS PAGE. Specific antibodies against HtrA[7] or the extracellular domain of E-cadherin (sc7870, Santa Cruz Biotechnology, Germany) were utilized to analyze the proteins in the Western blot. All experiments were carried out at least in triplicates.

### 1.4.2 Transmigration assay

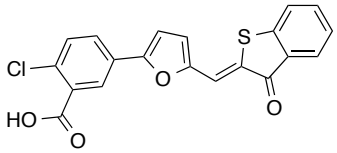
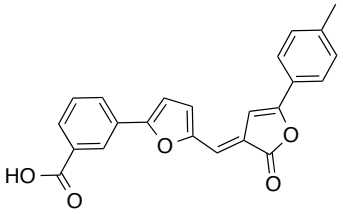
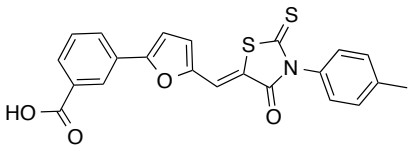
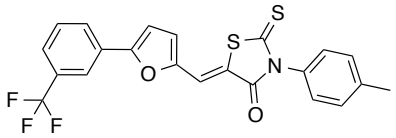
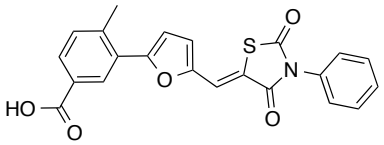
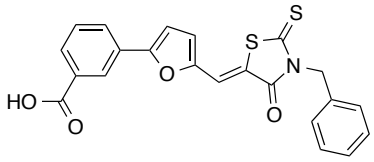
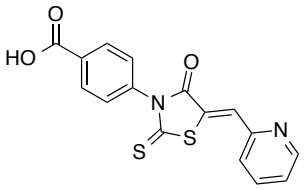
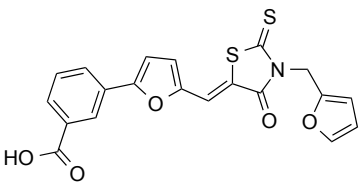
*Helicobacter pylori* strain P12 was grown under microaerophilic conditions at 37 °C on agar plates containing 10% horse serum. *Campylobacter jejuni* strain 81-176 was cultivated for 48 hours on *Campylobacter* blood-free selective Agar Base using *Campylobacter* growth supplement (Oxoid, Germany) at 37 °C (generated by CampyGen, Oxoid).

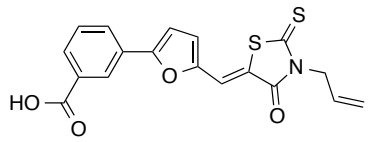
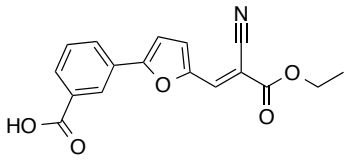
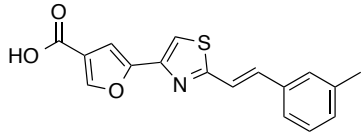
The HtrA inhibitory effect of the compounds in infection experiments was analyzed by performing transwell infection assays with polarized epithelial cells. To investigate the inhibitory potential, different concentrations of the compounds (5, and 10 µM) with solvent alone as control were added to the infected cells. Cell penetration of the bacteria in presence and absence of a potential inhibitor was detected and compared. For the infection assay, human gastric adenocarcinoma cell line MKN-28 was cultivated on transwell filters in RPMI1640 medium (Biochrom, Germany) containing 4 mM glutamine (Invitrogen, Germany) and 10% FCS (Biowest, France) under humidified conditions at 37°C. The cells on the transwell filters were incubated with bacteria at MOI 50 for the indicated periods of time per experiment. Quantification of the number of transmigrated *C. jejuni* and *H. pylori* was performed as described.[8] Briefly, the number of transmigrated bacteria was determined by taking aliquots from the basal filter chamber and defined as colony forming units (cfu). All infection assays were performed in triplicates and significance analyzed with Dunnett's test one-way ANOVA.

## 2. Supplementary data

### 2.1 Similarity searching

**Table S2.** Hit list based on compound **1a** as the query for similarity searching.

| Molecular ID | Molecular structure | Molecular weight (g/mol) | Inhibitory potential[a] | Binding potential[b] |
|---|---|---|---|---|
| **1b** |  | 383 | + | + |
| **1c** |  | 372 | + | + |
| **1d** |  | 421 | + | + |
| **1e** |  | 445 | - | - |
| **1f** |  | 405 | + | + |
| **1g** |  | 421 | + | - |
| **1h** |  | 342 | + | - |
| **1i** |  | 411 | + | + |

| | | | | |
|---|---|---|---|---|
| **1j** |  | 371 | - | + |
| **1k** |  | 311 | - | - |
| **1l** |  | 311 | + | + |

[a] E-cadherin cleavage assay; test compound concentration: 100 µM
[b] SPR binding assay; test compound concentration: 25 µM

## 2.1 Pharmacophore model

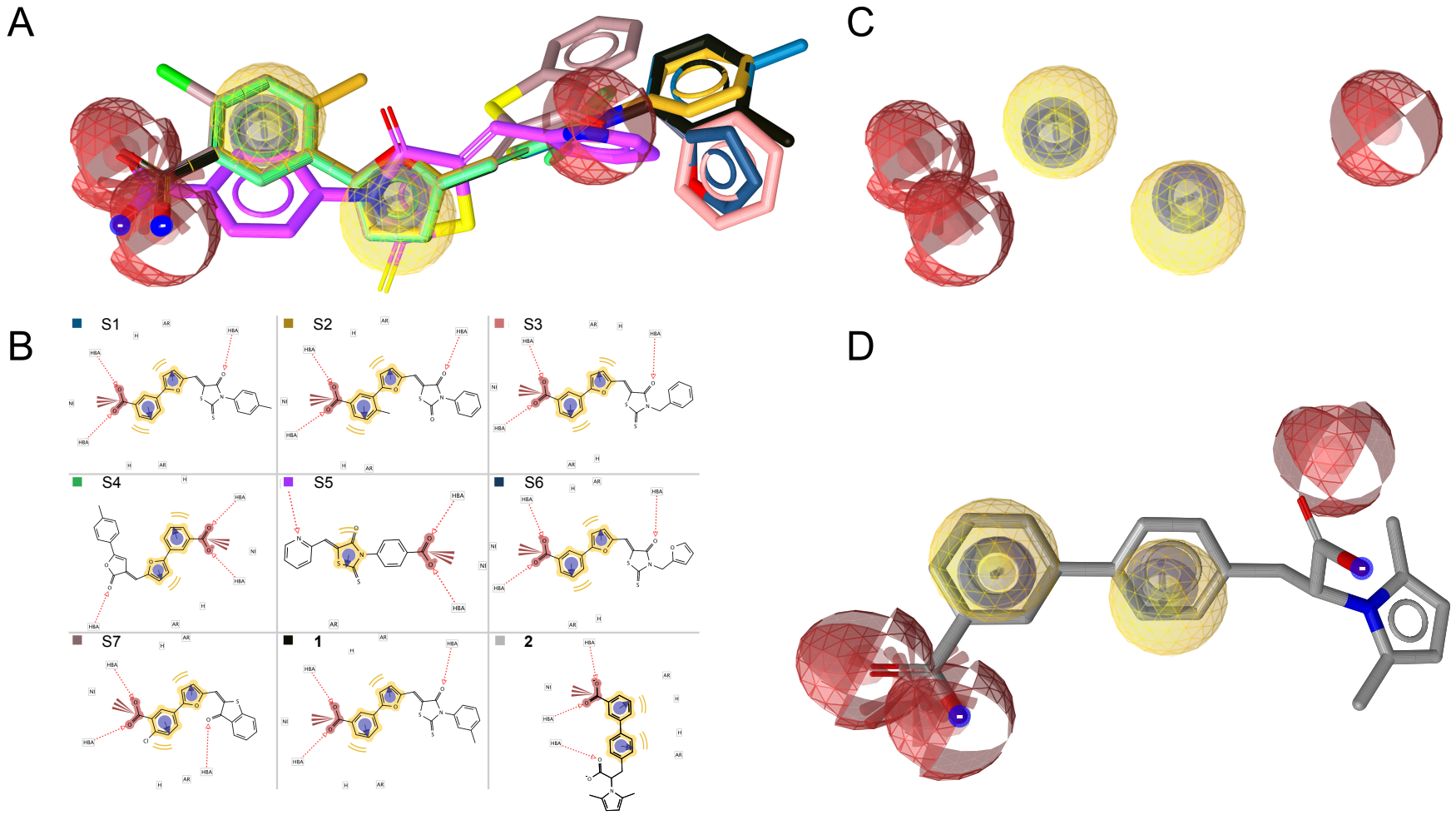



**Figure S1.** A) Flexible alignment of compounds **1a-d**, **1f-i** to generate a pharmacophore model; B) 2D pharmacophore depiction for compounds reported in the present study; C) 3D Pharmacophore model used for post-processing *de novo* designed structures suggested by DOGS; Aromatic interaction depicted as blue ring, hydrophobic interactions as yellow spheres, hydrogen bond acceptor as red spheres, and charged interactions as red lines; D) Overlay of compound **2** to the pharmacophore model, showing perfect feature match.

## 2.2 NMR

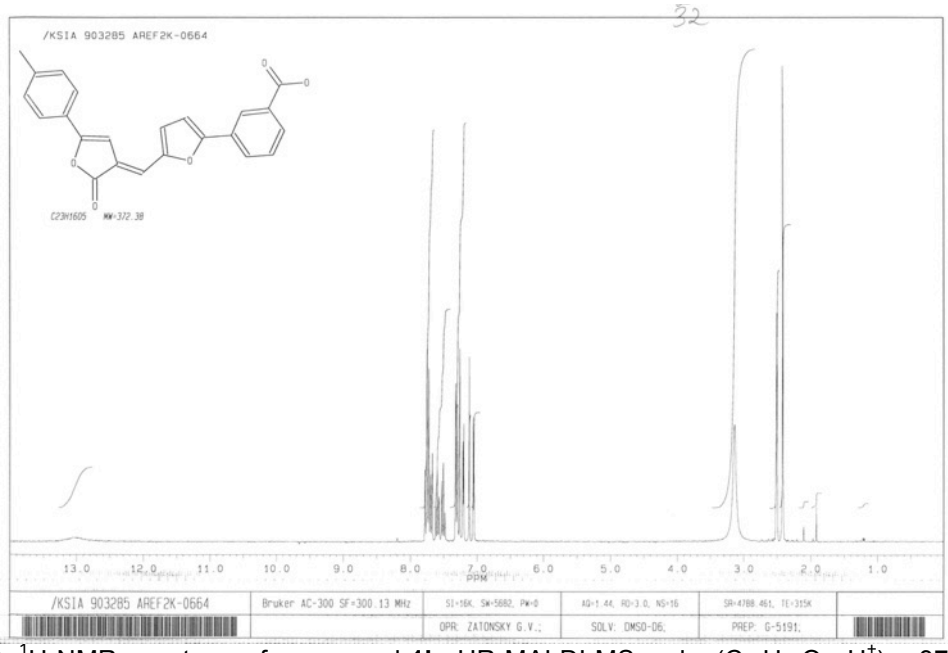



**Figure S2**. $^{1}$H NMR spectrum of compound **1b**. HR-MALDI-MS: calc. ($C_{23}H_{16}O_5$+$H^+$) = 372.1076 Da, found = 373.1076 Da

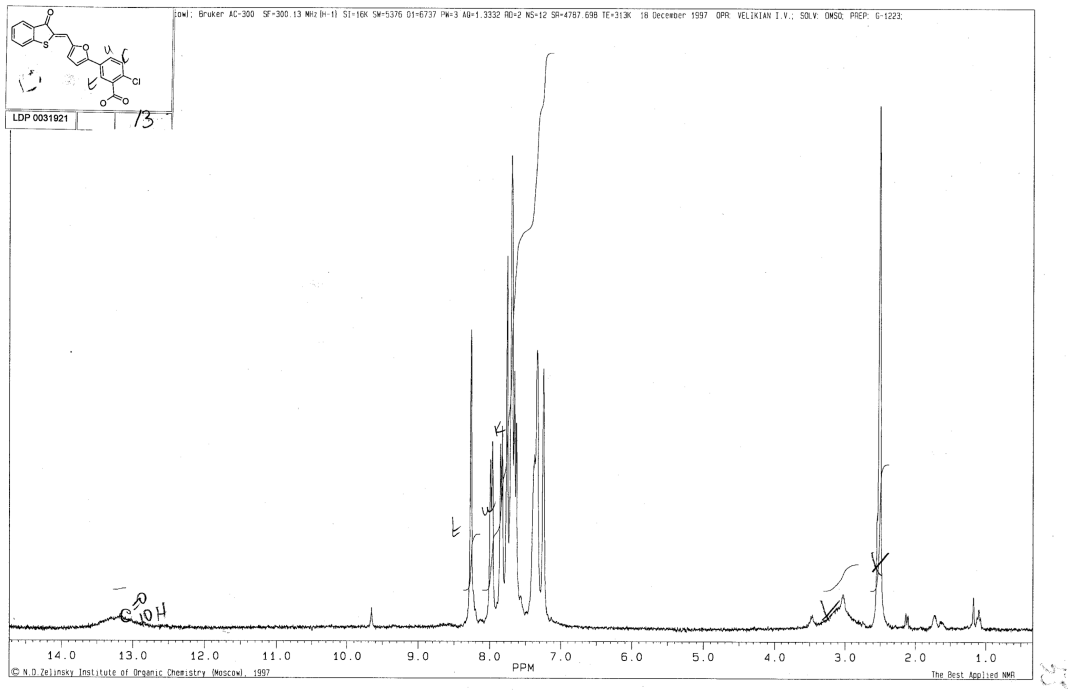



**Figure S3**. $^{1}$H NMR spectrum of compound **1c**. HR-MALDI-MS: calc. ($C_{20}H_{11}ClO_4S$+$H^+$) = 383.0145 Da, found = 383.0141 Da.

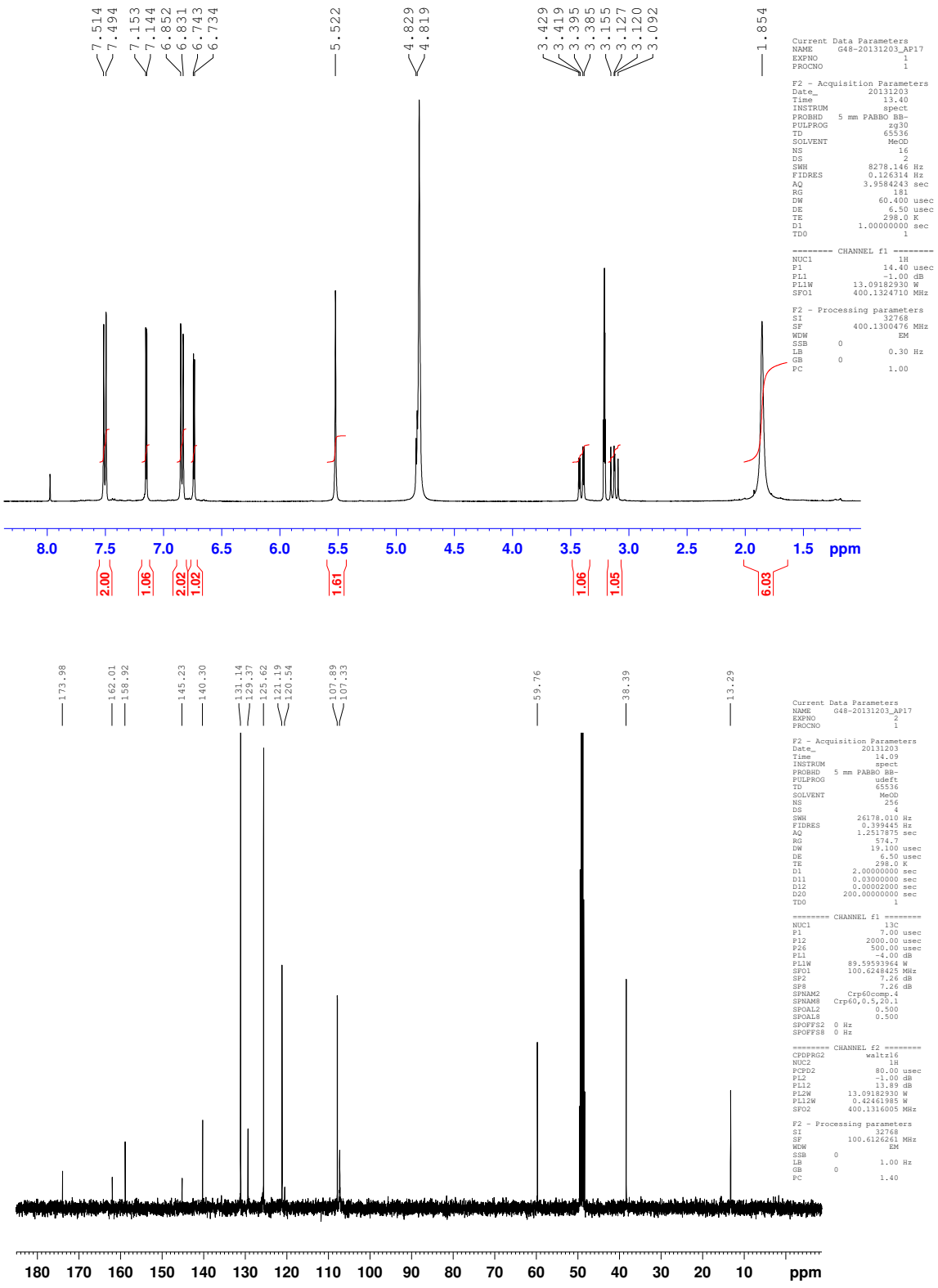



**Figure S4.** $^{1}H$ and $^{13}C$ NMR spectra for compound **2**.

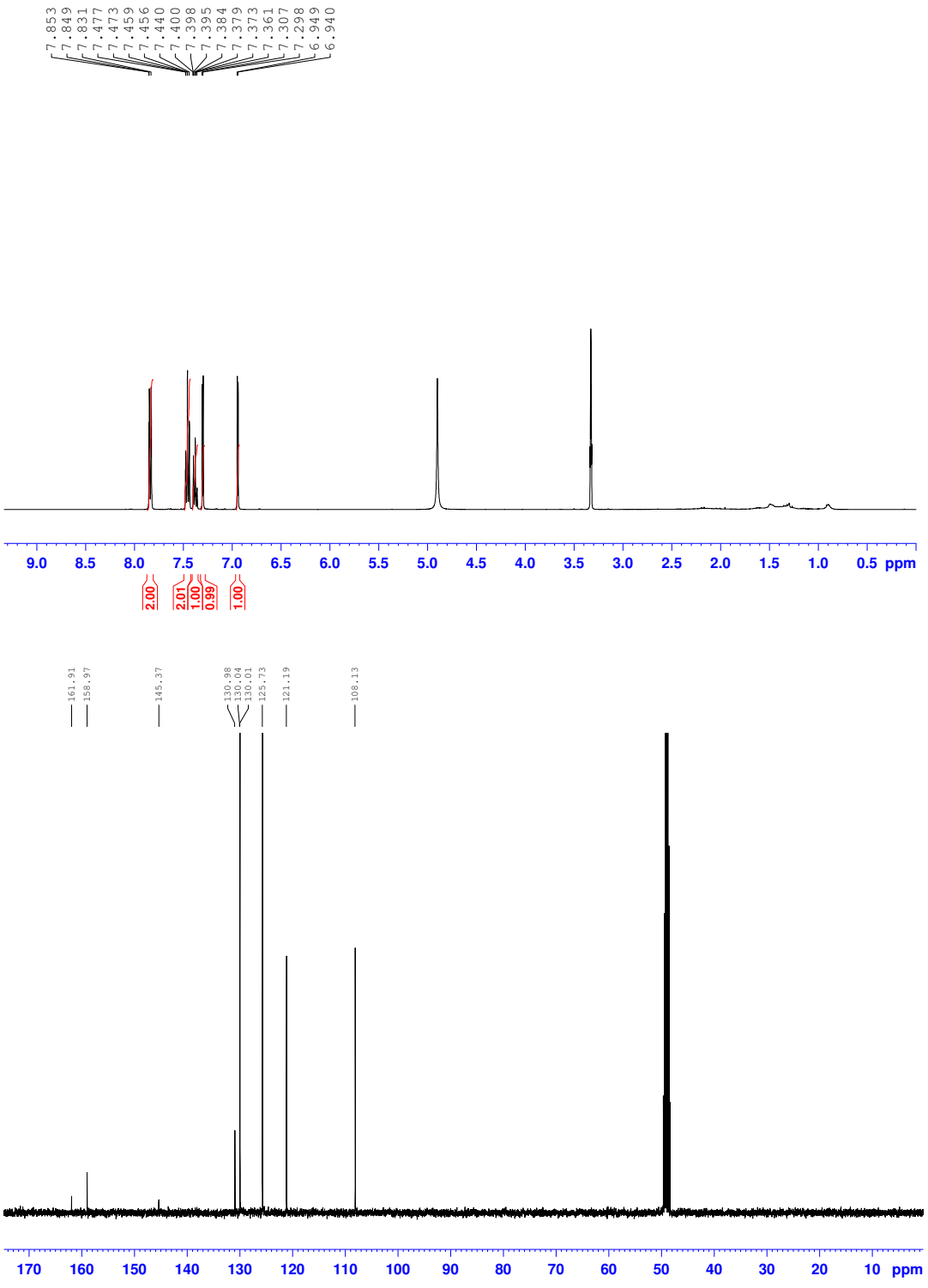



**Figure S5.** $^{1}H$ and $^{13}C$ NMR spectra for compound **3**.

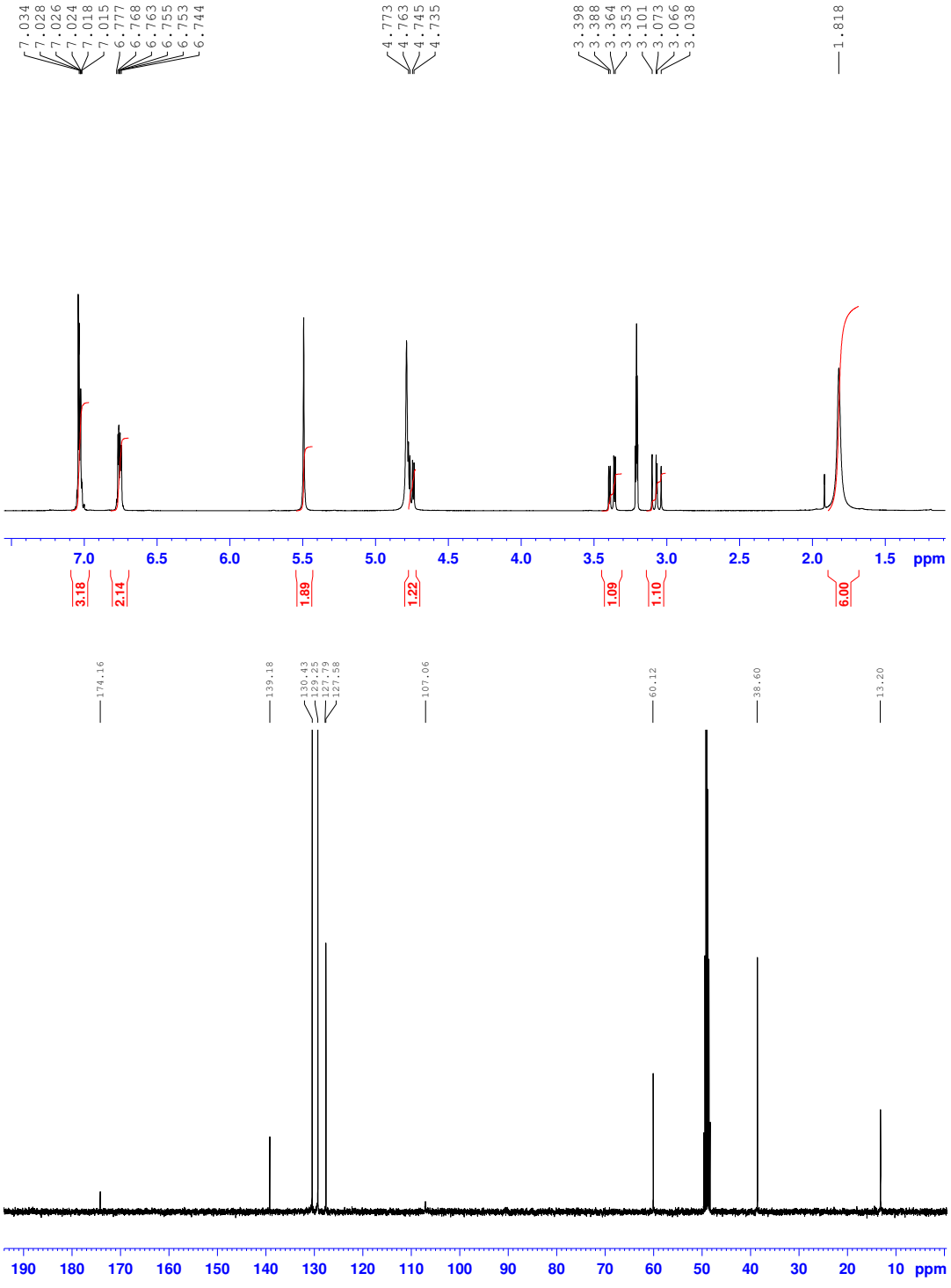



**Figure S6.** $^{1}H$ and $^{13}C$ NMR spectra for compound **4**.

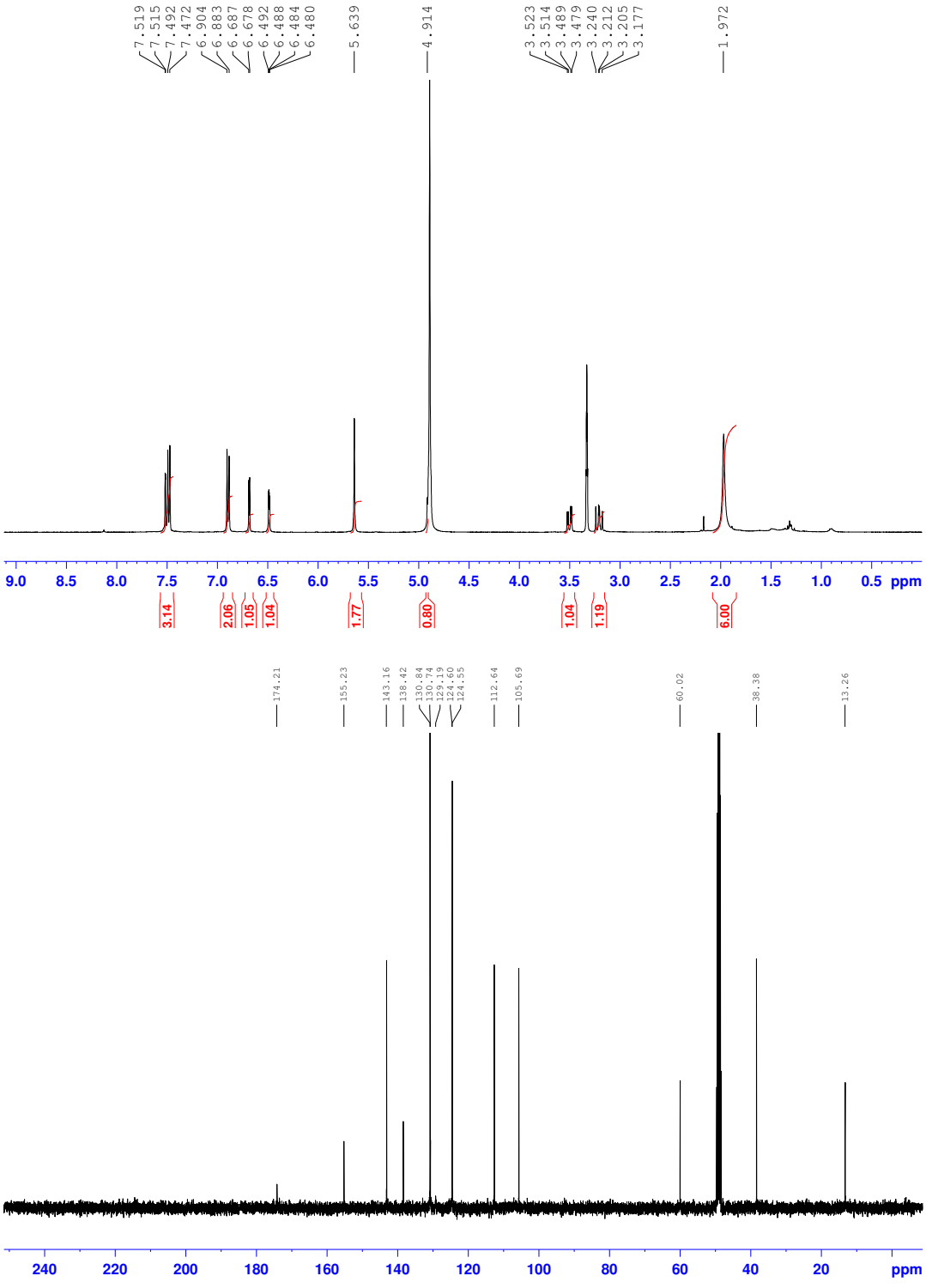



**Figure S7.** $^{1}H$ and $^{13}C$ NMR spectra for compound **5**.

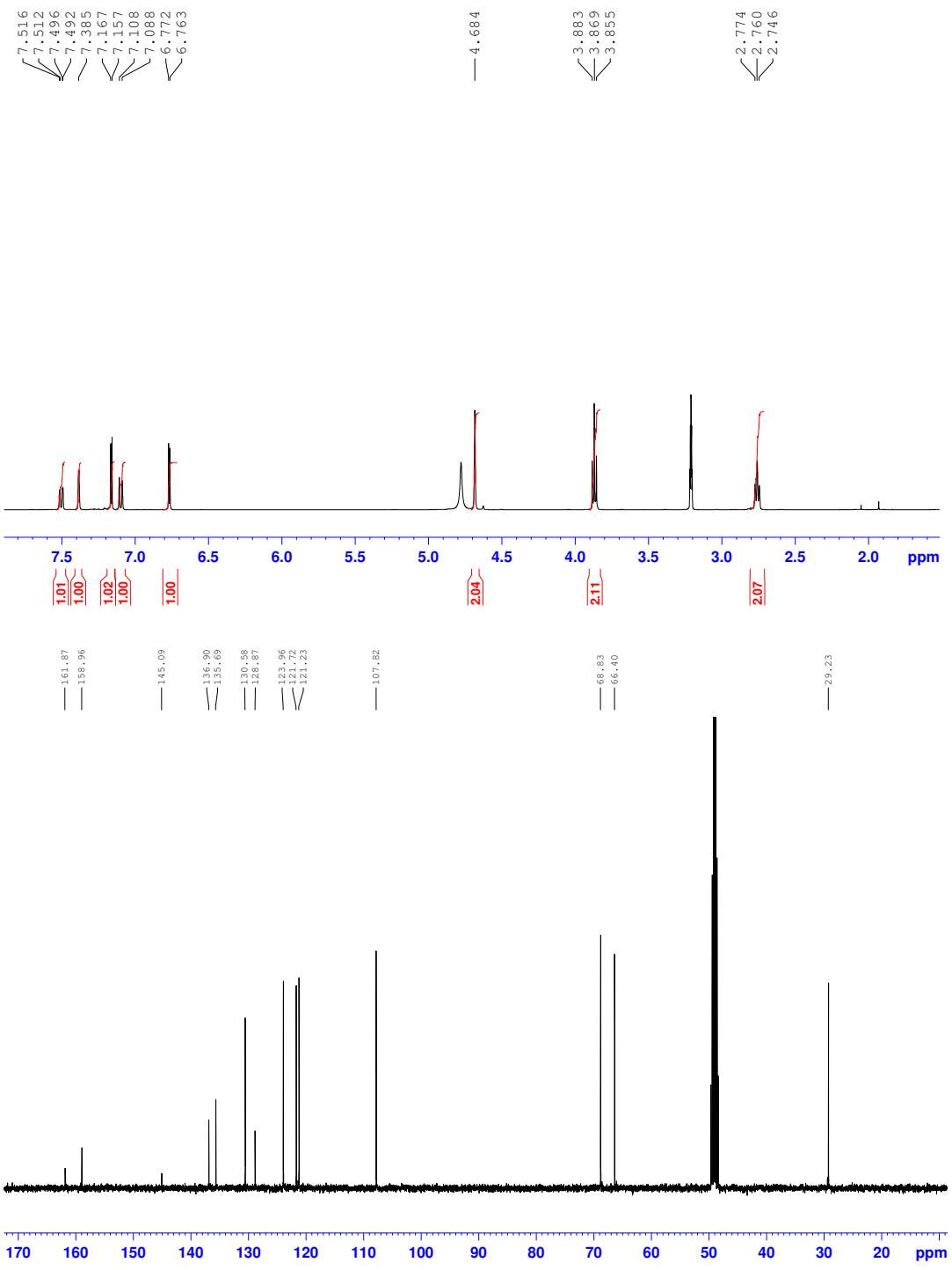



**Figure S8.** $^{1}H$ and $^{13}C$ NMR spectra for compound **6**.

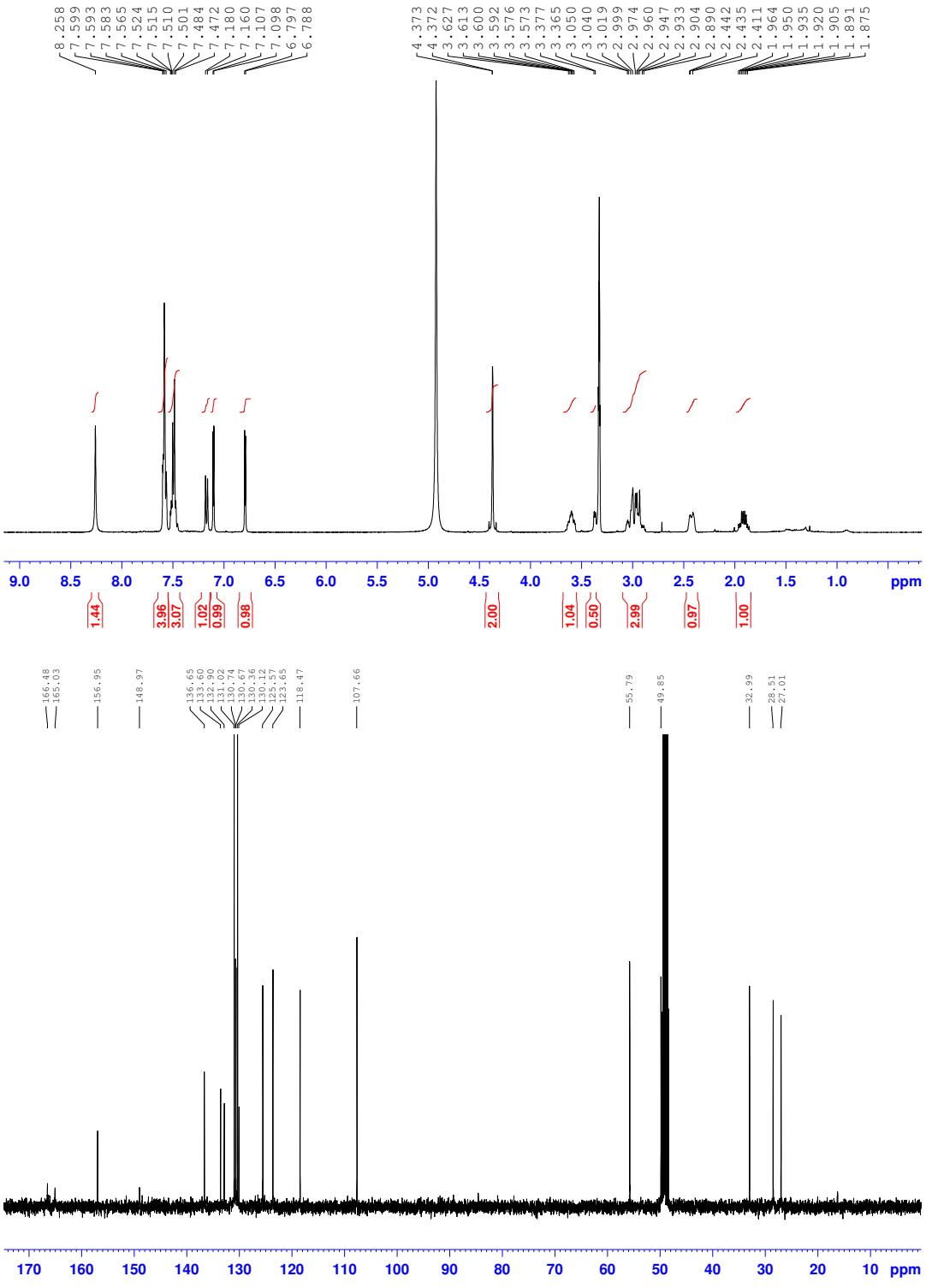



**Figure S9.** $^{1}H$ and $^{13}C$ NMR spectra for compound **7**.

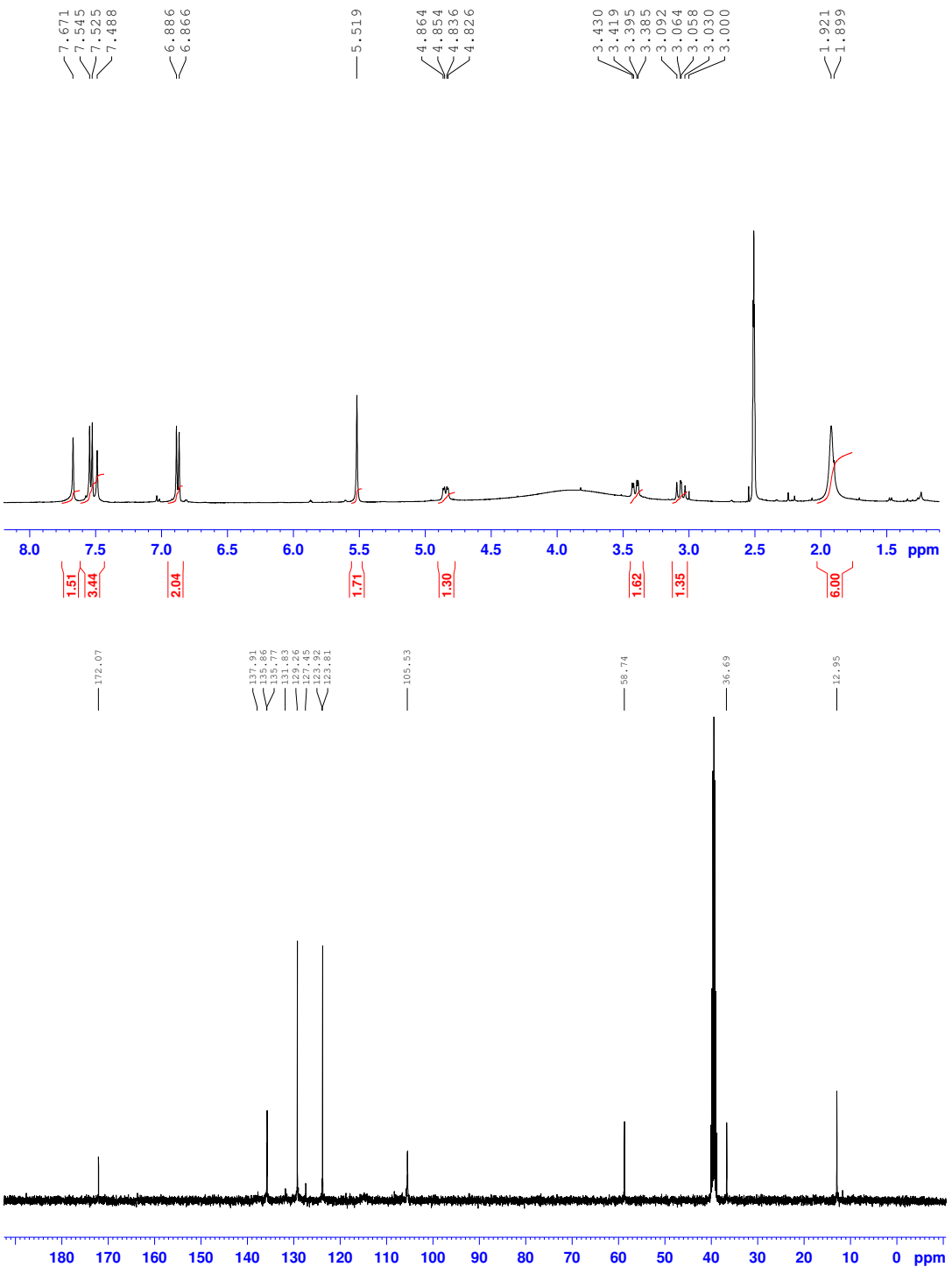



**Figure S10.** $^{1}H$ and $^{13}C$ NMR spectra for compound **8**.

### 2.3 Dynamic light scattering (DLS)

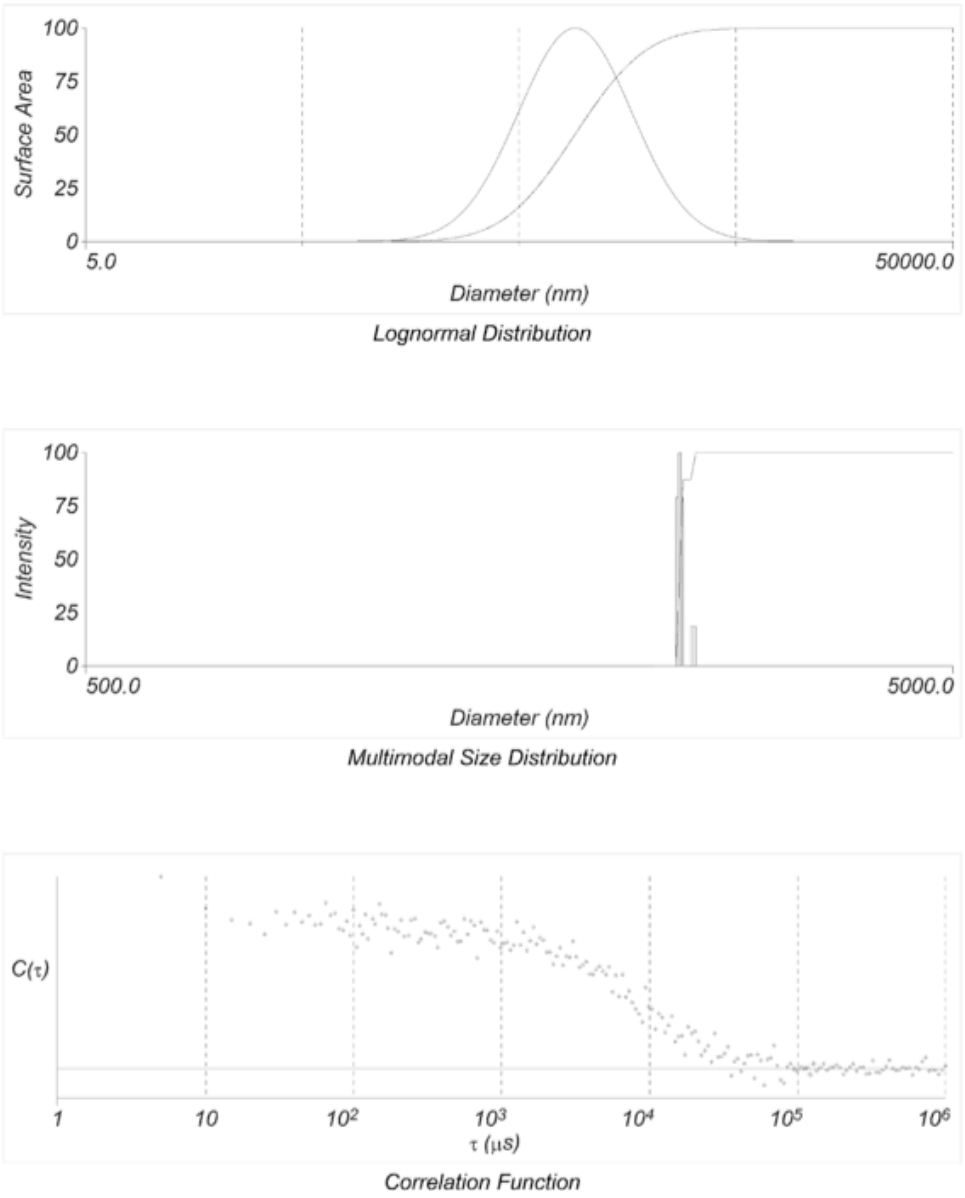



**Figure S11.** DLS data for compound **1a** at 50 μM.

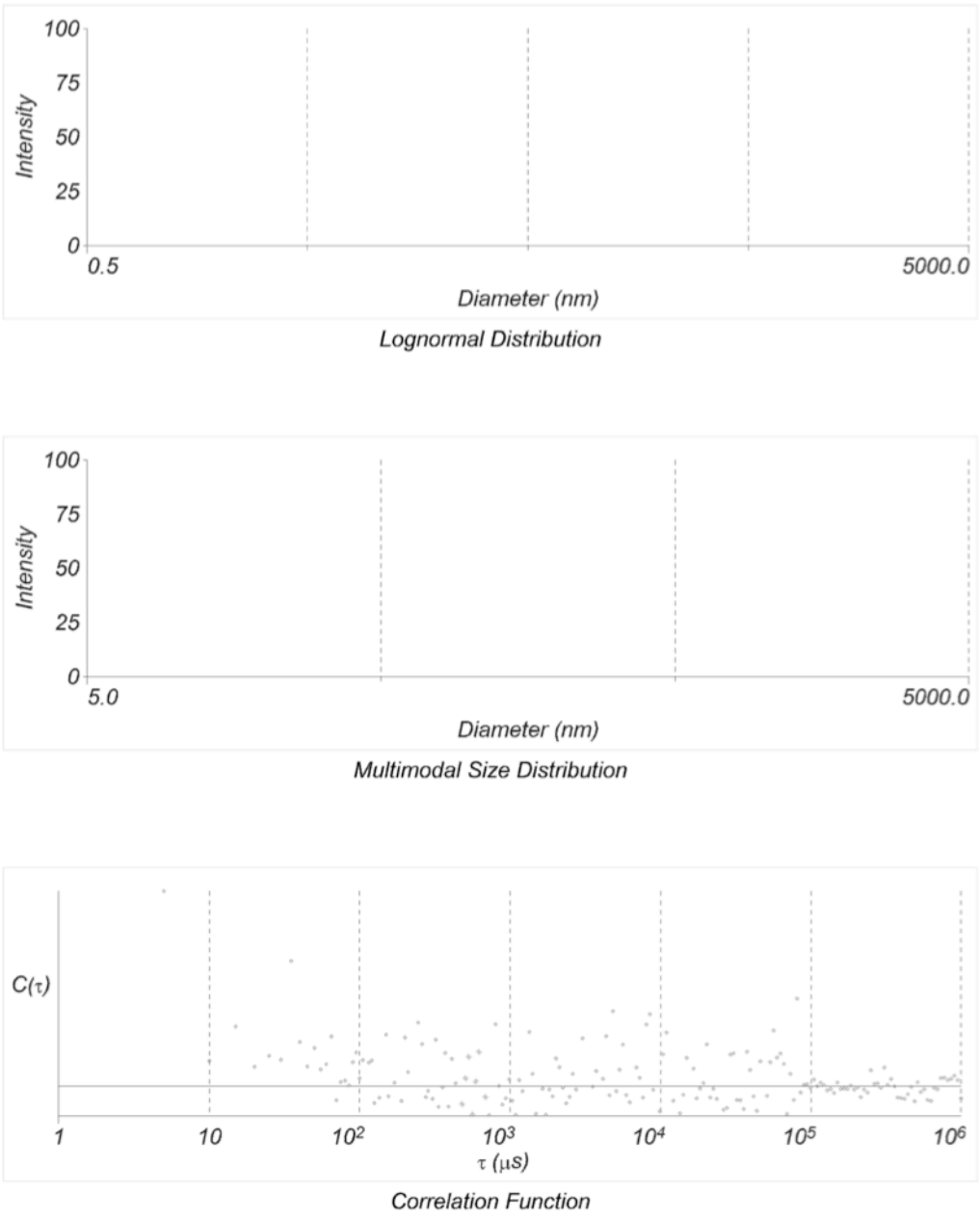
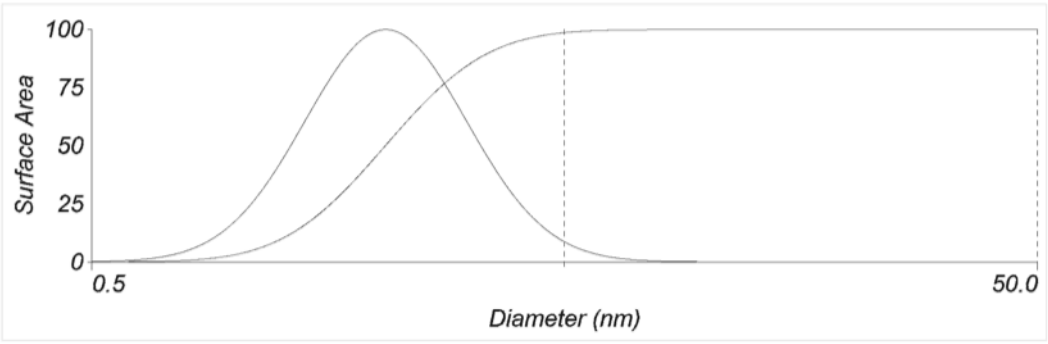
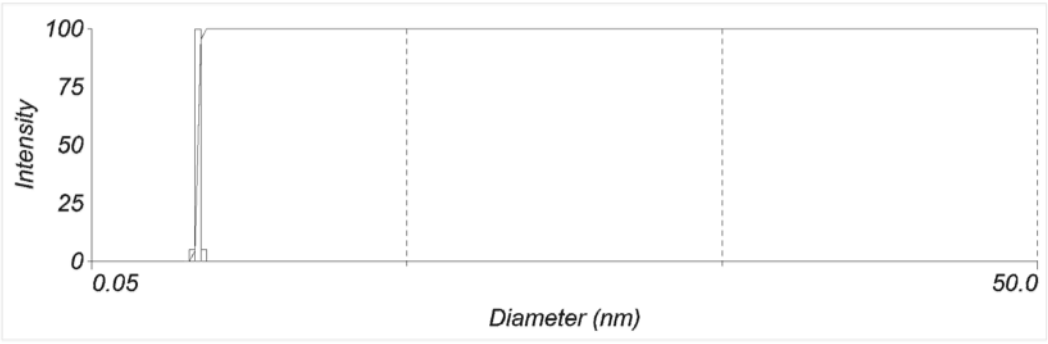


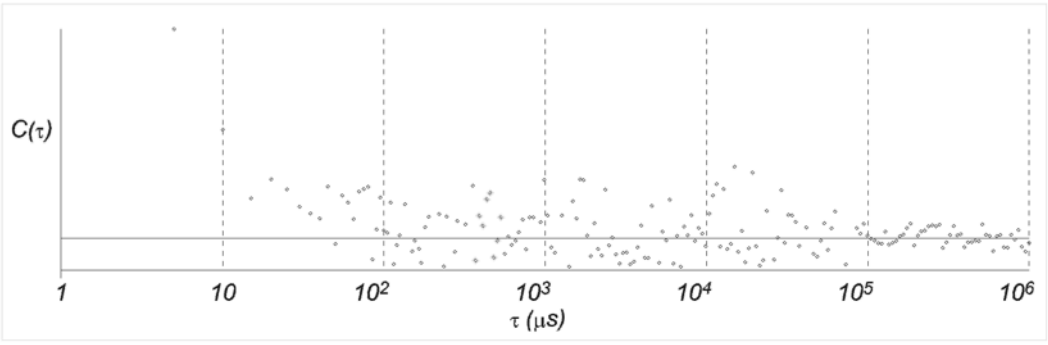

**Figure S12.** DLS data for compound **2** at 100 μM.




Lognormal Distribution

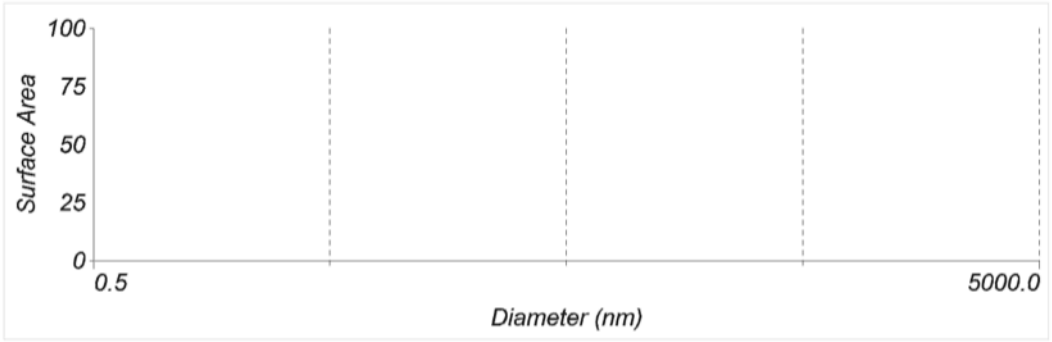



Multimodal Size Distribution

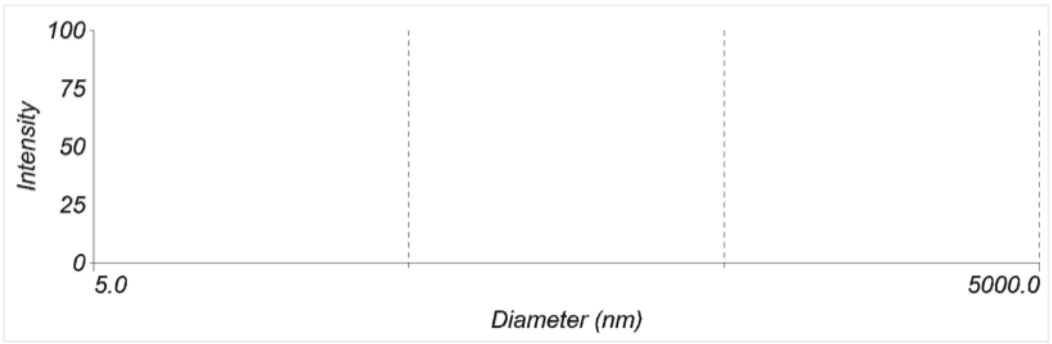



Correlation Function

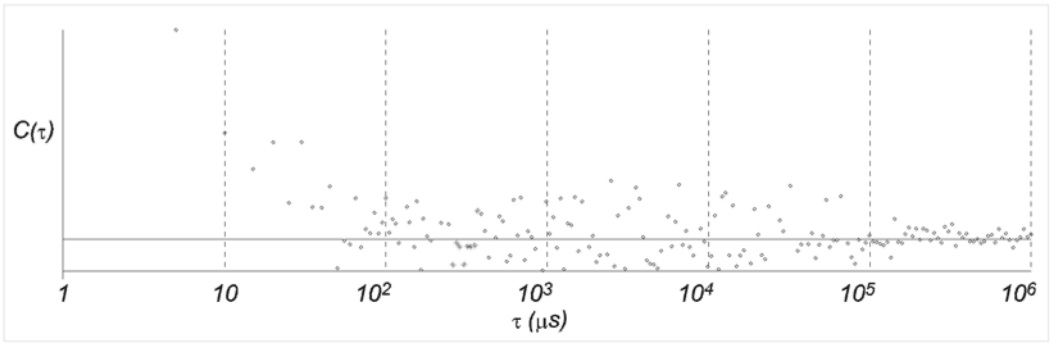
**Figure S13.** DLS data for compound **3** at 100 μM.




Lognormal Distribution

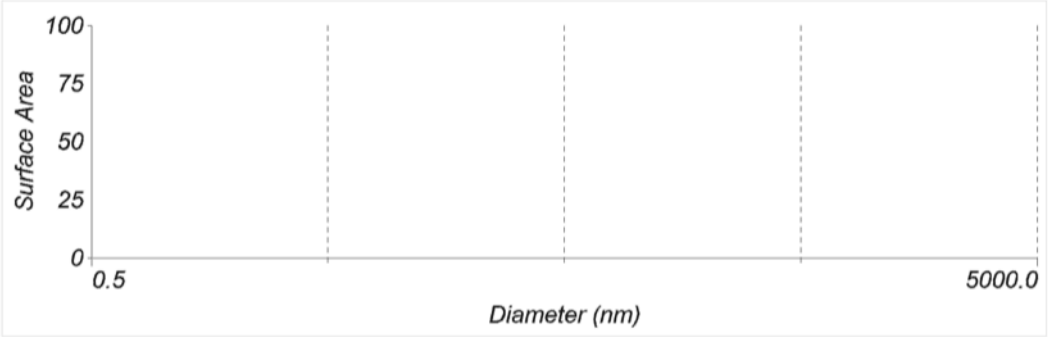


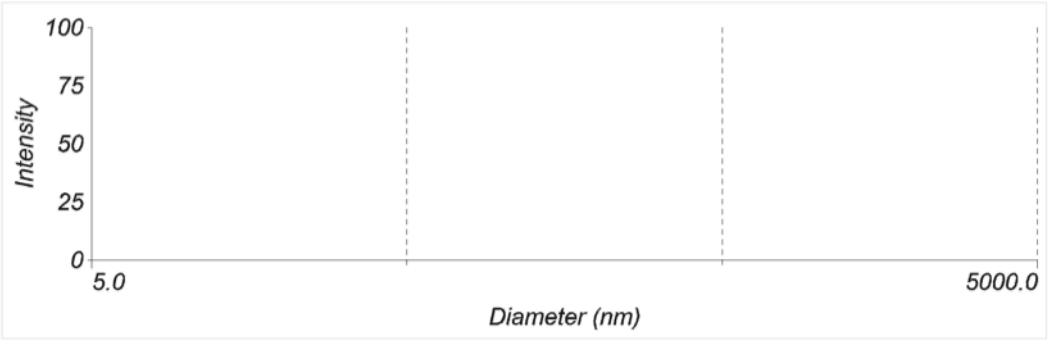

Multimodal Size Distribution




Correlation Function

**Figure S14.** DLS data for compound **4** at 100 μM.



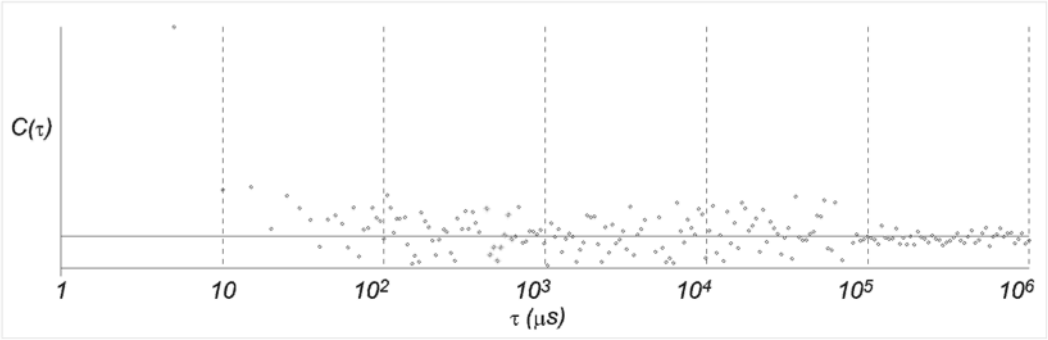




**Figure S15.** DLS data for compound **5** at 100 μM.

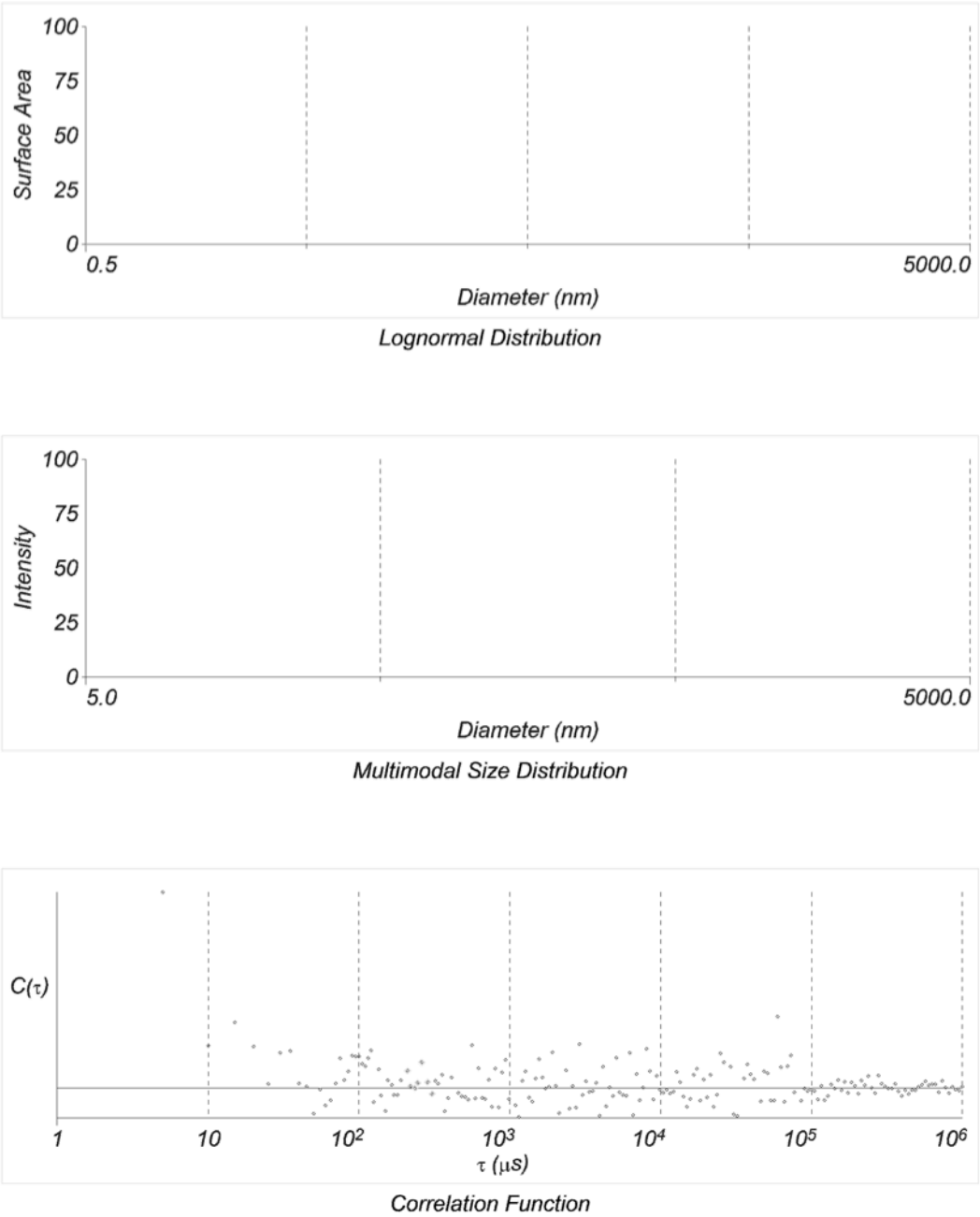
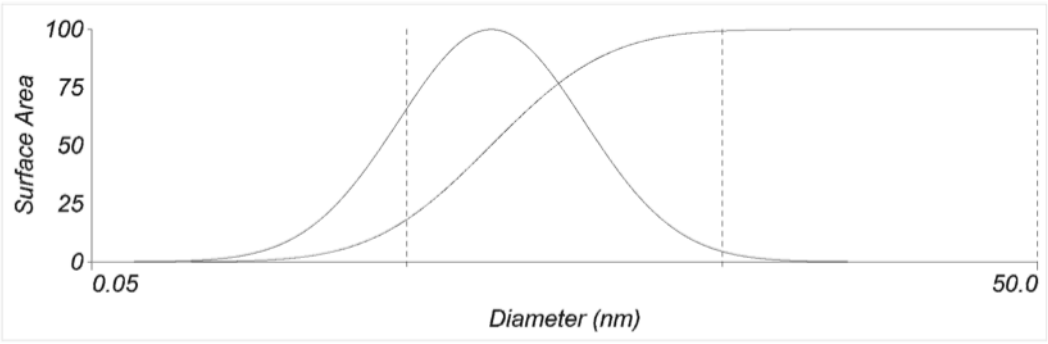
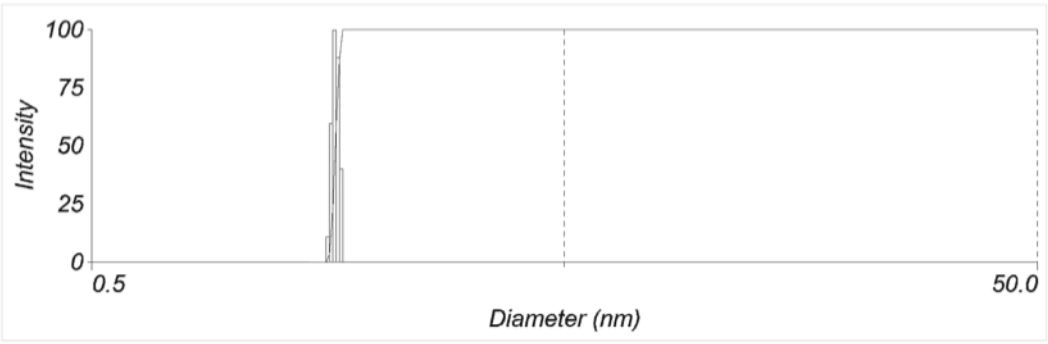


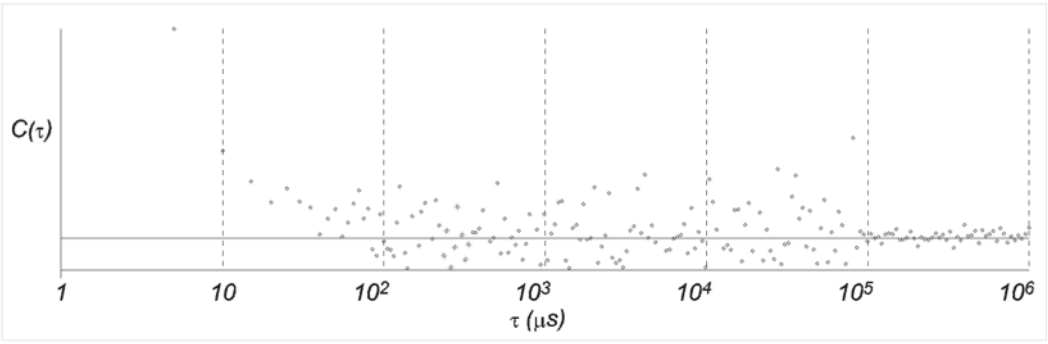

**Figure S16.** DLS data for compound **6** at 100 μM.




Lognormal Distribution

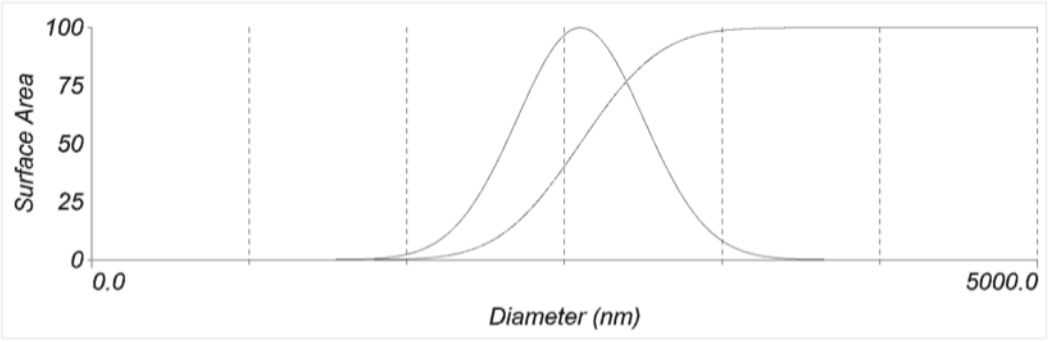



Multimodal Size Distribution

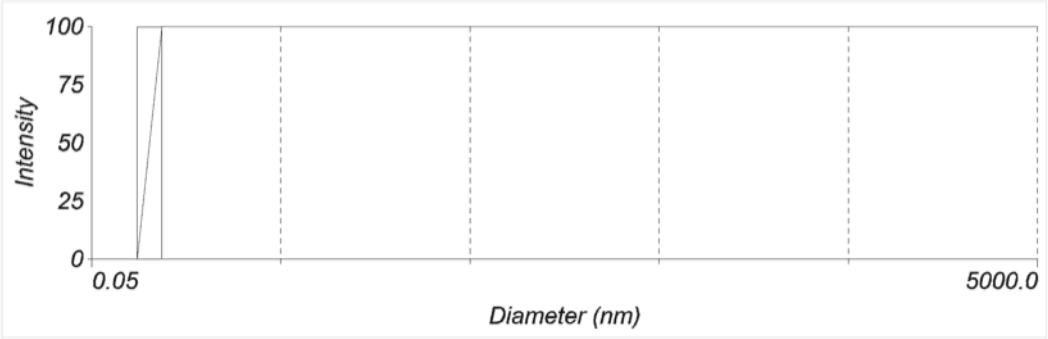



Correlation Function

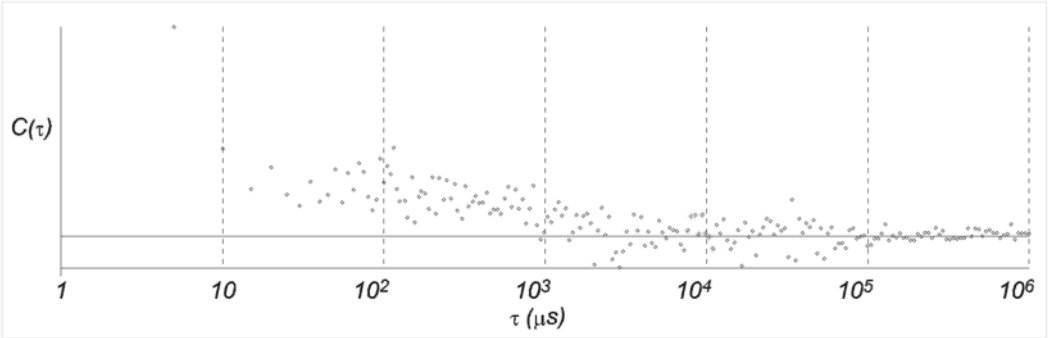
**Figure S17.** DLS data for compound **7** at 100 μM.




Lognormal Distribution




Multimodal Size Distribution




Correlation Function

**Figure S18.** DLS data for compound **8** at 100 μM.

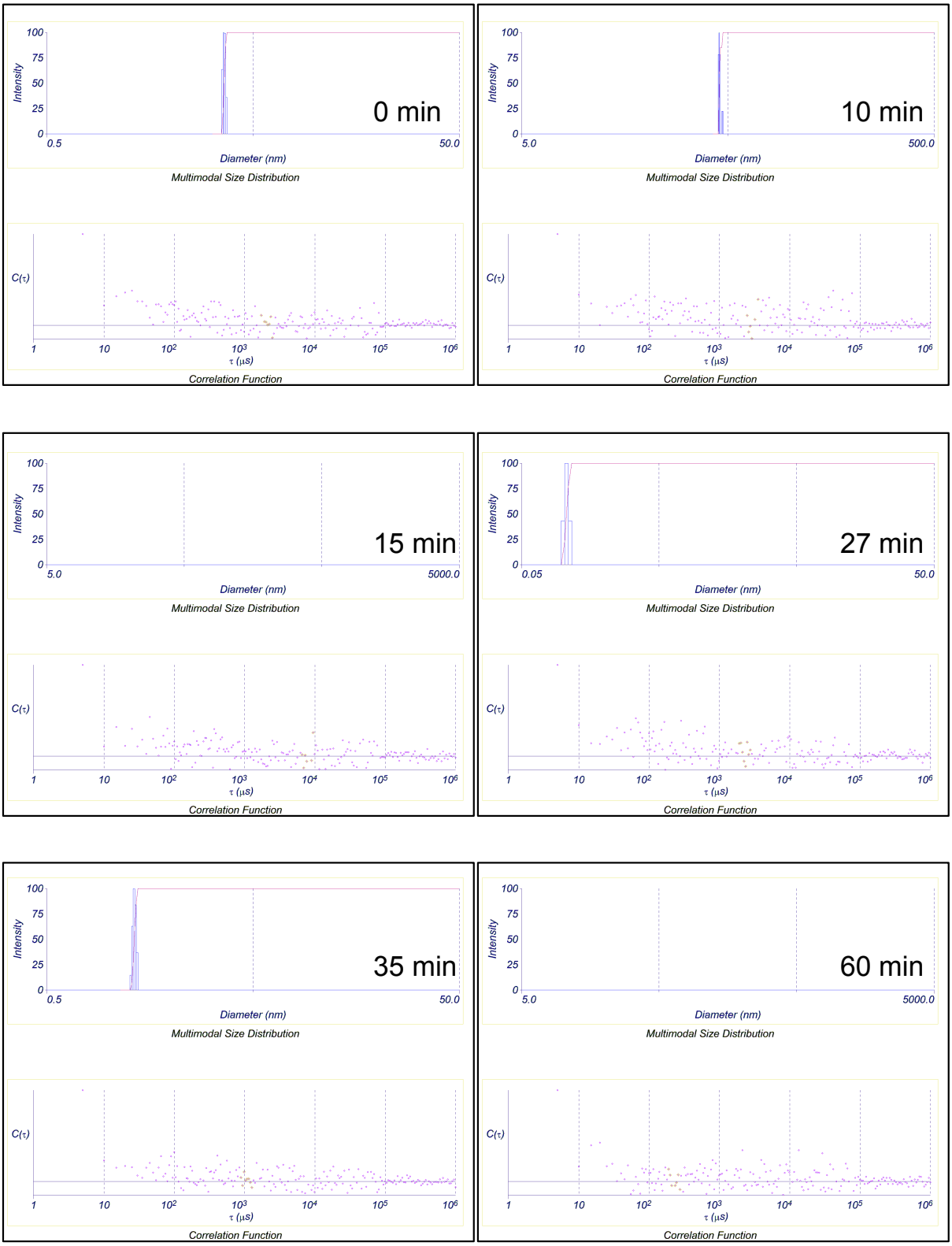



**Figure S19.** Kinetic solubility of **2** (200 µM) measured through DLS over the course of 1 hour in HEPES 10 mM.

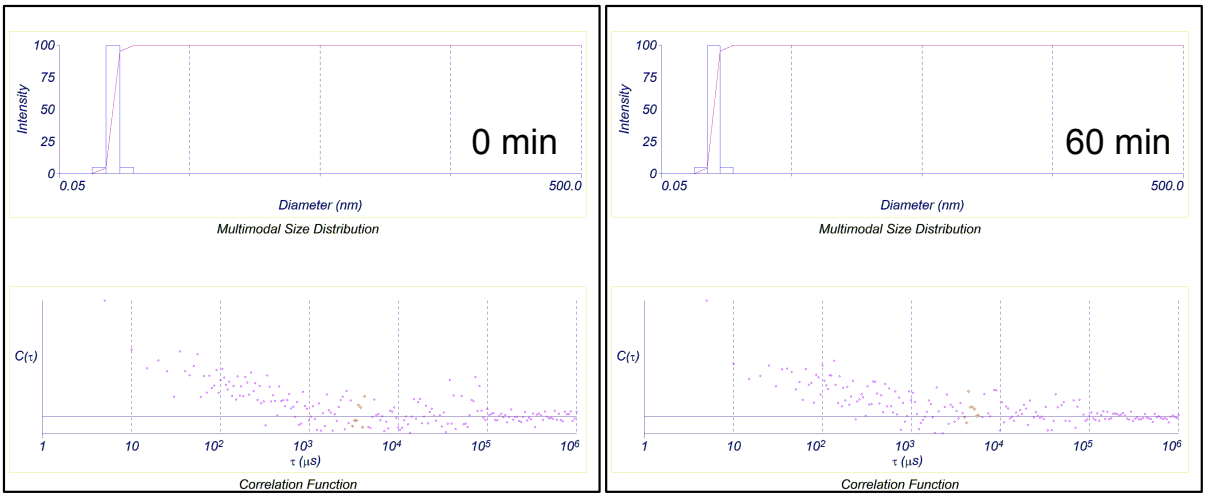



**Figure S20.** Kinetic solubility of **5** (194 μM) measured through DLS over the course of 1 hour in HEPES 10 mM.

### 2.4 SPR – examples

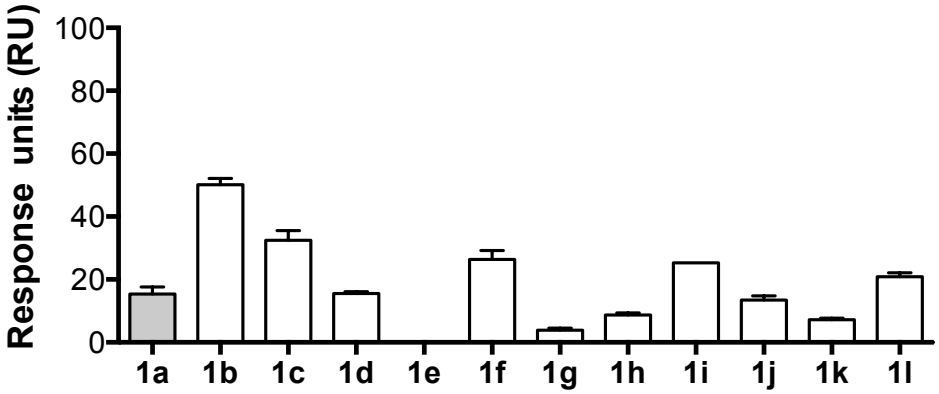



**Figure S21.** SPR screening of compound **1a** analogues **1b-l**. Response units are molecular weight corrected.

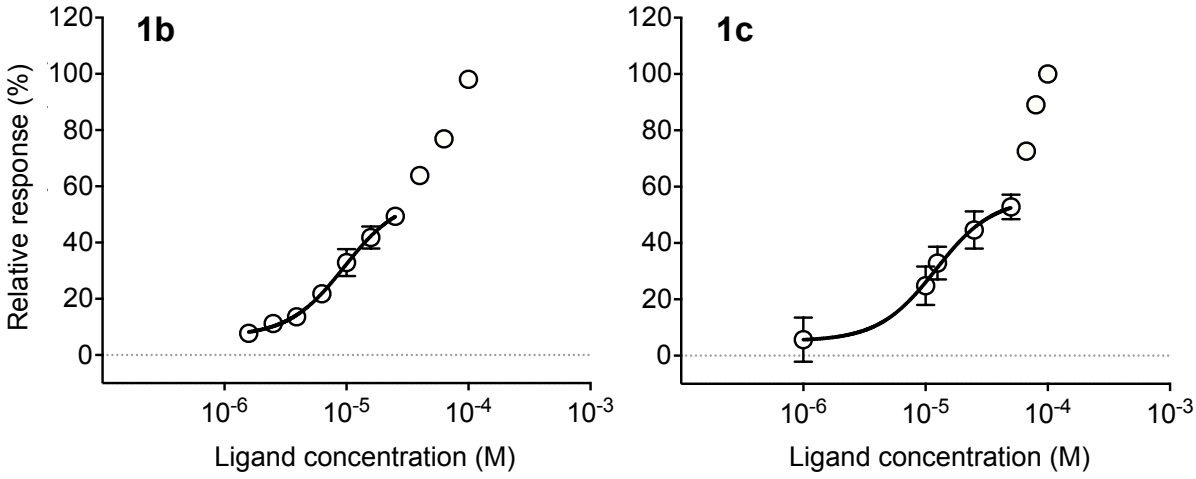



**Figure S22.** $K_D$ curves for compounds **1b-c** measured by SPR ($n$ = 3).

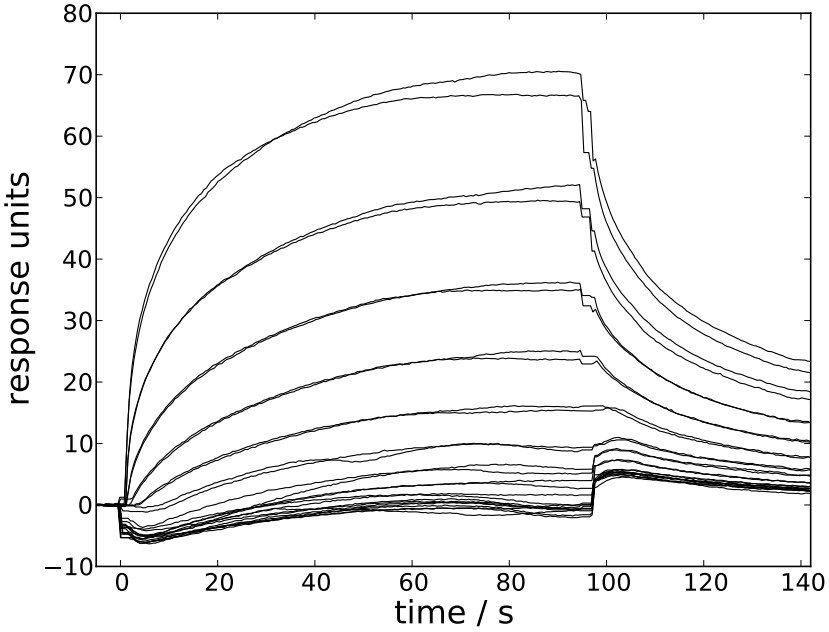



**Figure S23.** SPR sensorgram obtained for compound **2**.

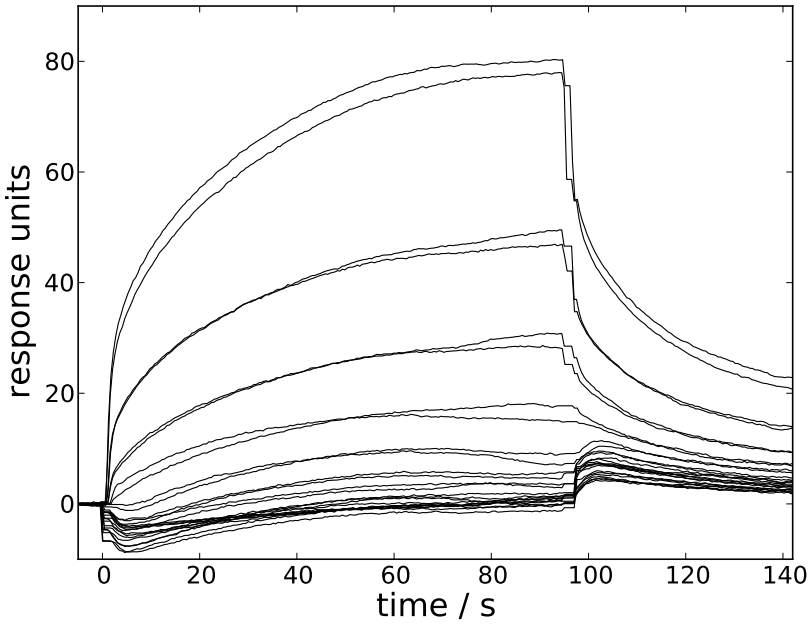



**Figure S24.** SPR sensorgram obtained for compound **4**.

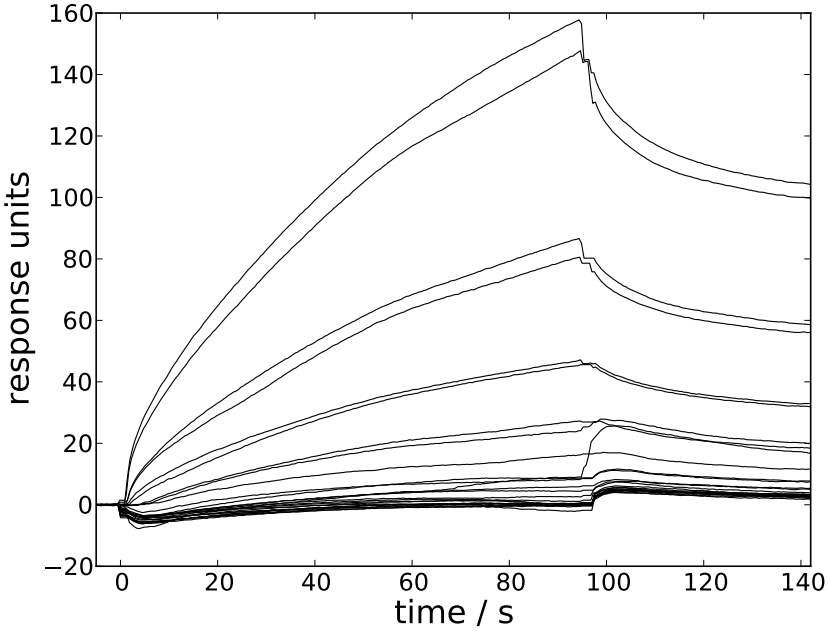



**Figure S25.** SPR sensorgram obtained for compound **8**.

**2.5 Western blots**

| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **E-cadherin** | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| **HtrA** | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **DMSO** | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Cpd. 1a [µM]** | - | - | - | 100 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Cpd. 2 [µM]** | - | - | - | - | 50 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Cpd. 3 [µM]** | - | - | - | - | - | 200 | 100 | 50 | 25 | 10 | - | - | - | - | - | - | - | - | - |
| **Cpd. 5 [µM]** | - | - | - | - | - | - | - | - | - | - | 100 | 50 | 25 | 10 | - | - | - | - | - |
| **Cpd. S1 [µM]** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 100 | 50 | 25 | 10 | - |

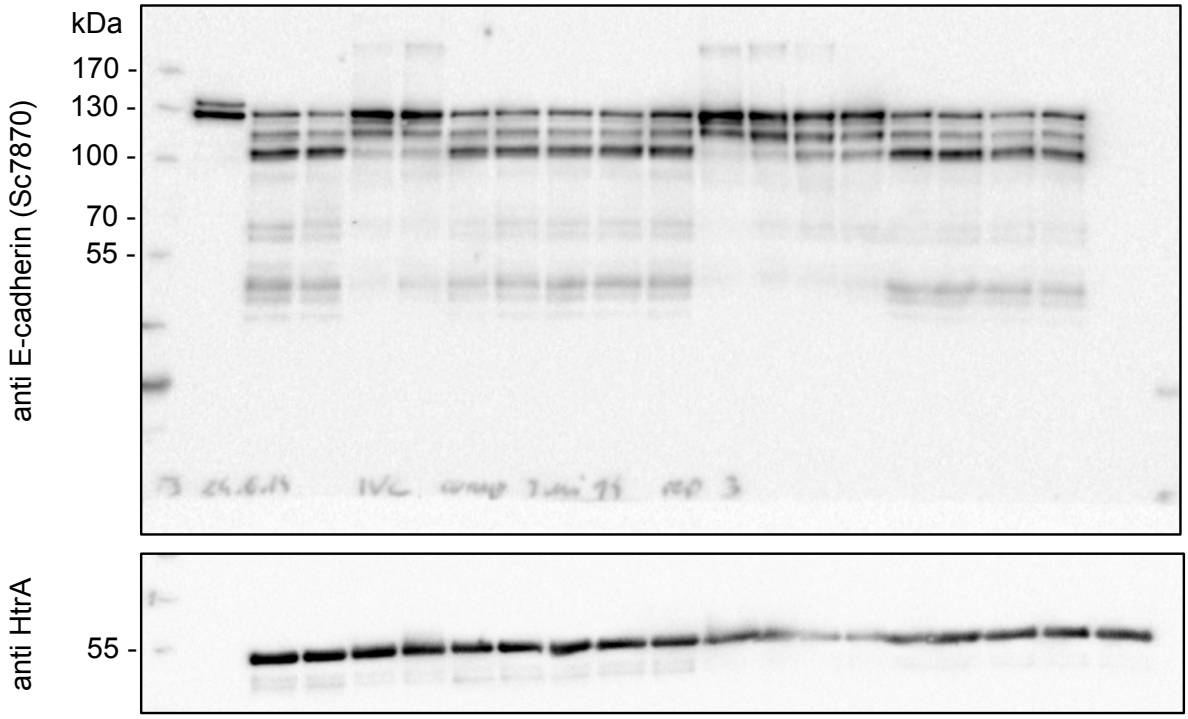



**Figure S26.** Example of a Western blot for compounds **3**, **5** and **S1**. Compound **S1** is poorly soluble in DMSO.

| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **E-cadherin** | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| **HtrA** | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Cpd. 1a [µM]** | - | - | 100 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Cpd. 2 [µM]** | - | - | - | 50 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Cpd. 6 [µM]** | - | - | - | - | 100 | 50 | 25 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Cpd. 7 [µM]** | - | - | - | - | - | - | - | - | 100 | 50 | 25 | 10 | - | - | - | - | - | - | - | - | - |
| **Cpd. 4 [µM]** | - | - | - | - | - | - | - | - | - | - | - | - | 100 | 50 | 25 | 10 | - | - | - | - | - |
| **Cpd. 5 [µM]** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 10 | 5 | 1 | 0.5 | - |

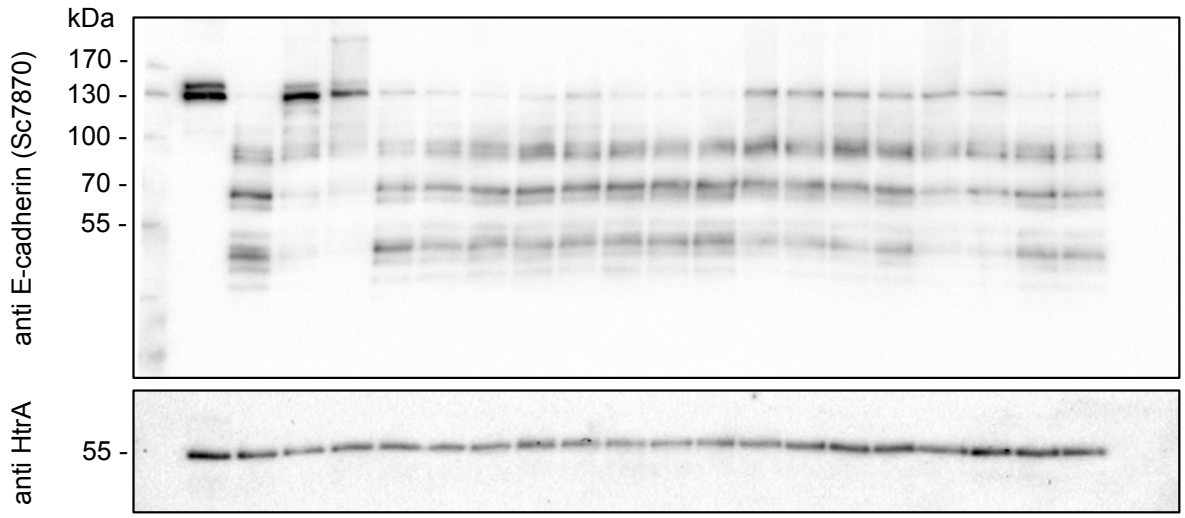



**Figure S27.** Example of a Western blot for compounds **4-7**.

| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| **E-cadherin** | + | + | + | + | + | + | + | + | + | + |
| **HtrA** | - | + | + | + | + | + | + | + | + | + |
| **DMSO** | - | - | + | - | - | - | - | - | - | - |
| **Cpd. 1a [µM]** | - | - | - | 100 | - | - | - | - | - | - |
| **Cpd. 2 [µM]** | - | - | - | - | 50 | - | - | - | - | - |
| **Cpd. 8 [µM]** | - | - | - | - | - | 100 | 50 | 25 | 10 | - |

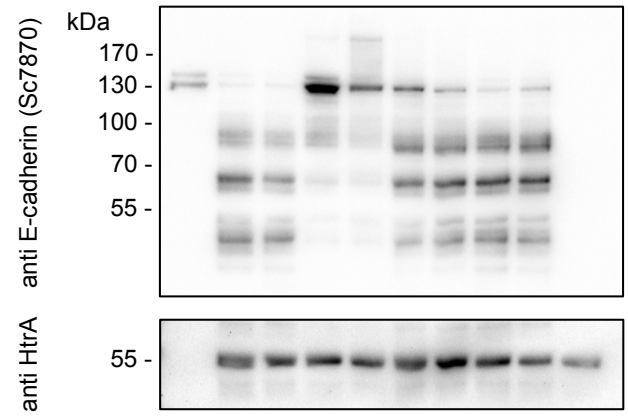



**Figure S28.** Example of a Western blot for compound **8**.

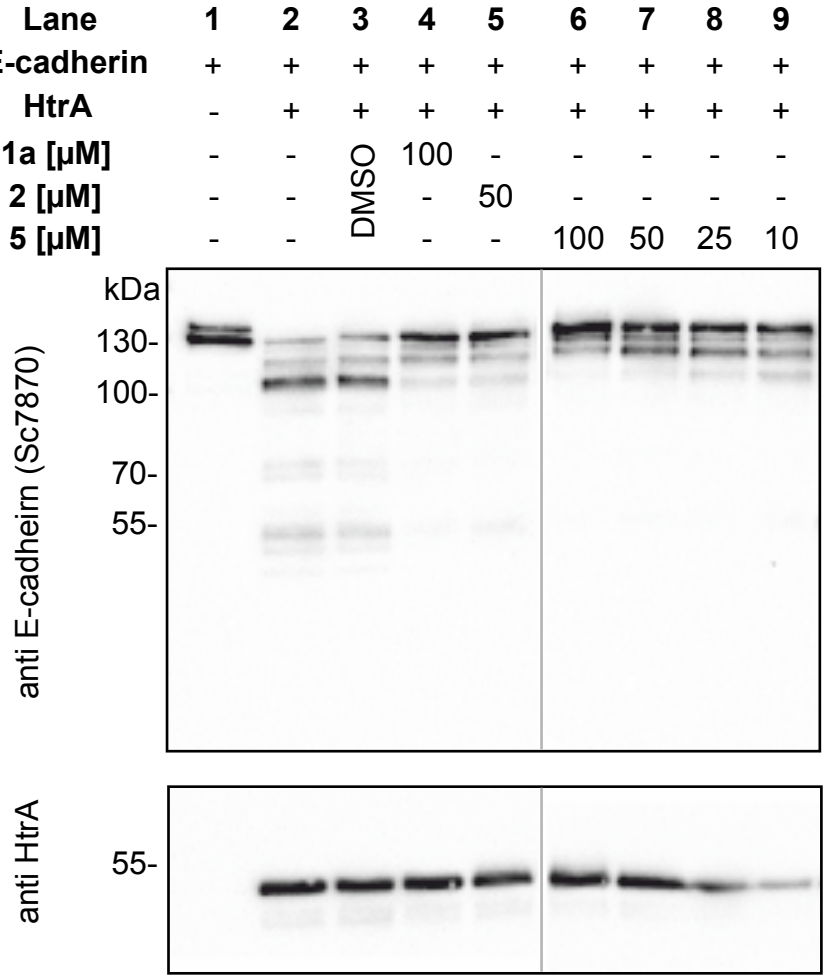
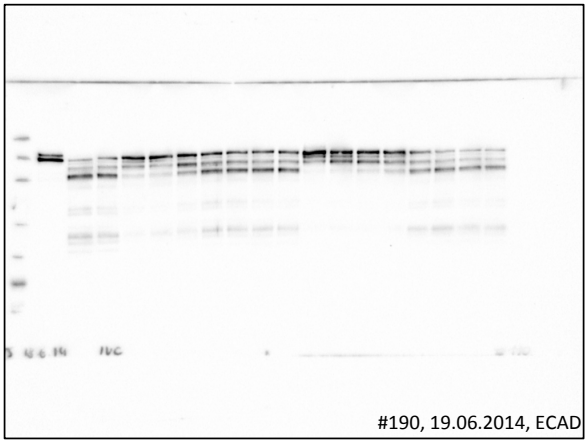
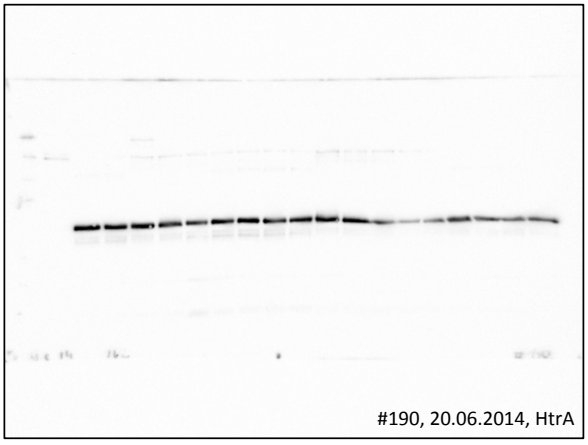



**Figure S29.** Western blot analysis of recombinant E-cadherin cleavage by *H. pylori* HtrA in the presence of compounds **1a**, **2** and **5** at different concentrations. Separate sections of the same membrane are shown (the orignal blots are shown below).



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