Supplemental Table 1. List of Primers, Constructs, and Strains Used in Study.

Gene	Forward Sequence	Reverse Sequence	Notes
ct431/clpP1_S92A	TTT AGC AGC AGC AAT GGG ATC TG	CCT GTA ACA ACT GTA GTC	Mutagenesis of ClpP1 serine active site
ct706/clpP2_S98A	ACA AGC CGC TGC AAT GGG AGC GC	CCA ATG CAG TAC GTA TTT ACA TCA CAG	Mutagenesis of ClpP1 serine active site
ct431/clpP1_6xH	ATC CCA CCG GTA TGC CTG AAG GGG AAA TGA TGC A	ATA TTC GGC CGT TAG TGA TGA TGA TGG TGA TGC AAG TCG TTA AAA GAG AAG AGA	Addition of 6xHis tag to ClpP1
ct706/clpP2_6xH	ACC CCA CCG GTA TGA CGT TGG TAC CAT ACG TTG T	ATA TTC GGC CGT TAG TGA TGA TGA TGG TGA TGA GAC GCA ATA CTC TTA TCT T	Addition of 6xHis tag to ClpP2
ct431/clpP1_GW	AAT TAA CAA GTT TGT ACA AAA AAG CAG GCT TTA TGC CTG AAG GGG AAA TGA TGC ATA AGT TGC AAG A	AAT TAC CAC TTT GTA CAA GAA AGC TGG GTT CAA GTC GTT AAA AGA GAA GAG AAT CCC ATC TAA CA	Addition of attB sites to ClpP1 for Gateway® Cloning
ct706/clpP2_GW	AAT TAA CAA GTT TGT ACA AAA AAG CAG GCT TTA TGA CGT TGG TAC CAT ACG TTG TTG AAG ACA CGG GT	AAT TAC CAC TTT GTA CAA GAA AGC TGG GTT AGA CGC AAT ACT CTT ATC TTT TGT CTC TTT AGC AGA	Addition of attB sites to ClpP2 for Gateway® Cloning
ct431/clpP1	AGA AGG AGA TAT AAC TAT GCC TGA AGG GGA AAT GAT GCA TAA G	GTG GTG GTG ATG GTG ATG GCC CAA GTC GTT AAA AGA GAA GAG AA TCC C	Cloning of ClpP1 into pLATE31
ct706/clpP2	AGA AGG AGA TAT AAC TAT GAC GTT GGT ACC ATA CGT TGT TGA AG	AGA AGG AGA TAT AAC TAT GAC GTT GGT ACC ATA CGT TGT TGA AG	Cloning of ClpP2 into pLATE31
ct431/clpP1_M6L/M7I	CTA TGA CAT CTT GCA ACT TAT GAA TCA GTT CCC CTT CAG GCA TAG TTA TAT C	ATA GAA AGT TGT TGG ATT CTC GTC G	Mutation of ClpP1 N-terminal Met residues for cloning into pLATE31
E. coli clpP/ NP_414971.1	AGA AGG AGA TAT AAC TAT GTC ATA CAG CGG CGA ACG AG	GTG GTG GTG ATG GTG ATG GCC ATT ACG ATG GGT CAG AAT CGA ATC G	Cloning of *E. coli* ClpP into pLATE31
ct431/clpP1_qPCR	GAT GCT GGG TTT GCT GTT TG	CAG ATC CCA TAG ATG CTG CTA AA	qPCR
ct706/clpP2_qPCR	GTT AGC GAT TTA CGA CAC CAT TC	CCC TTT GTC CCT GCA GAT AAT A	qPCR
ct707/clpX_qPCR	GGT TGC CGT CTA TAA CCA CTA TAA	AGA TCC TGT TGG GCC TAG TA	qPCR
ct286/clpC_qPCR	CTC TTC CTC CAT CAC TCC TAG A	AGG TTT ATC TCC GCC CAA AG	qPCR

Strain	Relevant genotype	Source or Reference
DHT1	F$^-$ *glnV44* (AS) *recA1 endA1 gyrA96* (Nal^R) *thi-1 hsdR17 spoT1 rfbD1 cya-854 ilv-691* ::Tn*10* (Tet^R)	(1)
XL1-Blue	*recA1 endA1 gyrA96* (Nal^R) *thi-1 hsdR17 supE44 relA1 lac*[F' *proAB lacI^{q}ZΔM15* Tn*10* (Tet^R)]	Stratagene (Agilent)
DH5α	*fhuA2* Δ(*argF-lacZ*)*U169 phoA glnV44* Φ*80* Δ(*lacZ*)*M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	New England Biolabs
dam-/dcm-	*ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 R*(*zgb210*::*Tn10*) *TetS endA1 rspL136* (Str^R)*dam13*::*Tn9* (Cam^R) *xylA-5 mtl-1 thi-1 mcrB1 hsdR2*	New England Biolabs
DH10β	Δ(*ara-leu*) *7697 araD139 fhuA* Δ*lacX74 galK16 galE15 ϕ80dlacZΔM15* (*e14-*) *recA1 relA1 endA1 nupG rpsL* (Str^R) *rph spoT1* Δ(*mrr-hsdRMS-mcrBC*)	New England Biolabs
BL21(DE3)	*fhuA2, [lon], ompT, gal, [dcm],* Δ*hsdS, λ DE3 (λ sBamHIo* Δ*EcoRI-B int::(lacI::PlacUV5::T7 gene1) i21* Δ*nin5)*	New England Biolabs

Construct Plasmids	Relevant genotype	ori	Source or Reference
pKT25	*aph* P$_{lac}$::*t25*	pACYC	(2)
pUT18C	*bla* P$_{lac}$::*t18*	ColE1	(2)
pDONR221	*aph attP1-[cat ccdB]-attP2*	ColE1	Invitrogen (Life Technologies)
pKT25-zip	*aph* P$_{lac}$::*t25-zip*	pACYC	(2)
pUT18C-zip	*bla* P$_{lac}$::*t18-zip*	ColE1	(2)
pST25-*clpP1*	*aadA* P$_{lac}$::*t25-clpP1*	p15A	This study
pST25-*clpP2*	*aadA* P$_{lac}$::*t25-clpP2*	p15A	This study
pUT18C-*clpP1*	*bla* P$_{lac}$::*t18-clpP1*	ColE1	This study
pUT18C-*clpP2*	*bla* P$_{lac}$::*t18-clpP2*	ColE1	This study
pTLR2-*clpP1(S92A) 6xH*::L2	*bla* P$_{tet}$ P$_{lac}$:: *clpP1_6xH*	ColE1	This Study
pTLR2-*clpP2(S98A) 6xH*::L2	*bla* P$_{tet}$ P$_{lac}$:: *clpP2_6xH*	ColE1	This study
pLATE31-*clpP1_6xH*	*bla* P$_{tet}$ P$_{lac}$:: *clpP1_6xH*	pMB1	This study
pLATE31-*clpP2_6xH*	*bla* P$_{tet}$ P$_{lac}$:: *clpP2_6xH*	pMB1	This study

1. **Dautin, N., G. Karimova, A. Ullmann, and D. Ladant.** 2000. Sensitive genetic screen for protease activity based on a cyclic AMP signaling cascade in *Escherichia coli*. J. Bacteriol. **182**: 7060-7066.
2. **Karimova, G., A. Ullmann, and D. Ladant.** 2001. Protein-protein interaction between *Bacillus stearothermophilus* tyrosyl-tRNA synthetase subdomains revealed by a bacterial two-hybrid system. J. Mol. Microbiol. Biotechnol. **3**: 73-82.

Supplemental Table 2. List of Buffers Used in Study

Buffer A: 25mM Tris (pH 7.5), 150mM NaCl, 10mM Imidazole, 10% glycerol
Buffer B: 25mM Tris (pH 7.5), 150mM NaCl, 300mM Imidazole, 10% glycerol.
Buffer C: 25mM Tris (pH 7.5), 150mM NaCl, 10% glycerol
Buffer D: 25mM Tris-HCl (pH 7.5), 5 mM KCl, 5 mM $MgCl_2$, 10% glycerol, 1 mM DTT
Buffer E: 50 mM Tris-HCl (pH 8), 200 mM KCl, 1 mM DTT
Buffer F: 50 mM Tris-HCl (pH 8), 200 mM KCl, 1 mM DTT, 0.2mM Sodium Citrate

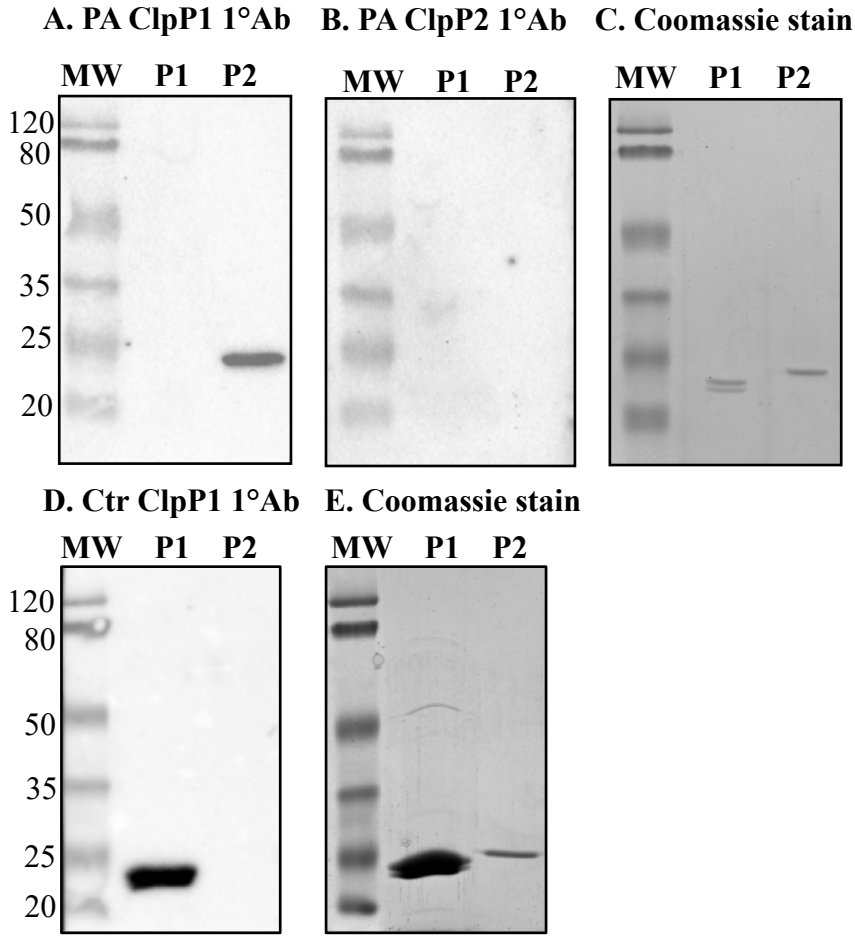


Figure S1. Verification of the ClpP specific antibodies. Western blots were run with purified, recombinant chlamydial Ctr ClpP1 (22 kDa) and Ctr ClpP2 (23 kDa). Ctr ClpP's were probed with antibodies raised against *P. aeruginosa* Pa ClpP1 (A), Pa ClpP2 (B), or antibodies raised against Ctr ClpP1 (D). Ctr ClpP1 and Ctr ClpP2 protein loading controls are shown in (C and E).

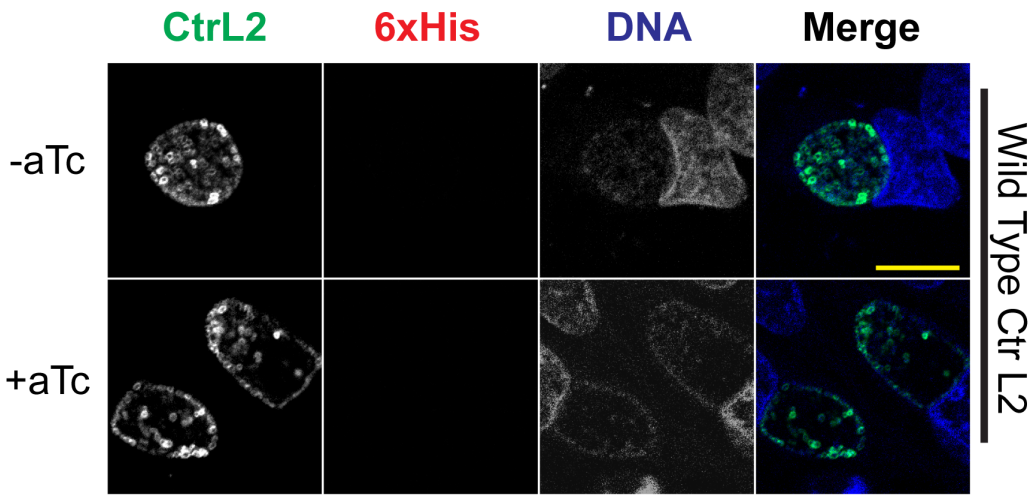


Figure S2: Anhydrotetracycline treatment of wild type *C. trachomatis* L2. Treatment with 10 nM aTc does not appear to negatively affect *Chlamydia*. Samples stained for major outer membrane protein (MOMP; green), 6xHis tag (red), and DNA (blue). Representative images of three independent experiments are presented. Scale bars are equal to 10 μm.

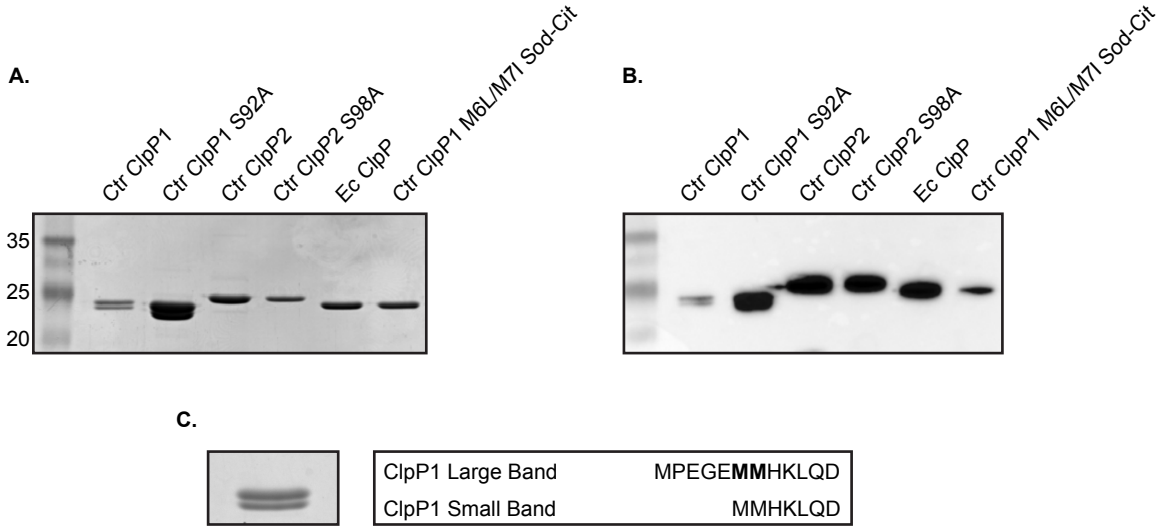
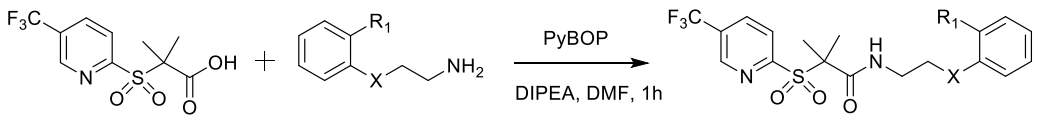


Figure S3. Analysis of purified, recombinant ClpP proteins. Representative purified proteins samples were run on 12% SDS-PAGE gels and then either stained with Coomassie Brilliant Blue or transferred to nitrocellulose for western blot with anti-6x His-tag antibodies (B). 500 ng was used for Ctr ClpP1 to view the "doublet" and 1 µg was run for the other proteins. Panel C shows the doublet formed by Ctr ClpP1 (resolved on a 15% SDS-PAGE gel). Note the absence of the doublet for ClpP1 M6L M7I (A). Both bands were excised and analyzed by mass spectrometry. The N-term peptides identified by mass spectrometry are listed to the right of the protein gel in C. Expected molecular weights (markers shown to the left of each panel) for the recombinant proteins: Ctr ClpP1 22 kDa, Ctr ClpP2 23 kDa, Ec ClpP 24 kDa.

Synthesis of ACP and analogues



ACP1a: X=CH_2, R_1=H
ACP1: X=S, R_1=H
ACP1b: X=S, R_1=Cl

General procedure. A solution of 0.16 mmol (50 mg) of 2-methyl-2-((5-(trifluoromethyl)pyridin-2-yl)sulfonyl)propanoic acid in anhydrous DMF (4 mL) was treated with a solution of PyBOP (87 mg, 0.17 mmol) in anhydrous DMF (1 mL) followed by the adition of DIPEA (83 µL) to activate the carboxylic acid functionality. Then, a solution of the desired amine (0.16 mmol) in anhydrous DMF (1 mL) was added dropwise. The resulting yellow solution was stirred at room temperature for 1 h. The DMF was removed and the crude product was absorbed onto silica gel and purified by a Teledyne CombiFlash Rf+ with a gradient of EtOAc-hexanes. Thin-layer chromatography was carried out on aluminum backed silica gel plates (Sorbtech Technologies), with visualization of components by UV light (254 nm).

Analysis of compound's purity was performed on an Agilent Technologies 1220 infinity LC HPLC system at wavelength of 220 nm with a C18 column (Diamonsil AAA 5u, 250 mm × 4.6 mm). Compounds were eluted with a gradient of acetronitrile-water from 5% to 95% over 25 min. All tested compounds have a purity ≥95%.

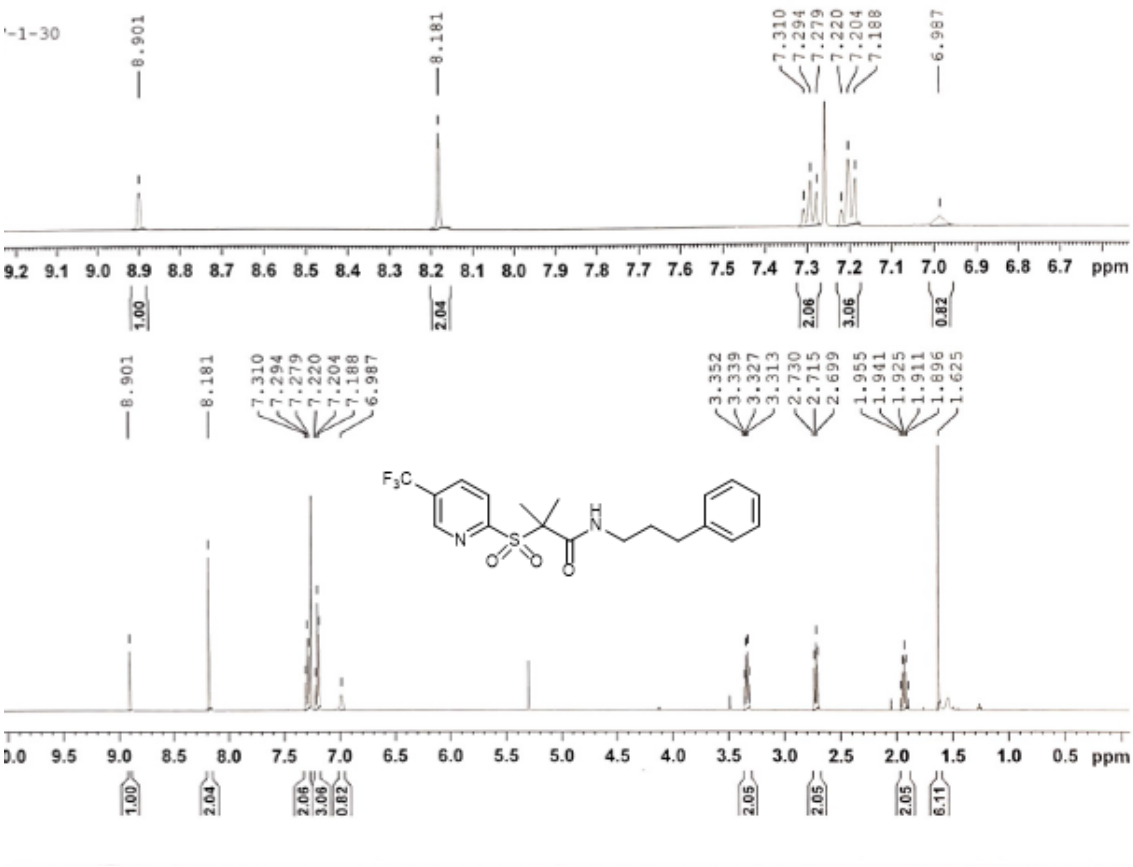
^{1}H NMR spectra were recorded on a Bruker 500 MHz spectrometer. The chemical shifts are reported in parts per million (ppm) relative to internal standard tetramethylsilane (TMS) or residual solvent peak and ^{13}C NMR (125 MHz) chemical shifts are reported in ppm with the solvents ($CDCl_3$: 77.16 ppm).

2-methyl-N-(3-phenylpropyl)-2-[[5-(trifluoromethyl)-2-pyridinyl]sulfonyl] propanamide (**ACP1a**). The compound was obtained as a white solid (52%yield). The obtained data matches the literature. Leung E, et al. Activators of cylindrical proteases as antimicrobials: identification and development of small molecule activators of ClpP protease. Chem. Biol. **2011**, 18, 1167–1178.

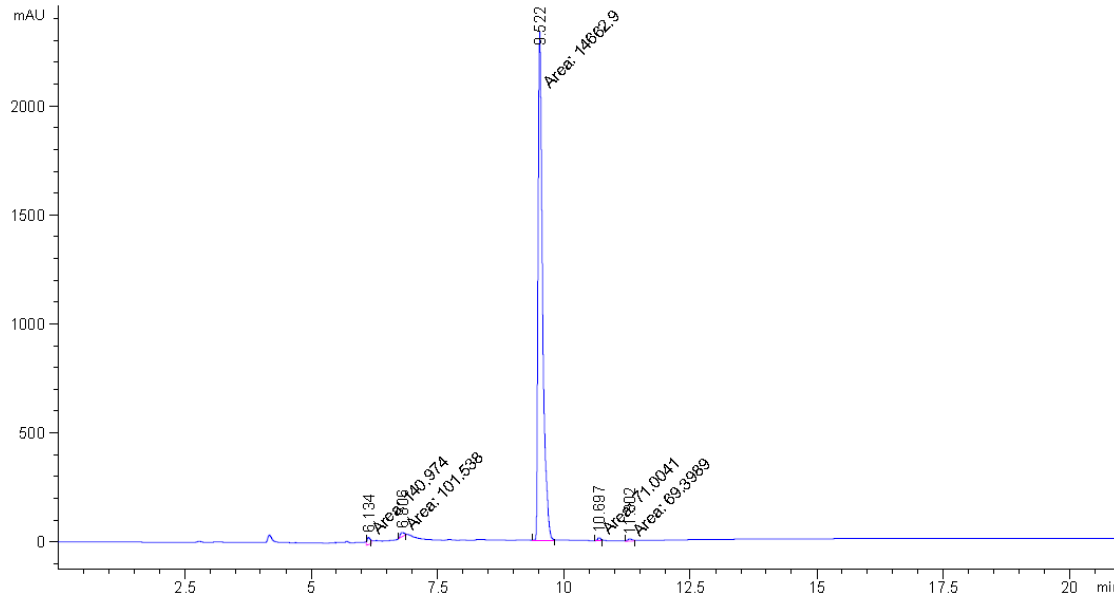
2-methyl-N-[2-(phenylthio)ethyl)]-2-[[5-(trifluoromethyl)-2-pyridiny]thio)]propanamide (**ACP1**). The title compound was obtained as a colorless oil (50 % yield) Rf: 0.39 (25 % v/v ethyl acetate in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.90 (1H, s), 8.20-8.18 (1H, d, J = 8 Hz), 8.15-8.13 (1H, d, J = 8.5 Hz), 7.40–7.39 (2H, m), 7.31–7.28 (2H, t, J = 7.5 Hz), 7.23–7.20 (1H, t, J = 7.5 Hz), 3.52–3.48 (2H, q, J = 6.5 Hz), 3.12-3.10 (2H, t, J = 6.5 Hz), 1.62 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.6, 158.1, 147.1, 135.5, 134.8, 130.1, 129.9, 126.6, 124.5, 121.2 (q, J=272.5 Hz), 67.7, 39.5, 32.8, 20.4. LCMS: single peak (254 and 285 nm), RT =2.71 min, MS (ESI^+) m/z = 433.0 $[M + H]^+$

N-[2-[(2-chlorophenyl)thio]ethyl]-2-methyl-2-[[5-(trifluoromethyl)-2-pyridinyl] sulfonyl]propanamide (**ACP1b**). The molecule was obtained in 44 % yield as white solid. Rf: 0.47 (25 % v/v ethyl acetate in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.92 (1H, s), 8.22-8.21 (1H, d, J = 8.5 Hz), 8.17-8.15 (1H, d, J = 8.5 Hz), 7.44–7.38 (2H, m), 7.26–7.21 (1H, t, J = 7.5 Hz), 7.17–7.14 (1H, t, J = 7.5 Hz), 3.54–3.50 (2H, q, J = 6.5 Hz), 3.15-3.13 (2H, t, J = 6.5 Hz), 1.64 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.9, 158.3, 147.3, 135.6, 134.6, 134.3, 130.2, 129.9, 127.58, 127.52, 124.6, 121.4 (q, J=272.5 Hz), 67.8, 39.4, 32.0, 20.6. LCMS: single peak (254 and 285 nm), RT =2.74 min, MS (ESI^+) m/z = 467.0 $[M + H]^+$

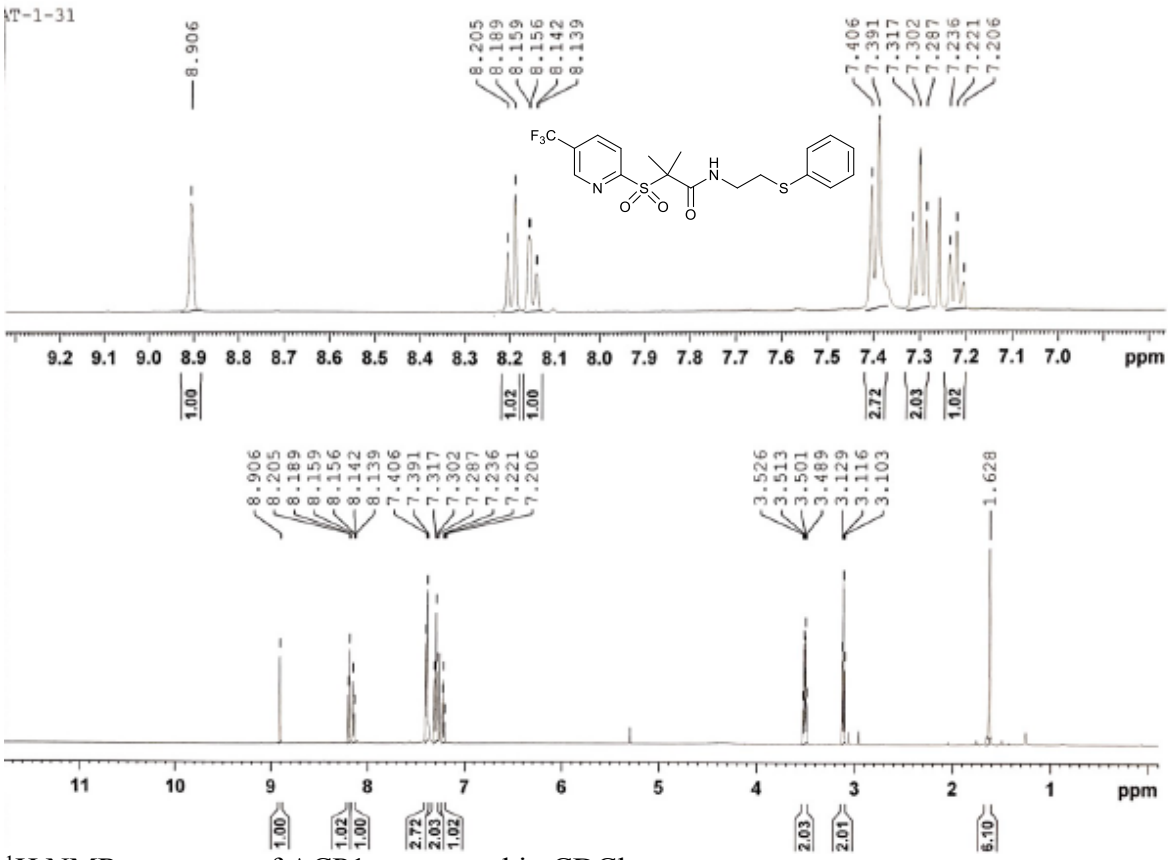
^{1}H NMR and ^{13}C NMR data of the compounds and analytical HPLC chromatogram.



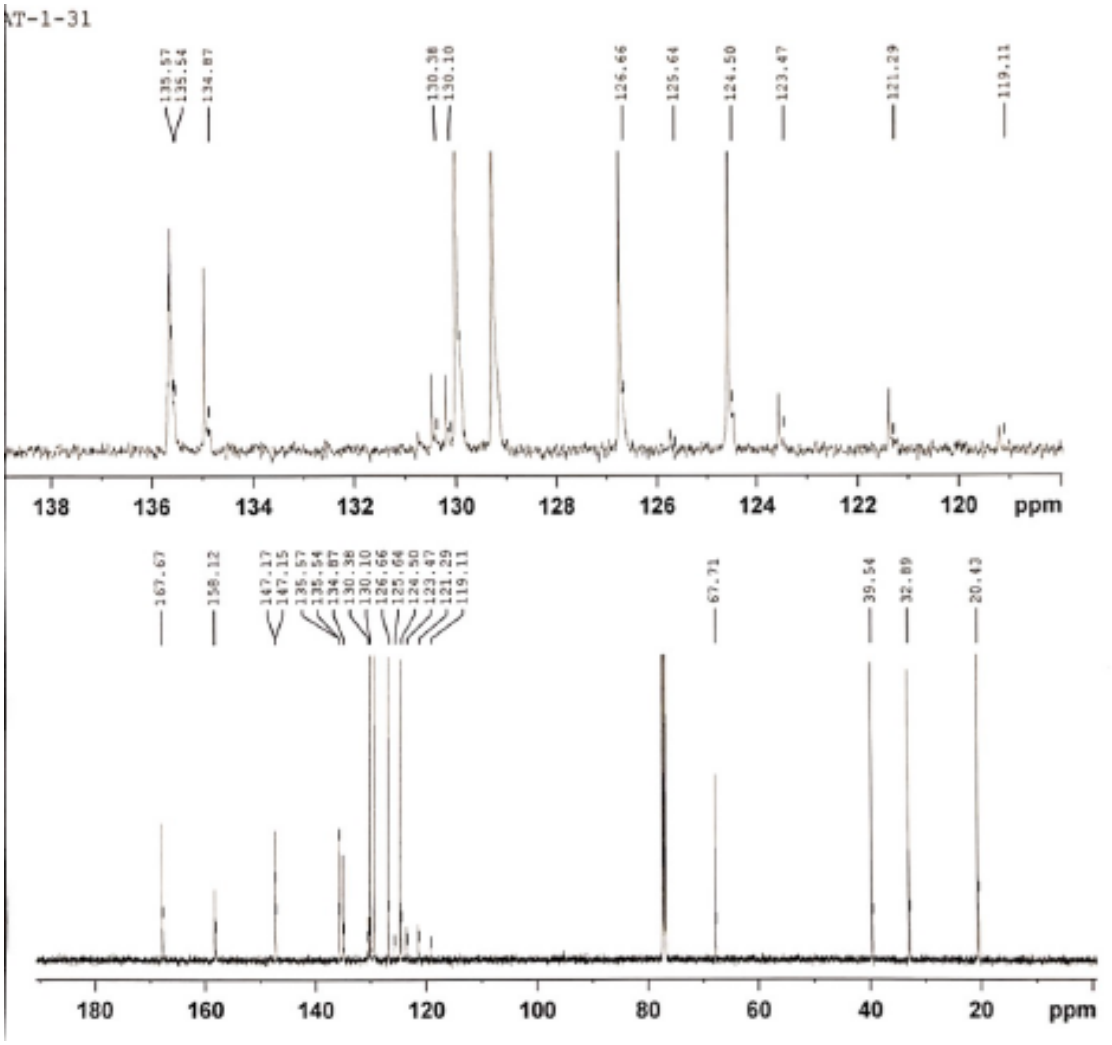
^{1}H NMR spectrum of ACP1a compound in $CDCl_3$.



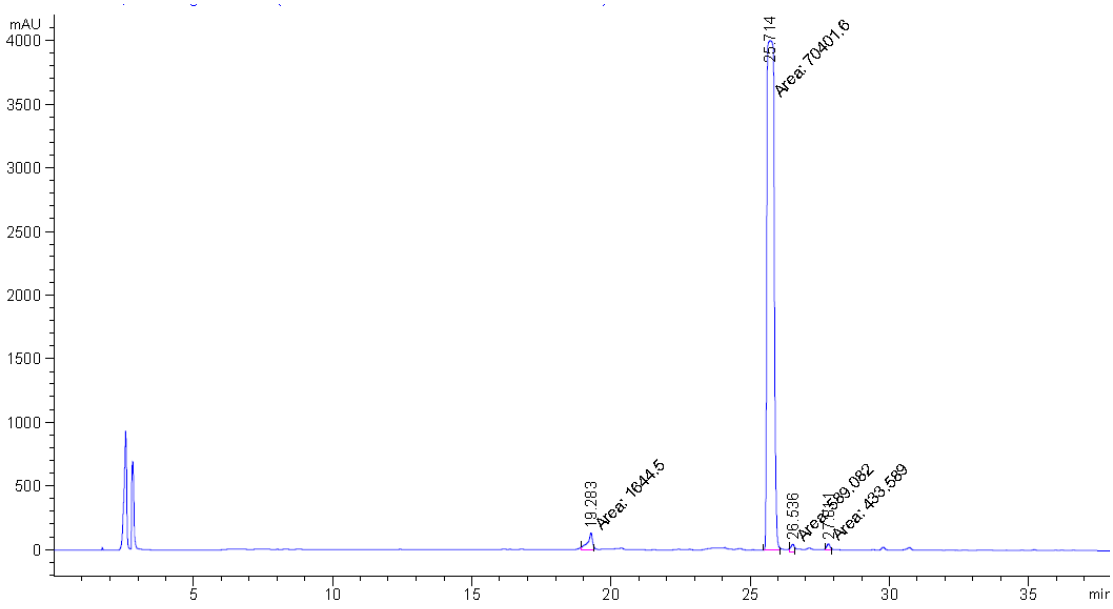
Analytical HPLC chromatogram of ACP1a (97.45% purity).



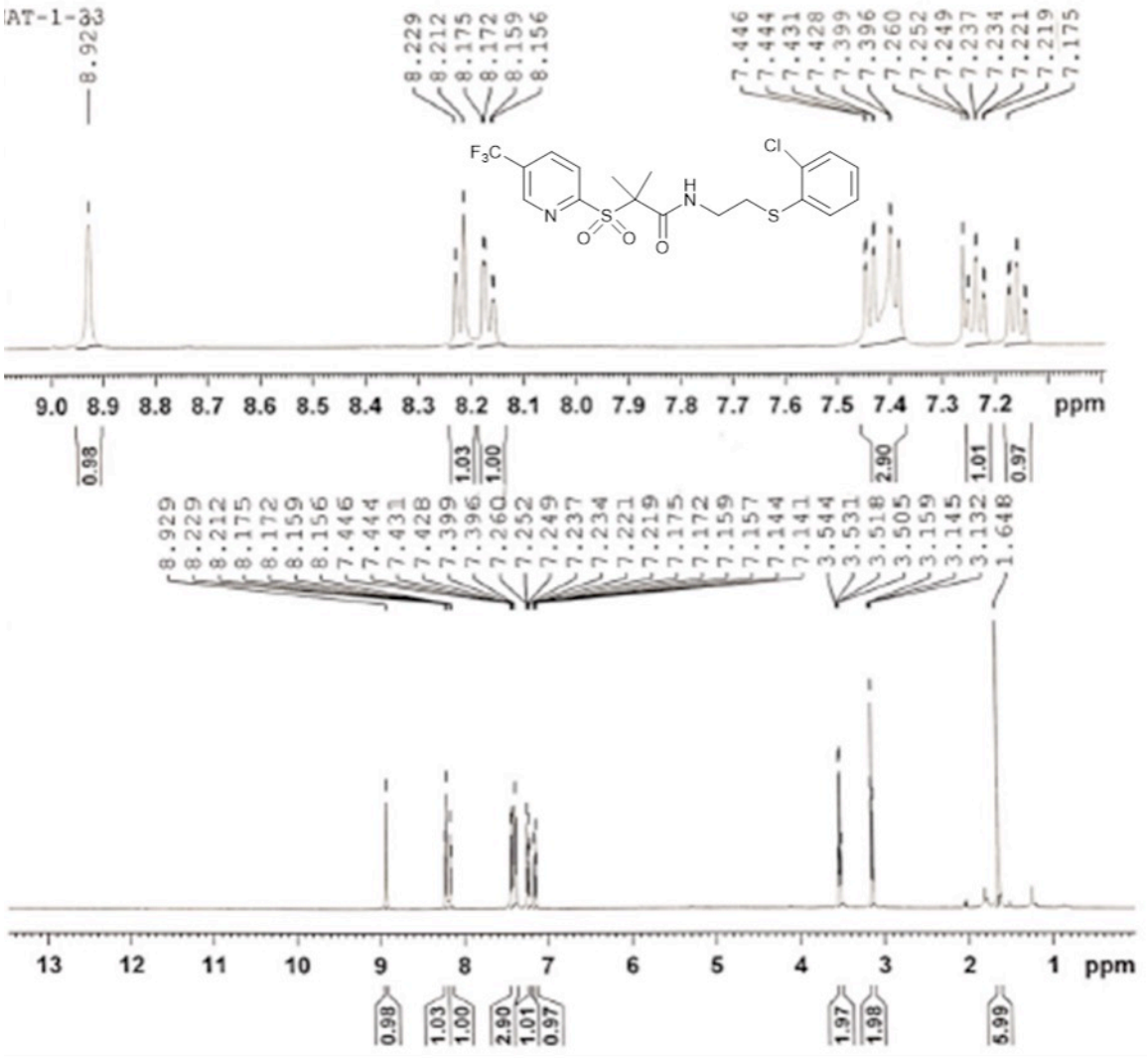
^{1}H NMR spectrum of ACP1 compound in $CDCl_3$.



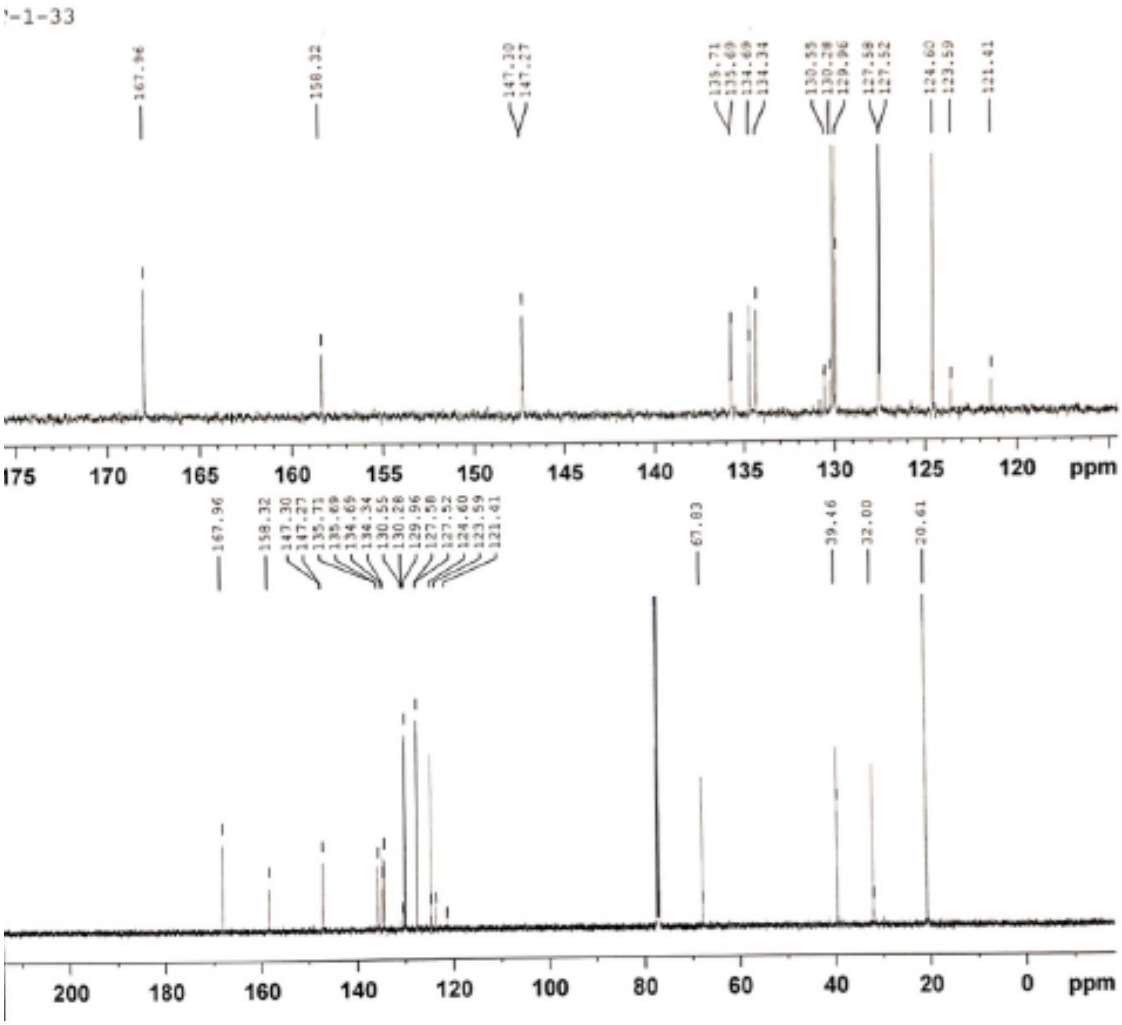
^{13}C NMR spectrum of ACP1 compound in $CDCl_3$.



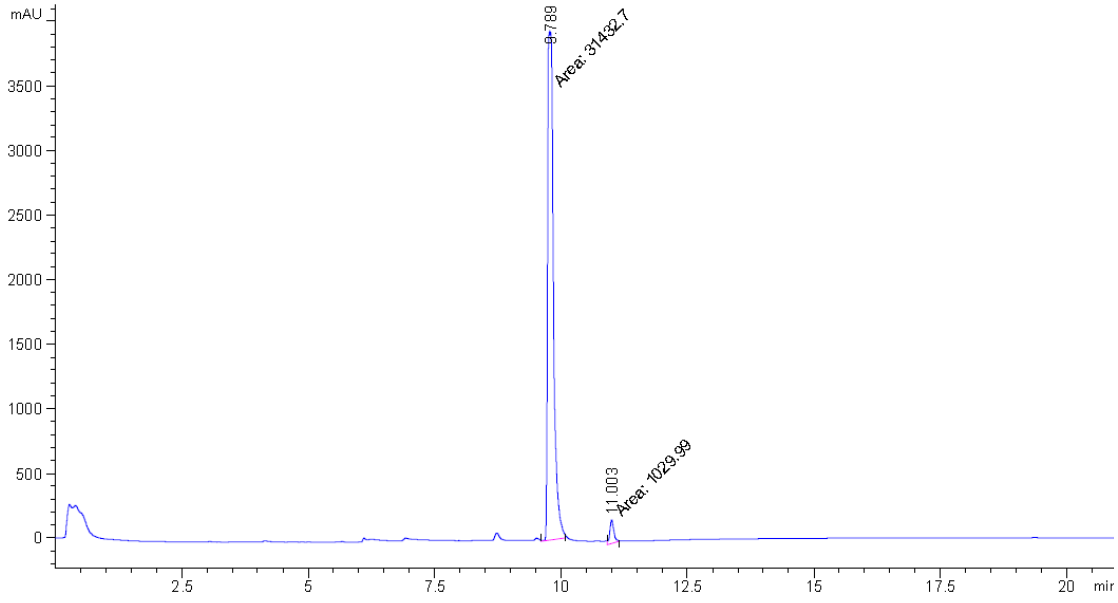
Analytical HPLC chromatogram of ACP1 (96.34% purity).



^{1}H NMR spectrum of ACP1b compound in $CDCl_3$.



^{13}C NMR spectrum of ACP1b compound in $CDCl_3$.



Analytical HPLC chromatogram of ACP1b (96.82% purity).