Supporting Information

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Experimental details

Solid Phase Peptide Synthesis

The resin (0.1 g) was first swelled by suspension in DCM for 30 minutes at room temperature and then drained. The resin was then washed with DMF (3 × 2 mL). Fmoc removal was accomplished with treatment of the resin by 2 mL of 20% piperidine in DMF and shaking at 200 rpm for 7.5 minutes. This process was then repeated once more for a total of 15 minutes deprotection time and then washed with DMF (3 × 2 mL). To couple each amino acid, Fmoc protected amino acids (2 equiv. relative to the resin loading) was dissolved in 2 mL DMF and mixed with 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU; 2 equiv.) and diisopropylethylamine (DIPEA; 2 equiv.). The solution was allowed to pre-activate for 1 minute prior to being added to the resin and shaken at 200 rpm for 1 hour at room temperature. After each coupling step, the resin was drained and washed with DMF (2 × 2 mL).

Final cleavage

The resin containing the final peptide product was washed with 5 mL diethyl ether and air dried for 10 minutes before transferring it into a 15 mL falcon tube. 3 mL of 2.5% water and 2.5% TIPS in TFA was then added and the tube was shaken for 3 hours. The cleaved peptide was filtered through a cotton ball into a 50 ml falcon tube. A cooled solution of diethyl ether:hexane (1:1, 45 ml, -20 °C) was added to the filtrate, and the peptide was allowed to precipitate in a freezer at -20 °C for 10 minutes. The 50 mL tube was centrifuged in a Beckman Coulter Allegra 6 centrifuge equipped with a GH3.8 rotor at 3000 rpm for 5 minutes to pellet the crude peptide. The supernatant was decanted off and the remaining crude peptide was dissolved in 10 mL ACN:H_2O (1:1) and lyophilized.

Biological Assays
Beta-Galactosidase Assays

An initial activation screening was performed at a high concentration (10 µM) for all CSP2 analogs. 198 µL bacterial culture were placed in triplicate to a 96-well plate containing 2 µL of CSP2 peptides and incubated at 37 °C for 30 minutes. A total of 2 µL of 20 µM solution of CSP1 (200 nM final concentration) were added in triplicate and served as the positive control for the group I strain (D39pcomX::lacZ), while 2 µL of 100 µM solution of CSP2 (1000 nM final concentration) were added as the positive control for the group II strain (TIGR4pcomX::lacZ). These concentrations were chosen to afford full activation of the QS circuit, as determined from the dose-dependent curves created for the native CSPs. 2 µL dimethyl sulfoxide (DMSO) were added in triplicate and served as the negative control. After the incubation time had elapsed, the absorbance at 600_{nm} was read. The wells were then treated with 20 µL 1% Triton X-100 in water for 30 minutes at 37 °C to lyse the bacterial cells. In a new plate, 100 µL Z-buffer solution (60.2 mM Na_2HPO_4, 45.8 mM NaH_2PO_4, 10 mM KCl, and 1.0 mM $MgSO_4$ in 18 MΩ H_2O; pH was adjusted to 7.0 and the buffer was sterilized before use) containing 2-Nitrophenyl-Beta-D-galactopyranoside (ONPG) at a final concentration of 0.4 mg/mL were added, followed by 100 µL lysate, and the plate was incubated for 3 hours at 37 ºC. After the incubation, the reaction was quenched by adding 50 µL of 1 M sodium carbonate solution, and the OD 420_{nm} and OD 550_{nm} were measured using a plate reader, allowing for the calculation of the activity in Miller units (see below). The final results were reported as percent activation, which is the ratio between the Miller units of the analog and that of the positive control.

$$Miller\ Unit = 1000 \times \frac{[Abs_{420} - (1.75 \times Abs_{550})]}{(t \times v \times Abs_{600})}$$

Abs_{420} is the absorbance of o-nitrophenol (ONP). Abs_{550} is the scatter from cell debris, which, when multiplied by 1.75 approximates the scatter observed at 420 nm. *t* is the duration of incubation with ONPG in minutes, *v* is volume of lysate in milliliters, and Abs_{600} reflects cell density.

EC_{50} Experiments

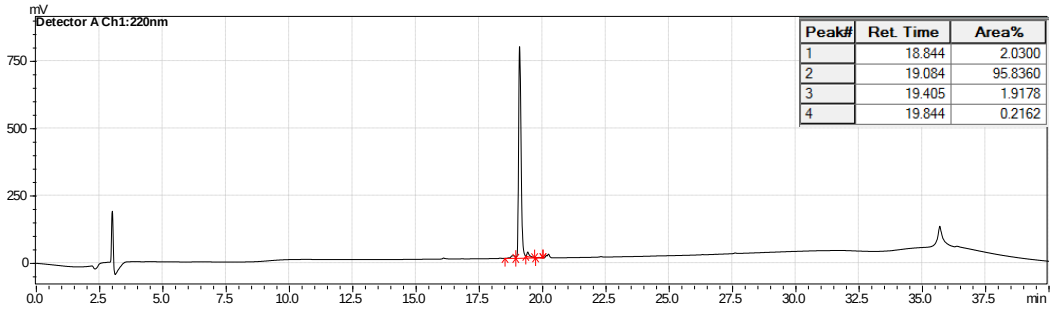
Analogs that exhibited high activity in the initial screening were further evaluated using a dose-dependent assay in which peptide stock solutions were diluted with DMSO in serial dilution and assayed as described above. The EC_{50} value was then determined through fitting using nonlinear regression with GraphPad Prism 5.

IC_{50} Experiments

Analogs that exhibited low *comX* activation in the initial screening were evaluated for competitive inhibition. The ability of synthesized CSP2 analogs to inhibit the expression of *comX* by outcompeting CSP for the receptor binding site was evaluated using the same assay conditions as described above, except that in the initial inhibition screening, the native CSP was added to every well in a set concentration (50 nM CSP1 for group I; 250 nM CSP2 for group II) that was chosen to afford full activation of the QS circuit, as determined from the dose-dependent curves created for the native CSPs. 2 µL of native CSP (5 µM solution of CSP1 for group I; 25 µM solution of CSP2 for group II) and 2 µL of 1 mM solution of CSP analogs were added to the same well in triplicate in a clear 96-well microtiter plate. 2 µL native CSP (5 µM solution of CSP1 for group I; 25 µM solution of CSP2 for group II) and 2 µL DMSO were added to the same well in triplicate and served as the positive control. 4 µL DMSO were added in triplicate and served as the negative control. Then, 196 µL bacterial culture were added to the wells and the plate was incubated at 37 ºC for 30 minutes. The procedure for lysis, incubation with ONPG and all the measurements were as described in the beta-galactosidase assay section above. Analogs that exhibited significant competitive inhibition in the initial screening were further evaluated using a dose-dependent assay where peptide stock solutions were diluted with DMSO in serial dilutions and assayed as described above. GraphPad Prism 5 was used to calculate the IC_{50} values, which are the concentration of an inhibitor where the response (or binding) is reduced by half.

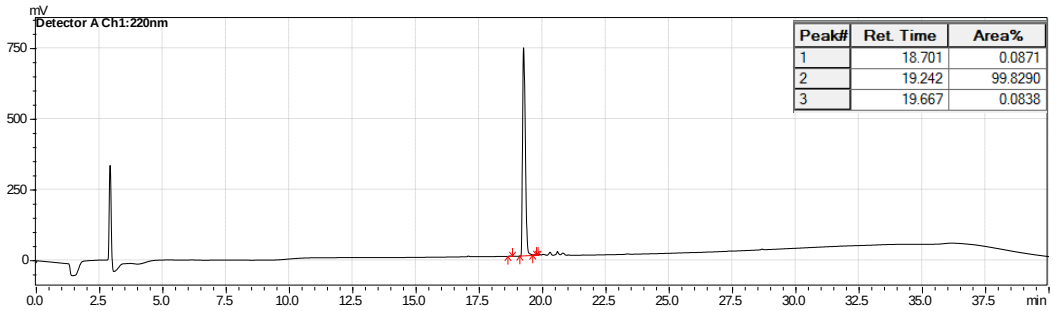
HPLC Traces

CSP2(15)-I4LI7F



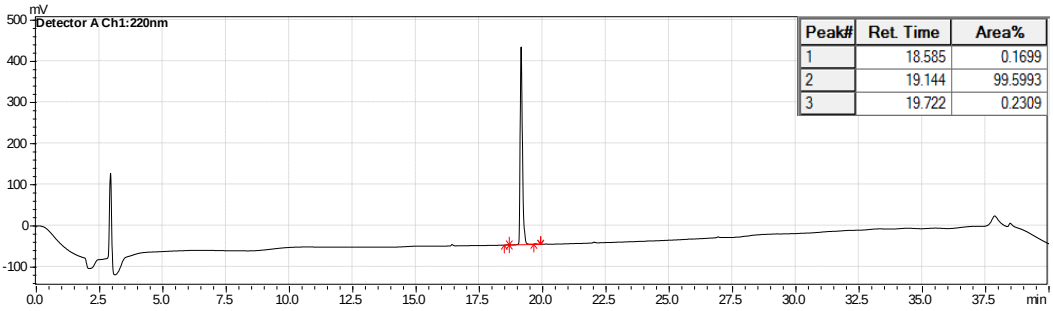
Peak#	Ret. Time	Area%
1	18.844	2.0300
2	19.084	95.8360
3	19.405	1.9178
4	19.844	0.2162

CSP2(15)-I4LL9R



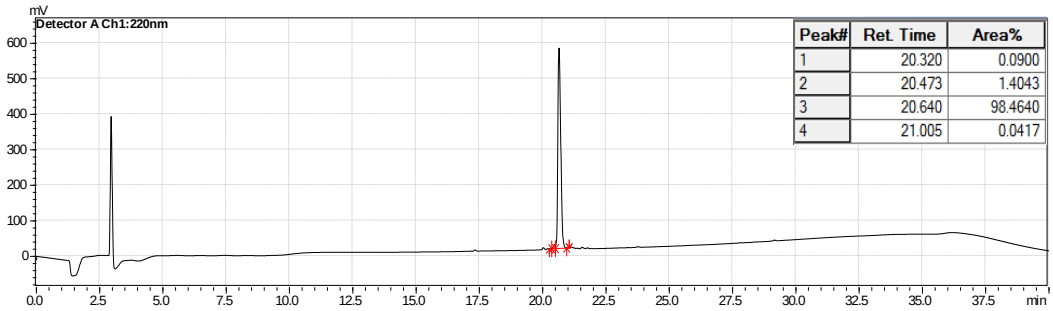
Peak#	Ret. Time	Area%
1	18.701	0.0871
2	19.242	99.8290
3	19.667	0.0838

CSP2(15)-I4LL12I



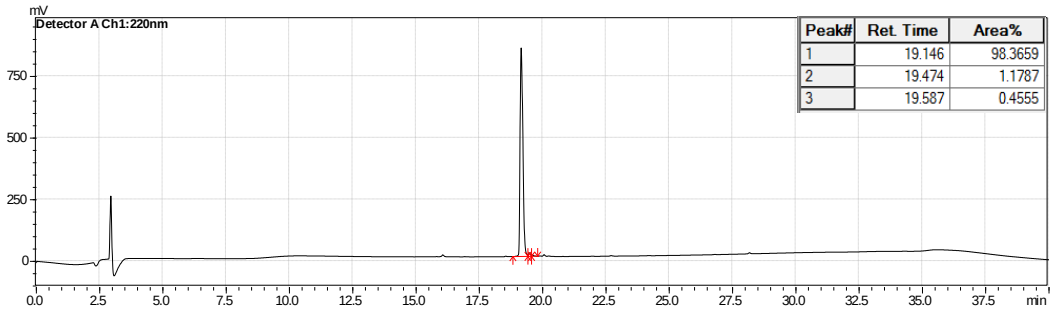
Peak#	Ret. Time	Area%
1	18.585	0.1699
2	19.144	99.5993
3	19.722	0.2309

CSP2(15)-I4LF13L



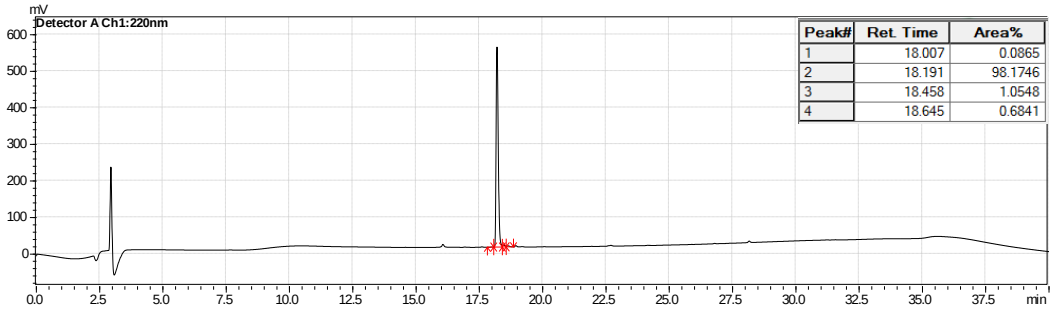
Peak#	Ret. Time	Area%
1	20.320	0.0900
2	20.473	1.4043
3	20.640	98.4640
4	21.005	0.0417

CSP2(15)-I4LL14Q



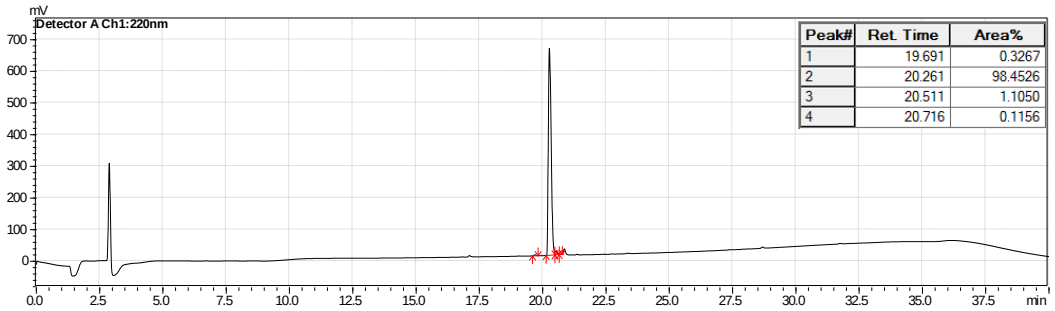
Peak#	Ret. Time	Area%
1	19.146	98.3659
2	19.474	1.1787
3	19.587	0.4555

CSP2(15)-I7FL9R



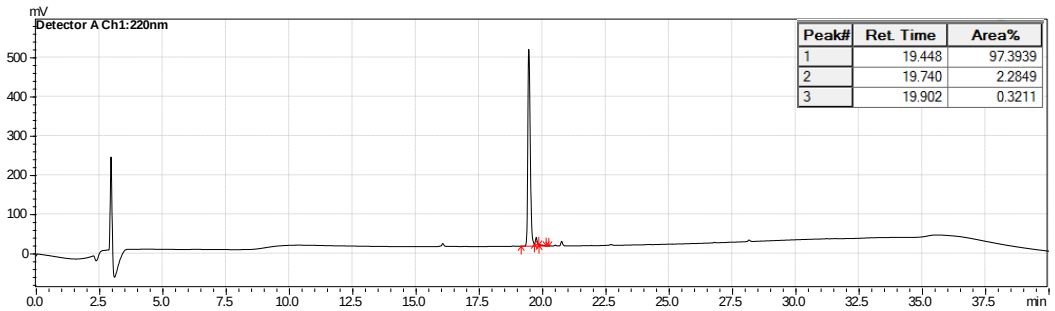
Peak#	Ret. Time	Area%
1	18.007	0.0865
2	18.191	98.1746
3	18.458	1.0548
4	18.645	0.6841

CSP2(15)-I7FL12I



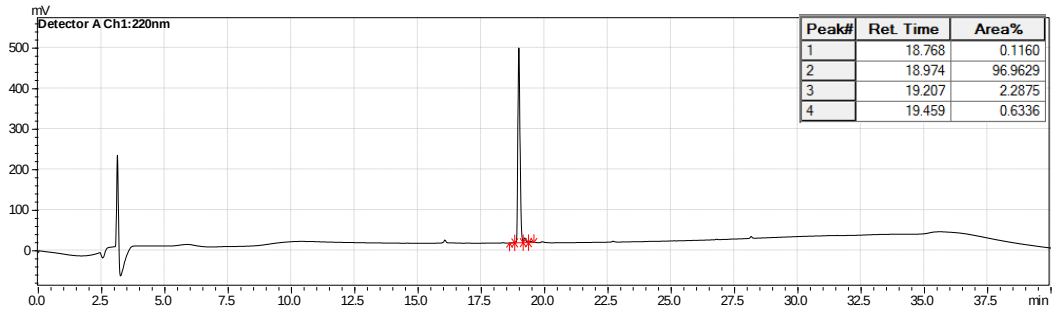
Peak#	Ret. Time	Area%
1	19.691	0.3267
2	20.261	98.4526
3	20.511	1.1050
4	20.716	0.1156

CSP2(15)-I7FF13L



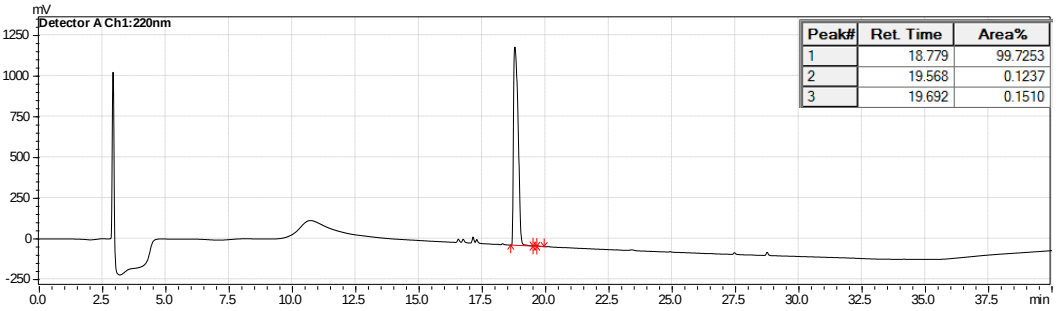
Peak#	Ret. Time	Area%
1	19.448	97.3939
2	19.740	2.2849
3	19.902	0.3211

CSP2(15)-I7FL14Q



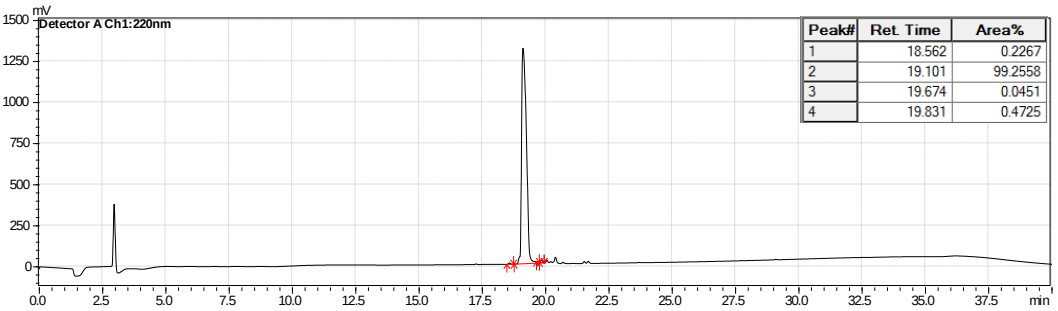
Peak#	Ret. Time	Area%
1	18.768	0.1160
2	18.974	96.9629
3	19.207	2.2875
4	19.459	0.6336

CSP2(15)-L9RL12I



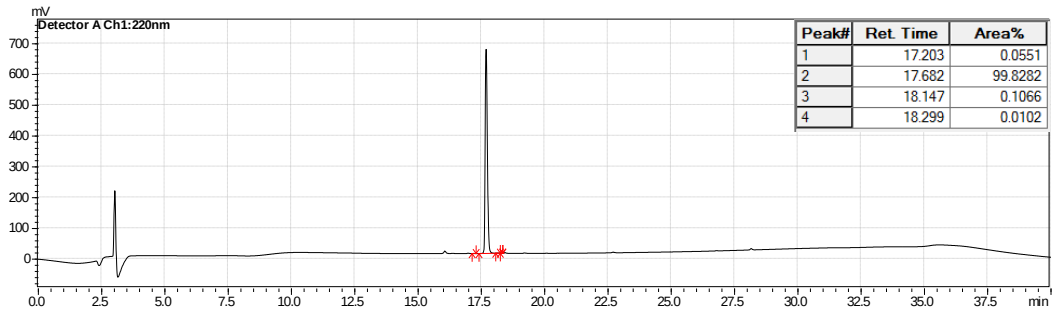
Peak#	Ret. Time	Area%
1	18.779	99.7253
2	19.568	0.1237
3	19.692	0.1510

CSP2(15)-L9RF13L



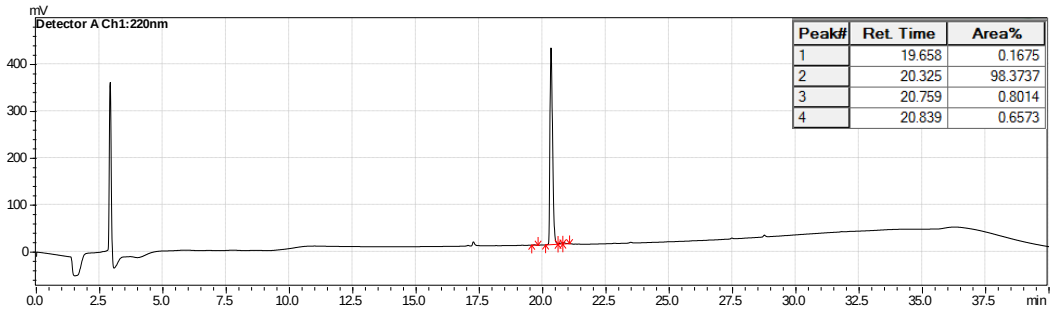
Peak#	Ret. Time	Area%
1	18.562	0.2267
2	19.101	99.2558
3	19.674	0.0451
4	19.831	0.4725

CSP2(15)L9RL14Q



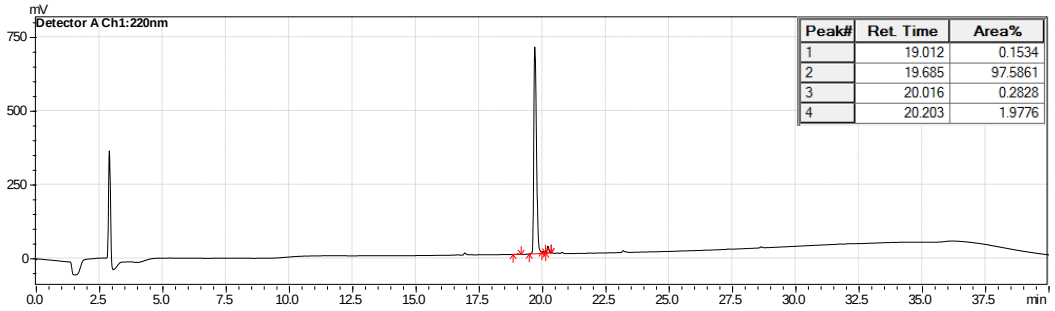
Peak#	Ret. Time	Area%
1	17.203	0.0551
2	17.682	99.8282
3	18.147	0.1066
4	18.299	0.0102

CSP2(15)-L12IF13L



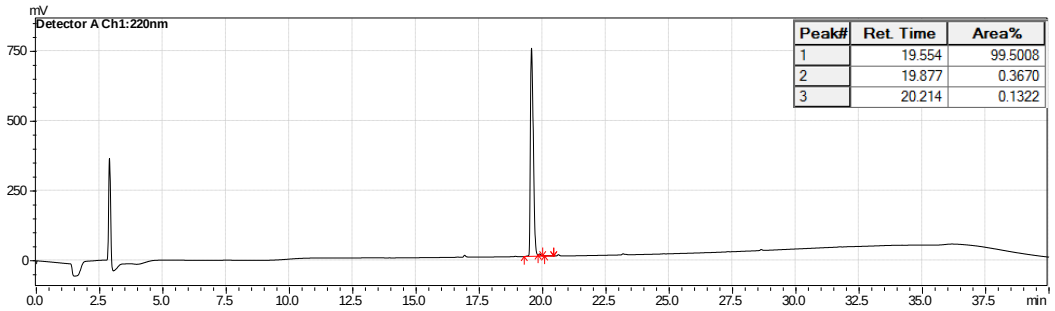
Peak#	Ret. Time	Area%
1	19.658	0.1675
2	20.325	98.3737
3	20.759	0.8014
4	20.839	0.6573

CSP2(15)-L12IL14Q



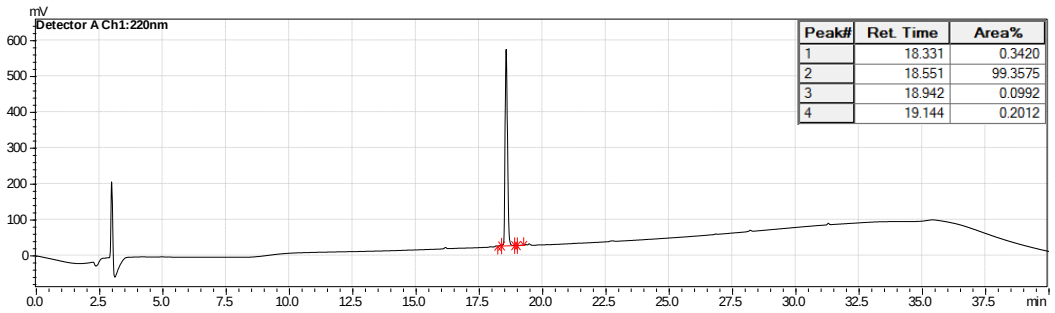
Peak#	Ret. Time	Area%
1	19.012	0.1534
2	19.685	97.5861
3	20.016	0.2828
4	20.203	1.9776

CSP2(15)-F13LL14Q



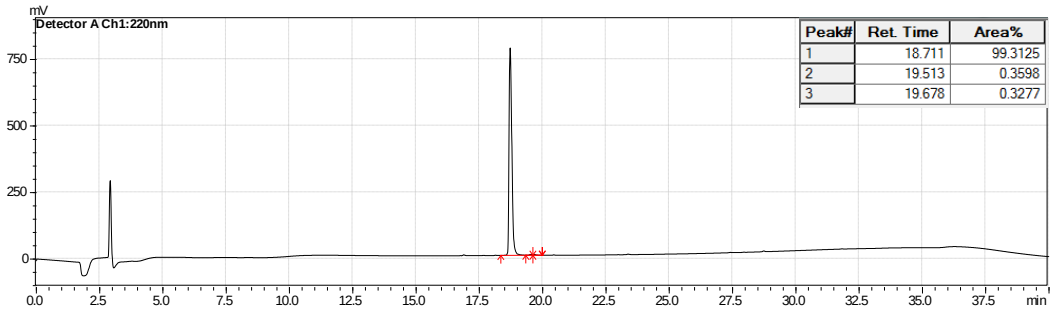
Peak#	Ret. Time	Area%
1	19.554	99.5008
2	19.877	0.3670
3	20.214	0.1322

CSP2(15)-I4LL9RF13L



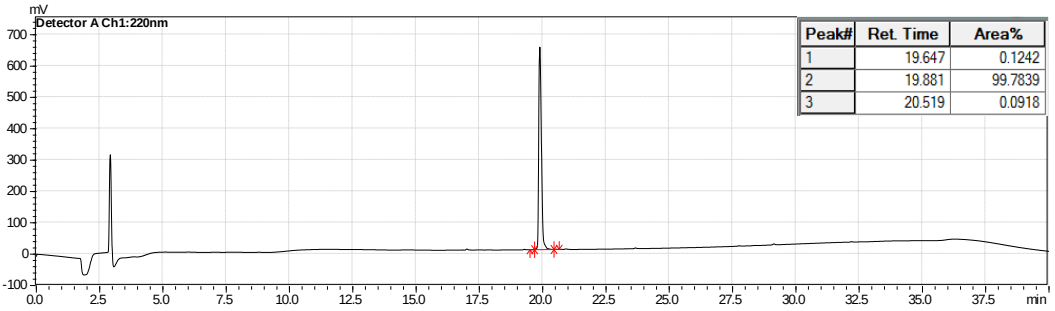
Peak#	Ret. Time	Area%
1	18.331	0.3420
2	18.551	99.3575
3	18.942	0.0992
4	19.144	0.2012

CSP2(15)-I4LL9RL14Q



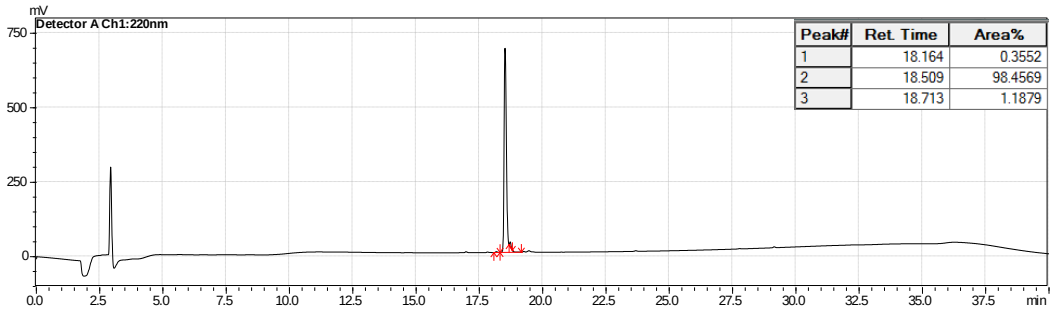
Peak#	Ret. Time	Area%
1	18.711	99.3125
2	19.513	0.3598
3	19.678	0.3277

CSP2(15)-I4LF13LL14Q



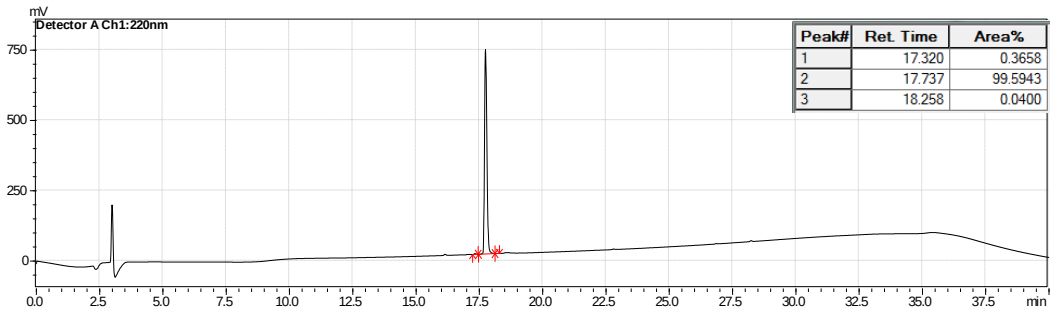
Peak#	Ret. Time	Area%
1	19.647	0.1242
2	19.881	99.7839
3	20.519	0.0918

CSP2(15)-L9RF13LL14Q



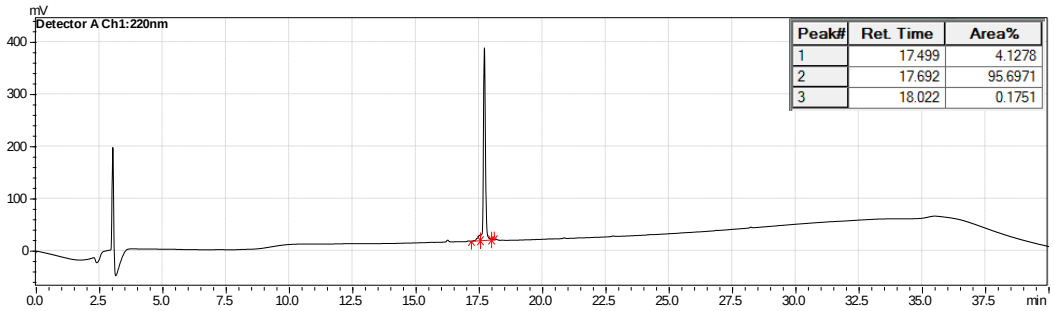
Peak#	Ret. Time	Area%
1	18.164	0.3552
2	18.509	98.4569
3	18.713	1.1879

CSP2(15)-I4LL9RF13LL14Q



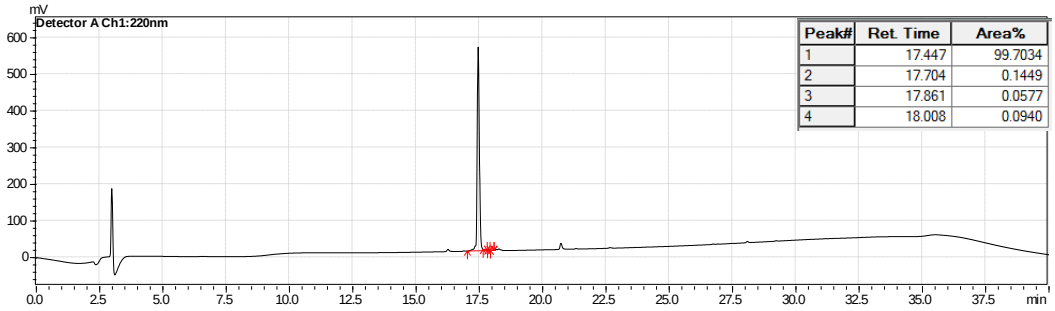
Peak#	Ret. Time	Area%
1	17.320	0.3658
2	17.737	99.5943
3	18.258	0.0400

CSP2(15)-I4LR6KL9RF13LL14Q



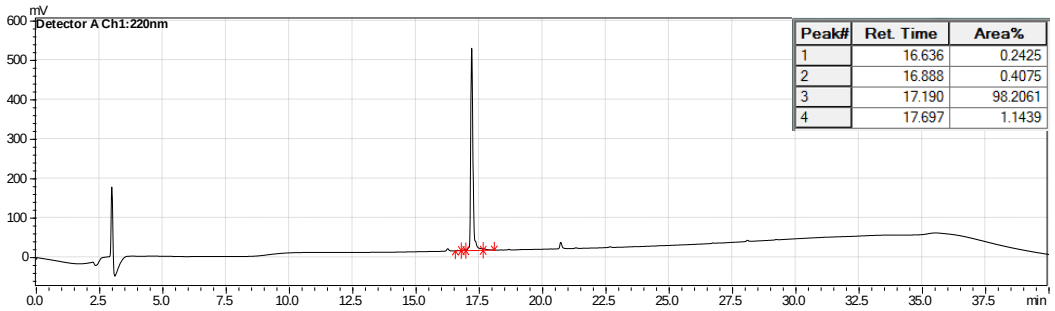
Peak#	Ret. Time	Area%
1	17.499	4.1278
2	17.692	95.6971
3	18.022	0.1751

CSP2(15)-I4LI7FL9RF13LL14Q



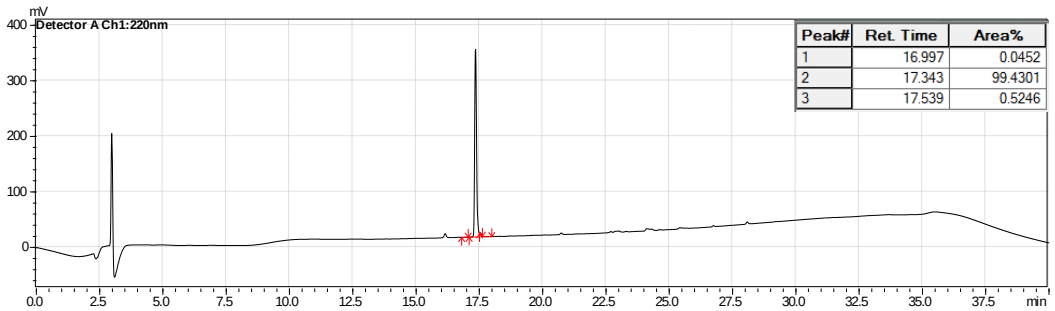
Peak#	Ret. Time	Area%
1	17.447	99.7034
2	17.704	0.1449
3	17.861	0.0577
4	18.008	0.0940

CSP2(15)-I4LI8FL9RF13LL14Q



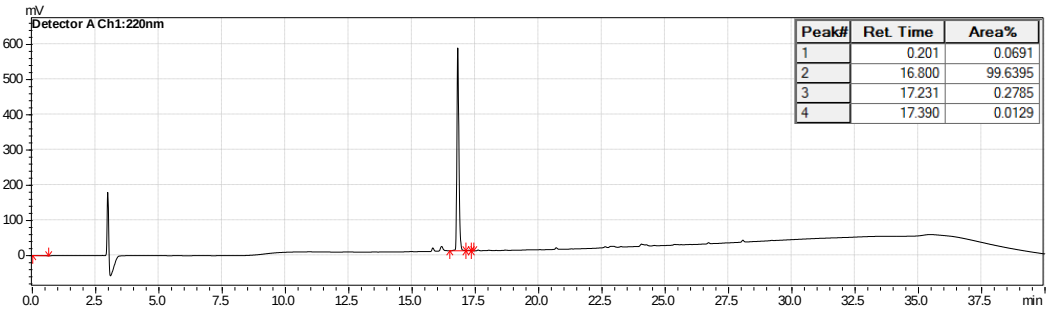
Peak#	Ret. Time	Area%
1	16.636	0.2425
2	16.888	0.4075
3	17.190	98.2061
4	17.697	1.1439

CSP2(15)-I4LL9RL12IF13LL14Q



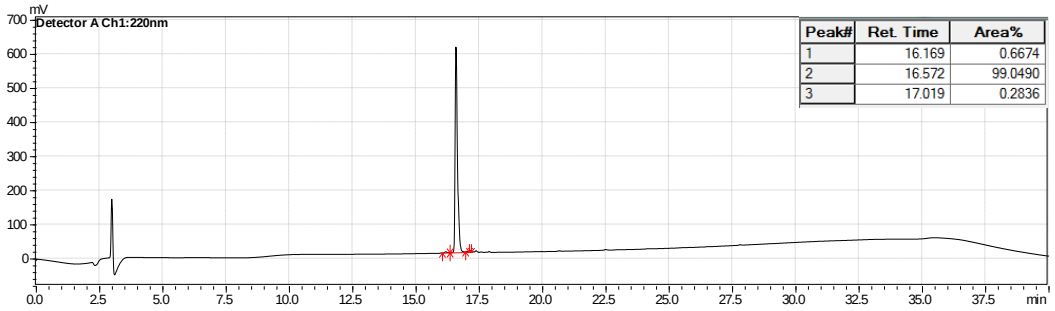
Peak#	Ret. Time	Area%
1	16.997	0.0452
2	17.343	99.4301
3	17.539	0.5246

CSP2-E1AL9RL14Q



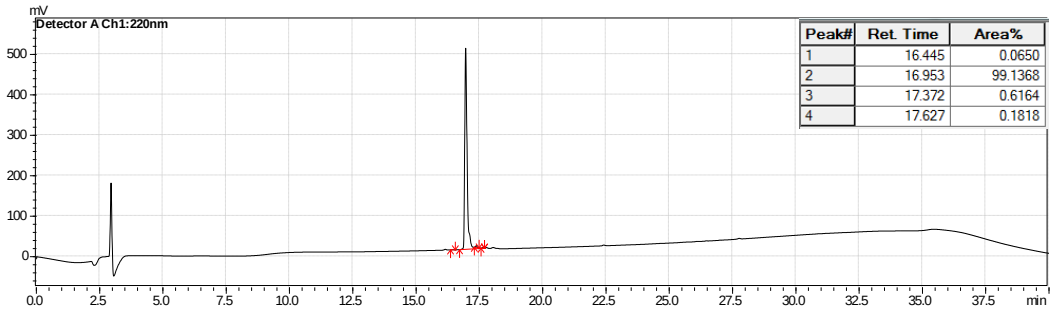
Peak#	Ret. Time	Area%
1	0.201	0.0691
2	16.800	99.6395
3	17.231	0.2785
4	17.390	0.0129

CSP2-E1AL9Rd10L14Q



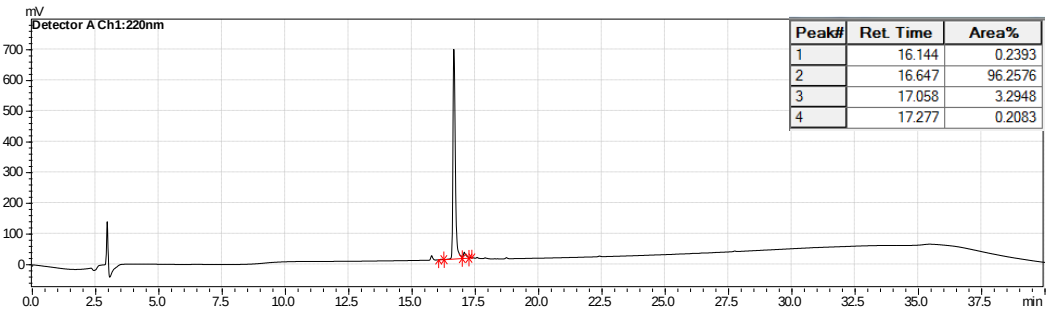
Peak#	Ret. Time	Area%
1	16.169	0.6674
2	16.572	99.0490
3	17.019	0.2836

CSP2-E1AI4LL9RL14Q



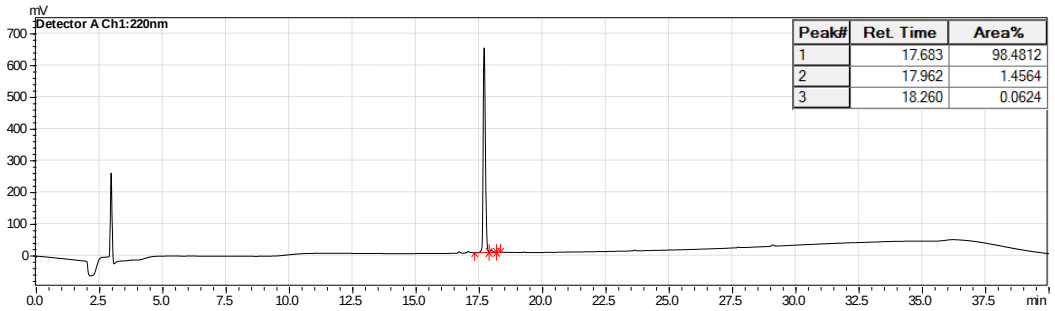
Peak#	Ret. Time	Area%
1	16.445	0.0650
2	16.953	99.1368
3	17.372	0.6164
4	17.627	0.1818

CSP2-E1AI4LL9Rd10L14Q



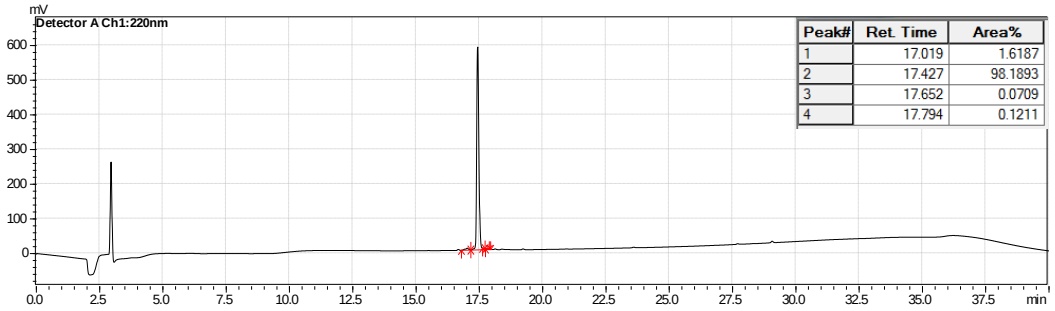
Peak#	Ret. Time	Area%
1	16.144	0.2393
2	16.647	96.2576
3	17.058	3.2948
4	17.277	0.2083

CSP2-E1AI4LL9RF13LL14Q



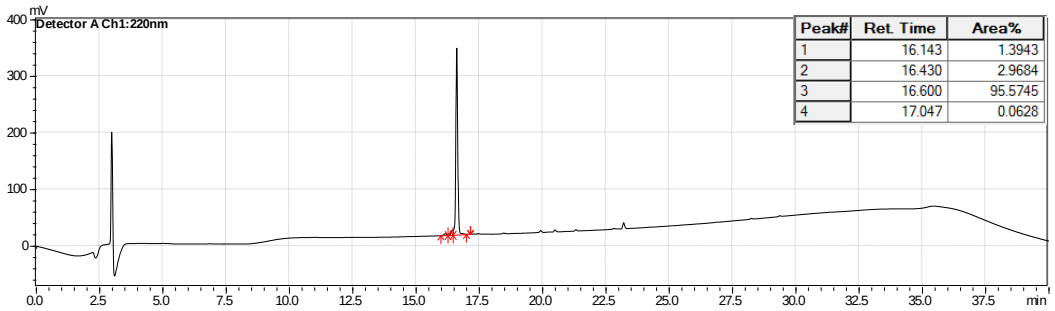
Peak#	Ret. Time	Area%
1	17.683	98.4812
2	17.962	1.4564
3	18.260	0.0624

CSP2-E1AI4LL9Rd10F13LL14Q



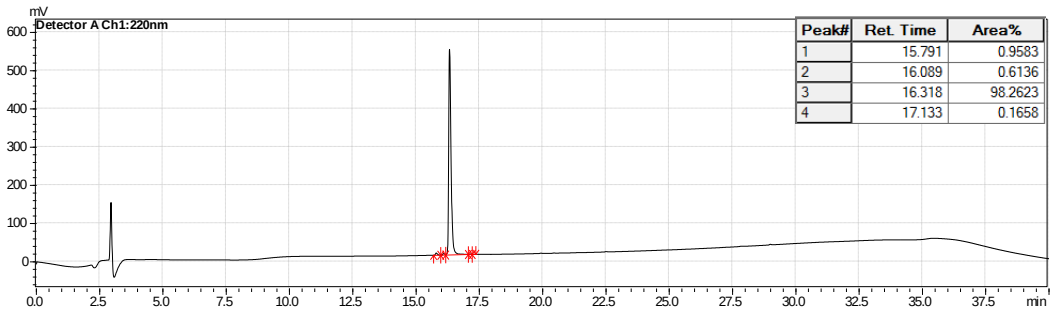
Peak#	Ret. Time	Area%
1	17.019	1.6187
2	17.427	98.1893
3	17.652	0.0709
4	17.794	0.1211

CSP2-E1AI4LI8FL9RF13LL14Q



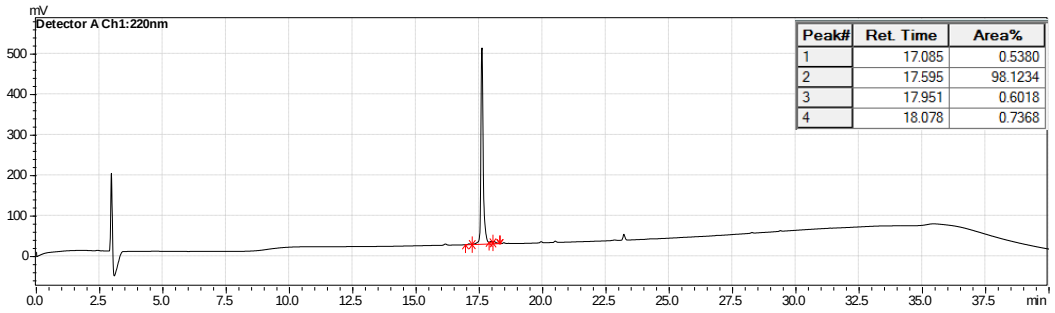
Peak#	Ret. Time	Area%
1	16.143	1.3943
2	16.430	2.9684
3	16.600	95.5745
4	17.047	0.0628

CSP2-E1AI4LI8FL9Rd10F13LL14Q



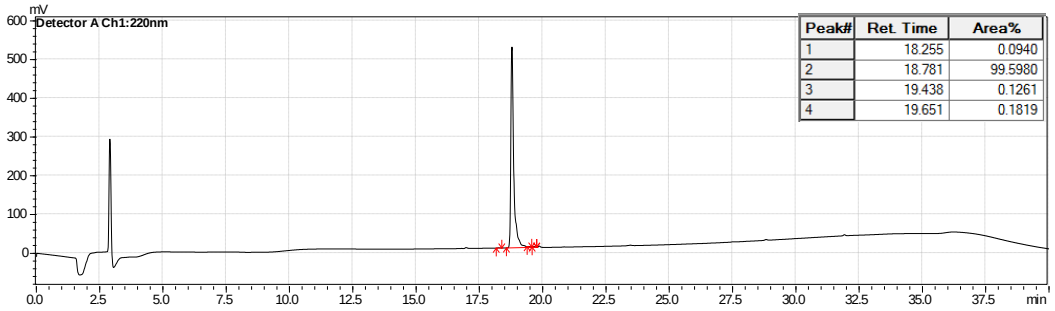
Peak#	Ret. Time	Area%
1	15.791	0.9583
2	16.089	0.6136
3	16.318	98.2623
4	17.133	0.1658

CSP2-E1AI4Nvad10



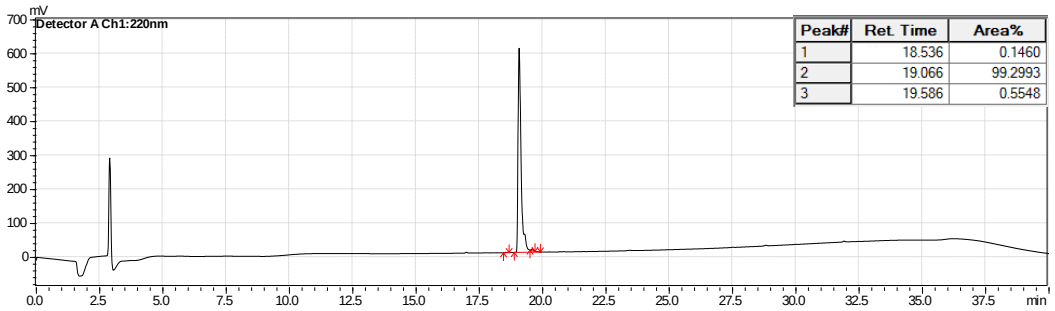
Peak#	Ret. Time	Area%
1	17.085	0.5380
2	17.595	98.1234
3	17.951	0.6018
4	18.078	0.7368

CSP2-E1AI4Nled10



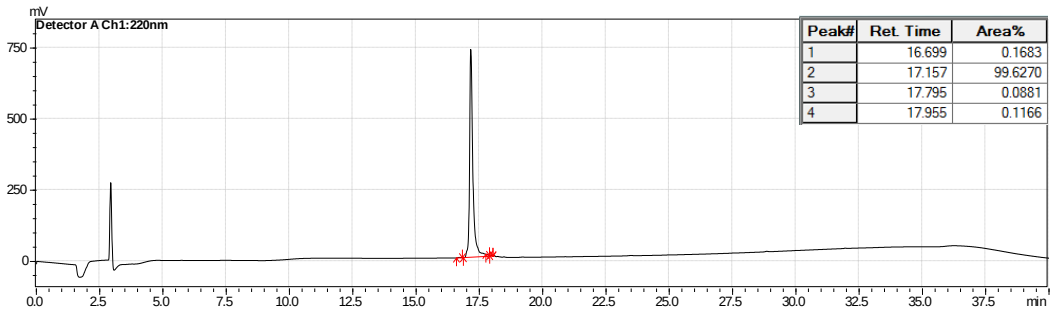
Peak#	Ret. Time	Area%
1	18.255	0.0940
2	18.781	99.5980
3	19.438	0.1261
4	19.651	0.1819

CSP2-E1AI4HLeud10



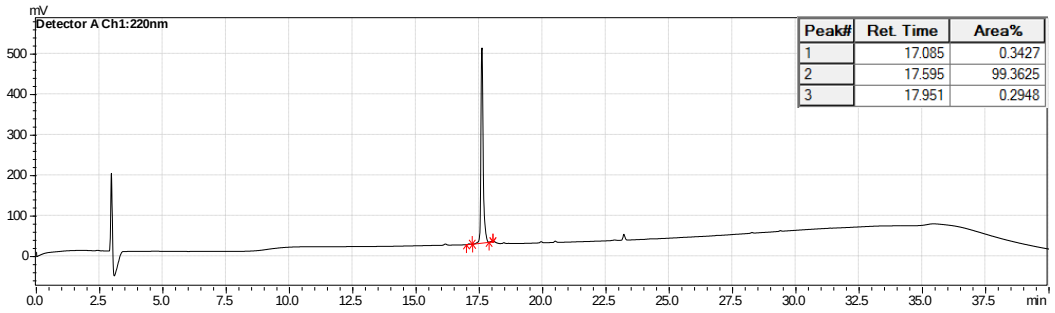
Peak#	Ret. Time	Area%
1	18.536	0.1460
2	19.066	99.2993
3	19.586	0.5548

CSP2-E1AI4NvaI8FL9RF13LL14Q



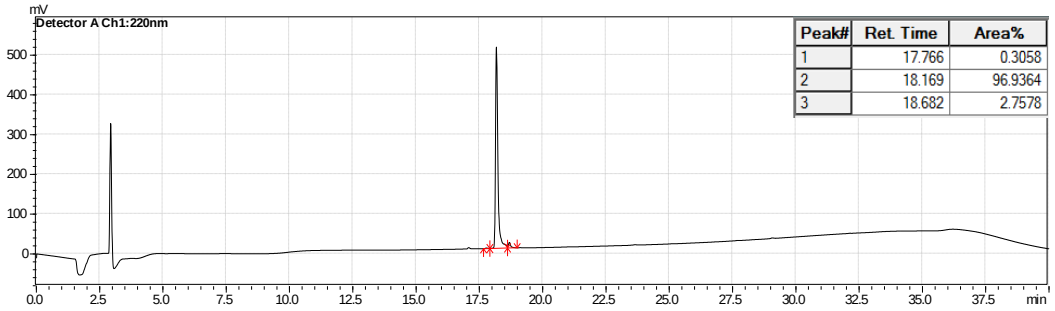
Peak#	Ret. Time	Area%
1	16.699	0.1683
2	17.157	99.6270
3	17.795	0.0881
4	17.955	0.1166

CSP2-E1AI4NvaI8FL9Rd10F13LL14Q



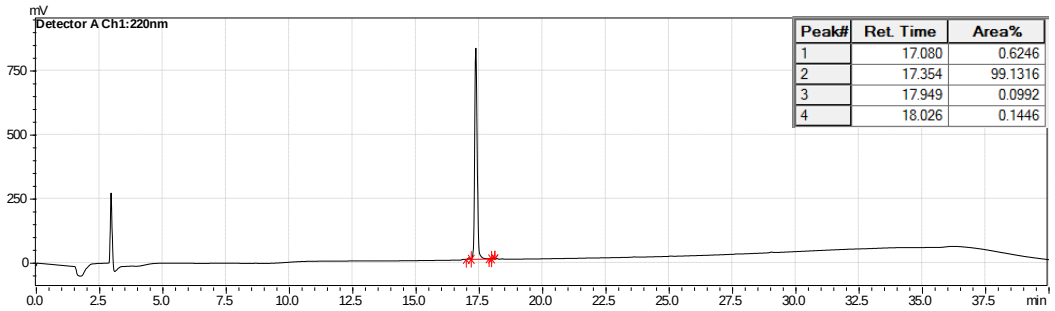
Peak#	Ret. Time	Area%
1	17.085	0.3427
2	17.595	99.3625
3	17.951	0.2948

CSP2-E1AI4NvaI8Fd10



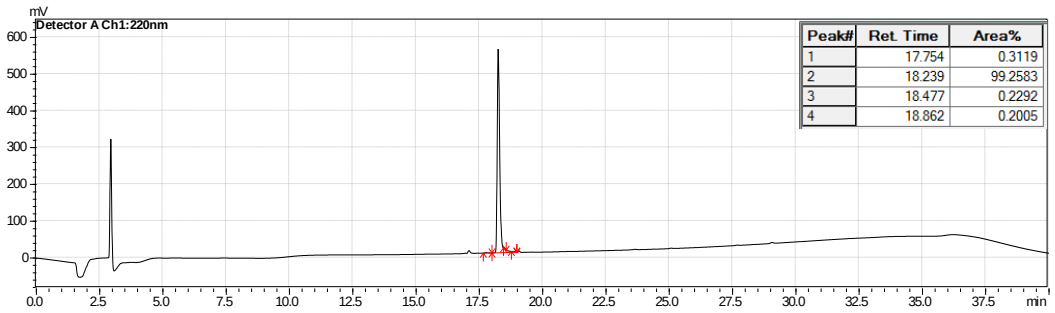
Peak#	Ret. Time	Area%
1	17.766	0.3058
2	18.169	96.9364
3	18.682	2.7578

CSP2-E1AI4NvaL9Rd10



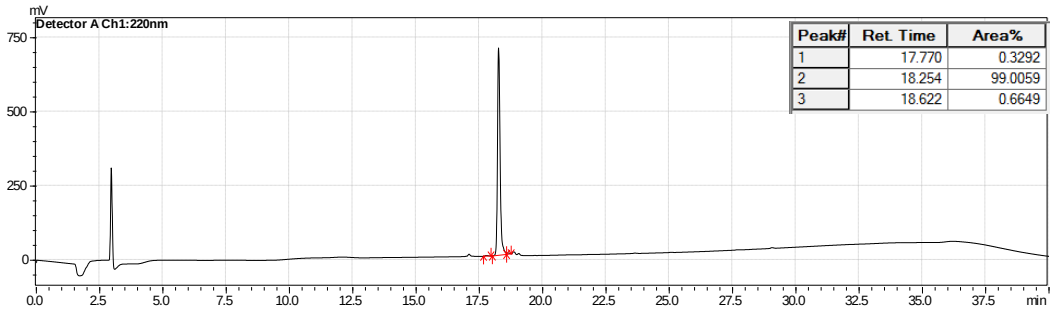
Peak#	Ret. Time	Area%
1	17.080	0.6246
2	17.354	99.1316
3	17.949	0.0992
4	18.026	0.1446

CSP2-E1AI4Nvad10F13L



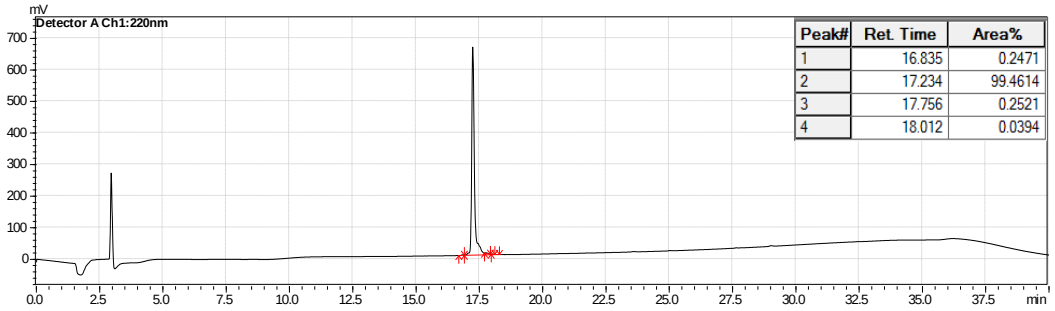
Peak#	Ret. Time	Area%
1	17.754	0.3119
2	18.239	99.2583
3	18.477	0.2292
4	18.862	0.2005

CSP2-E1AI4Nvad10L14Q



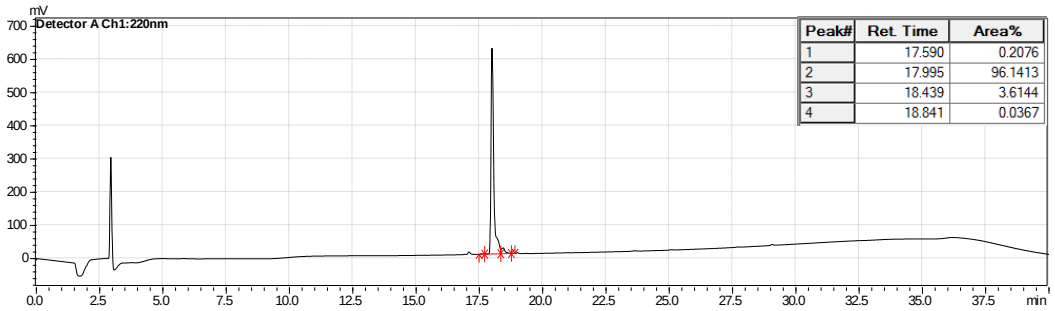
Peak#	Ret. Time	Area%
1	17.770	0.3292
2	18.254	99.0059
3	18.622	0.6649

CSP2-E1AI4NvaI8FL9Rd10



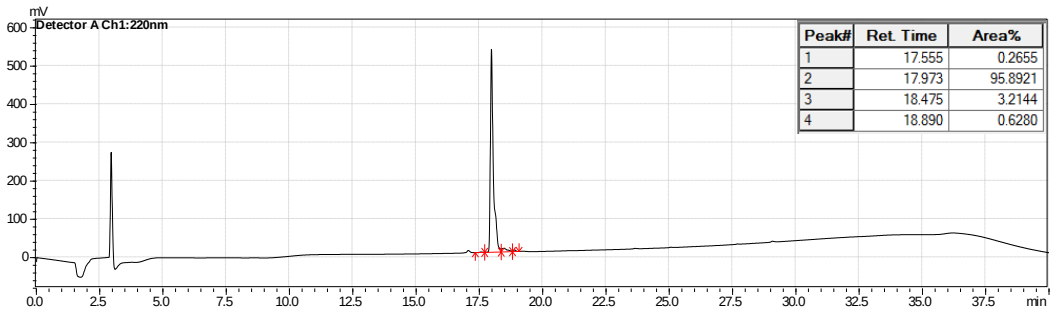
Peak#	Ret. Time	Area%
1	16.835	0.2471
2	17.234	99.4614
3	17.756	0.2521
4	18.012	0.0394

CSP2-E1AI4NvaI8Fd10F13L



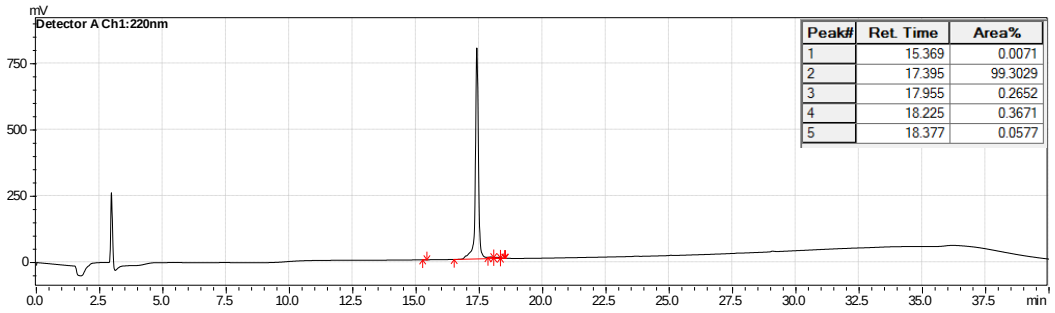
Peak#	Ret. Time	Area%
1	17.590	0.2076
2	17.995	96.1413
3	18.439	3.6144
4	18.841	0.0367

CSP2-E1AI4NvaI8Fd10L14Q



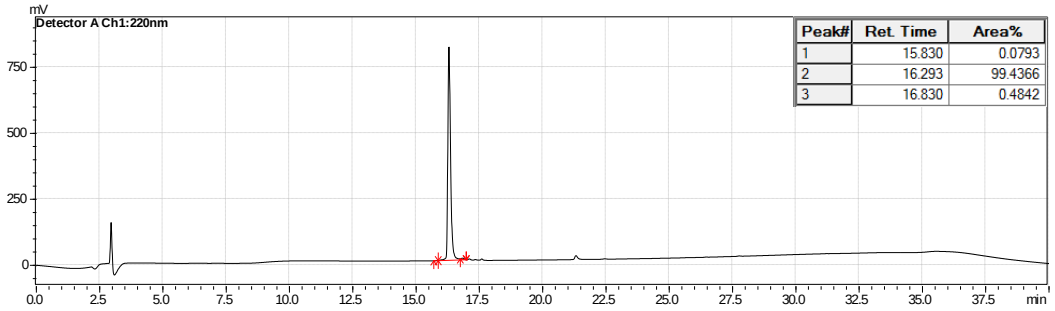
Peak#	Ret. Time	Area%
1	17.555	0.2655
2	17.973	95.8921
3	18.475	3.2144
4	18.890	0.6280

CSP2-E1AI4NvaL9Rd10F13L



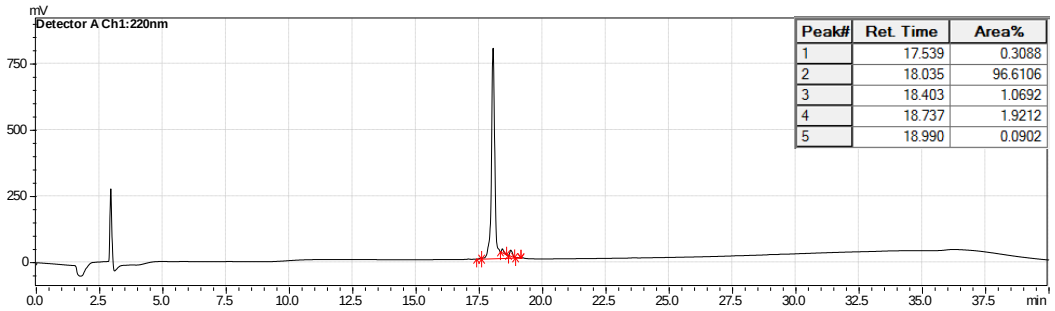
Peak#	Ret. Time	Area%
1	15.369	0.0071
2	17.395	99.3029
3	17.955	0.2652
4	18.225	0.3671
5	18.377	0.0577

CSP2-E1AI4NvaL9Rd10L14Q



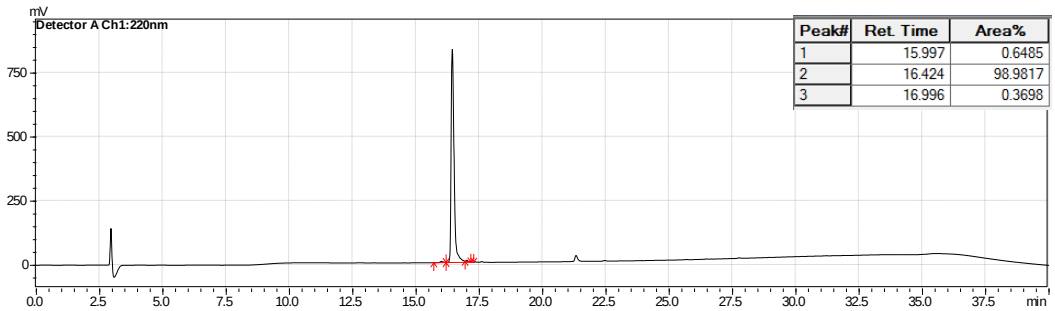
Peak#	Ret. Time	Area%
1	15.830	0.0793
2	16.293	99.4366
3	16.830	0.4842

CSP2-E1AI4Nvad10F13LL14Q



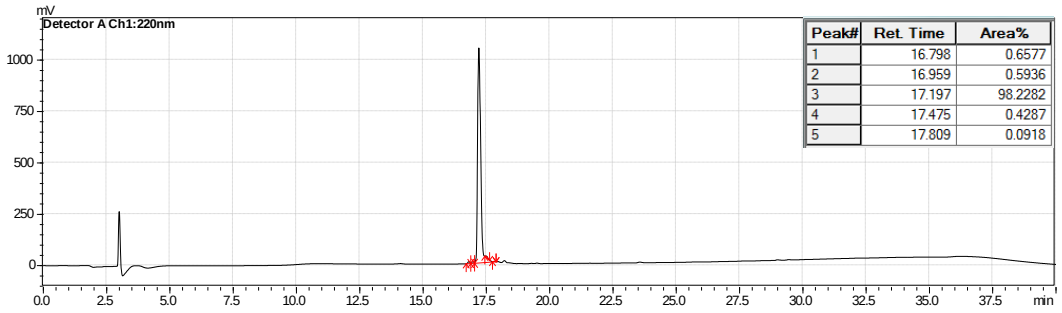
Peak#	Ret. Time	Area%
1	17.539	0.3088
2	18.035	96.6106
3	18.403	1.0692
4	18.737	1.9212
5	18.990	0.0902

CSP2-E1AI4NvaI8FL9Rd10L14Q



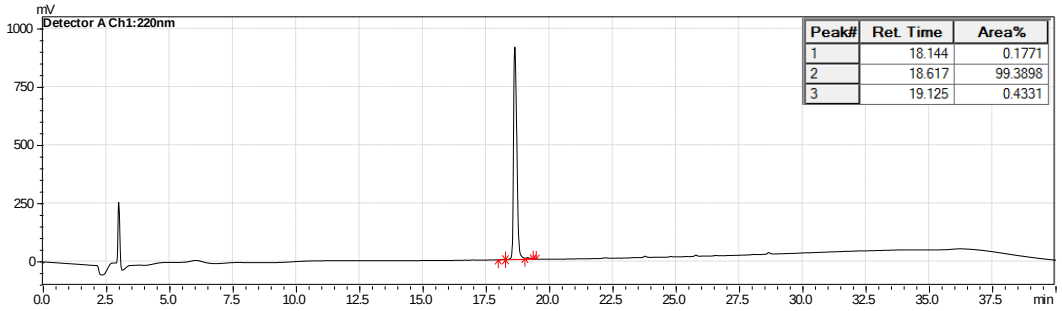
Peak#	Ret. Time	Area%
1	15.997	0.6485
2	16.424	98.9817
3	16.996	0.3698

CSP2-E1AI4NvaI8FL9Rd10F13L



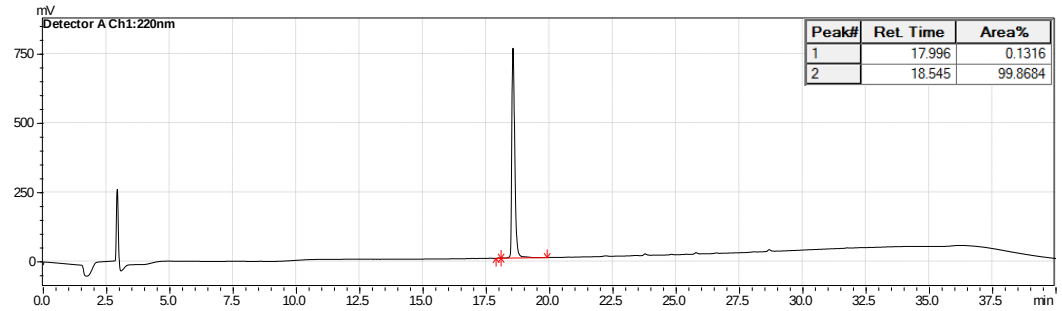
Peak#	Ret. Time	Area%
1	16.798	0.6577
2	16.959	0.5936
3	17.197	98.2282
4	17.475	0.4287
5	17.809	0.0918

CSP2-E1Ad10L14Q



Peak#	Ret. Time	Area%
1	18.144	0.1771
2	18.617	99.3898
3	19.125	0.4331

CSP2-E1AI4Ld10L14Q



Peak#	Ret. Time	Area%
1	17.996	0.1316
2	18.545	99.8684

MS and HPLC data for CSP2 analogs

Table S-1. MS and HPLC data for CSP2(15) analogs.

Compound Name	Calc. EM MH_2^{2+}	Obs. EM MH_2^{2+}	Purity (%)
CSP2(15)-I4LI7F	979.0495	979.0511	>96
CSP2(15)-I4LL9R	983.5658	983.5678	>99
CSP2(15)-I4LL12I	962.0573	962.0558	>99
CSP2(15)-I4LF13L	945.0651	945.0669	>98
CSP2(15)-I4LL14Q	969.5445	969.5459	>98
CSP2(15)-I7FL9R	1000.5580	1000.5534	>98
CSP2(15)-I7FL12I	979.0495	979.0463	>98
CSP2(15)-I7FF13L	962.0573	962.0576	>97
CSP2(15)-I7FL14Q	986.5367	986.5376	>96
CSP2(15)-L9RL12I	983.5658	983.5390	>99
CSP2(15)-L9RF13L	966.5736	966.5752	>99
CSP2(15)-L9RL14Q	991.0530	991.0545	>99
CSP2(15)-L12IF13L	948.0886	948.0899	>98
CSP2(15)-L12IL14Q	969.5445	969.5478	>97
CSP2(15)-F13LL14Q	952.5524	952.5543	>99
CSP2(15)-I4LL9RF13L	966.0722	966.0735	>99
CSP2(15)-I4LL9RL14Q	991.0530	991.0525	>99
CSP2(15)-I4LF13LL14Q	952.5524	952.5542	>99
CSP2(15)-L9RF13LL14Q	974.0608	974.0622	>98
CSP2(15)-I4LL9RF13LL14Q	990.0469	990.0482	>99
CSP2(15)-I4LR6KL9RF13LL14Q	960.0578	960.0545	>95
CSP2(15)-I4LI7FL9RF13LL14Q	991.0530	991.0563	>99
CSP2(15)-I4LI8FL9RF13LL14Q	991.0530	991.0568	>98
CSP2(15)-I4LL9RL12IF13LL14Q	974.0608	974.0612	>99

Table S-2. MS and HPLC data for CSP2 analogs.

Compound Name	Calc. EM MH_2^{2+}	Obs. EM MH_2^{2+}	Purity (%)
CSP2-E1AL9RL14Q	1090.1452	1090.1468	>99
CSP2-E1AL9Rd10L14Q	1090.1452	1090.1469	>99
CSP2-E1AI4LL9RL14Q	1090.1452	1090.1475	>99
CSP2-E1AI4LL9Rd10L14Q	1090.1452	1090.1448	>96
CSP2-E1AI4LL9RF13LL14Q	1073.1531	1073.1548	>98
CSP2-E1AI4LL9Rd10F13LL14Q	1073.1531	1073.1536	>98
CSP2-E1AI4LI8FL9RF13LL14Q	1090.1452	1090.1415	>95
CSP2-E1AI4LI8FL9Rd10F13LL14Q	1090.1452	1090.1475	>98
CSP2-E1AI4Nvad10	1054.1417	1054.1411	>98
CSP2-E1AI4Nled10	1061.1495	1061.1460	>98
CSP2-E1AI4HLeud10	1068.1573	1068.1591	>99
CSP2-E1AI4NvaI8FL9RF13LL14Q	1083.1374	1083.1342	>99
CSP2-E1AI4NvaI8FL9Rd10F13LL14Q	1083.1374	1083.1343	>99
CSP2-E1AI4NvaI8Fd10	1071.1339	1071.1326	>96
CSP2-E1AI4NvaL9Rd10	1075.6502	1075.6531	>99
CSP2-E1AI4Nvad10F13L	1037.1495	1037.1521	>99
CSP2-E1AI4Nvad10L14Q	1061.6189	1061.6192	>99
CSP2-E1AI4NvaI8FL9Rd10	1092.1384	1092.1370	>99
CSP2-E1AI4NvaI8Fd10F13L	1054.1417	1054.1432	>96
CSP2-E1AI4NvaI8Fd10L14Q	1078.6211	1078.6198	>96
CSP2-E1AI4NvaL9Rd10F13L	1058.6580	1058.6596	>99
CSP2-E1AI4NvaL9Rd10L14Q	1083.1374	1083.1358	>99
CSP2-E1AI4Nvad10F13LL14Q	1044.6367	1044.6340	>96
CSP2-E1AI4NvaI8FL9Rd10L14Q	1100.1296	1100.1308	>98
CSP2-E1AI4NvaI8FL9Rd10F13L	1054.6501	1054.6525	>99
CSP2-E1Ad10L14Q	1068.6367	1068.6392	>99
CSP2-E1AI4Ld10L14Q	1068.6367	1068.6395	>99

Bioassay Initial Screening Results

***S. pneumoniae* D39pcomX::lacZ (ComD1)**

Agonism assays were performed at 10 µM concentration. CSP1 was used as the positive control (100%) while DMSO as the negative control (0%). Percent (%) *comX* activation was measured by normalizing the Miller units obtained for each peptide to that of CSP1. All peptides were screened in triplicates over three separate trials. Error bars indicate standard error of the mean of nine values.

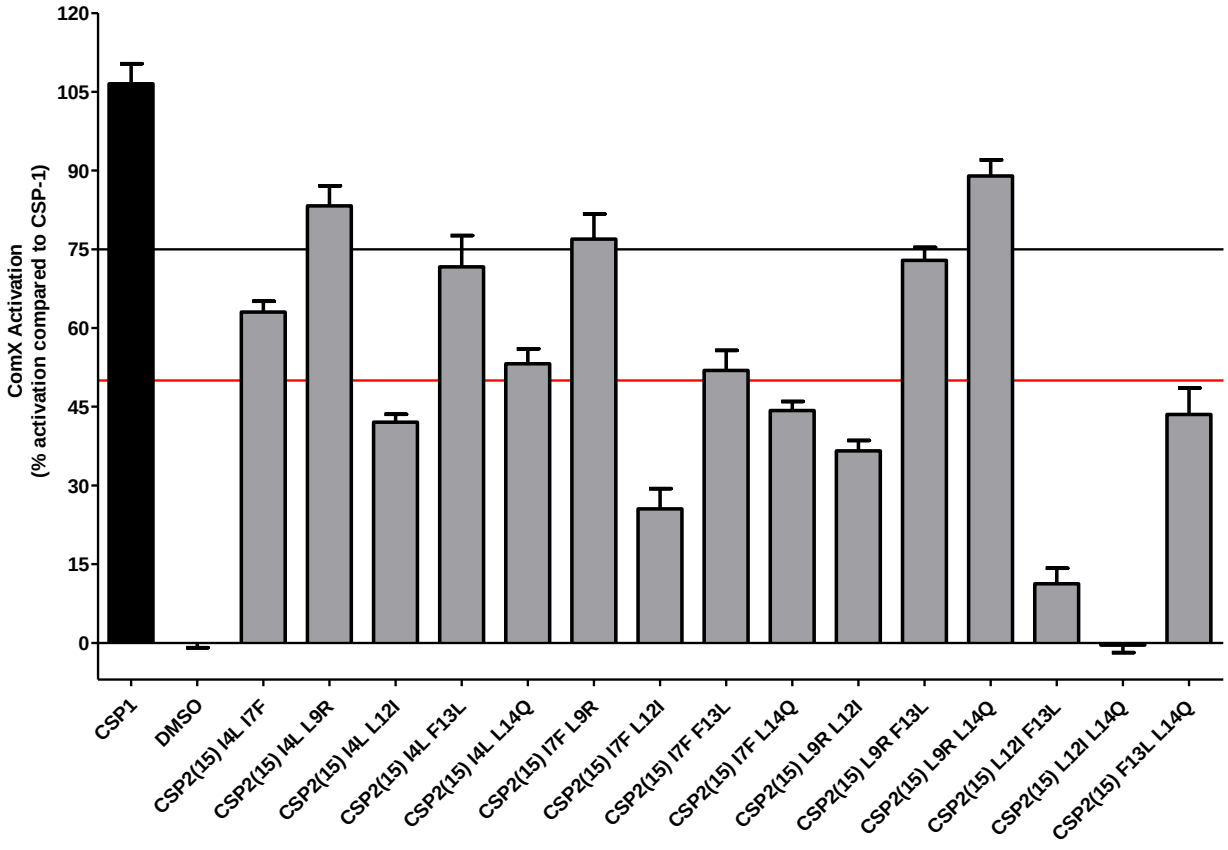


Figure S-1. Primary agonism screening assay data for the CSP2(15) double mutation library. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50} while peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

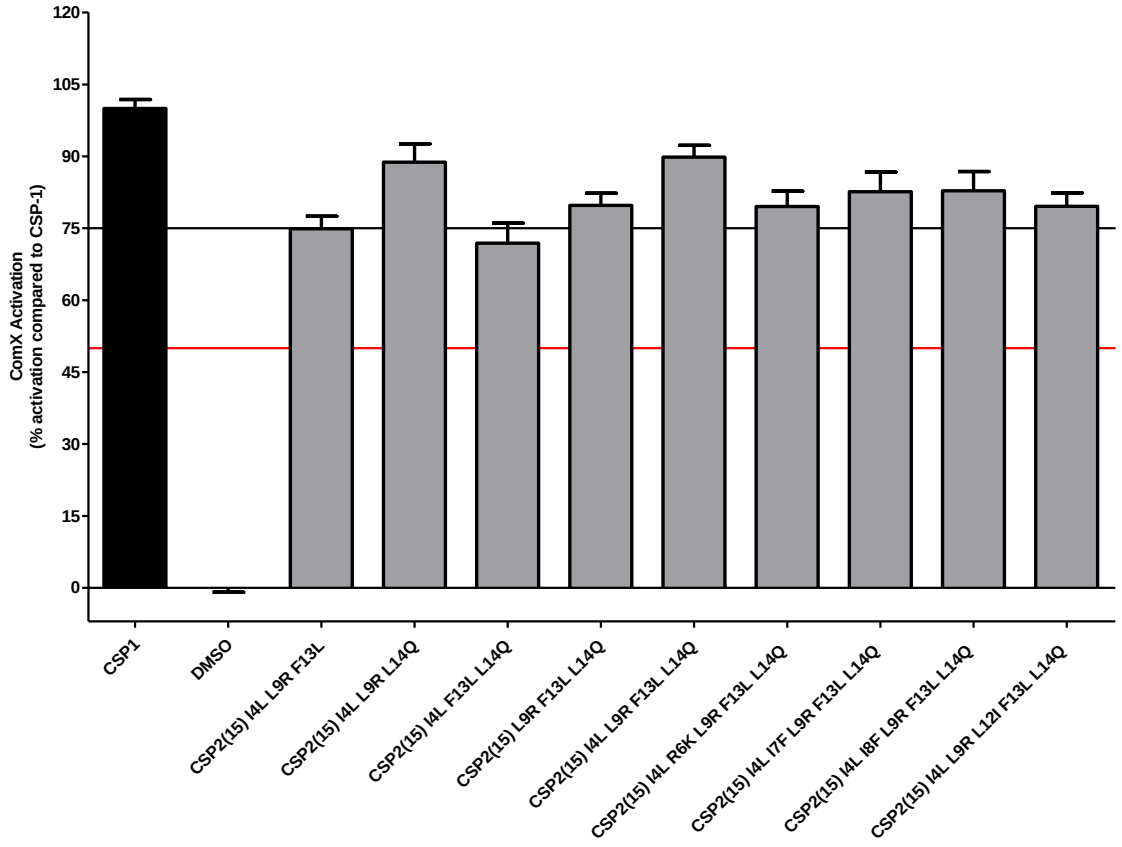


Figure S-2. Primary agonism screening assay data for the CSP2(15) triple, quadruple and quintuple mutation library. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50}.

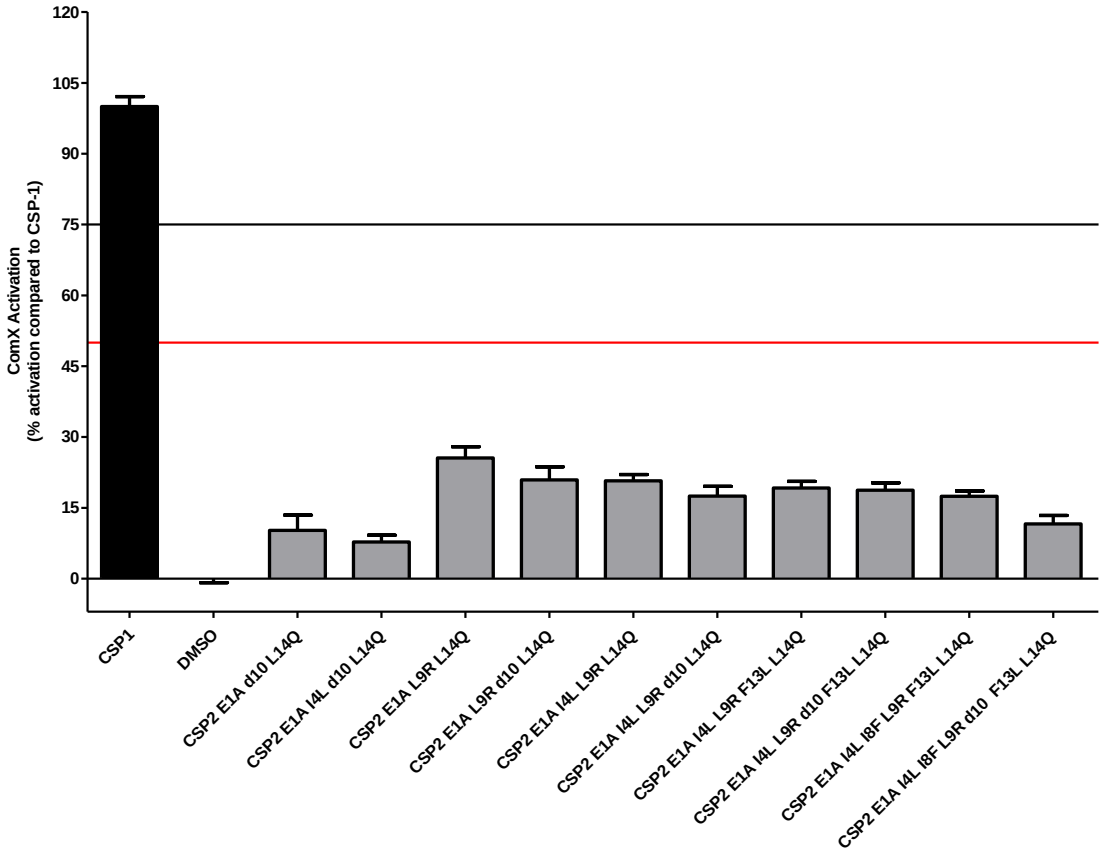


Figure S-3. Primary agonism screening assay data for the CSP2 E1A and d10 modification library. Peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

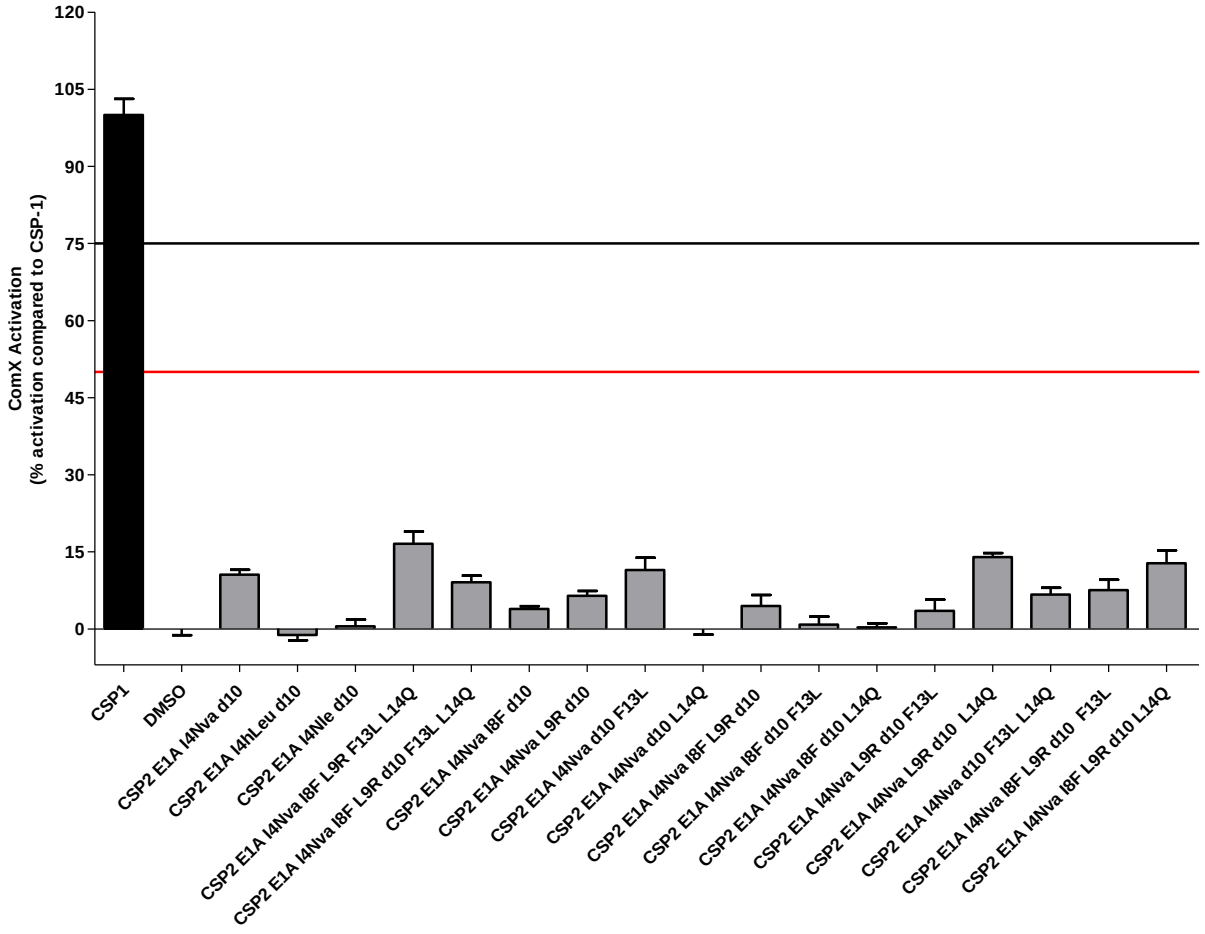


Figure S-4. Primary agonism screening assay data for the CSP2 non-proteogenic modification library. Peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

Antagonism assays were performed at 10 µM concentration of peptides against 50 nM concentration of CSP1. CSP1 (50 nM) was used as the positive control (100%) while DMSO as the negative control (0%). Percent (%) *comX* activation was measured by normalizing the Miller units obtained for each peptide to that of CSP1. All peptides were screened in triplicates over three separate trials. Error bars indicate standard error of the mean of nine values.

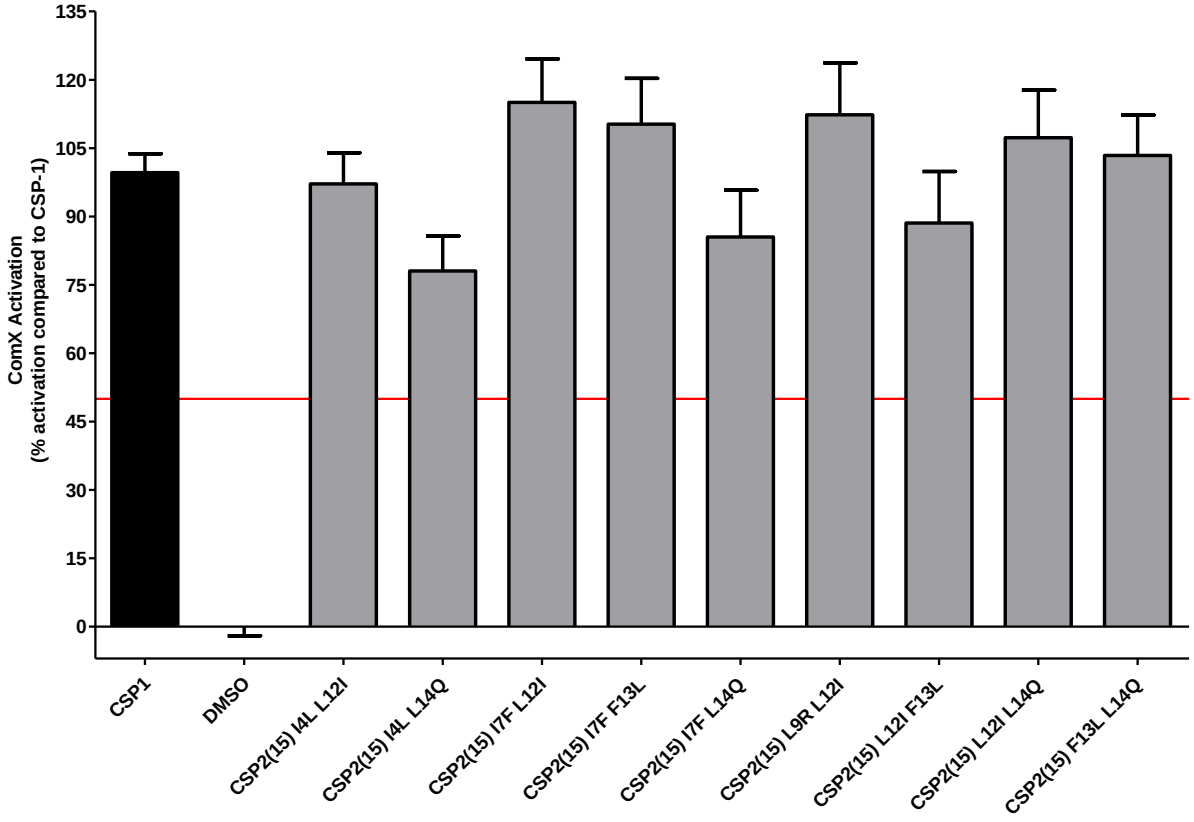


Figure S-5. Primary antagonism screening assay data for the CSP2(15) double mutation library. None of the analogs exhibited less than 50% activation.

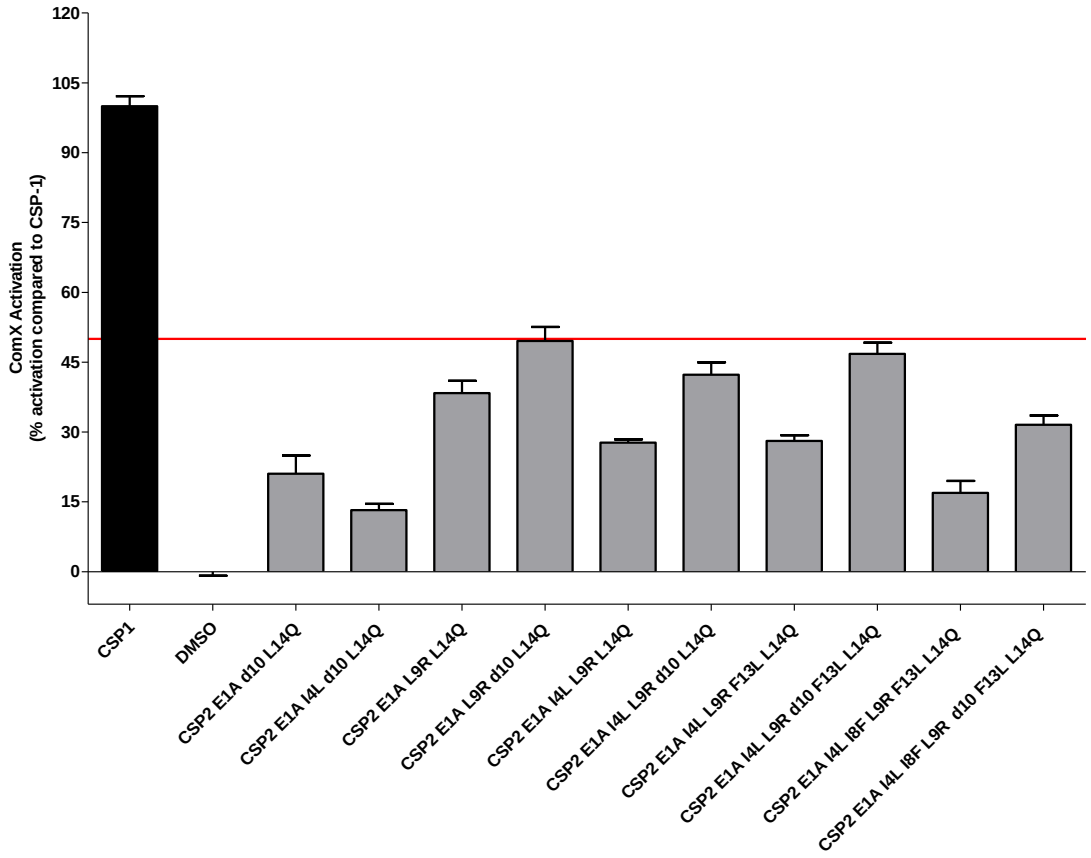


Figure S-6. Primary antagonism screening assay data for the CSP2 E1A and d10 modification library. Peptides that exhibited less than 50% activation were further evaluated to determine their IC_{50}.

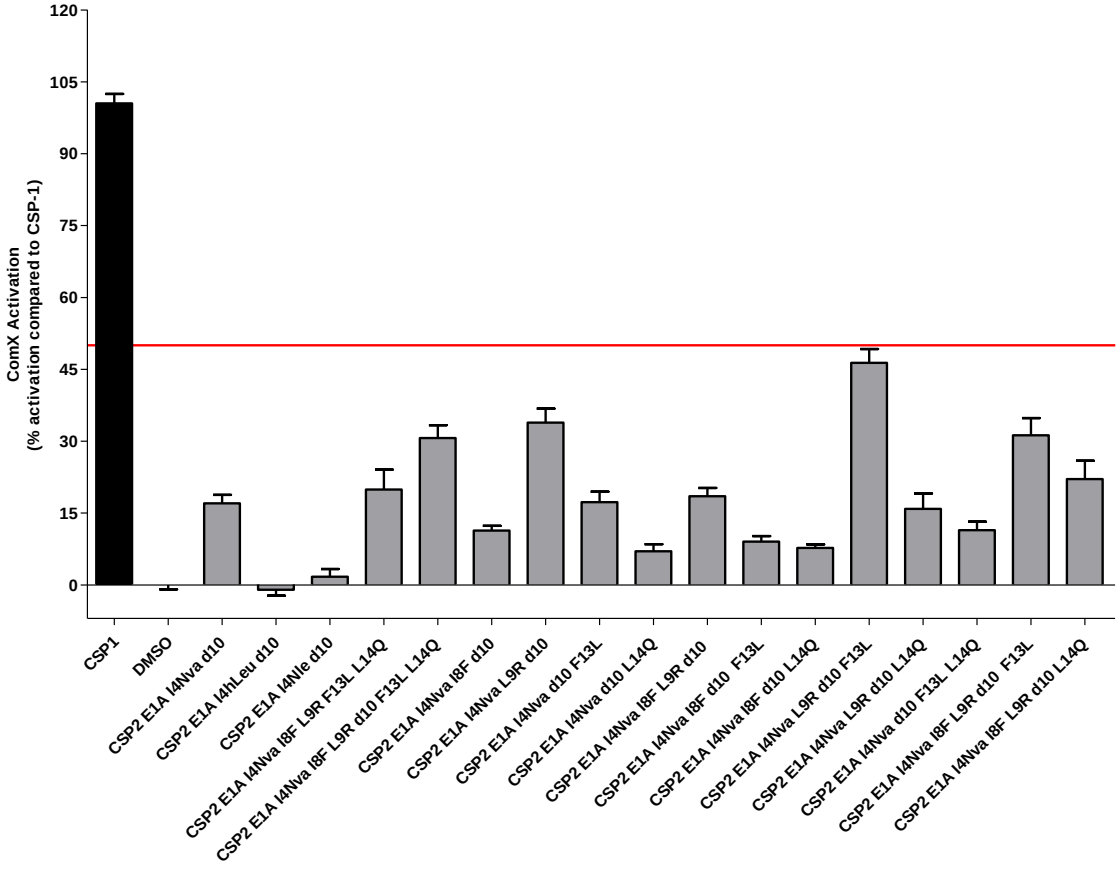


Figure S-7. Primary antagonism screening assay data for the non-proteogenic modification of CSP2 libraries. Peptides that exhibited less than 50% activation were further evaluated to determine their IC_{50}.

***S. pneumoniae* TIGR4pcomX::lacZ (ComD2)**

Agonism assays were performed at 10 µM concentration. CSP2 was used as the positive control (100%) while DMSO as the negative control (0%). Percent (%) *comX* activation was measured by normalizing the Miller units obtained for each peptide to that of CSP2. All peptides were screened in triplicates over three separate trials. Error bars indicate standard error of the mean of nine values.

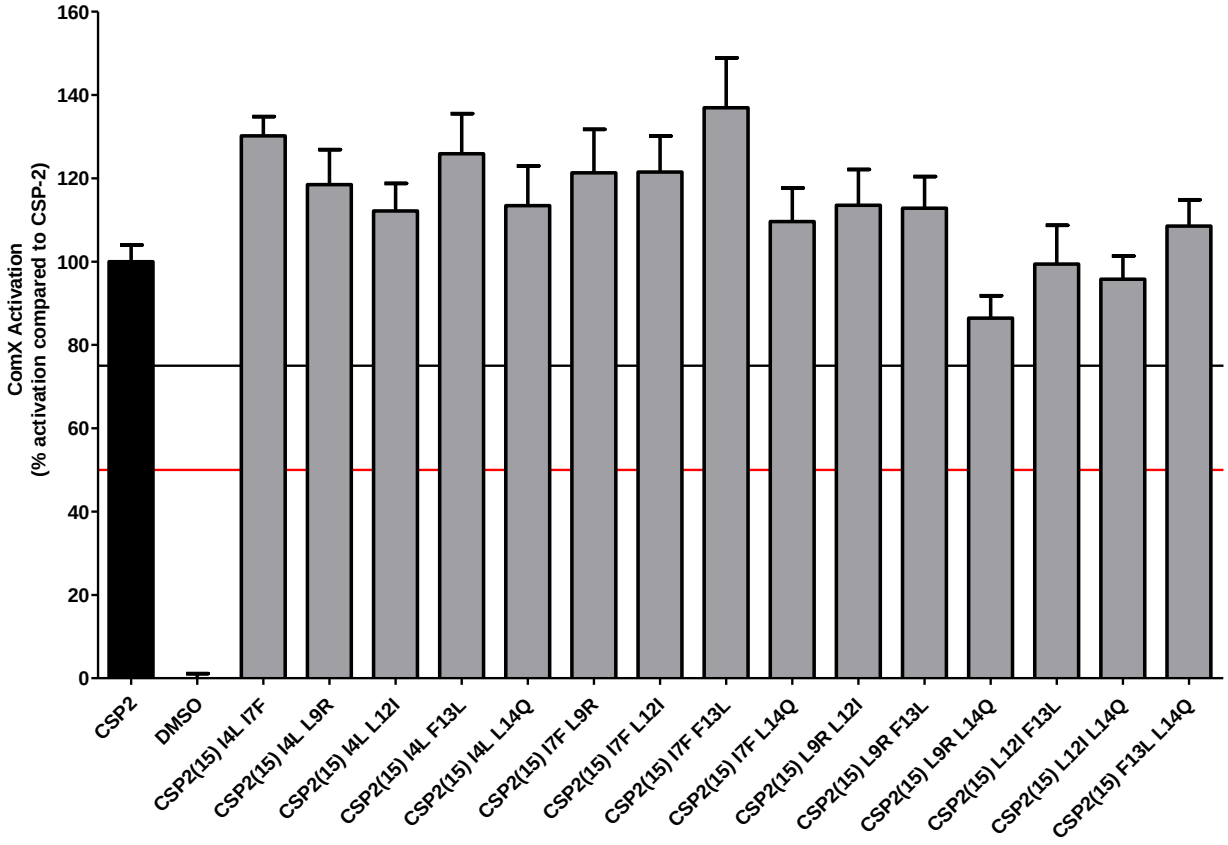


Figure S-8. Primary agonism screening assay data for the CSP2(15) double mutation library. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50}.

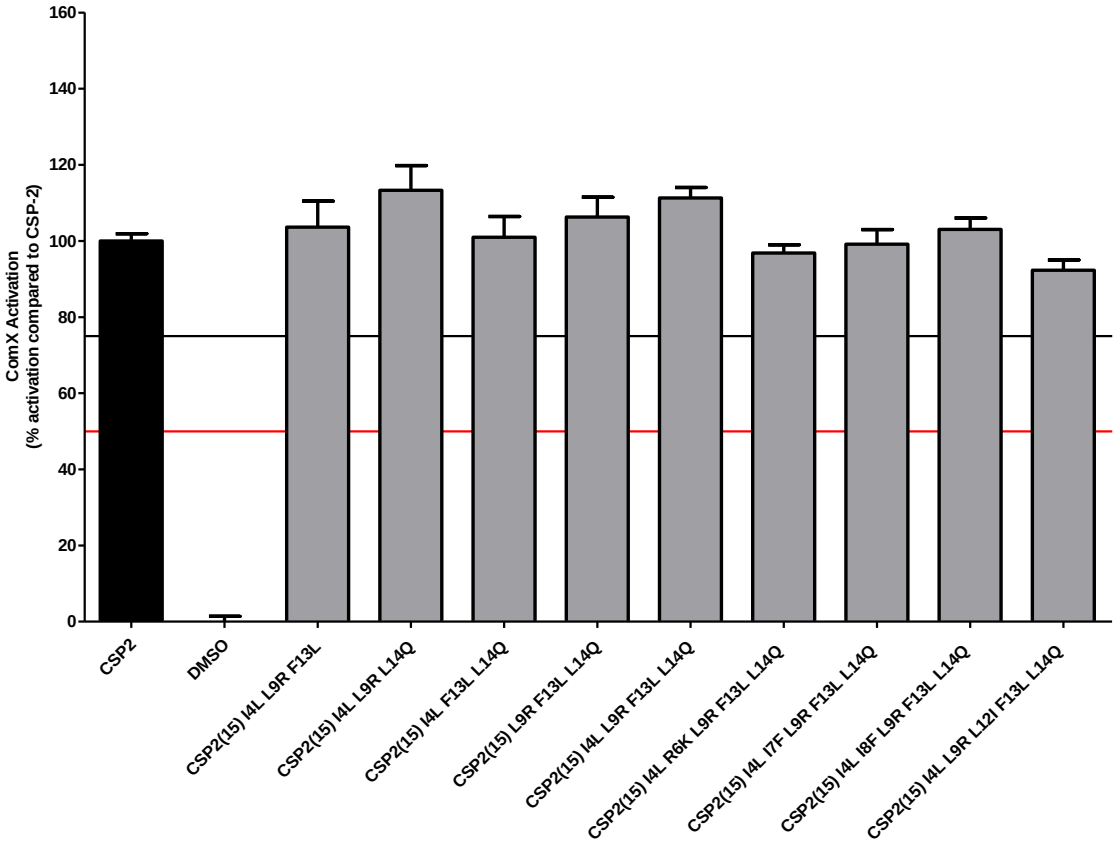


Figure S-9. Primary agonism screening assay data for the CSP2(15) triple, quadruple and quintuple mutation library. Peptides that exhibited over 75% activation were further evaluated to determine their EC_{50}.

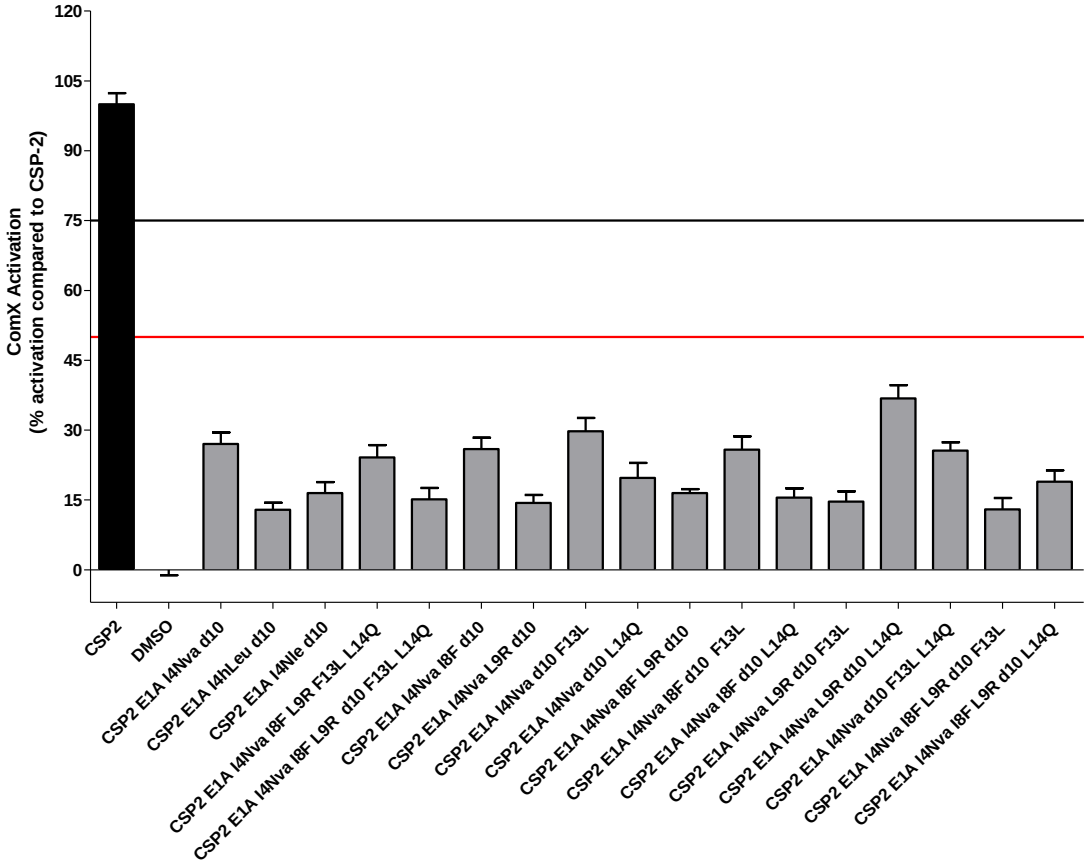


Figure S-10. Primary agonism screening assay data for the CSP2 non-proteogenic modification library. Peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

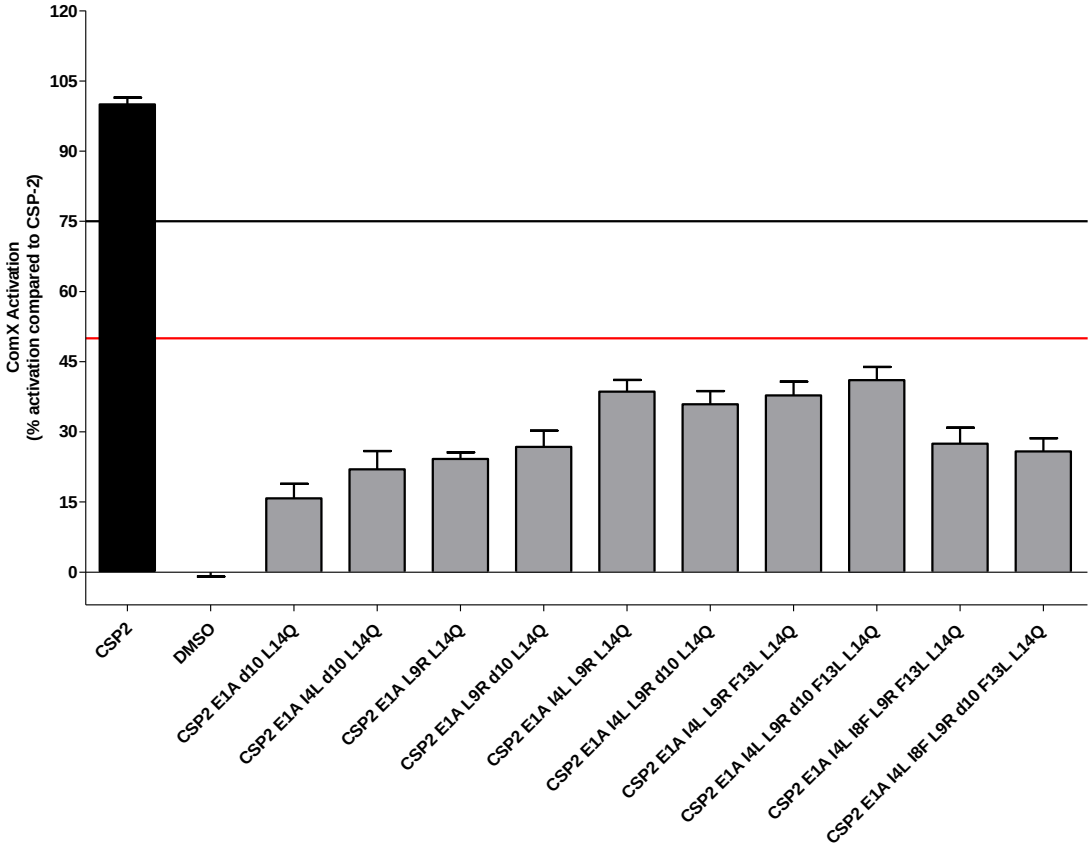


Figure S-11. Primary agonism screening assay data for the CSP2 E1A and d10 modification library. Peptides that exhibited less than 50% activation were evaluated as potential competitive inhibitors.

Antagonism assays were performed at 10 µM concentration of peptides against 250 nM concentration of CSP2. CSP2 (250 nM) was used as the positive control (100%) while DMSO as the negative control (0%). Percent (%) *comX* activation was measured by normalizing the Miller units obtained for each peptide to that of CSP2. All peptides were screened in triplicates over three separate trials. Error bars indicate standard error of the mean of nine values.

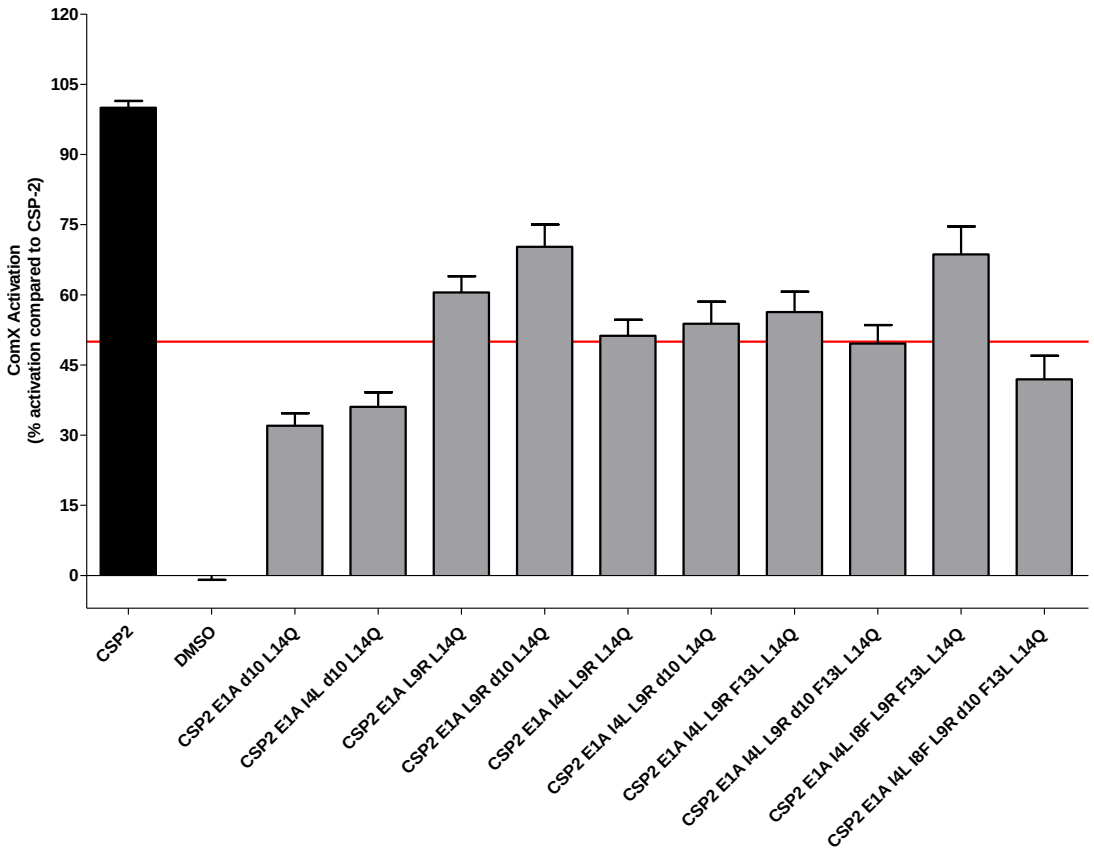


Figure S-12. Primary antagonism screening assay data for the CSP2 E1A and d10 modification library. Peptides that exhibited less than 50% activation were further evaluated to determine their IC_{50}.

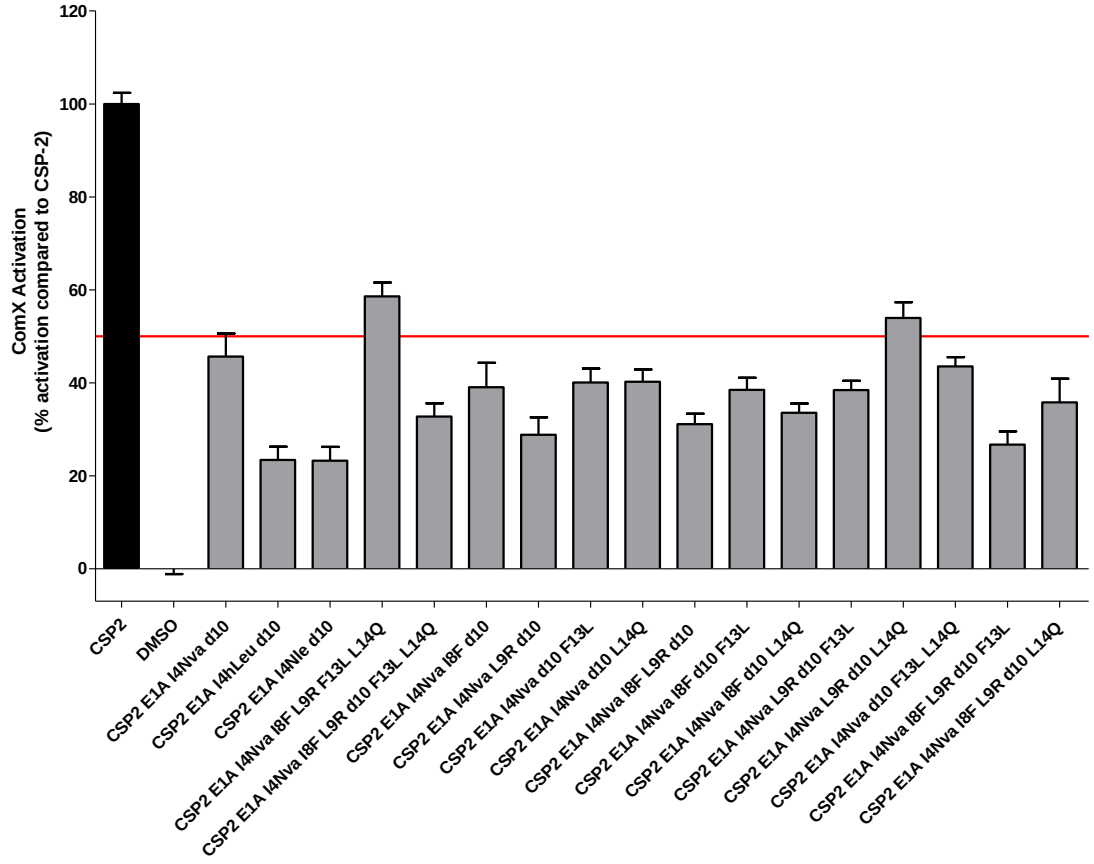
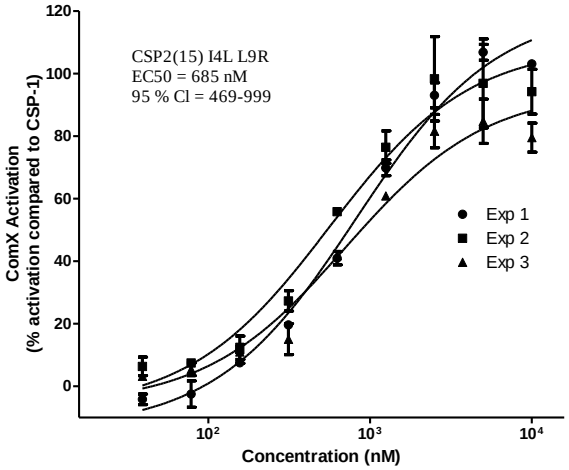
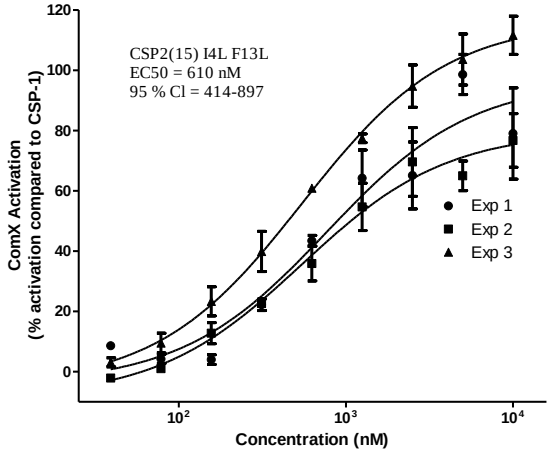


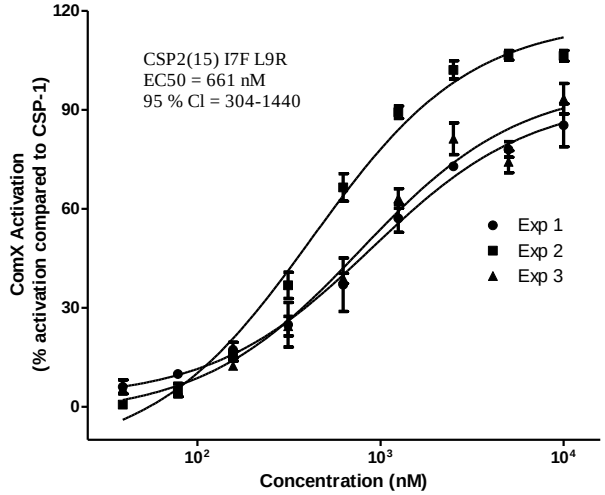
Figure S-13. Primary antagonism screening assay data for the CSP2 non-proteogenic modification library. Peptides that exhibited less than 50% activation were further evaluated to determine their IC_{50}.

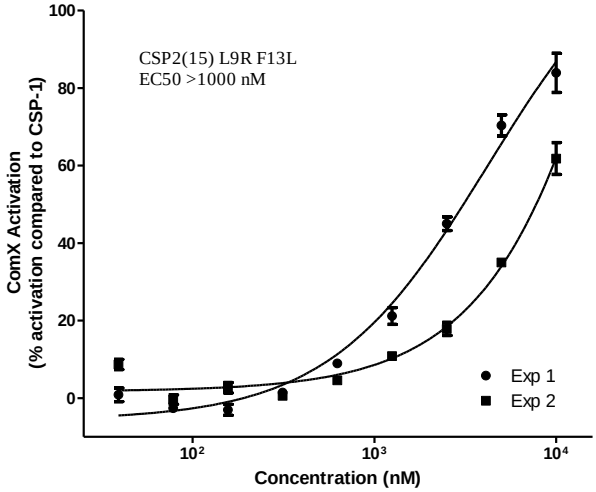
Dose Response Curves

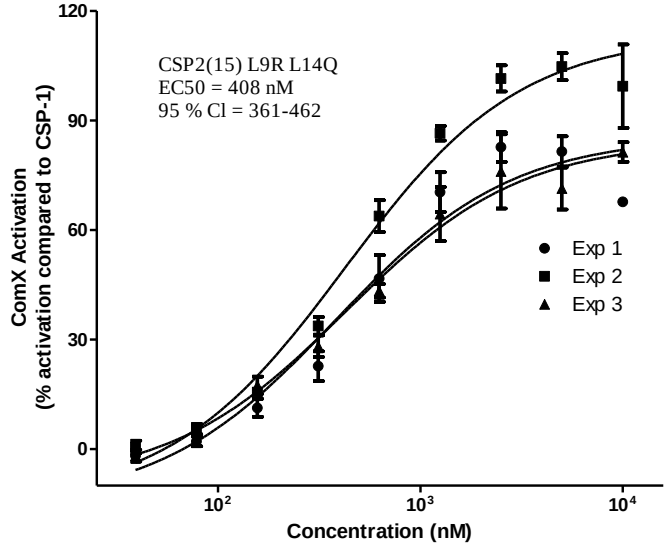
***S. pneumoniae* D39pcomX::lacZ (ComD1)**

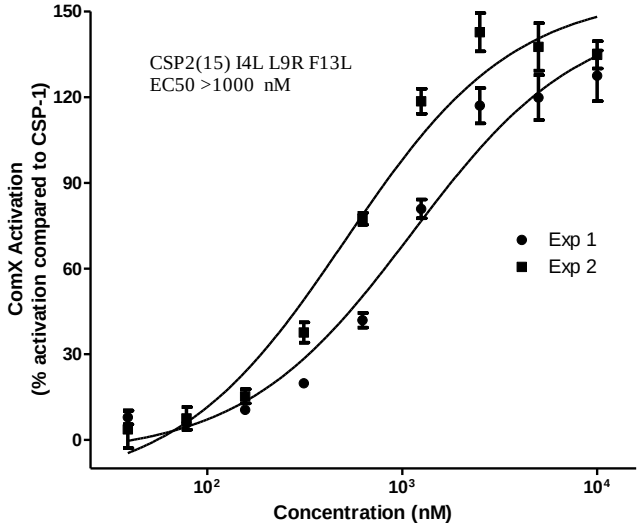


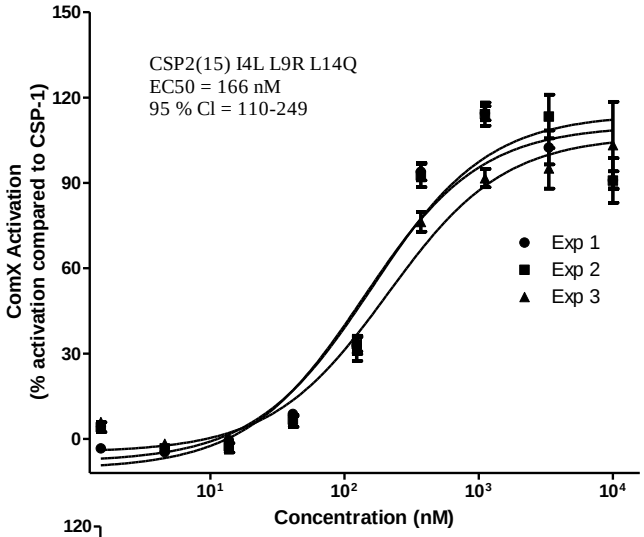


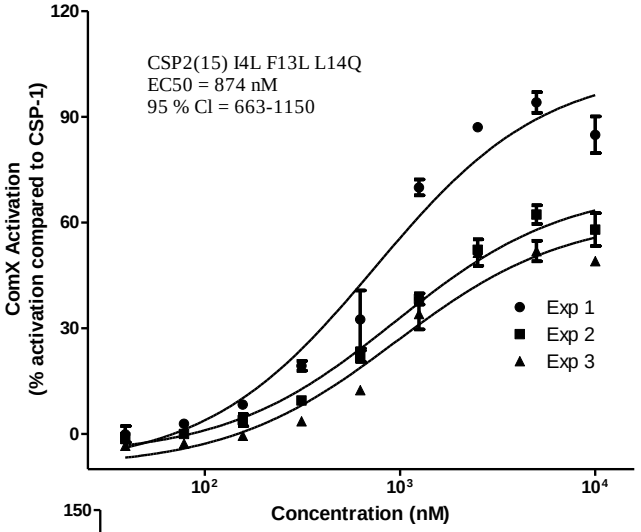


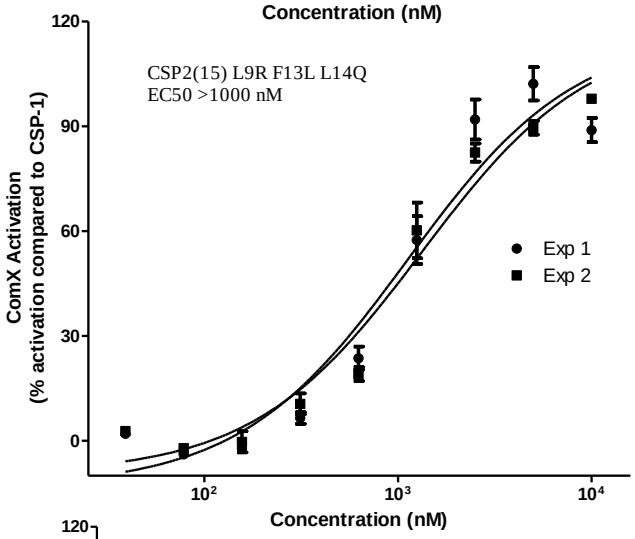


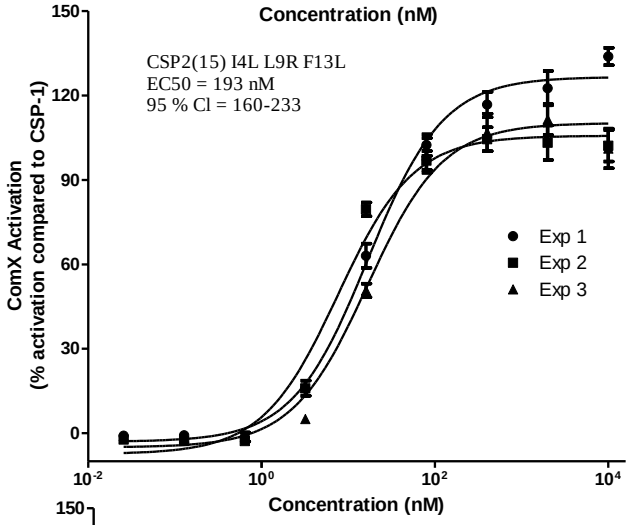


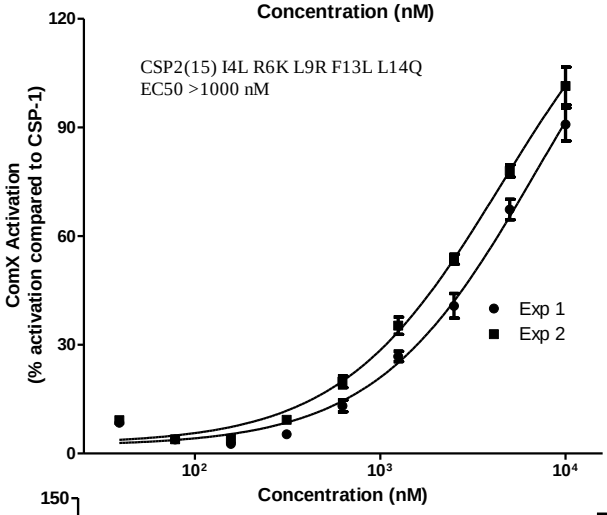


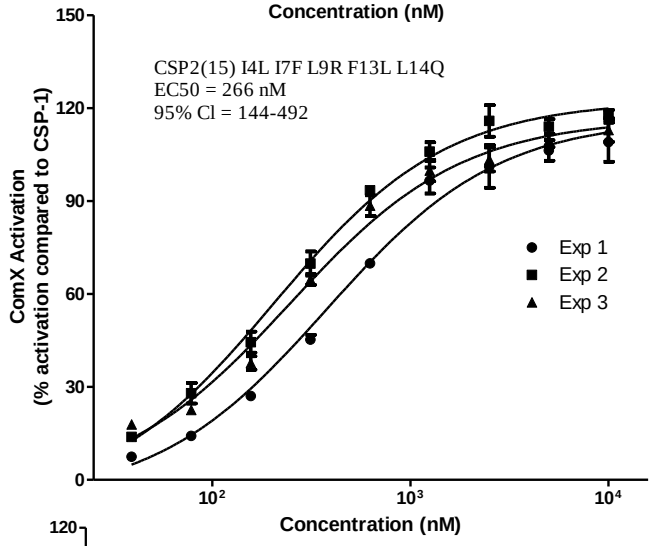

CSP2(15) I4L L9R L14Q
EC50 = 166 nM
95 % Cl = 110-249
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

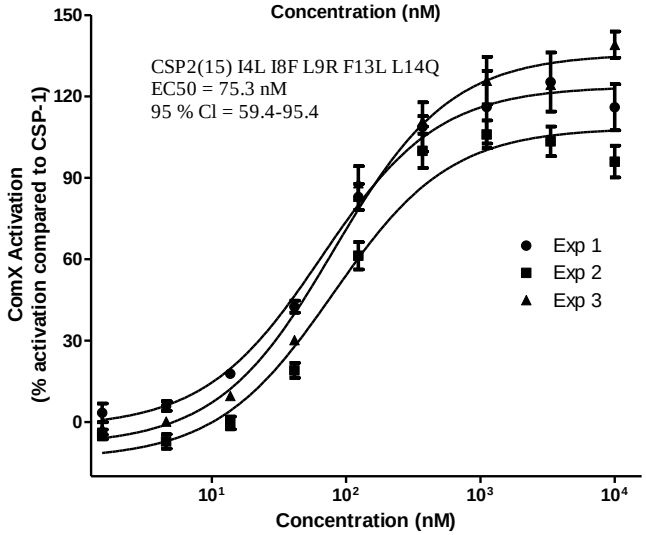

CSP2(15) I4L F13L L14Q
EC50 = 874 nM
95 % Cl = 663-1150
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

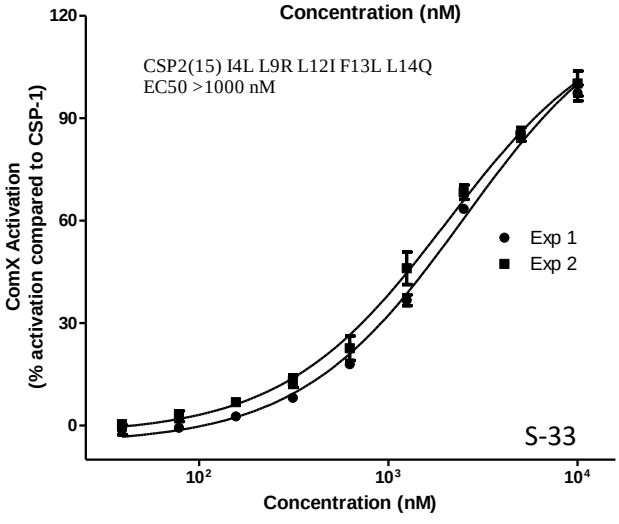

CSP2(15) L9R F13L L14Q
EC50 >1000 nM
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)
Exp 1
Exp 2

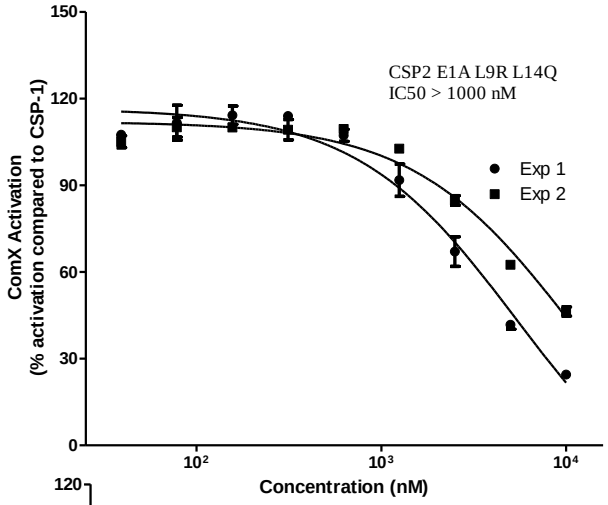

CSP2(15) I4L L9R F13L
EC50 = 193 nM
95 % Cl = 160-233
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

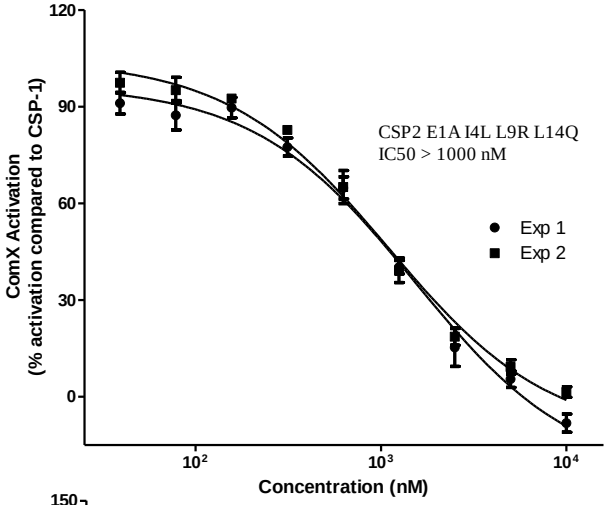

CSP2(15) I4L R6K L9R F13L L14Q
EC50 >1000 nM
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)
Exp 1
Exp 2

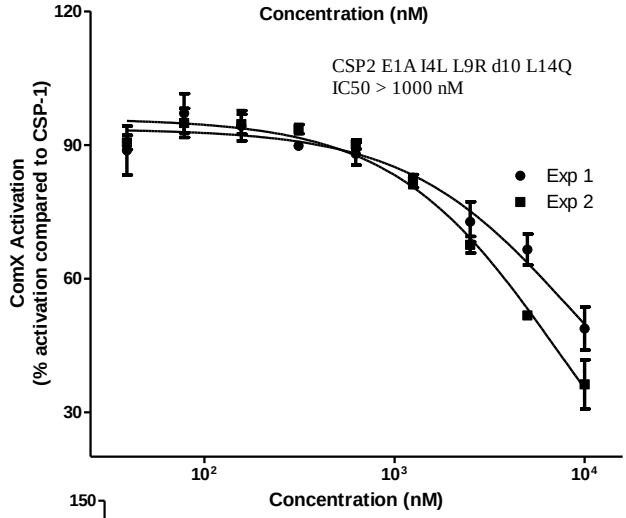

CSP2(15) I4L I7F L9R F13L L14Q
EC50 = 266 nM
95% Cl = 144-492
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

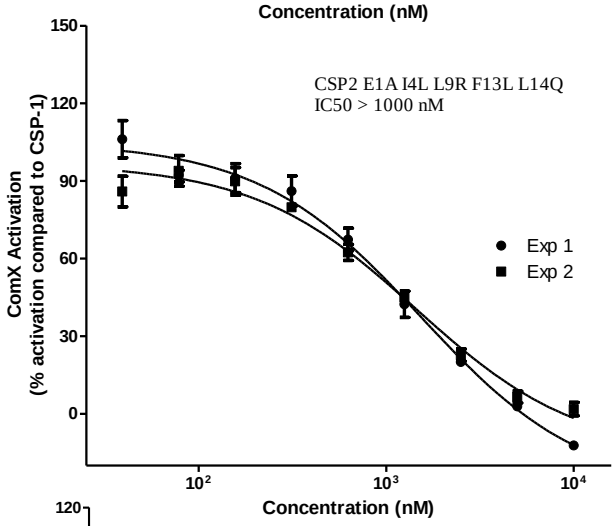

CSP2(15) I4L I8F L9R F13L L14Q
EC50 = 75.3 nM
95 % Cl = 59.4-95.4
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)
Exp 1
Exp 2
Exp 3

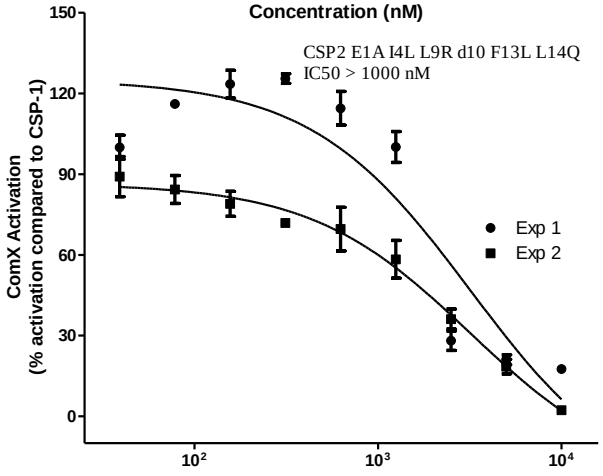

CSP2(15) I4L L9R L12I F13L L14Q
EC50 >1000 nM
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)
Exp 1
Exp 2

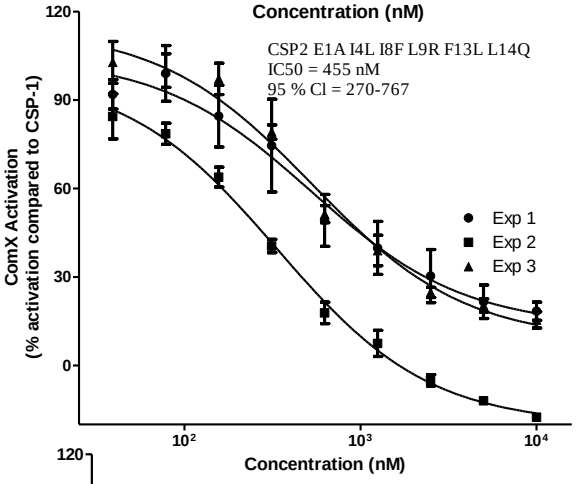

CSP2 E1A L9R L14Q
IC50 > 1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

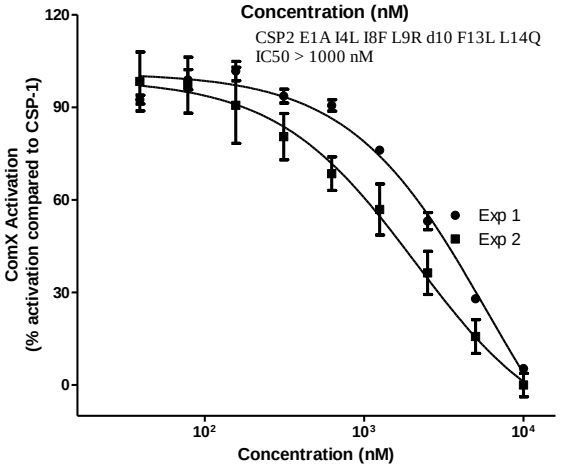

CSP2 E1A I4L L9R L14Q
IC50 > 1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

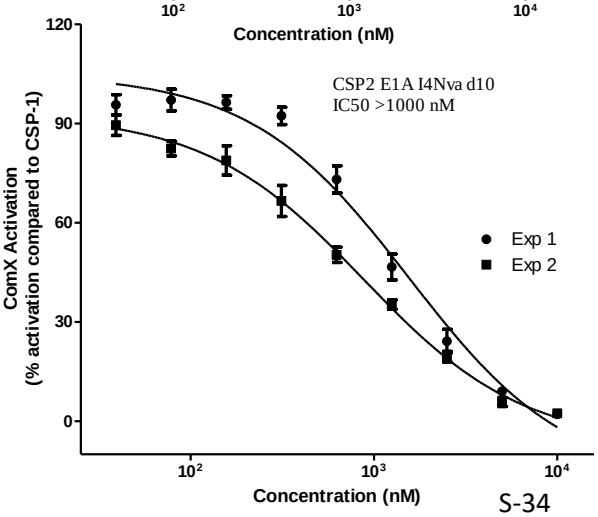

CSP2 E1A I4L L9R d10 L14Q
IC50 > 1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

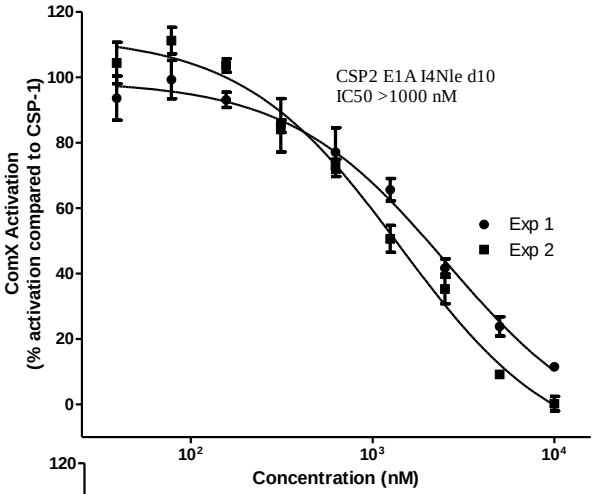

CSP2 E1A I4L L9R F13L L14Q
IC50 > 1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

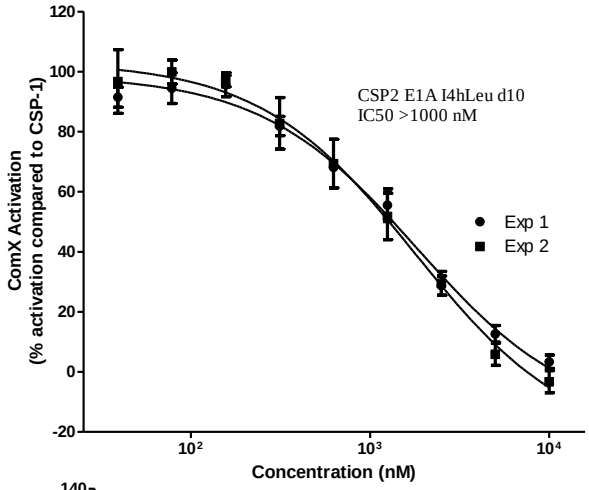

CSP2 E1A I4L L9R d10 F13L L14Q
IC50 > 1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

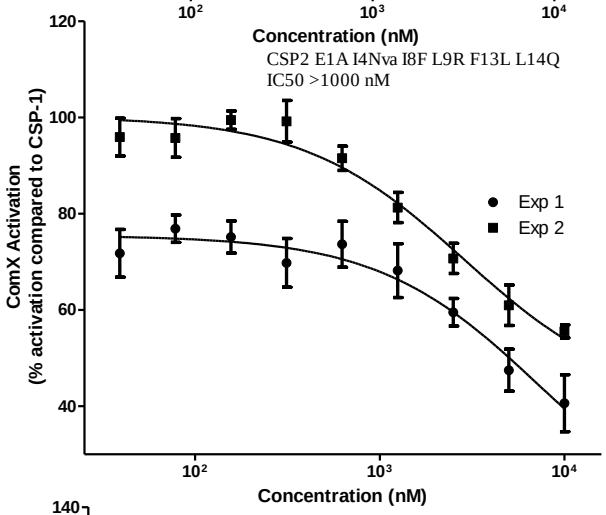

CSP2 E1A I4L I8F L9R F13L L14Q
IC50 = 455 nM
95 % CI = 270-767
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

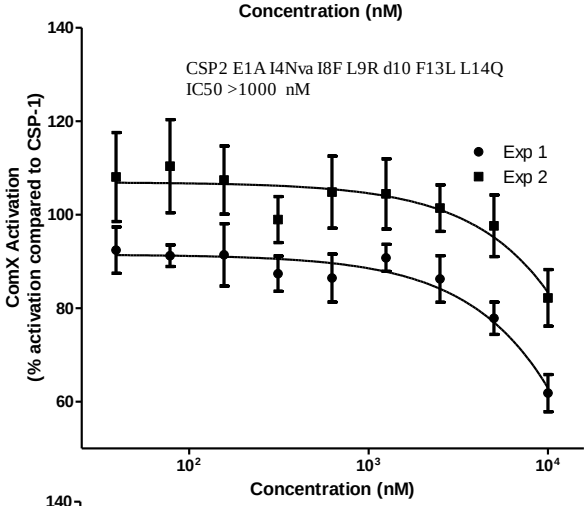

CSP2 E1A I4L I8F L9R d10 F13L L14Q
IC50 > 1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

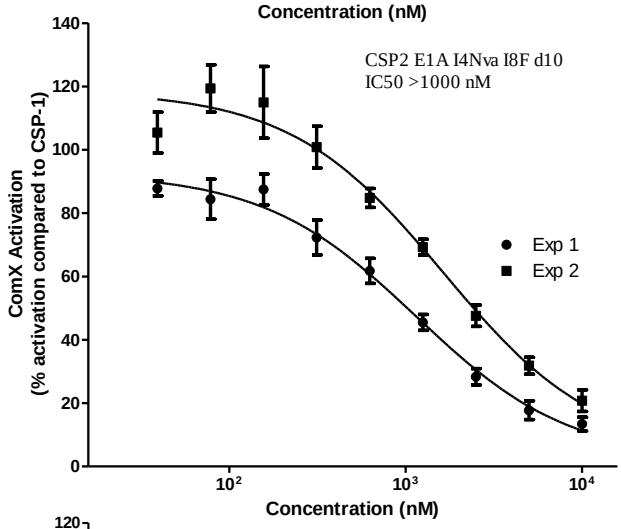

CSP2 E1A I4Nva d10
IC50 >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

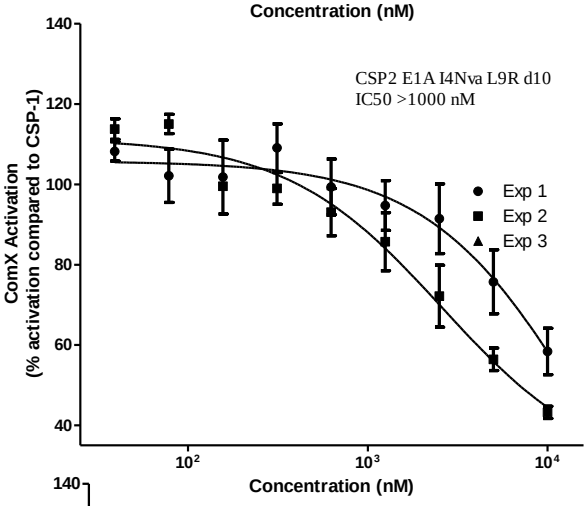

CSP2 E1A I4Nle d10
IC50 >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

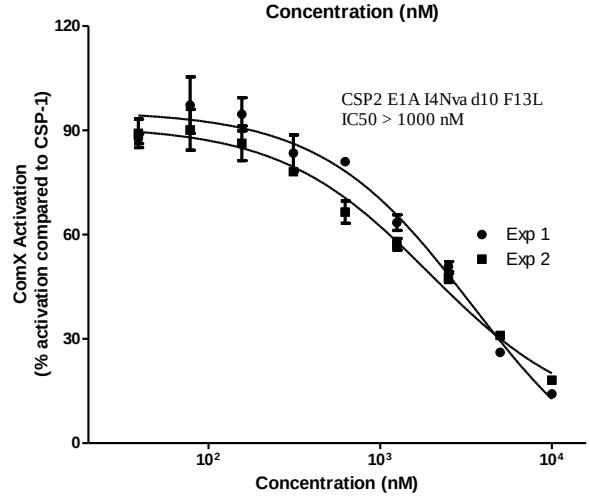

CSP2 E1A I4hLeu d10
IC50 >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

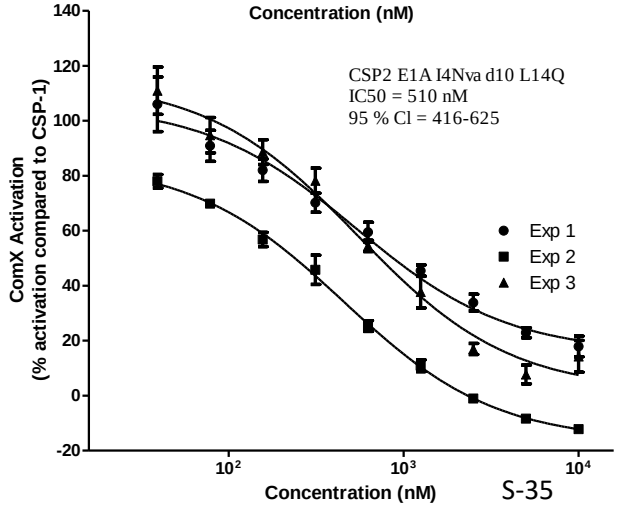

CSP2 E1A I4Nva I8F L9R F13L L14Q
IC50 >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

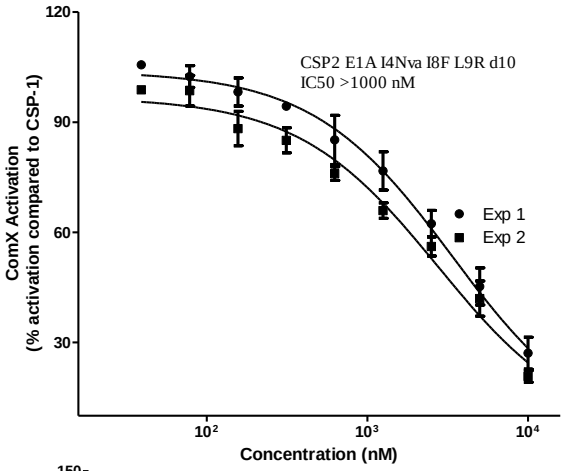

CSP2 E1A I4Nva I8F L9R d10 F13L L14Q
IC50 >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

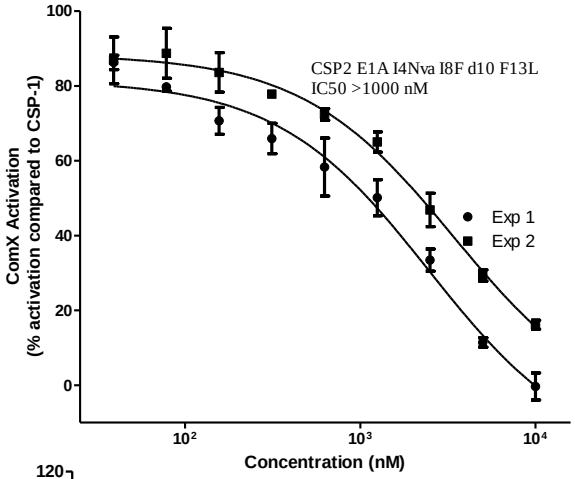

CSP2 E1A I4Nva I8F d10
IC50 >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

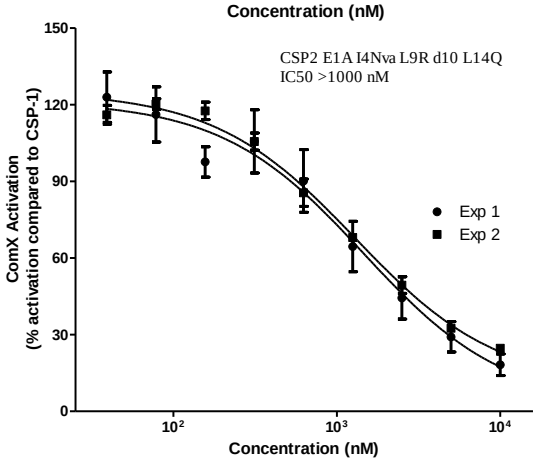

CSP2 E1A I4Nva L9R d10
IC50 >1000 nM
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

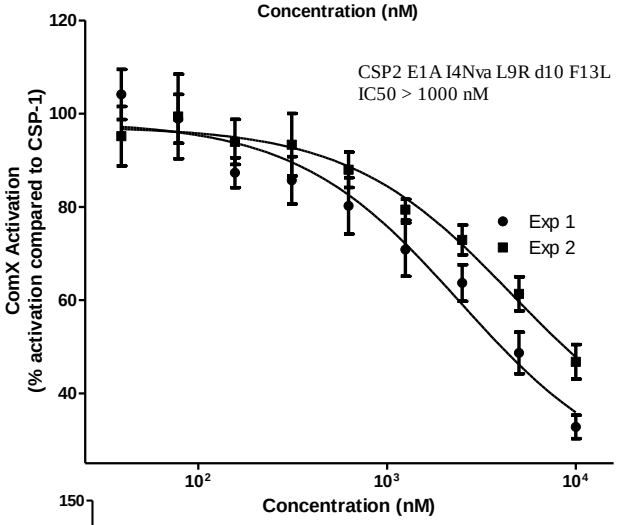

CSP2 E1A I4Nva d10 F13L
IC50 > 1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

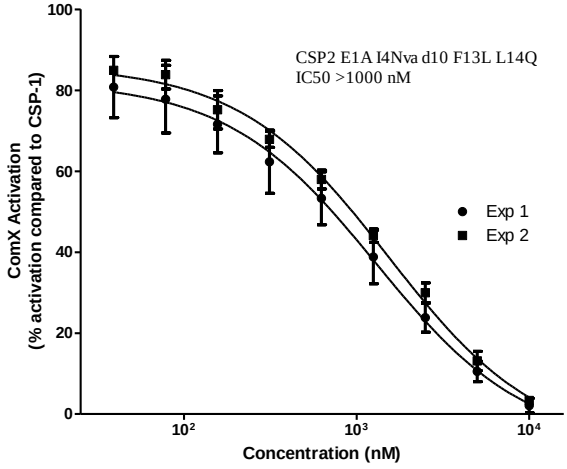

CSP2 E1A I4Nva d10 L14Q
IC50 = 510 nM
95 % Cl = 416-625
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

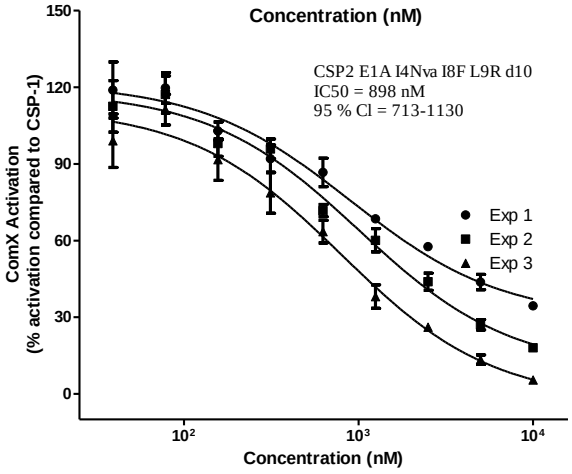

CSP2 E1A I4Nva I8F L9R d10
IC50 >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

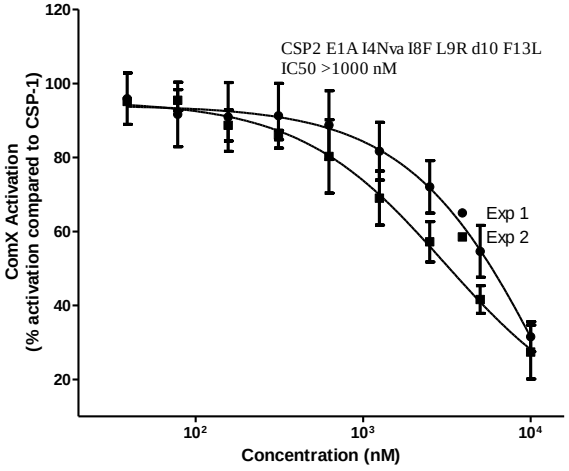

CSP2 E1A I4Nva I8F d10 F13L
IC50 >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

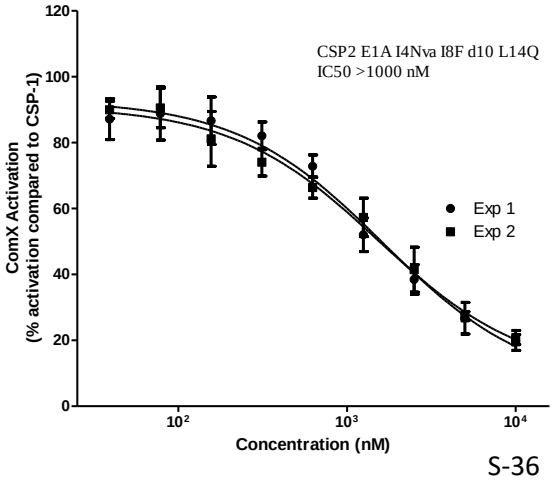

CSP2 E1A I4Nva L9R d10 L14Q
IC50 >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

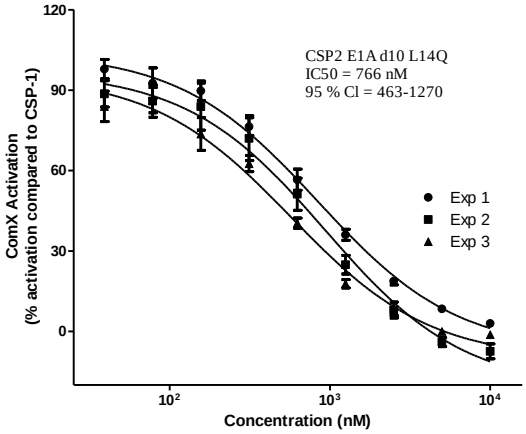

CSP2 E1A I4Nva L9R d10 F13L
IC50 > 1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

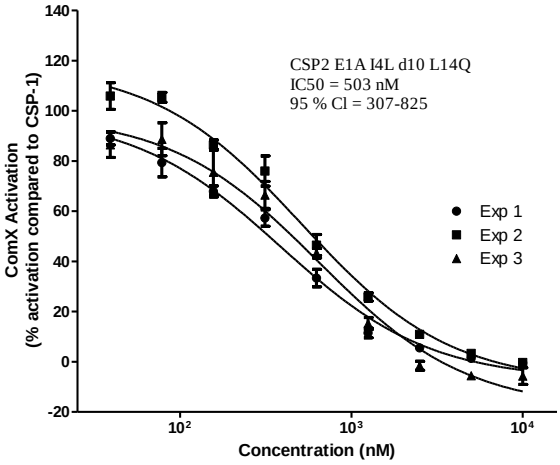

CSP2 E1A I4Nva d10 F13L L14Q
IC50 >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)


CSP2 E1A I4Nva I8F L9R d10
IC50 = 898 nM
95 % CI = 713-1130
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

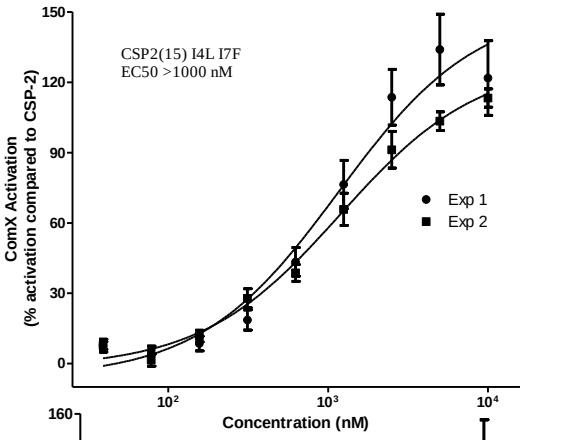

CSP2 E1A I4Nva I8F L9R d10 F13L
IC50 >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

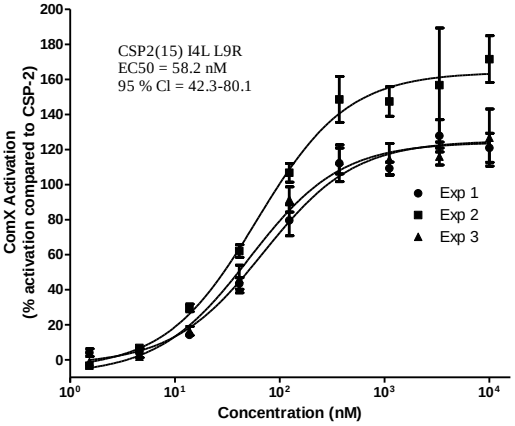

CSP2 E1A I4Nva I8F d10 L14Q
IC50 >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

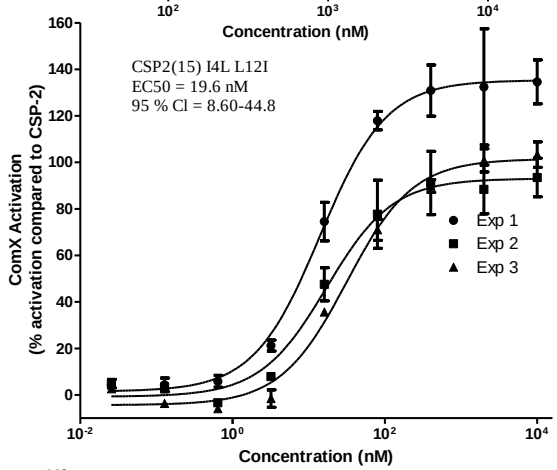

CSP2 E1A d10 L14Q
IC50 = 766 nM
95 % Cl = 463-1270
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

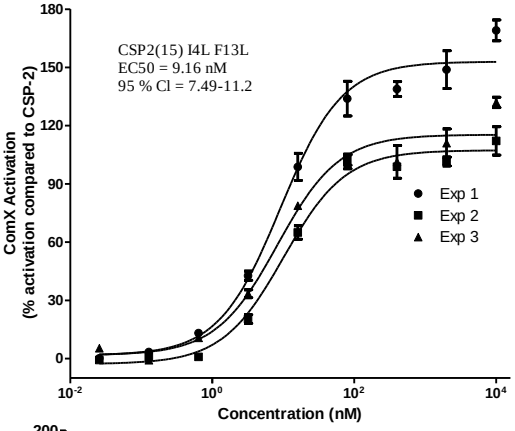

CSP2 E1A I4L d10 L14Q
IC50 = 503 nM
95 % Cl = 307-825
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-1)
Concentration (nM)

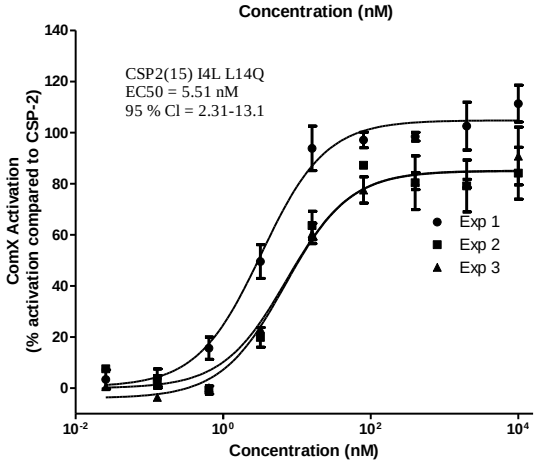
S. pneumoniae TIGR4pcomX::lacZ (ComD2)

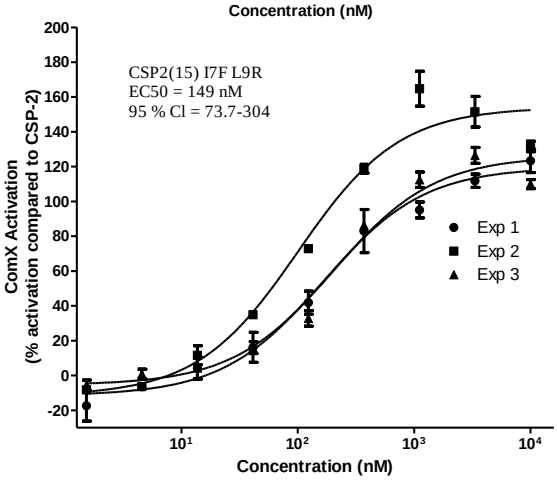


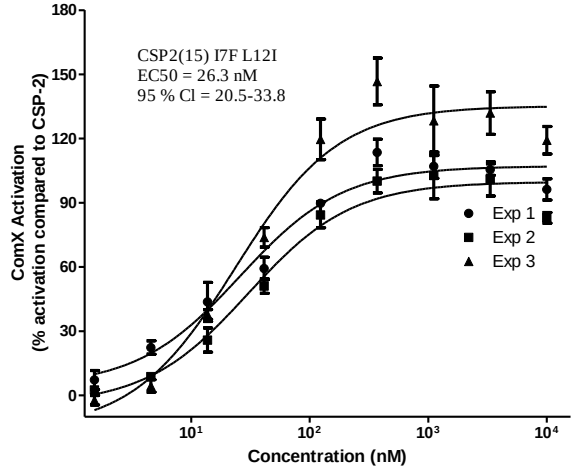


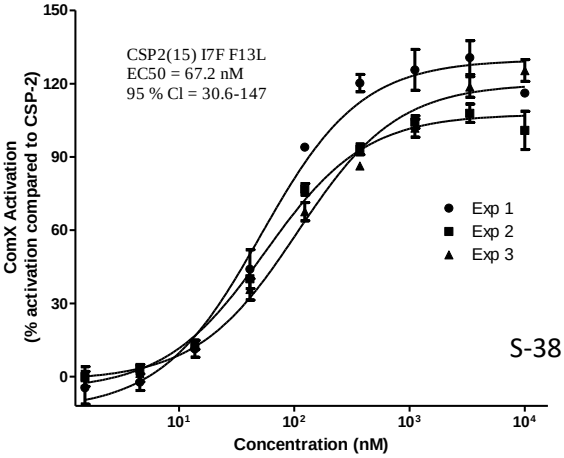


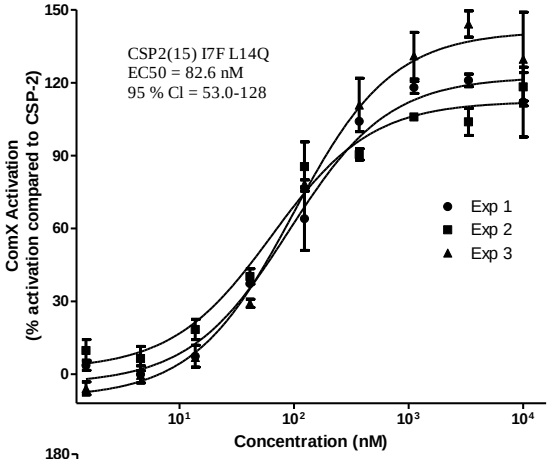


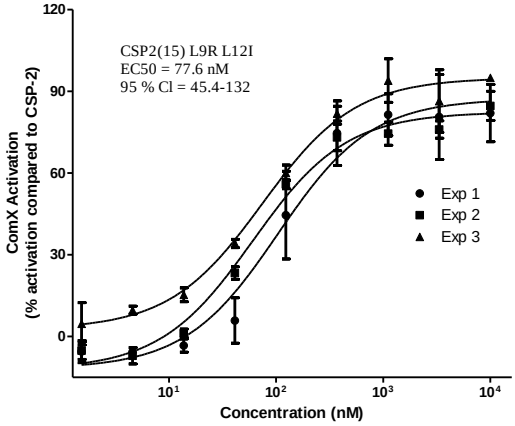


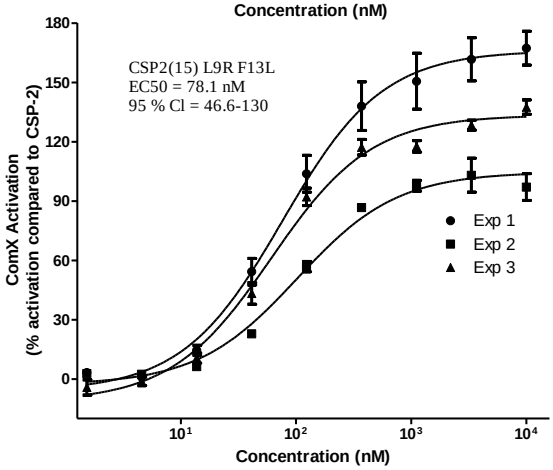


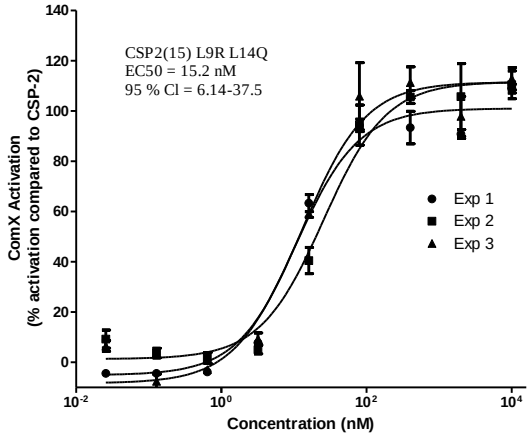


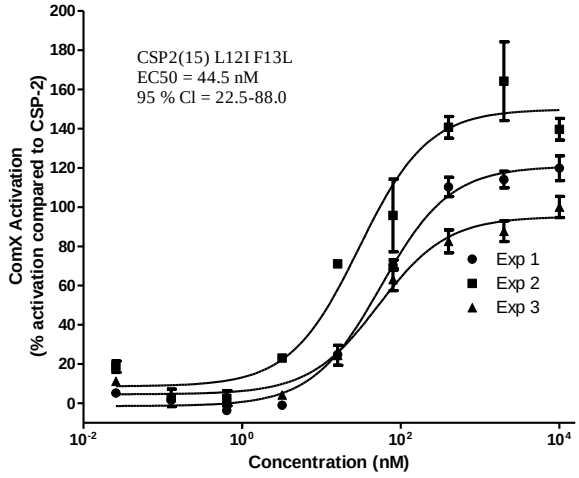


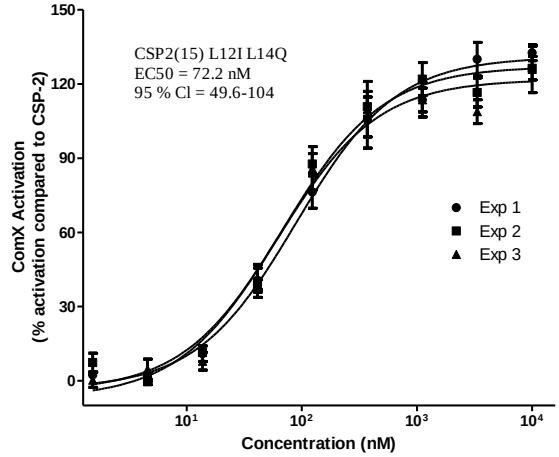

CSP2(15) I7F L14Q
EC50 = 82.6 nM
95 % CI = 53.0-128
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

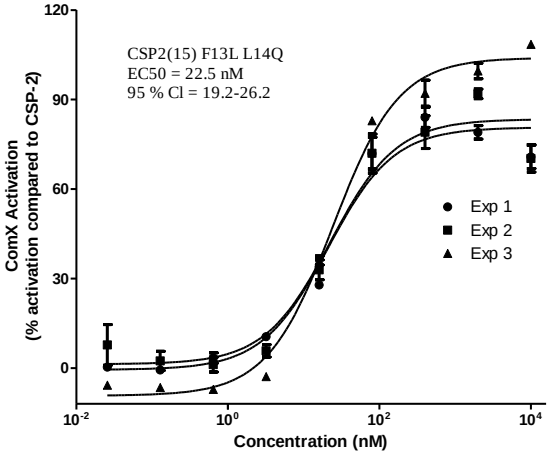

CSP2(15) L9R L12I
EC50 = 77.6 nM
95 % CI = 45.4-132
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

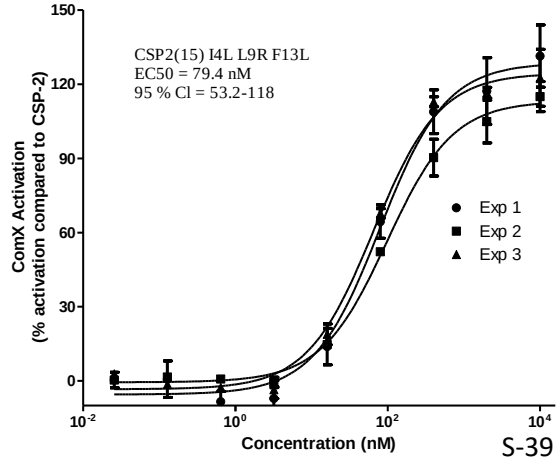

CSP2(15) L9R F13L
EC50 = 78.1 nM
95 % CI = 46.6-130
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

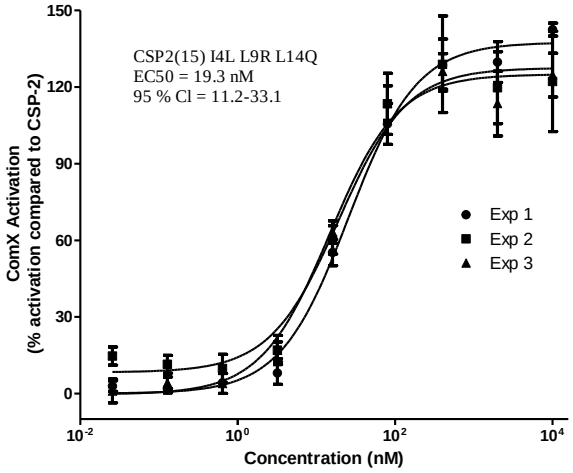

CSP2(15) L9R L14Q
EC50 = 15.2 nM
95 % CI = 6.14-37.5
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

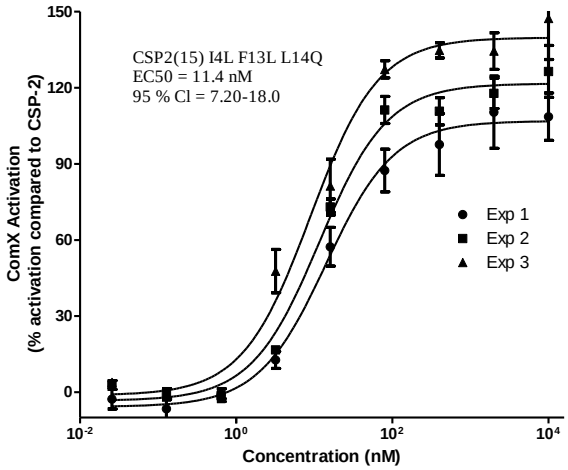

CSP2(15) L12I F13L
EC50 = 44.5 nM
95 % CI = 22.5-88.0
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

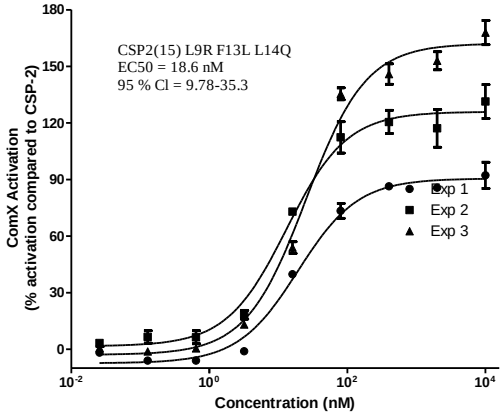

CSP2(15) L12I L14Q
EC50 = 72.2 nM
95 % CI = 49.6-104
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

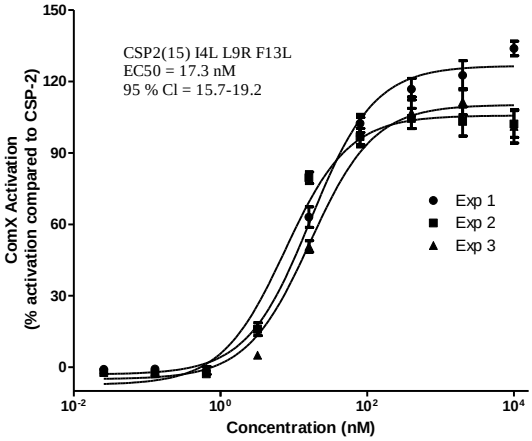

CSP2(15) F13L L14Q
EC50 = 22.5 nM
95 % CI = 19.2-26.2
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

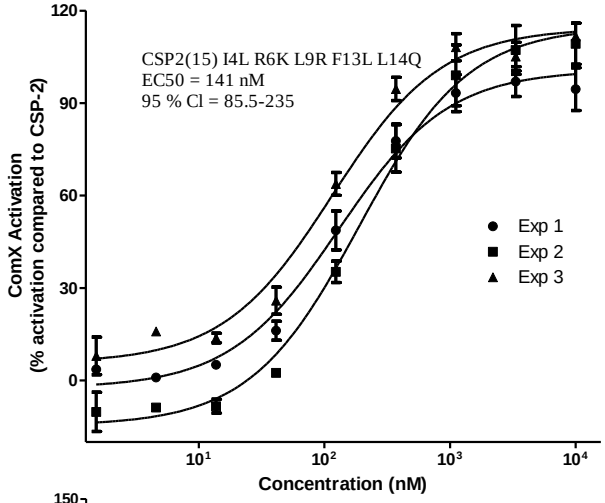

CSP2(15) I4L L9R F13L
EC50 = 79.4 nM
95 % CI = 53.2-118
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

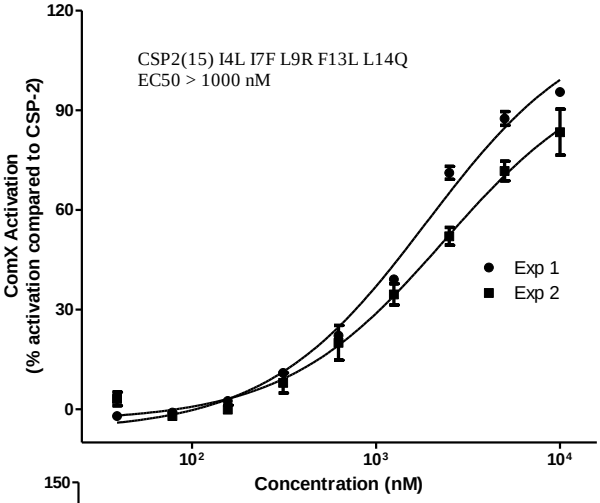

CSP2(15) I4L L9R L14Q
EC50 = 19.3 nM
95 % CI = 11.2-33.1
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

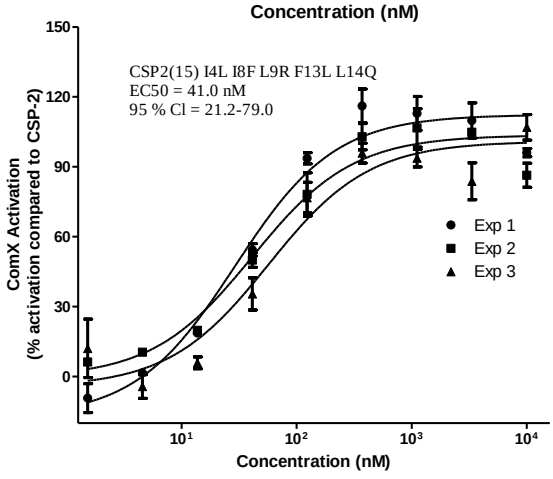

CSP2(15) I4L F13L L14Q
EC50 = 11.4 nM
95 % CI = 7.20-18.0
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

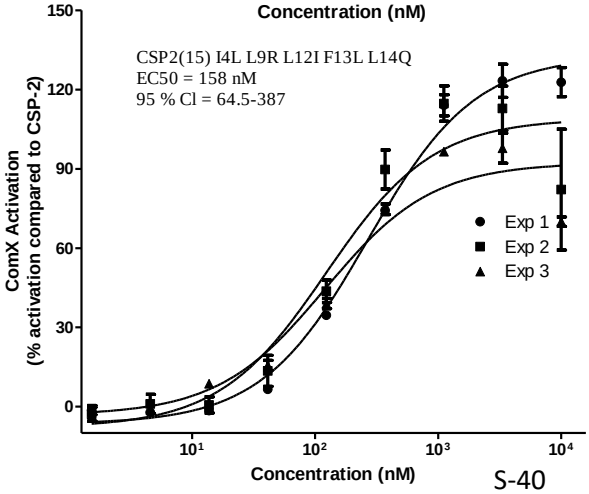

CSP2(15) L9R F13L L14Q
EC50 = 18.6 nM
95 % CI = 9.78-35.3
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

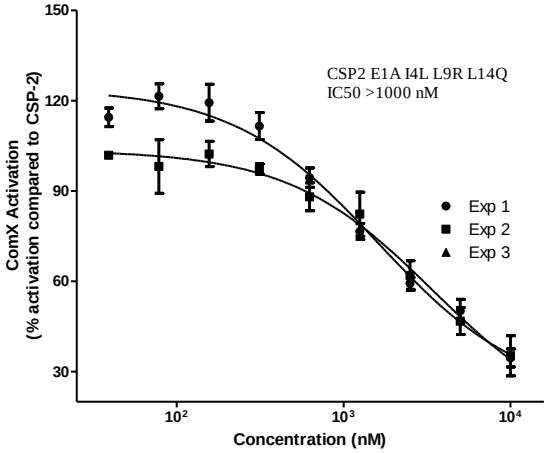

CSP2(15) I4L L9R F13L
EC50 = 17.3 nM
95 % CI = 15.7-19.2
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

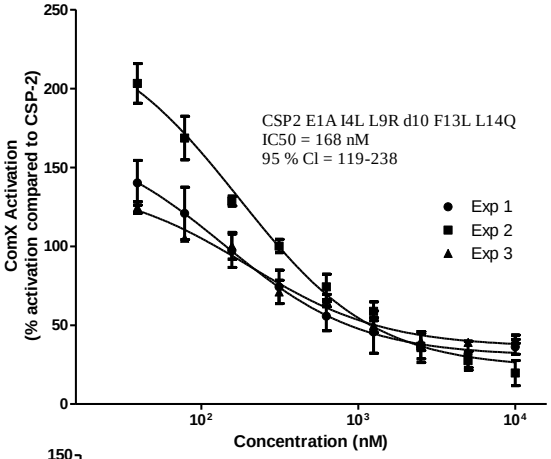

CSP2(15) I4L R6K L9R F13L L14Q
EC50 = 141 nM
95 % CI = 85.5-235
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

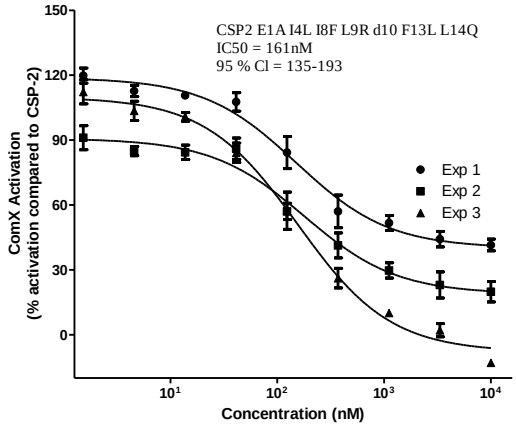

CSP2(15) I4L I7F L9R F13L L14Q
EC50 > 1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

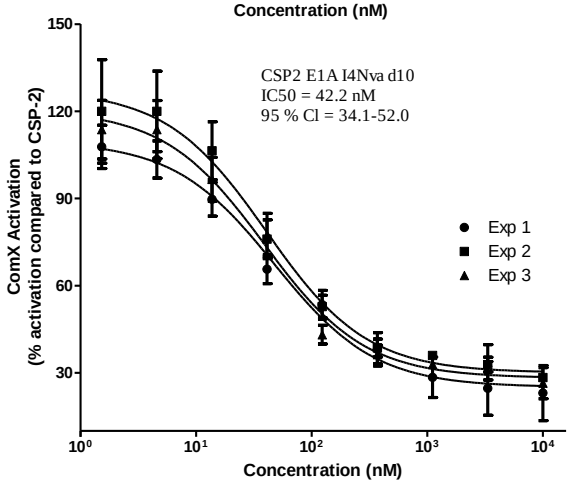

CSP2(15) I4L I8F L9R F13L L14Q
EC50 = 41.0 nM
95 % CI = 21.2-79.0
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

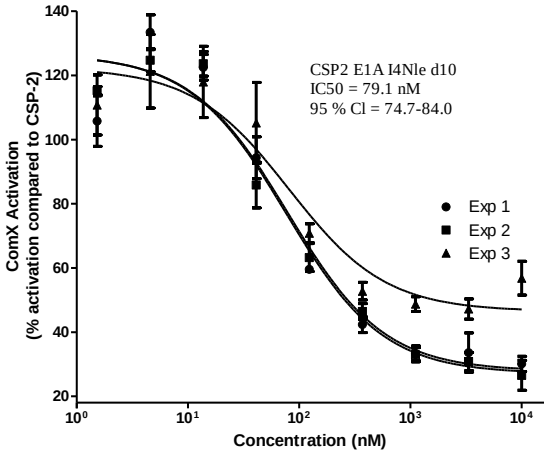

CSP2(15) I4L L9R L12I F13L L14Q
EC50 = 158 nM
95 % CI = 64.5-387
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

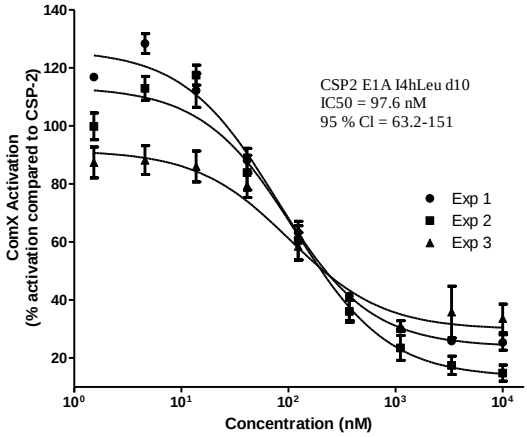

CSP2 E1A I4L L9R L14Q
IC50 >1000 nM
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

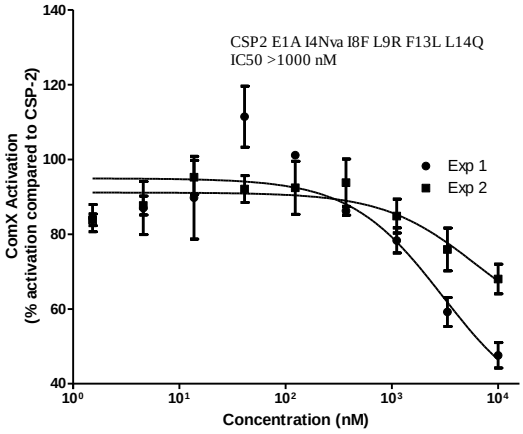

CSP2 E1A I4L L9R d10 F13L L14Q
IC50 = 168 nM
95 % Cl = 119-238
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

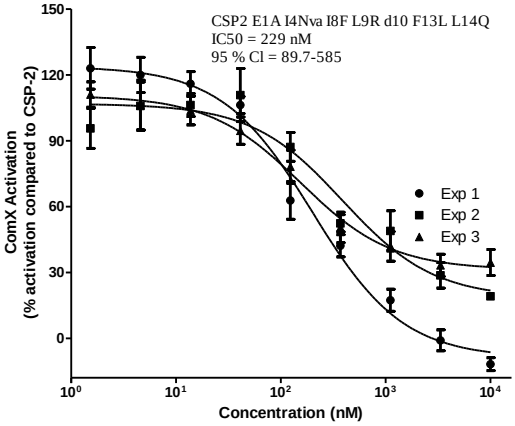

CSP2 E1A I4L I8F L9R d10 F13L L14Q
IC50 = 161nM
95 % Cl = 135-193
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

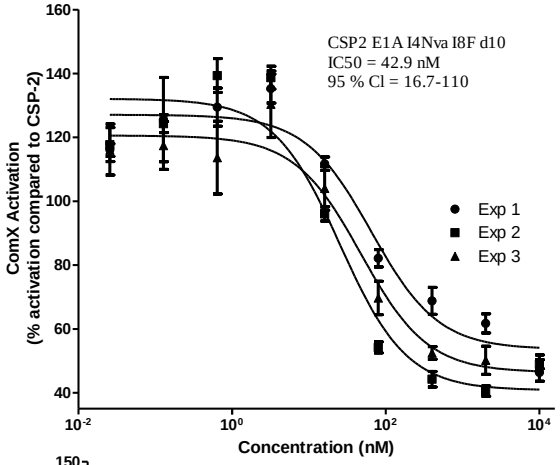

CSP2 E1A I4Nva d10
IC50 = 42.2 nM
95 % Cl = 34.1-52.0
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

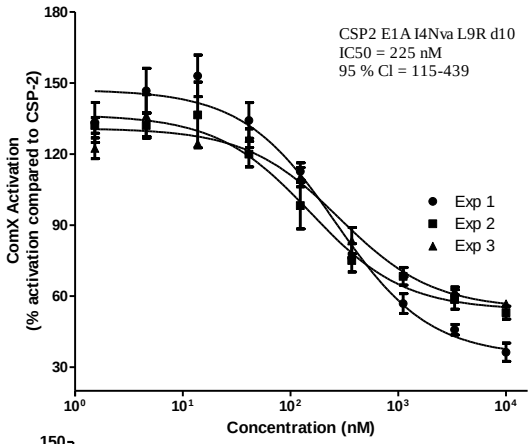

CSP2 E1A I4Nle d10
IC50 = 79.1 nM
95 % Cl = 74.7-84.0
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

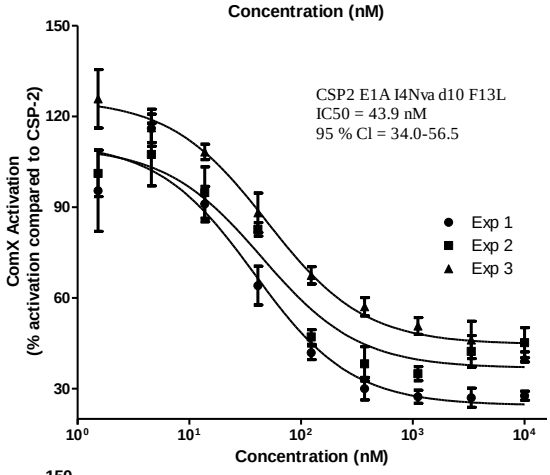

CSP2 E1A I4hLeu d10
IC50 = 97.6 nM
95 % Cl = 63.2-151
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

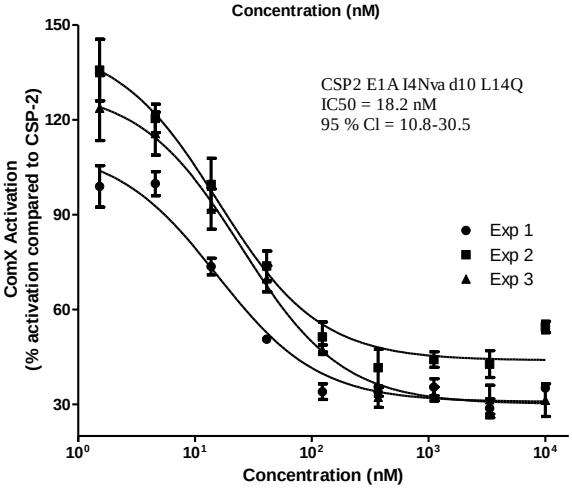

CSP2 E1A I4Nva I8F L9R F13L L14Q
IC50 >1000 nM
Exp 1
Exp 2
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

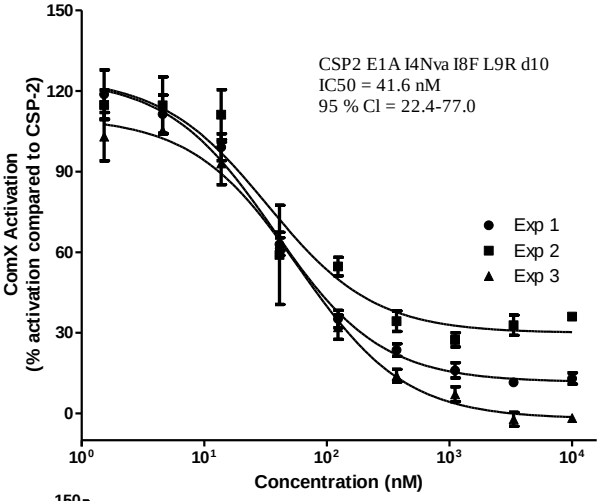

CSP2 E1A I4Nva I8F L9R d10 F13L L14Q
IC50 = 229 nM
95 % Cl = 89.7-585
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

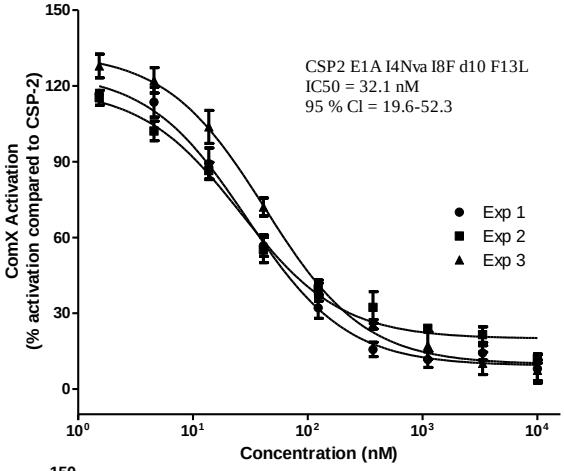

CSP2 E1A I4Nva I8F d10
IC50 = 42.9 nM
95 % CI = 16.7-110
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

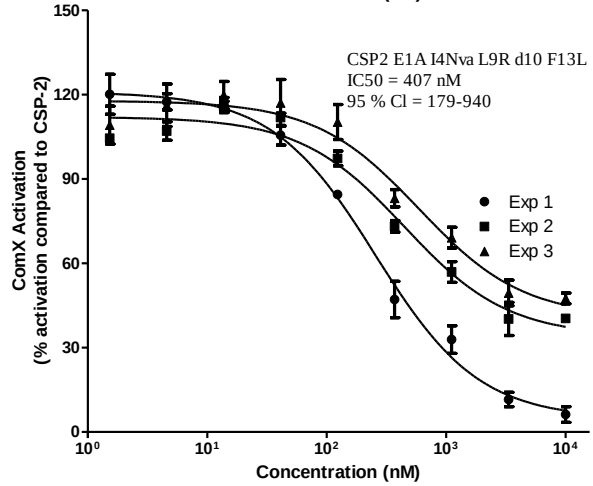

CSP2 E1A I4Nva L9R d10
IC50 = 225 nM
95 % CI = 115-439
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

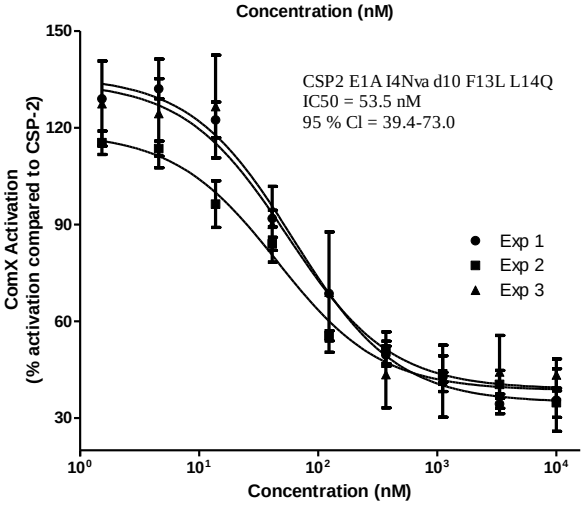

CSP2 E1A I4Nva d10 F13L
IC50 = 43.9 nM
95 % CI = 34.0-56.5
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

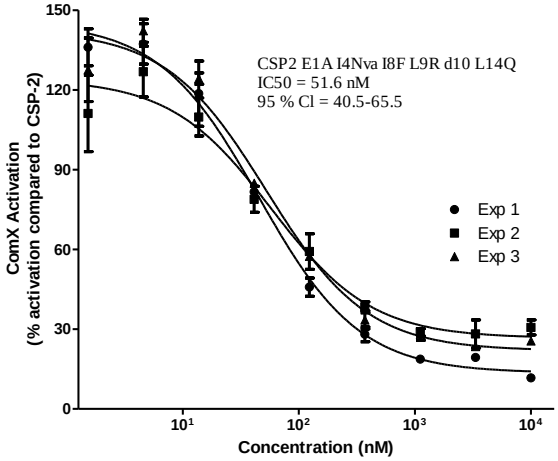

CSP2 E1A I4Nva d10 L14Q
IC50 = 18.2 nM
95 % CI = 10.8-30.5
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

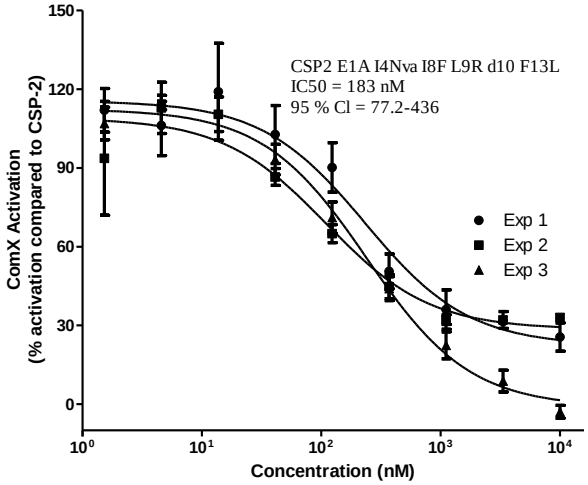

CSP2 E1A I4Nva I8F L9R d10
IC50 = 41.6 nM
95 % CI = 22.4-77.0
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

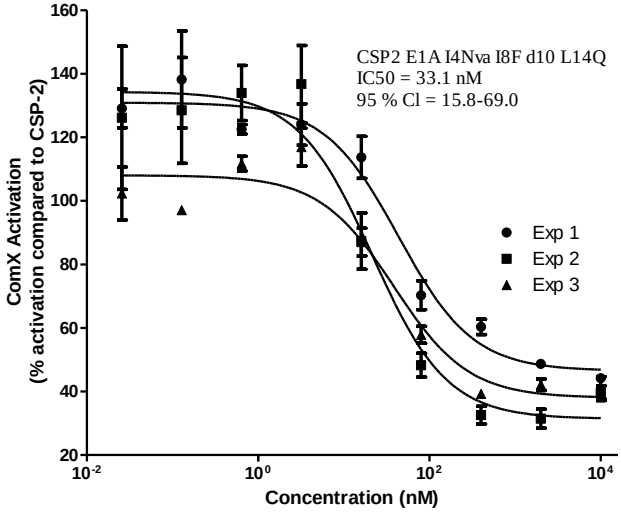

CSP2 E1A I4Nva I8F d10 F13L
IC50 = 32.1 nM
95 % CI = 19.6-52.3
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

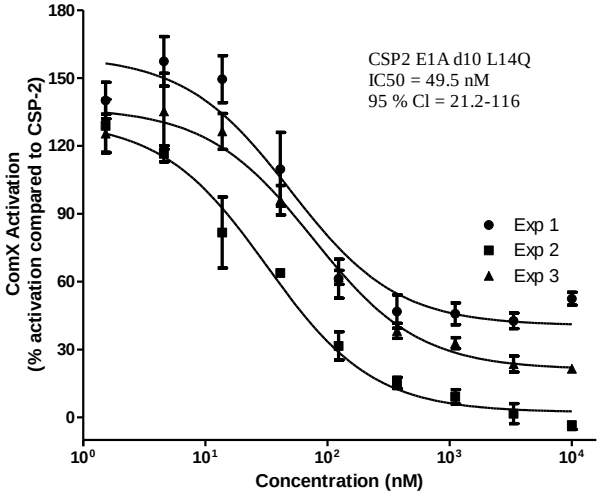

CSP2 E1A I4Nva L9R d10 F13L
IC50 = 407 nM
95 % CI = 179-940
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)

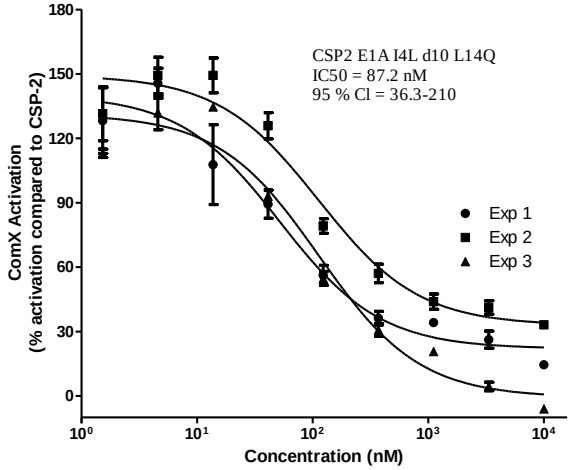

CSP2 E1A I4Nva d10 F13L L14Q
IC50 = 53.5 nM
95 % CI = 39.4-73.0
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)


CSP2 E1A I4Nva I8F L9R d10 L14Q
IC50 = 51.6 nM
95 % Cl = 40.5-65.5
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)


CSP2 E1A I4Nva I8F L9R d10 F13L
IC50 = 183 nM
95 % Cl = 77.2-436
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)


CSP2 E1A I4Nva I8F d10 L14Q
IC50 = 33.1 nM
95 % Cl = 15.8-69.0
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)


CSP2 E1A d10 L14Q
IC50 = 49.5 nM
95 % Cl = 21.2-116
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)


CSP2 E1A I4L d10 L14Q
IC50 = 87.2 nM
95 % Cl = 36.3-210
Exp 1
Exp 2
Exp 3
ComX Activation
(% activation compared to CSP-2)
Concentration (nM)