Supplementary Material (ESI) for Molecular BioSystems

Supplementary Information

Bivalent inhibitors of the tyrosine kinases ABL and SRC: Determinants of potency and selectivity

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Contents

I. Primers Used for Overlap Extension PCR of AGT Constructs

AGT(PP1)
Step 1
FWD: 5'- CCG CGT AAC CGT CCG CGT CTG AGC GGT AGC GGC GAC AAA GAT TGC GAA ATG AAA -3'
RVS: 5'- GGG GAC CAC TTT GTA CAA GAA AGC TGG GTA CTA CAG ACC CGG TTT ACC-3'

Step 2
FWD: 5'- GGGG ACA AGT TTG TAC AAA AAA GCA GGC TTC GCG CCG CCG CTG CCG CCG CGT AAC CGT CCG CGT CTG-3'
RVS: 5'- GGG GAC CAC TTT GTA CAA GAA AGC TGG GTA CTA CAG ACC CGG TTT ACC-3'

AGT(PP2)
Step 1
FWD: 5'- CCG CGT AAC CGT CCG CGT CTG AGC GGT AGC GGC AGC GGT AGC GGC AGC GGT GAC AAA GAT TGC GAA ATG AAA -3'
RVS: 5'- GGG GAC CAC TTT GTA CAA GAA AGC TGG GTA CTA CAG ACC CGG TTT ACC-3'

Step 2
FWD: 5'- GGGG ACA AGT TTG TAC AAA AAA GCA GGC TTC GTG TCT CTG GCG CGT CGT CCG CTG CCG CCG CTG CCG CGT CTG AGC GGT AGC GGC-3'
RVS: 5'- GGG GAC CAC TTT GTA CAA GAA AGC TGG GTA CTA CAG ACC CGG TTT ACC-3'

AGT(PP3)
Step 1
FWD: 5'- CCG CTG CCG CCG CTG CCG CCG AGC GGT AGC GGC GAC AAA GAT TGC GAA ATG AAA -3'
RVS: 5'- GGG GAC CAC TTT GTA CAA GAA AGC TGG GTA CTA CAG ACC CGG TTT ACC-3'

Step 2
FWD: 5'- GGGG ACA AGT TTG TAC AAA AAA GCA GGC TTC CGT GCG GCG CGT CCG CTG CCG CCG CTG CCG CCG-3'
RVS: 5'- GGG GAC CAC TTT GTA CAA GAA AGC TGG GTA CTA CAG ACC CGG TTT ACC-3'

AGT(PP4)

Step 1
FWD: 5'- TAT AGC CCG CCG CCG CCG CCG AGC GGT AGC GGC GAC AAA GAT TGC GAA ATG AAA-3'
RVS: 5'- GGG GAC CAC TTT GTA CAA GAA AGC TGG GTA CTA CAG ACC CGG TTT ACC-3'

Step 2
FWD: 5'- GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT CGC GCC GAC CTA TAG CCC GCC GCC GCC GCC G-3'
RVS: 5'- GGG GAC CAC TTT GTA CAA GAA AGC TGG GTA CTA CAG ACC CGG TTT ACC-3'

II. Primers Used for Cloning of SRC and HCK SH3 Domain

SRC SH3

FWD: 5'- GGGG ACA AGT TTG TAC AAA AAA GCA GGC TTC GTC ACC ACT TTC GTG GCT CTC
RVS: 5'- GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC CTA GGG CGC GAC ATA GTT ACT GGG

HCK SH3

FWD: 5'- GGGG ACA AGT TTG TAC AAA AAA GCA GGC TTC ATC ATC GTG GTT GCC CTG TAT
RVS: 5'- GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC CTA AAC GCG GGC GAC ATA GTT GCT

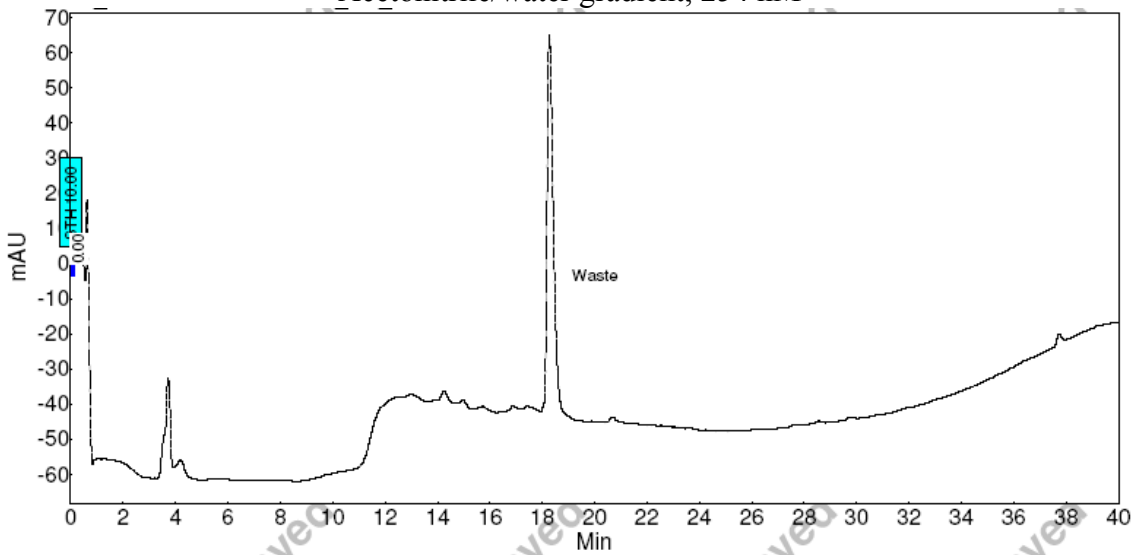
III. Analytical HPLC Traces of Compounds 1,2,3,4,5, and 6

General Analytical HPLC Conditions for compounds 1-3 C_{18} column (250 x 4.6 mm), Acetonitrile/Water-0.1% CF_3CO_2H gradient: 1:99 to 100:0 over 40 min. Methanol/Water–0.1% CF_3CO_2H gradient: 1:99 to 100:0 over 40 min. Flow rate = 1mL/min; 254 nM detection for 45 min

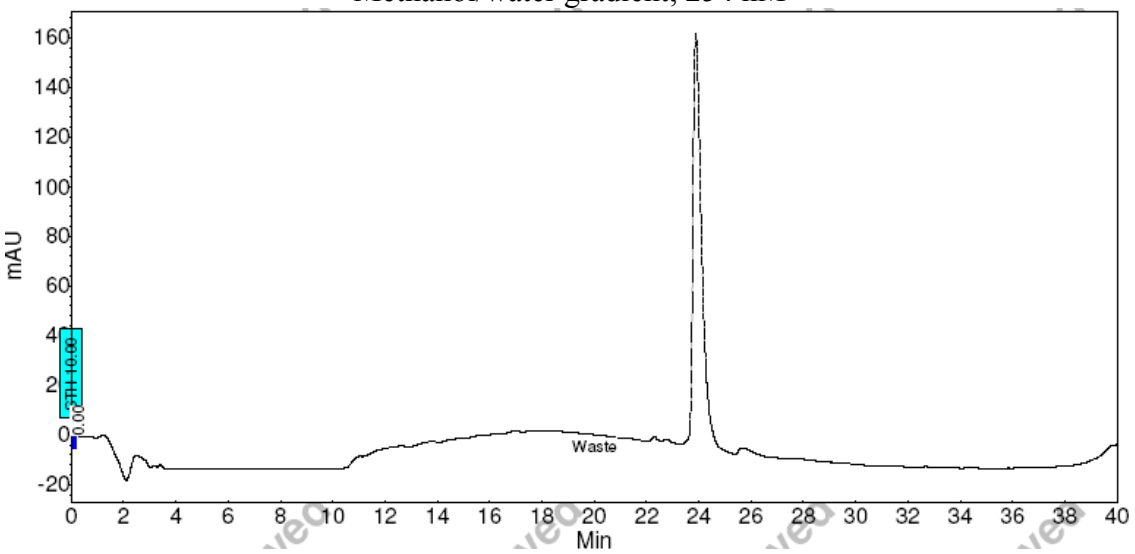
General Analytical HPLC Conditions for compounds 4-6 C_{18} column (150 x 2.1 mm), Acetonitrile/Water-0.1% CF_3CO_2H gradient: 1:99 to 100:0 over 30 min. Methanol/Water–0.1% CF_3CO_2H gradient: 1:99 to 100:0 over 30 min. Flow rate = 1mL/min; 254 nM detection for 30 min.

Analytical HPLC traces for compound 1

Acetonitrile/water gradient; 254 nM

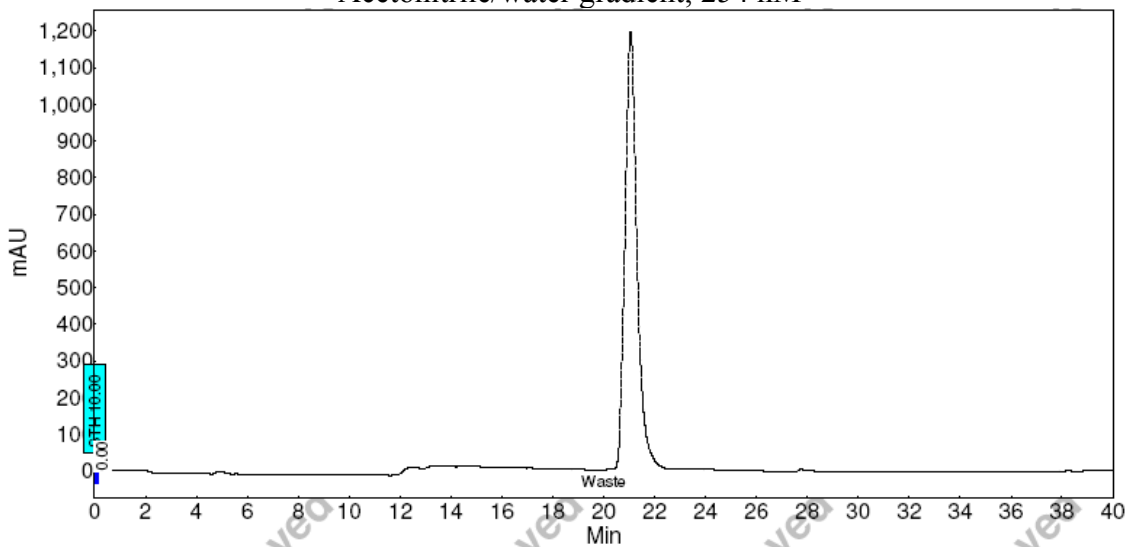


Methanol/water gradient; 254 nM

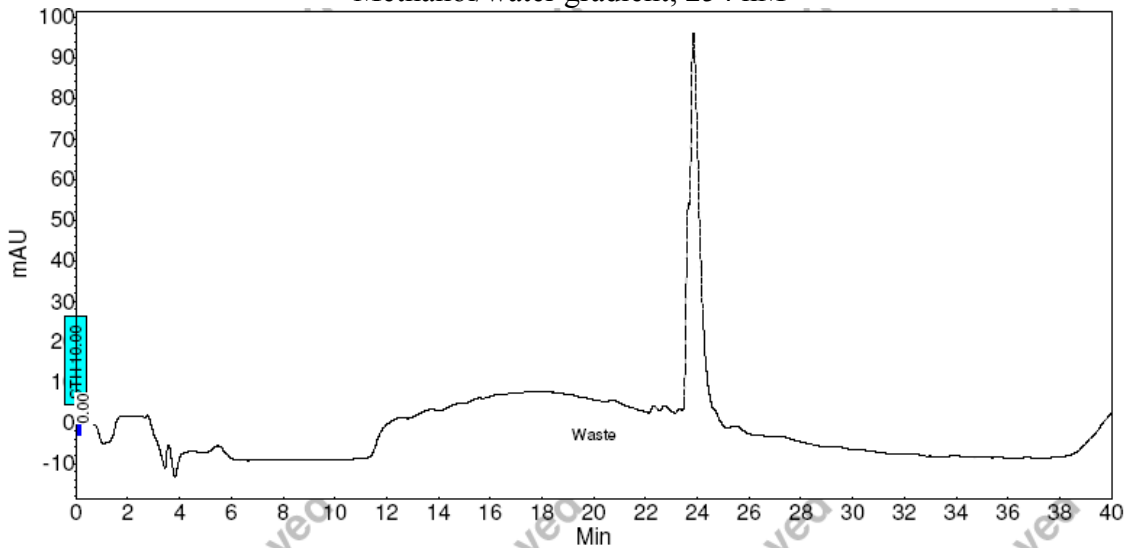


Analytical HPLC traces for compound 2

Acetonitrile/water gradient; 254 nM

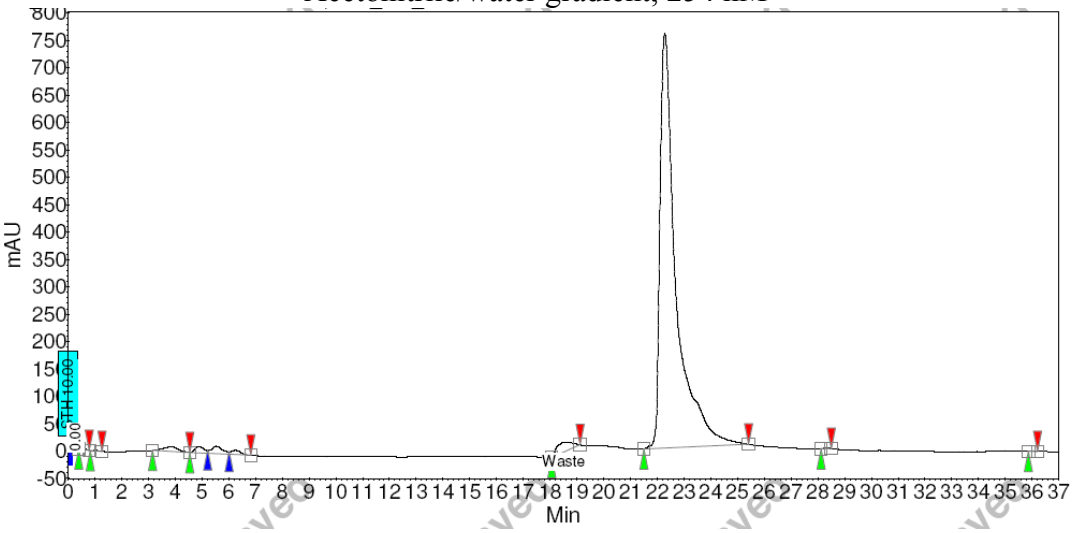


Methanol/water gradient; 254 nM



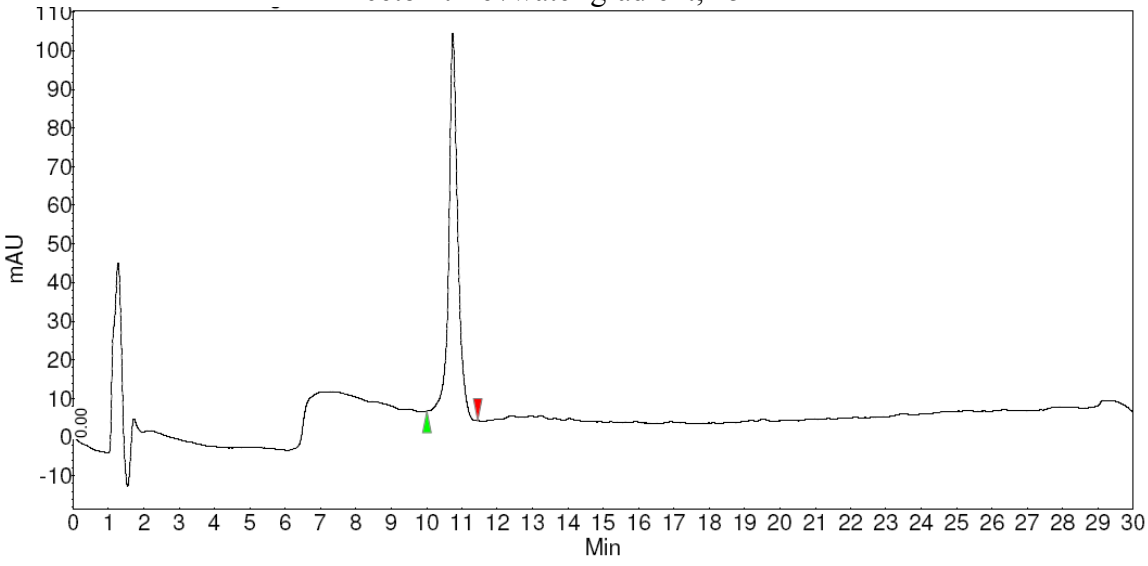
Analytical HPLC trace for compound 3

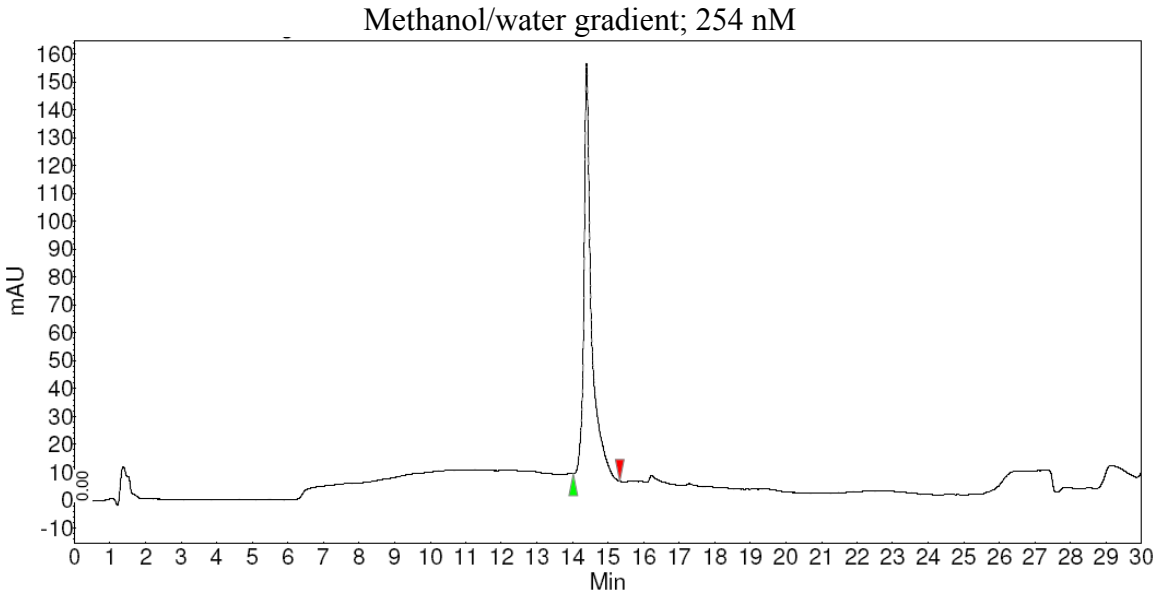
Acetonitrile/water gradient; 254 nM



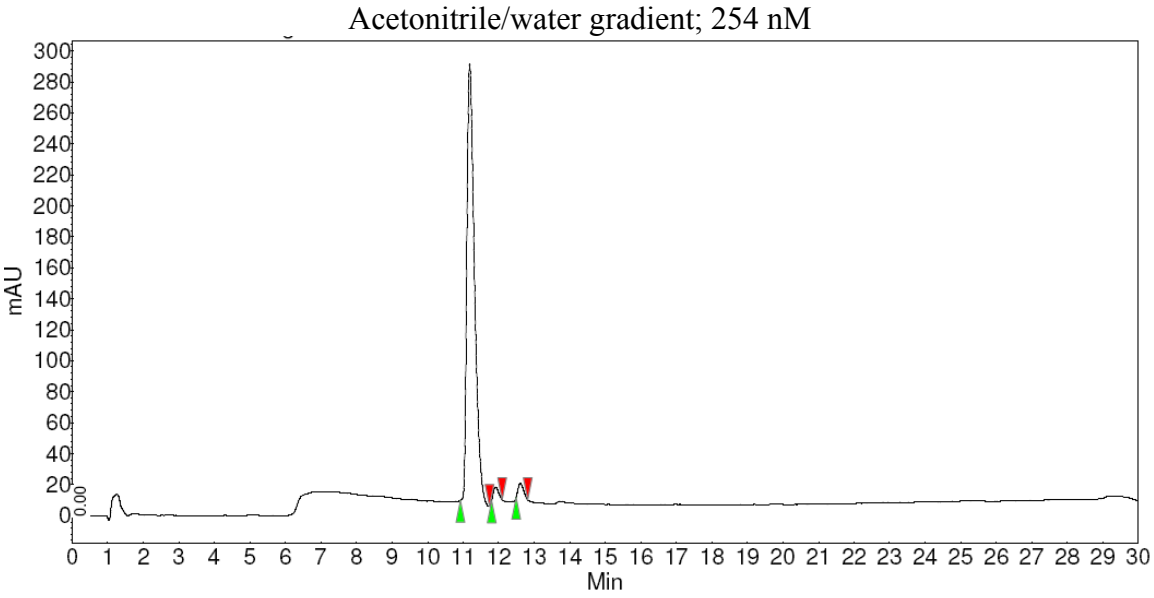
Analytical HPLC traces for compound 4

Acetonitrile /water gradient; 254 nM

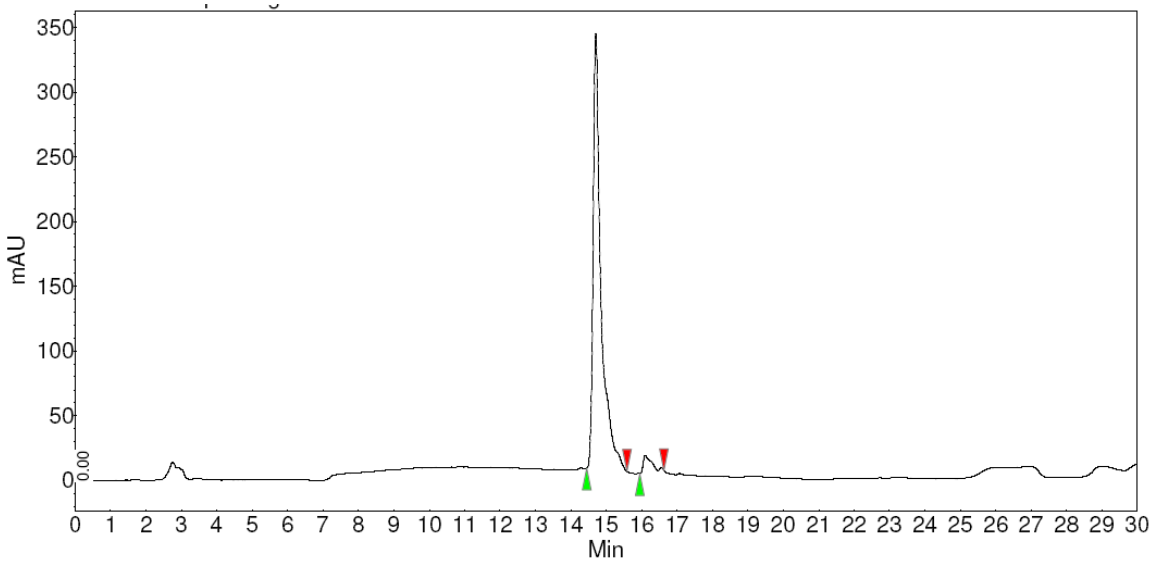




Analytical HPLC traces for compound 5

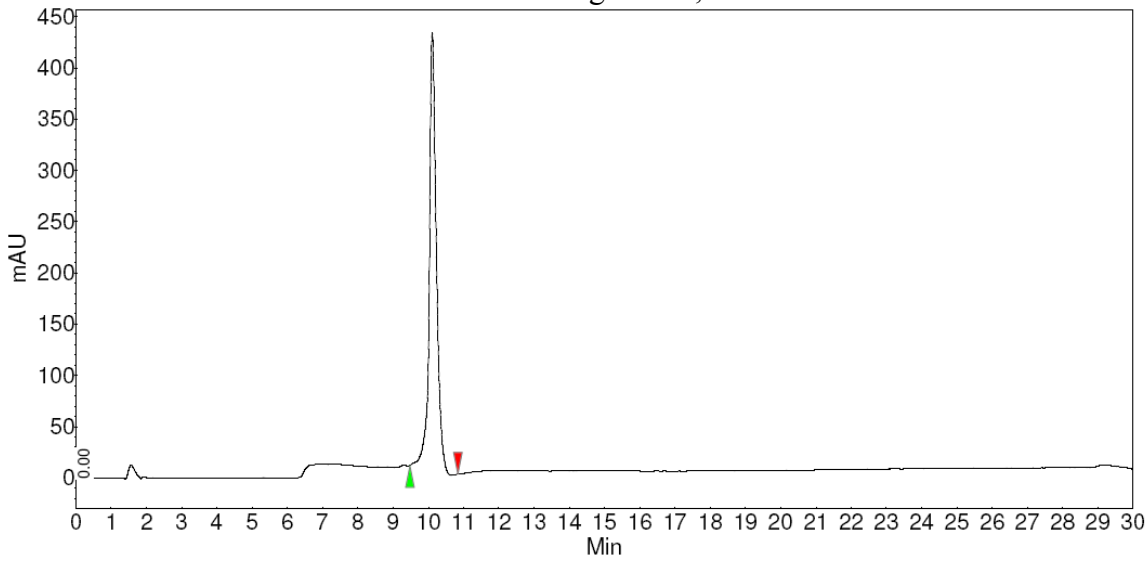


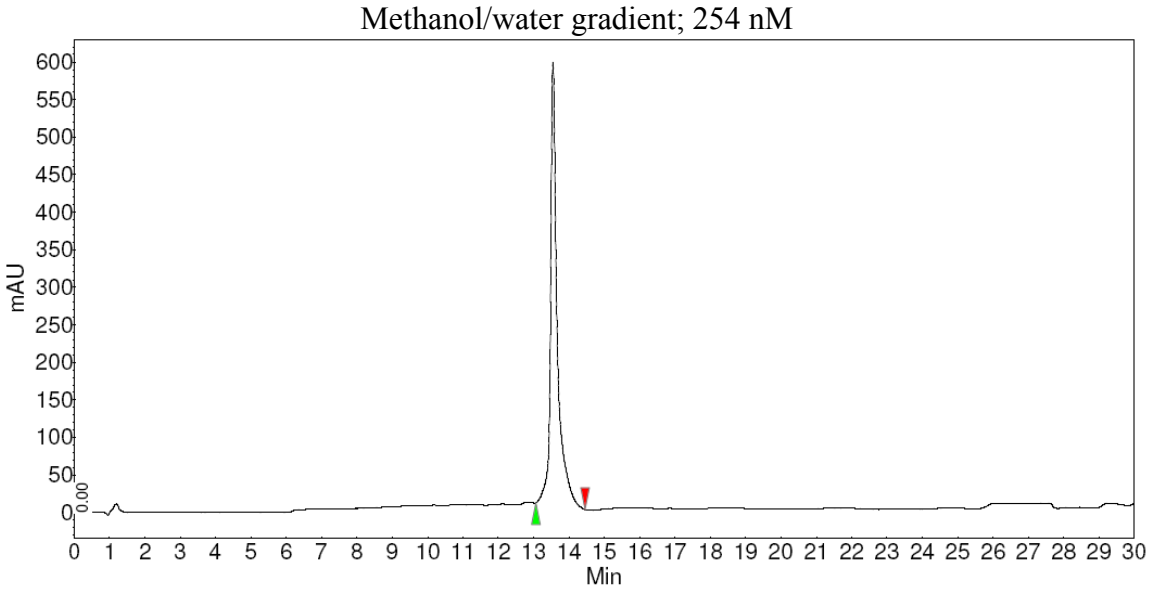
Methanol/water gradient; 254 nM



Analytical HPLC traces for compound 6

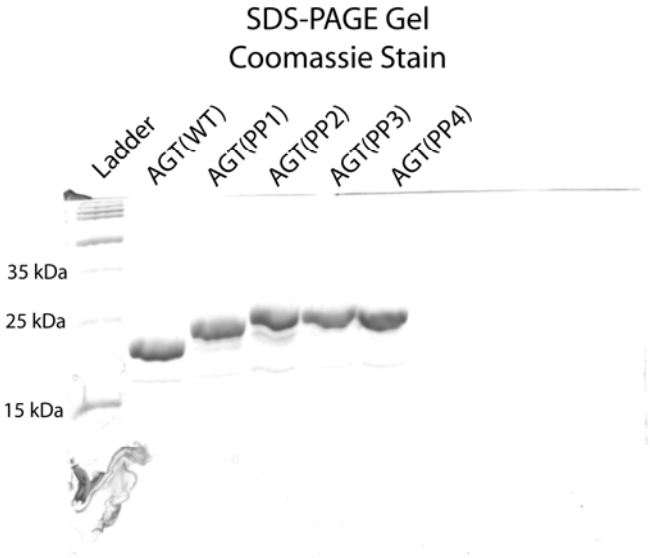
Acetonitrile/water gradient; 254 nM



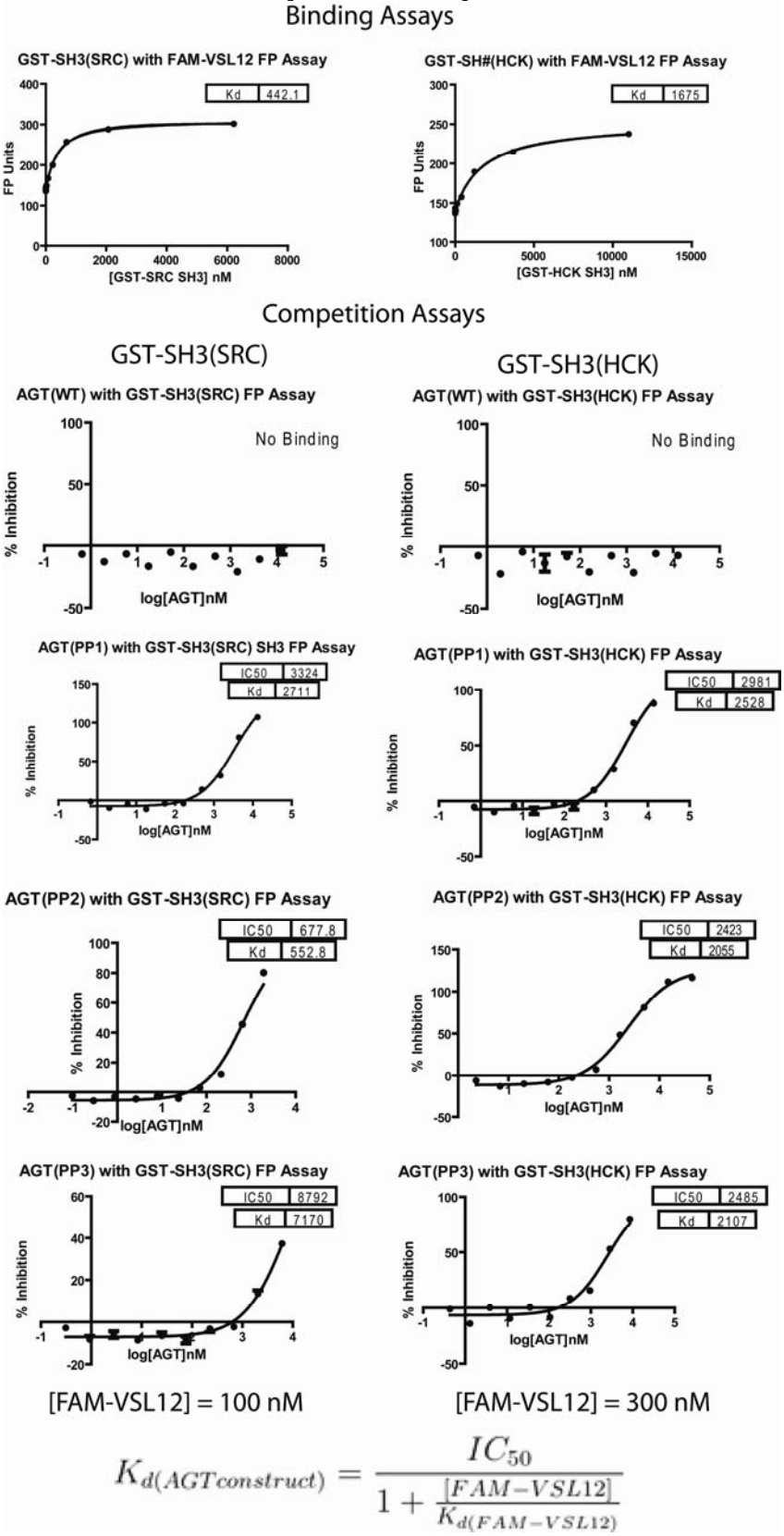


IV. SDS-PAGE Gel of AGT Constructs

To verify protein purity, AGT constructs were run on a 15% SDS-Page gel and stained with coomassie (gel below). 2.5 µg of protein was loaded in each well.



V. Fluorescence Polarization Assay Data and Equation



$$K_{d(AGTconstruct)} = \frac{IC_{50}}{1 + \frac{[FAM-VSL12]}{K_{d(FAM-VSL12)}}}$$

All FP assays contained two control wells. One with no GST-SH3 to give the minimum mP units, the other with no AGT construct to give maximal mP units. The minimum mP units where treated as background and subtracted from all assay data points. The mP units from the AGT titration wells were divided by the maximal signal to give the percent activity. This was subtracted from 100 to give percent inhibition which was plotted against log of AGT concentration to give the above plots.

Data was analyzed using Prism Graphpad software and implied K_d values were determined using non-linear regression analysis. K_d values were calculated from the observed IC_{50}s by dividing by 1 plus the concentration of FAM-VSL12 over the known K_d of FAM-VSL12 for the GST-SH3 domain. This relationship is described in the above equation.