Structure, Volume *25*

Supplemental Information

KRAS G12C Drug Development: Discrimination between Switch II Pocket Configurations Using Hydrogen/Deuterium-Exchange Mass Spectrometry

Jia Lu, Rane A. Harrison, Lianbo Li, Mei Zeng, Sudershan Gondi, David Scott, Nathanael S. Gray, John R. Engen, and Kenneth D. Westover

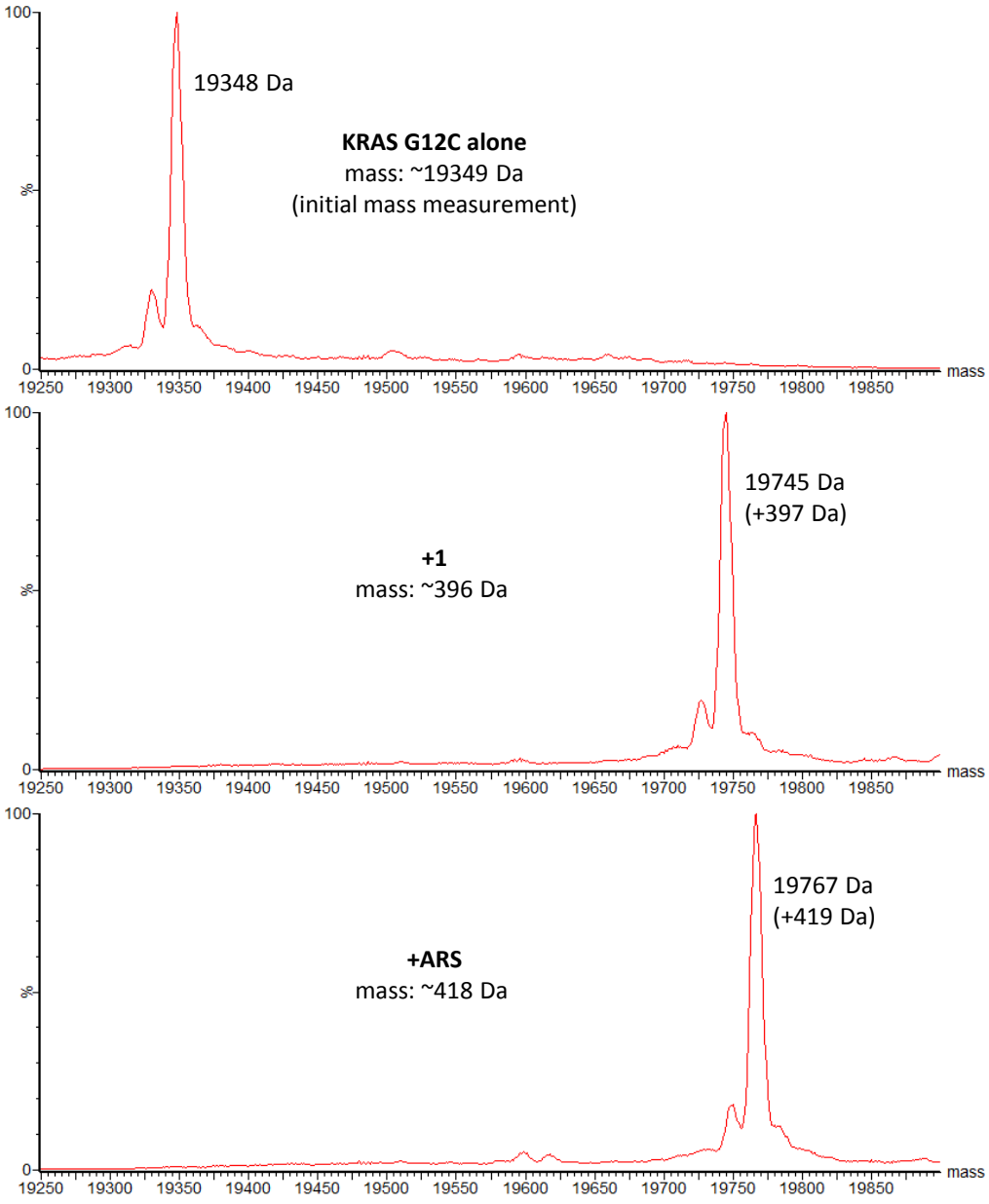
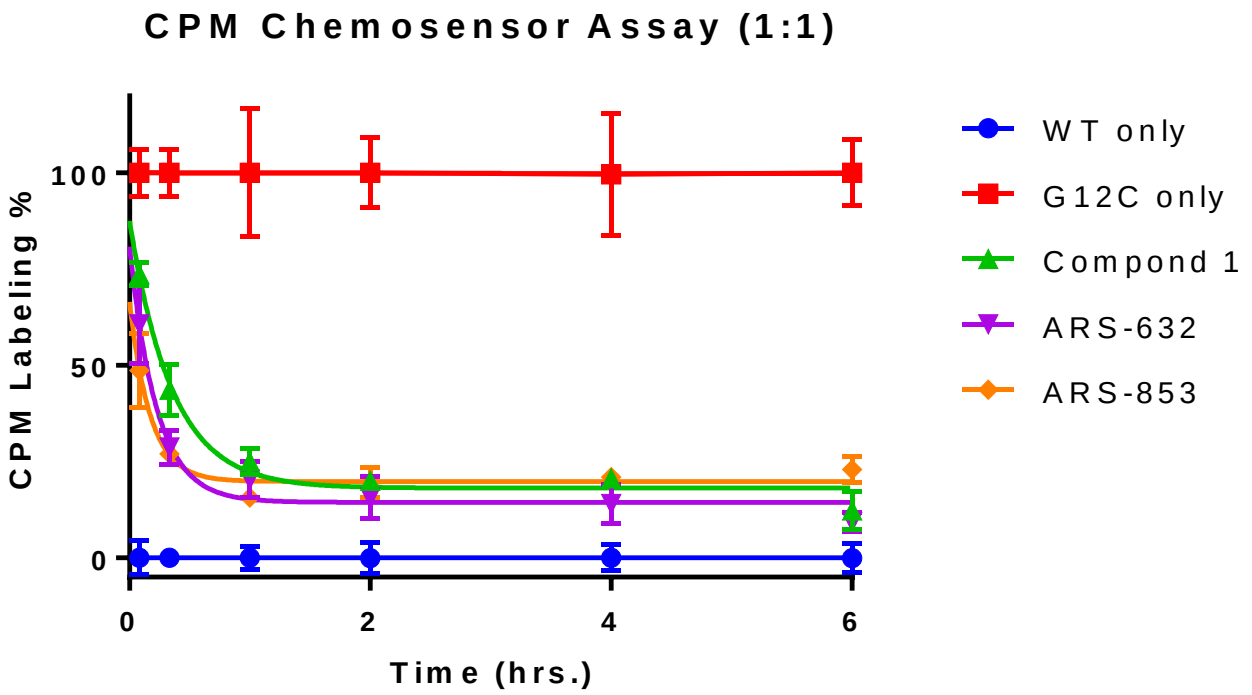


Figure S-1 Intact Mass Spectrometry of KRAS G12C control (upper panel), KRAS G12C labeled with 1 (middle), or ARS-632 (bottom), Related to Figure 2. The shift in molecular weight corresponds to compound molecular weight.



Compound	$T_{½}$ (hrs)	95% CI (hrs)
1	0.25	0.1839 - 0.3714
ARS-632	0.16	0.1037 - 0.3193
ARS-853	0.12	0.07169 - 0.3649

Figure S-2 Chemosensor Assay, Related to STAR Methods. Cysteine-reactive compound 7-diethylamino-3-(4-maleimidophenyl) or CPM was used to monitor the rate of reaction between compounds and G12C protein. CPM exhibits enhanced fluorescence after reacting with a thiol group. Upon covalent labeling of Cys12 in KRAS G12C, the thiol group is no longer able to react with CPM and the fluorescent signal deceases. GDP-loaded KRas G12C was incubated with an 1:1 ratio of **1** (green), ARS-632 (magenta) orARS-853 (orange) for the indicated length of time. WT (blue) and G12C only (red) were used as negative and positive control, respectively. The rate of half CPM labeling were determined by Graphpad Prism 7.01 (GraphPad Software, Inc., La Jolla, CA). All measurements were performed in triplicate and represented as AVG ± SD.

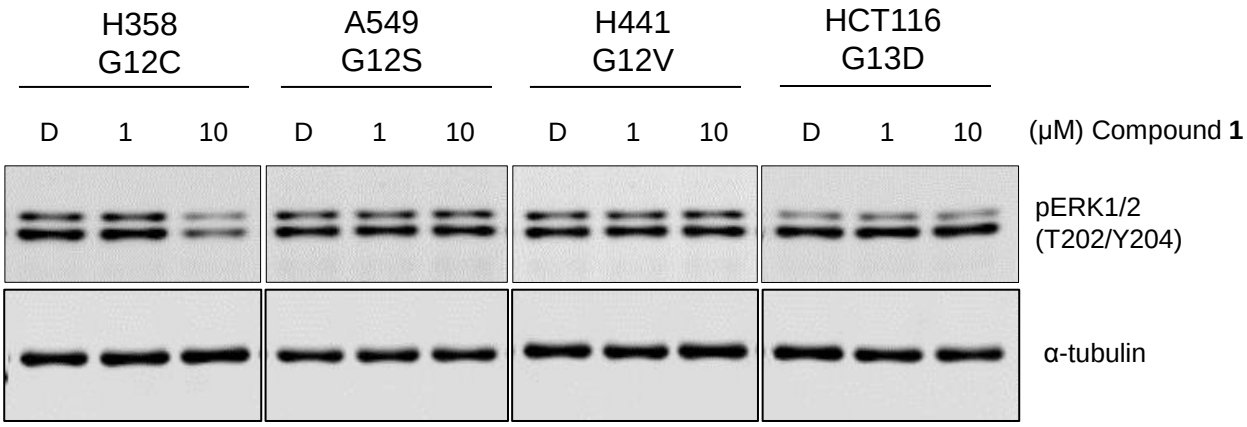


Figure S-3. Impact of compound 1 on pERK level in different cell lines, Related to Figure 2. KRAS G12C mutant cell line H358 and non-G12C mutant cell lines (A549, H441 and HCT116) were treated with compound **1** for 6 hours at two concentrations (1 and 10 μM). Phosphorylation of ERK1/2 was determined by immunoblotting.

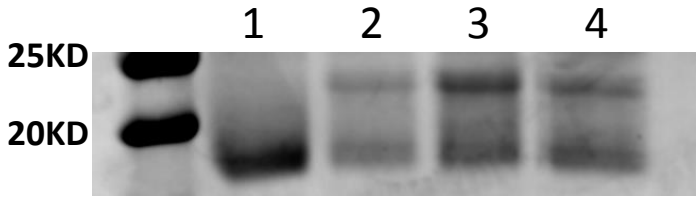


Figure S-4. Compound-dependent shift of electrophoretic gel mobility of KRAS G12C, Related to Figure 2. Purified recombinant KRAS G12C protein was incubated with 2:1 stoichiometric ratio of compound to protein overnight at room temperature and subjected to SDS-gel. Lane1: G12C only; Lane2: compound ***1***; Lane3: ARS-632; Lane4: ARS-853.

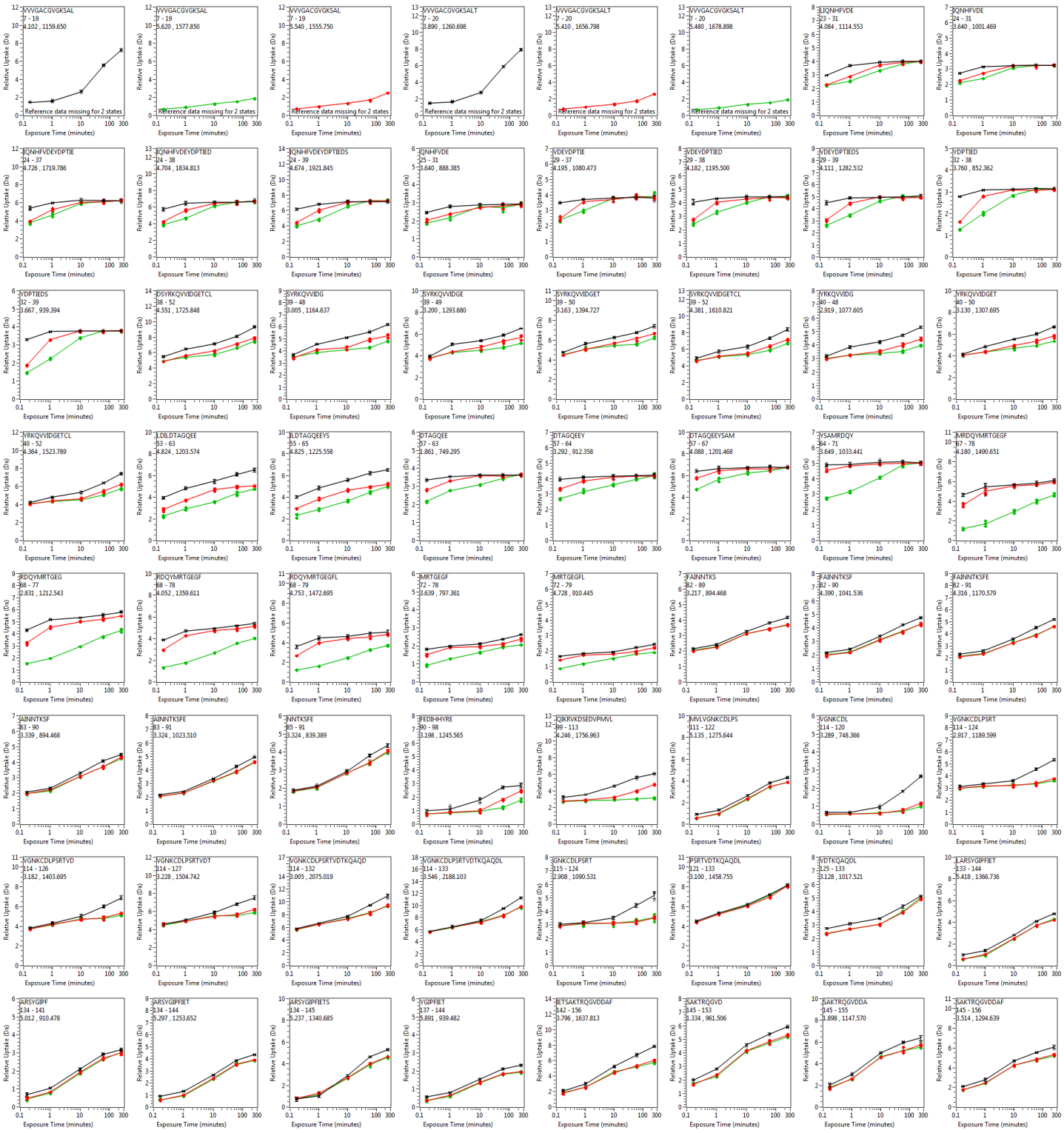
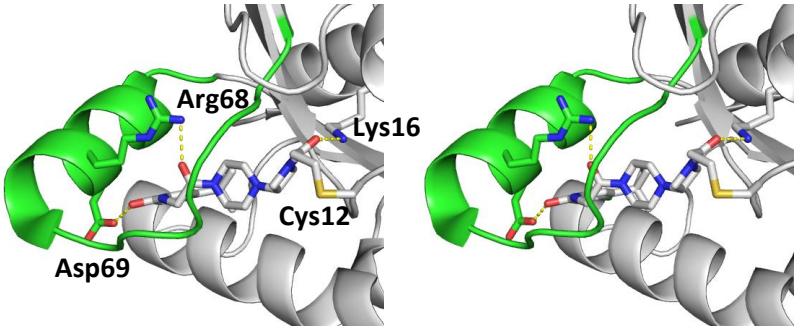


Figure S-5. HDX-MS time course for all peptides derived from compound-exposed KRAS G12C, Related to Figure 3.

A.



B.

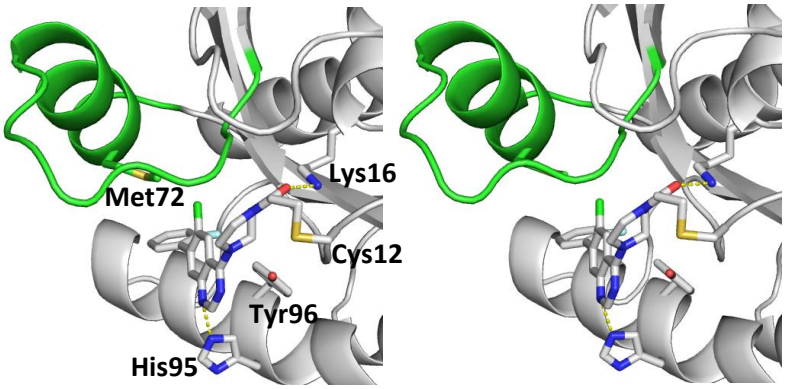
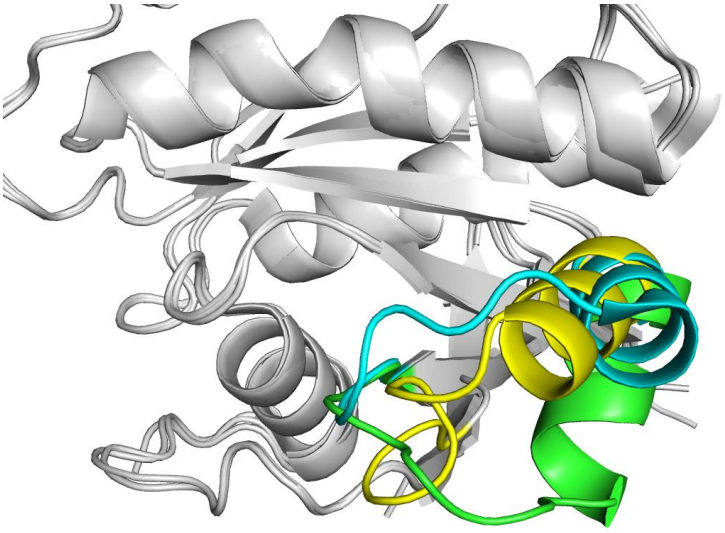


Figure S-6. Stereo imagines of KRAS G12C in complex with ARS-853 (A), or ***1*** (B), Related to Figure 4. Switch II is in green and critical interacting residues shown as sticks. Hydrogen bonds shown as yellow dash lines.



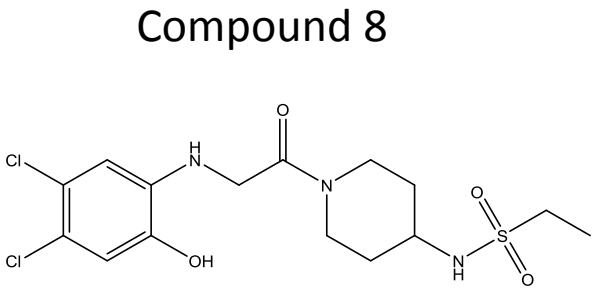
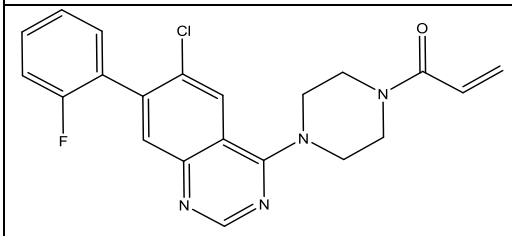
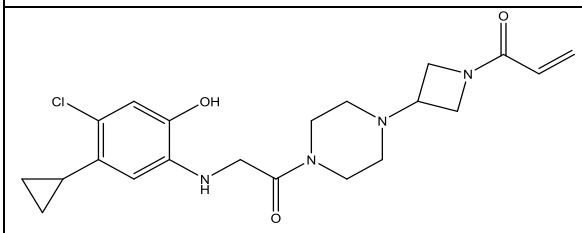
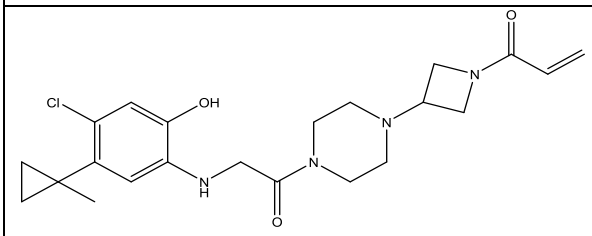


Figure S-7. Comparison of Switch II region between Compound *1* (green), **ARS-853** (cyan) **and Compound 8** (yellow, from Ostrem et al., 2013). Related to Figure 4.

Structure	Name	Reference
 **1**	1-(4-(6-chloro-7-(2-fluorophenyl)quinazolin-4-yl)piperazin-1-yl)prop-2-en-1-one	Patent: WO2015054572 Example 81
 ARS-632	1-(3-(4-((4-chloro-5-cyclopropyl-2-hydroxyphenyl)glycyl)piperazin-1-yl)azetidin-1-yl)prop-2-en-1-one	Patent: WO2014152588 Example V-35
 ARS-853	1-(3-(4-((4-chloro-2-hydroxy-5-(1-methylcyclopropyl)phenyl)glycyl)piperazin-1-yl)azetidin-1-yl)prop-2-en-1-one	Patent: WO2014152588 Example V-64

Supplemental Table S-1, Related to Figure 1. Compound ***1***, ARS-632 and ARS-853 with structure and reference.