Supplementary
Supplementary Figure 1a – Mass shift of WT EGFR in the presence of CNX17 after a 60 minute incubation at 10X of CNX17 to WT EGFR protein
Supplementary Figure 1b – Mass shift of WT EGFR in the presence of CNX17 after a 60 minute incubation at 10X of CNX17 to WT EGFR protein
Supplementary Figure 2 – shows the percent CNX-17 and 70 remaining after incubation with 500-fold excess of GSH (5 mM) and analyzed via MS for compound remaining compared to the zero minute time point.
Supplementary Figure 3: CNX-17 Irreversibly Inhibits the Molecular Target EGFR Within 10 Minutes: A431 cells were serum starved overnight and treated for time shown with 1uM of either CNX-17. Compound containing media was replaced with compound free media. Prior to collecting samples at the indicated time, cells were stimulated with EGF (100ng/ml) for 10 min.
Supplementary Figure 4: A431 cells were serum starved overnight and treated for an hour with 1μM of either CNX-17 or CNX-70. Compound containing media was replaced with compound free media. Prior to collecting samples at the indicated time of washout or after preincubation with compound, cells were stimulated with EGF (100ng/ml) for 10 min. Prolonged pharmacodynamic effect of CNX-17 inhibition of EGFR phosphorylation was observed 8h after compound removal (n=2).
Supplementary Figure 5. Dose dependent signaling inhibition by CNX-17 and CNX-70 in A) A431 cells, B) HCC827 cells and C) H1975 cells. Cells were seeded in 12 well plates and serum starved overnight. Cells were then treated for an hour with indicated concentrations of either CNX-17 or CNX-70. For A431 cells, samples were treated with EGF (100 ng/mL) for 15 min. Inhibition was determined by inhibition of pEGFR (Y1068), pAKT, ppS6 and pERK as indicated (n=3)
Figure 5A
A431 (Egfr\textsuperscript{WT})
Figure 5B
HCC827 (Egfr^{DelE746-A750})

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Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications
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Figure 5C
H1975 (Egfr<sup>L858R/T790M</sup>)

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**pEgfr**

**Total Egfr**

**pAkt**

**Total Akt**

**pS6RP**

**Total S6RP**

**pERK**

**Total ERK**
Supplementary Figure 6. Dose response inhibition by CNX-17 and CNX-70 in A431, H1975 and HCC827 cell proliferation. Cells were seeded at 3000 cells per well and treated with indicated concentrations of either CNX-17 and CNX-70 for 72 hrs. Cell viability was determined by CellTiter Glo. n = 6. Average data with standard deviation is shown.
Supplementary Figure 7: 3D model of CNX17 (pink color) bonded to C773 in the EGFR ATP binding site. The pyrimidine ring forms a hydrogen bond interaction with the backbone of M769 as shown by the yellow dash line. An irreversible EGFR inhibitor shown in green (from 2J5F) was superimposed to the docked CNX17 structure.