Fig. S1. ASK120067 treatment inhibits activation of EGFR and downstream signaling and induces apoptosis in PC-9 cells. a ASK120067 inhibited the phosphorylation of EGFR-Tyr1068 and its downstream signaling proteins AKT and ERK in PC-9 cells. b Apoptosis rate of PC-9 cells was evaluated by flow cytometry after treatment with increasing concentrations of ASK120067 for 24 to 72 h. Data are presented as the mean ± SEM, and the significance of differences was determined by Student's t test (*p < 0.05, **p < 0.01).
**Fig. S2** Characterization of ASK120067- or osimertinib resistant NCI-H1975 cell lines. 

**a** ASK120067-resistant cell populations (67R) or osimertinib-resistant cell populations (AZDR) and the parental NCI-H1975 cells were treated with the indicated concentrations of ASK120067 or osimertinib for 72 h. 

**b** 67R or AZDR and the parental NCI-H1975 cells were treated with the indicated concentrations of osimertinib or ASK120067 for 72 h. Viable cells were measured by the SRB assay and plotted relative to the untreated controls. 

**c** Mutation status of the 790 codon and 858 codon of EGFR in NCI-H1975, 67R and AZDR cells were tested by whole-exome sequencing (WES).
**Supplementary Figure 3**

**A**

**B**

**Fig. S3** ASK120067-resistant NCI-H1975 cells exhibited high Ack1 phosphorylation levels and growth dependence on Ack1.  

**a** Phosphorylation levels of 71 kinases in parental NCI-H1975 cells and 67R cells were tested and compared using human tyrosine kinase phosphorylation array.  

**b** Growth curves of ASK120067-resistant NCI-H1975 cells after Ack1 knockdown were assessed by SRB assay. Data are shown as the mean ± SEM, and the significance of differences was calculated by Student's *t* test (*p* < 0.05, **p** < 0.01).
Fig. S4 ASK120067-resistant cells exhibited apoptotic resistance to ASK120067 treatment. 67R and the parental NCI-H1975 cells were treated with ASK120067 for 48 h, and then apoptosis rates were evaluated by flow cytometry. Data are presented as the mean ± SEM, and the significance of differences was determined by Student's t test (*p < 0.05, **p < 0.01).
**Fig. S5** Combination of ASK120067 with either dasatinib (a) or bosutinib (b) partially restored the apoptosis-inducing activity of ASK120067-resistant cells to ASK120067 treatment. Data are plotted as the mean ± SD, and the significance of differences was evaluated by Student's t test (*p < 0.05, **p < 0.01).
Fig. S6 Comparison of the *in vivo* antitumor efficacy of ASK120067 in an NCI-H1975 xenograft model and ASK120067-resistant xenograft models. Data are plotted as the mean ± SD, and the significance of differences was evaluated by Student's *t* test (*p* < 0.05, **p** < 0.01).