Supplementary Figure Legend

Supplementary Fig. S1. Effect of EZN-3920 on HER3 protein level and apoptosis in 15PC3 cells with lipofection. 15PC3 cells were treated with EZN-3920 with lipofection and measured for protein levels and apoptosis after 48 hrs. (A) Western analysis of HER3 and p-AKT protein levels. (B) Caspase 3/7 activity. Data are mean ± SD (n = 3).

Supplementary Fig. S2. Effect of EZN-3920 on HER3 mRNA level in 15PC3 cells without lipofection. 15PC3 were treated with indicated amount of EZN-3920 or a control simply dissolved in saline and measured for HER3 mRNA levels after 48 hrs. Data are shown as means ± SD (n = 3).

Supplementary Fig. S3. Effect of C5.5-labeled EZN-3920 on HER3 mRNA in mouse liver. Nude mice were dosed on day 1 with 30 mg/kg C5.5-labeled EZN-3920. Livers were harvested after 24 hrs. Data are mean ± SEM (n = 8). *p < 0.05 compared with saline group.

Supplementary Fig. S4. EZN-3920 inhibited gefitinib-induced HER3 expression in HCC827 xenograft model. Tumor bearing mice were administered 30 mg/kg EZN-3920 or EZN-SCR (q3.5dx4, i.v.) (n = 8 per group). Twelve hours prior to the harvesting of tumors, 15 mg/kg gefitinib (qd×10, p.o.) was administered. Mice were sacrificed and tumors harvested for mRNA analysis by qRT-PCR method.

Supplementary Fig. S5. Effect of EZN-3920 on HER3 mRNA in various resistant cell lines.
Cells were treated with indicated amount of EZN-3920 dissolved in saline and measured for HER3 mRNA levels after 48 hrs. (A) HCC827 and gefitinib resistant clones generated in Enzon. (B) PC9 and its gifitinib-resistant clone. (C) BT474M1 and its trastuzumab-resistant clone. (D) HCC827 and gefitinib-resistant clone GR6. Data are shown as means ± SD (n = 3).

**Supplementary Fig. S6. Effect of EZN-3920 on HER3 expression and downstream signaling molecules.** FaDu head and neck cancer cells were plated and treated with EZN-3920 or EZN-SCR. Forty hours later, cells were treated with or without 80 ng/ml neuregulin for one hour and then harvested for Western analysis of different proteins as indicated.