Screening and discovery of nitro-benzoxadiazole compounds activating epidermal growth factor receptor (EGFR) in cancer cells

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Legends to Figures

Supplementary Fig. 1. Diagram of DNA cloning of domain-specific regions of human sEGFR.

Each DNA sequence coding one of the four sEGFR domains was amplified by PCR and cloned into pENTR/D-Topo vector to create entry clones in the *Escherichia coli* DH5α strain. The resulting recombinant plasmids carrying the domains I, II or IV, and designated DI, DII, and DIV respectively (the region DIII was impossible to clone), fused with the N-terminal His-tag of the destination vector pDEST17, were transferred into the *E. coli* BL21 AI strain for overexpression and purification of proteins.

Supplementary Fig. 2. Kinetics of protein tyrosine phosphorylation in breast cancer cells exposed to NSC 228155.

The MDA MB468 cells were grown in DMEM with 10% FBS, deprived of serum for 24 h, and exposed to 100 μM of the compound for a specified length of time. Changes in tyrosine phosphorylation and abundance of EGFR were assessed by immunoblotting with anti-pTyr P100, anti-pEGFR-Y1068, and anti-EGFR (cytoplasmic domain) antibodies.

Supplementary Fig. 3. Dose-dependent effect of NSC 228155 and determination of the EC_{50} value in MDA MB468 cells.

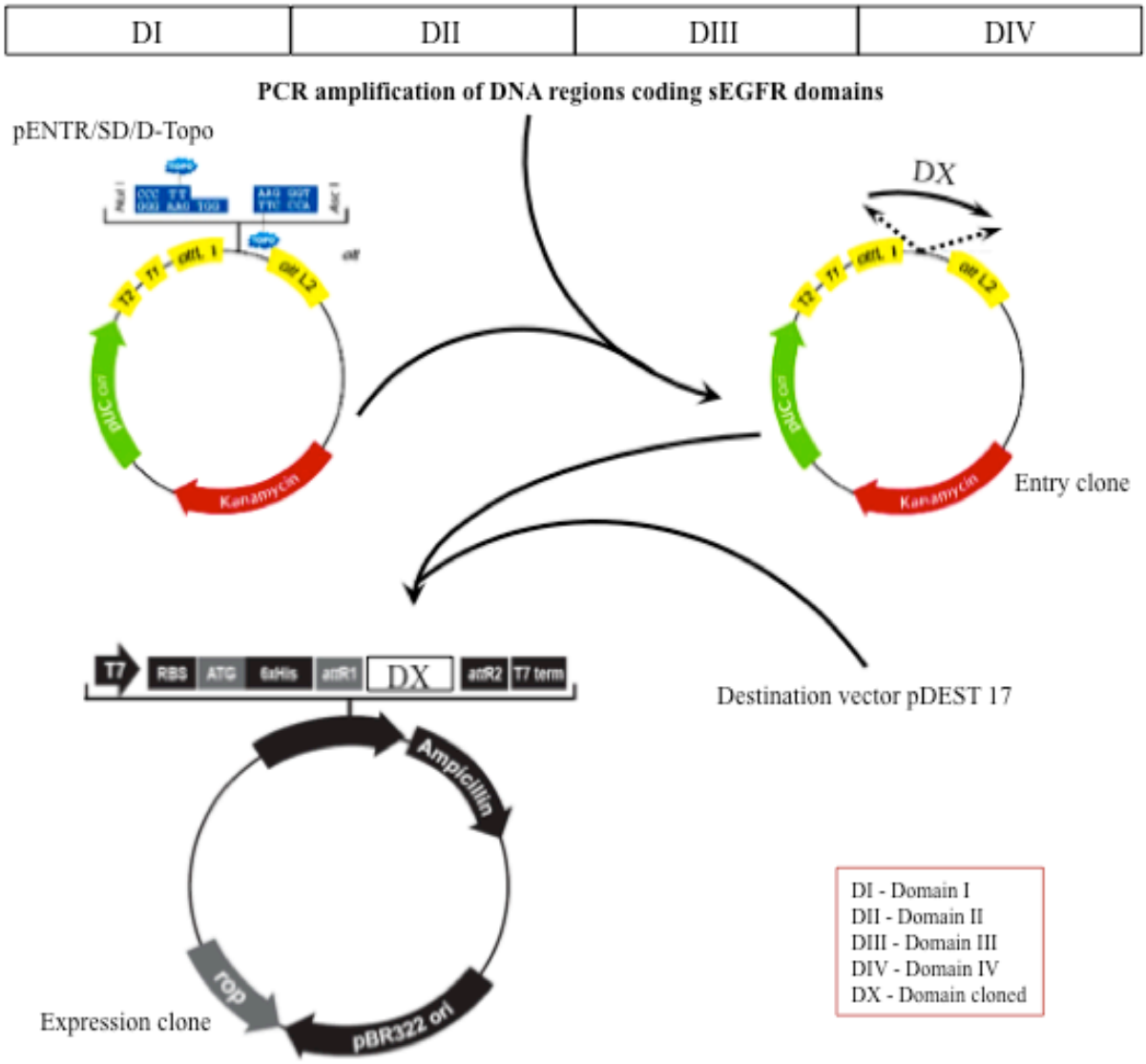
The cells were grown in DMEM and, after serum-starvation for 24 h, were incubated with different concentrations of NSC 228155 for 1 h. **a**, immundetection with anti-phospho-EGFR pY1068 and anti-EGFR antibodies. The fluorescence intensity data were collected and quantified with Odyssey software (Li-COR). **b**, EGFR phosphorylation plots were generated by fitting the fluorescence intensity, measured at each treatment time point relative to the untreated cells, using Origin software (OriginLab Corporation).

Supplementary Fig. 4. Dose-response of NSC 228155 and EGF-induced signaling in MDA MB468 cells.

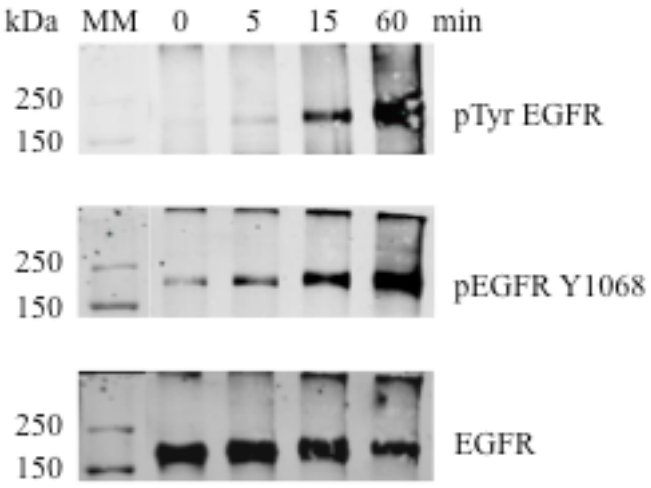
The cells were serum-starved for 4 hours, and then exposed to increasing doses of NSC 228155 (from 0.1 to 10 μM) for 10 or 30 minutes, or to increasing doses of EGF (from 5 to 500 nM) for 10 minutes. After stimulation, total cell lysates were subjected to SDS-PAGE. Phosphorylated ERK1/2 (Thr202/Tyr204) and EGFR (Tyr1173) were detected using selective antibodies followed by chemiluminescence. Anti-ERK2 antibody was used to monitor gel loading and to evaluate the signal ratio of protein bands.

a, Representative immunoblots of ligand-induced ERK1/2, and EGFR phosphorylation in MDA MB 468 cells. Phosphorylated ERK 1/2 (**b**) and EGFR (**c**) signals were quantified and normalized *versus* ERK2. In each case, the maximum response was arbitrarily taken to be 100%. The data are means +/- SEM from 3 independent experiments.

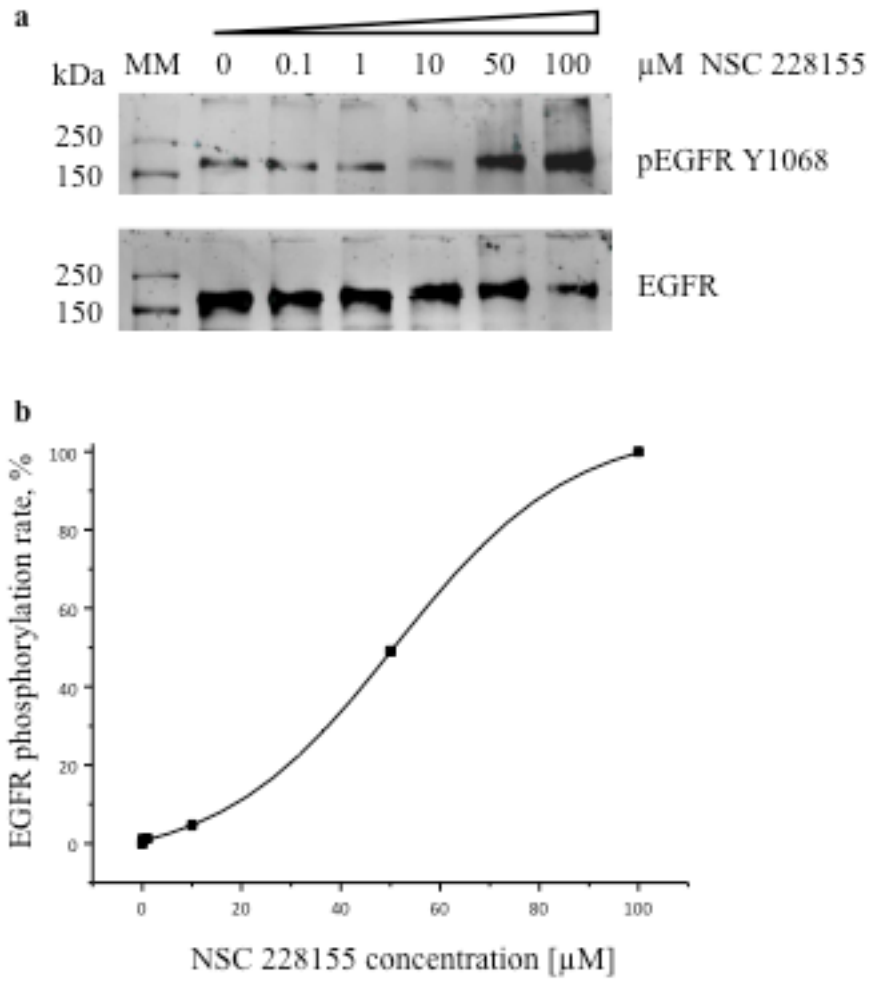
Supplementary Fig. 5. Tyrosine phosphorylation at EGFR in NSCLC-N6-L16 cells treated with NSC 228155.



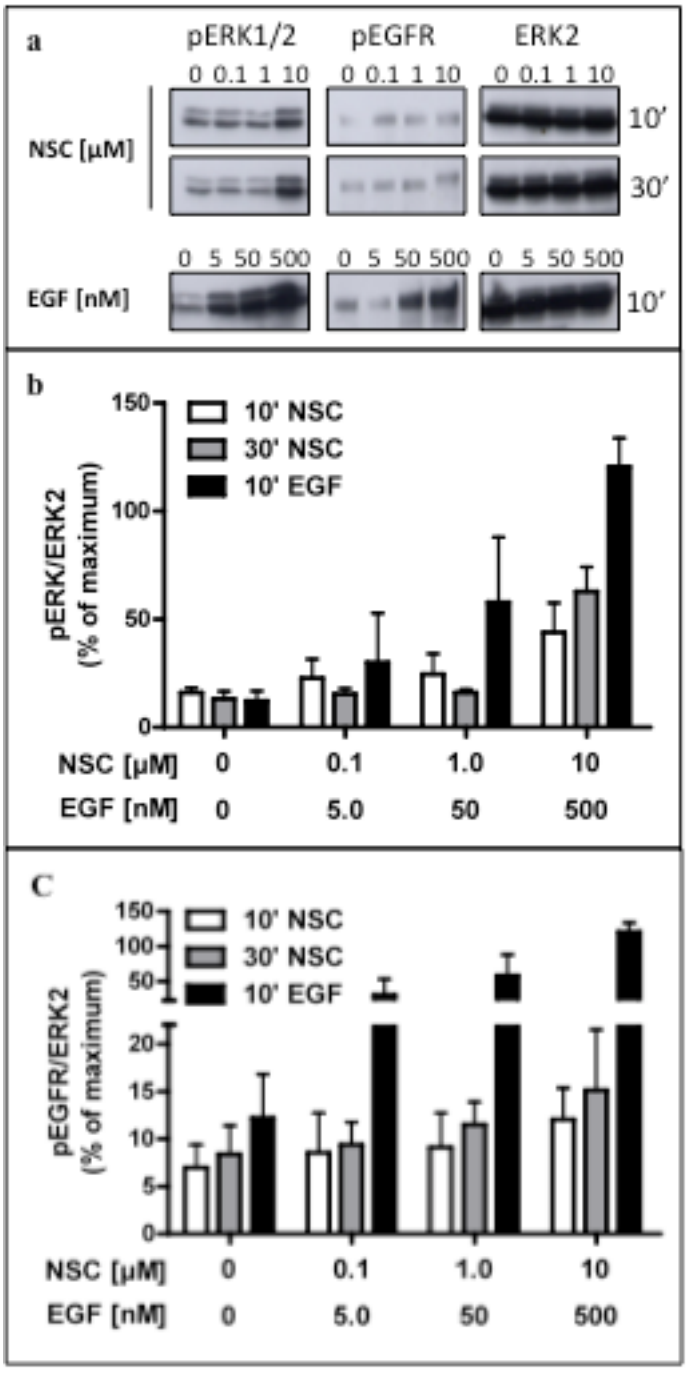
Supplementary Fig. 1.



Supplementary Fig. 2.



Supplementary Fig. 3.



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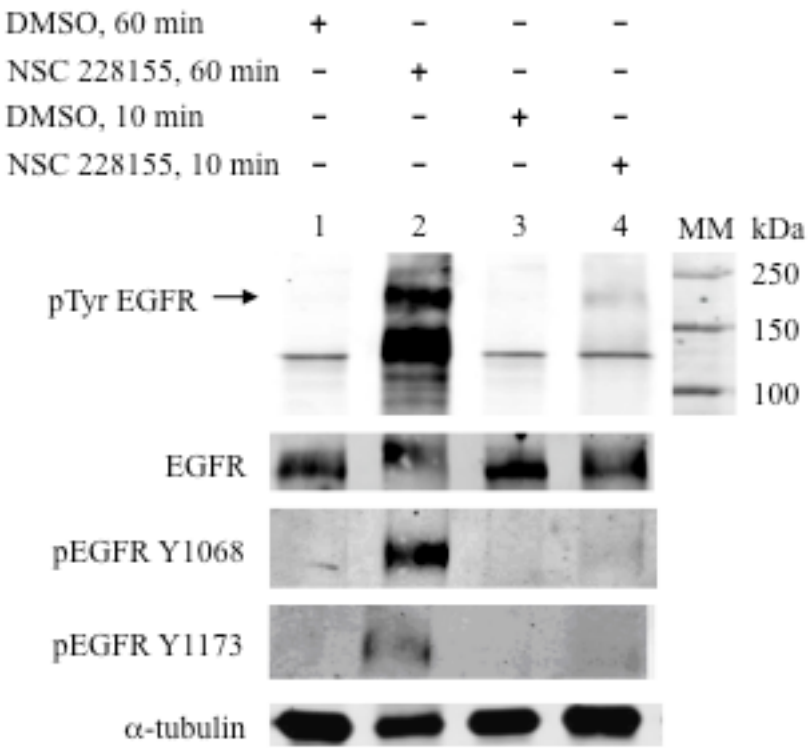

Supplementary Fig. 5.