

Design and evaluation of PEGylated liposomal formulation of a novel multi-kinase inhibitor for enhanced chemosensitivity and inhibition of metastatic pancreatic ductal adenocarcinoma

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Methods

Molecular docking

We performed a molecular docking analysis to investigate the binding conformation of N19 in cMET and EGFR. Kinase inhibitors can be roughly grouped into three types (I, II, and III).^{1,2} Type-I inhibitors target the active DFG-in conformation and compete with ATP. Type II inhibitors bind the inactive DFG-out conformation.² The inactive DFG-out conformation creates an additional pocket (called back pocket) adjacent the ATP-binding site.² Shapes of type II inhibitors are long and slender to simultaneously occupy the ATP-binding site and the back

pocket by a head group and a tail group, respectively. The tail group is hydrophobic and forms additional interactions with the back pocket.^{2, 3} N19 is more likely to be a Type-I inhibitor because its shape is not slender and lacks a hydrophobic tail group. However, cMET and EGFR structures that are currently available in the Protein Data Bank have multiple DFG-conformations. For cMET and EGFR, we modeled the protein structure in with a preference for the active DFG-in conformation using a homology-modeling approach.⁴ The structure (PDB code 2PVF) of FGFR2 with a sequence identity of 57% was selected as a structure template because it is in the active DFG-in conformation and contains an ATP analog, ACP. Additionally, docking was completed directly on well-resolved X-ray structures for cMET and EGFR using PDB codes 3CCN and 3W33 with 1.9 Å and 1.7 Å resolution, respectively. These structures were resolved in the presence of cofactors allowing excellent grid mapping. The residues within 10 Å around the ATP analog defined the binding site. Then, N19 was docked into the binding site using XP Glide docking on Schrodinger. XP Glide uses additional precision settings and provides interaction profiles between compounds and protein residues. XP Glide uses well-documented scoring function to determine intermolecular energies, including electrostatic, van der Waals, and hydrogen-bonding interactions. Our previous studies using XP Glide has had excellent results for drug binding and development with structure-function studies.⁵⁻¹⁰ In addition, XP Glide has been successfully applied to discover new inhibitors and binding sites for several proteins.¹¹⁻¹⁴

Supplementary Figures:

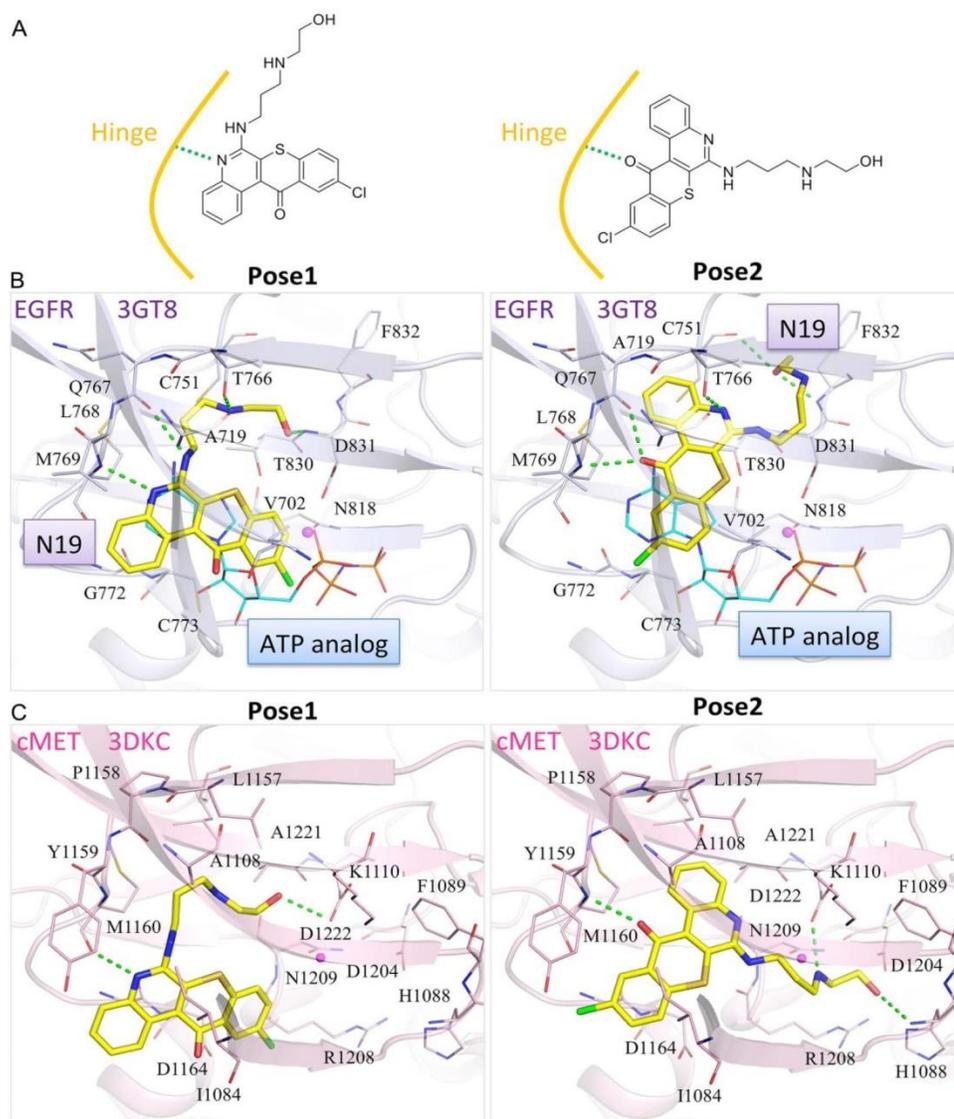


Figure S1. Binding confirmation of N19 in cMET and EGFR. A. Schematic representation of the protein binding pocket position relative to the N19 compound with pose1 and pose2 for orientation of the nitrogen versus ketone oxygen to the pocket hinge point. B. EGFR binding poses for N19 are given, where the ring and chain groups of N19 form 3 and 2 hydrogen bonds with EGFR residues, respectively. C. Similarly, the cMET binding poses for N19 are given, where the very similar hydrogen-bonding interactions are occurring. In both B and C panels,

hydrogen bonds between residues and N19 are represented as light green dashes. The ring group also makes stable van der Waals interactions with the binding site, which are depicted.

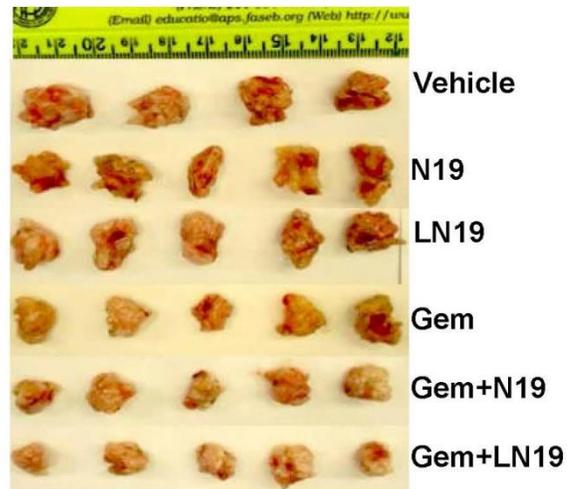


Figure S2: Representative examples of tumors treated with indicated groups.

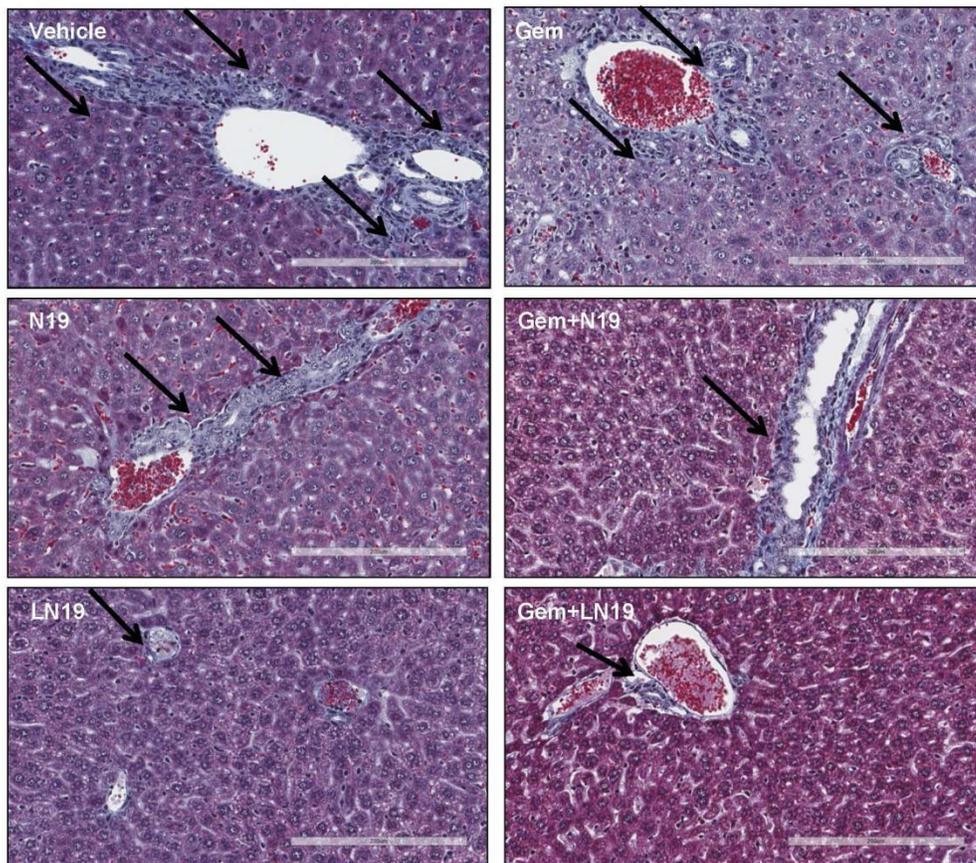


Figure S3: Immunohistochemistry of liver tissue for trichrome staining after 2x/wk for three weeks of treatment with indicated groups. Scale bars=200 μ m.

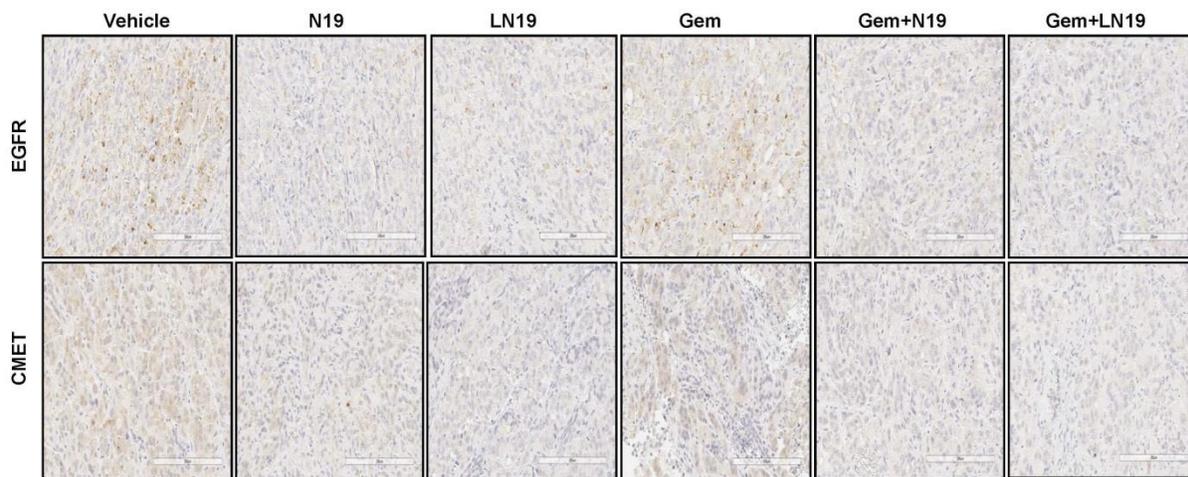


Figure S4: N19 inhibits both EGFR and cMET in PDAC tumors. IHC staining with anti-EGFR (top panel) and anti-cMET (bottom panel) of tumor tissues 2x/wk for three weeks treated with indicated groups. Scale bars=200 μ m.

Table S1: Primers used in the study

cMET-F	5'CATGCCGACAAGTGCAGTA3'
cMET-R	5'TCTTGCCATCATTGTCCAAC3'
EGFR -F	5'GCGTCTCTTGCCGGAATGT3'
EGFR-R	5'GGCTCACCTCCAGAAGGTT3'

Table S2. Docking results for N19 with cMET and EGFR in multiple poses.

Compounds	Protein	Docking Score (kcal/mol)	Pose Rank
N19 (pose1)	cMET	-5.87	3
N19 (pose2)	cMET	-5.3	4
Decoy Cmpd*	cMET	-4.0	5
N19 (pose1)	EGFR	-7.03**	1

N19 (pose2)	EGFR	-6.89	2
Decoy Cmpd*	EGFR	-3.51	6

*Decoy compounds were randomly selected for a counter screen on the efficacy of the N19 binding affinity for the EGFR and cMET receptors, which does indicate superiority over the randomly selected decoy compound by >30-1000X depending on the pairing.

**Best docked compound pose with the highest affinity.

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