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2-arylamino-6-ethynylpurines as Potent Irreversible Inhibitors of the Mitotic Kinase Nek2

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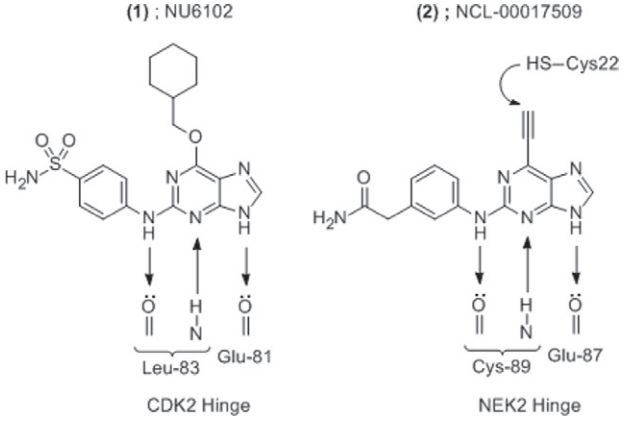
The serine/threonine kinase Nek2 is of considerable interest as a potential drug target for cancer, owing to its important role in the cellular mitotic machinery and abnormal expression in a number of human malignancies.[1] As a consequence, efforts directed towards the design and synthesis of Nek2 inhibitors have resulted in the identification of both ATP-competitive and irreversible inhibitors of this kinase.[2,3] Screening of a series of purine derivatives originally developed as CDK2 inhibitors, identified a number of compounds with modest Nek2-inhibitory activity (*e.g.* NU6102 [**1**]; CDK2, IC_{50}= 5.0 nM; Nek2, IC_{50}= 12 μM).[4] Further studies, guided by crystal structures of Nek2 in complex with a range of purine-based inhibitors, enabled the design of potent irreversible inhibitors as exemplified by the 6-ethynylpurine derivative NCL-00017509 (**2**). Importantly, the purine heterocycle of **2** maintains the key triplet of hydrogen bond interactions with the kinase hinge region, and positions the 6-ethynyl substituent proximal to Cys22, thereby facilitating a covalent Michael reaction with the thiol.



Enzyme kinetic studies demonstrated a time-dependent inhibition of Nek2 by **2** and related compounds, consistent with the irreversible nature of their interaction within the ATP-binding domain. A crystal structure of **2** in complex with Nek2 confirmed covalent modification of Cys22 thiol leading to an adduct in which a CH=CH group links the purine to Cys22-S. The activity of **2** as a potent kinase-selective irreversible Nek2 inhibitor (IC_{50} = 56 nM), combined with promising drug-like properties, prompted further investigations with this chemotype. The synthesis, structure-activity relationships, and structural biology of selected 2-arylamino-6-ethynylpurine Nek2 inhibitors will be discussed.

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Effect of Ketoconazole Coadministration On Lenvatinib (E7080) Exposure in Healthy Volunteers

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Background: Lenvatinib (L), an orally administered tyrosine kinase inhibitor targeting VEGFR1−3, FGFR1−4, PDGFRβ, RET, and KIT, is currently being studied in patients with solid tumors in doses of 24 mg daily. This study in volunteers assessed the influence of simultaneous CYP3A4 and Pgp inhibition using ketoconazole (K) on L pharmacokinetic (PK) parameters.
Material and Methods: This was a randomized, 2-period, crossover study in 15 males, 3 females. Subjects were randomized to 1 of 2 treatment sequences (placebo [P]/K or K/P). K (400 mg) or P was orally administered once daily for the first 4 days of each treatment period. On the fifth day, in addition to K or P, 5 mg L was administered orally. K or P administration then continued for 13 additional days. There was a 2-week washout between periods. Sixteen subjects were in the PK data set.
Results: PK and statistical results are summarized in the table. Results indicate that systemic exposure to L increases slightly (15%-19%) with coadministration of K; however, the 90% confidence interval (CI) for AUC is within the prespecified bioequivalence interval of 80%-125%. C_{max} slightly exceeded the upper CI bound (ie, 134%). No change in t_{max}, t_{lag}, or $t_{1/2}$ was observed.

Table: Pharmacokinetic and statistical results following oral administration of test and reference treatments

PK Parameters	Test L+K		Reference L+P		Geometric LS
	Mean (SD)	Geometric LS Mean	Mean (SD)	Geometric LS Mean	Mean Ratio % (90% CI)
C_{max} (ng/mL)	54.56 (20.30)	52.44	45.08 (11.70)	44.18	118.69 (105.34–133.74)
$AUC_{(0-t)}$ (ng·h/mL)	661.00 (146.92)	652.58	571.19 (99.26)	569.85	114.52 (108.21–21.20)
$AUC_{(0-inf)}$ (ng·h/mL)	675.69 (147.63)	667.37	584.31 (100.85)	582.80	114.51 (108.48–120.88)

LS, least squares.

Thirteen of 18 subjects experienced treatment-emergent adverse events (TEAEs) (11 mild, 2 moderate, 0 severe or serious). Only 4 subjects reported treatment-related TEAEs. Three were in the L+K cohort (1 each for nausea, increased ALT/AST, or dysmenorrhea) and the fourth was in the L+P cohort (dry mouth and dyspnoea).
Conclusions: K coadministration only slightly (15%-19%) increases systemic exposure to L. Since no change was observed in $t_{1/2}$, t_{max}, or t_{lag}, the slight increase in L systemic exposure is probably related to a decrease in first-pass metabolism.

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A Novel Combinatorial Treatment for Glioblastoma of Temozolomide and JLK1486

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Background: Glioblastoma multiforme (GBM) is a grade IV brain tumor characterized by a heterogeneous population of cells that are highly invasive and resistant to chemotherapeutic treatments. The current standard of care, comprised of surgical resection followed by radiation and chemotherapy, only provides GBM patients with a 12−14 month survival period. Our lab is currently investigating the potential therapeutic combination of temozolomide, the chemotherapeutic agent currently used to treat GBM patients, with a novel agent, JLK1486, to determine if this combination decreases or inhibits tumor recurrence *in vitro*.
Materials and Methods: GBM neurospheres and primary lines were treated with single or combinatorial treatments of temozolomide (TMZ) and/or JLK1486. Spheres were counted on day 7 to determine initial sphere formation, re-counted on day 14 to determine recovery, and were then pH dissociated and counted on day 21 to determine secondary sphere formation.
Results: Our lab found that neither TMZ nor JLK1486 alone eliminated secondary sphere formation. When cells were treated with a single dose of both TMZ+JLK1486 on day 0 secondary sphere formation was not inhibited. However, when cells where treated with TMZ+JLK1486 on day 0 and then dosed a second time with JLK1486 on day 7, secondary sphere formation was significantly reduced in double versus single agent treated cells.