Electronic Supplementary Material (ESI) for MedChemComm.

Supplementary Information

In depth analysis of kinase cross screening data to identify chemical starting points for inhibition of the Nek family of kinases

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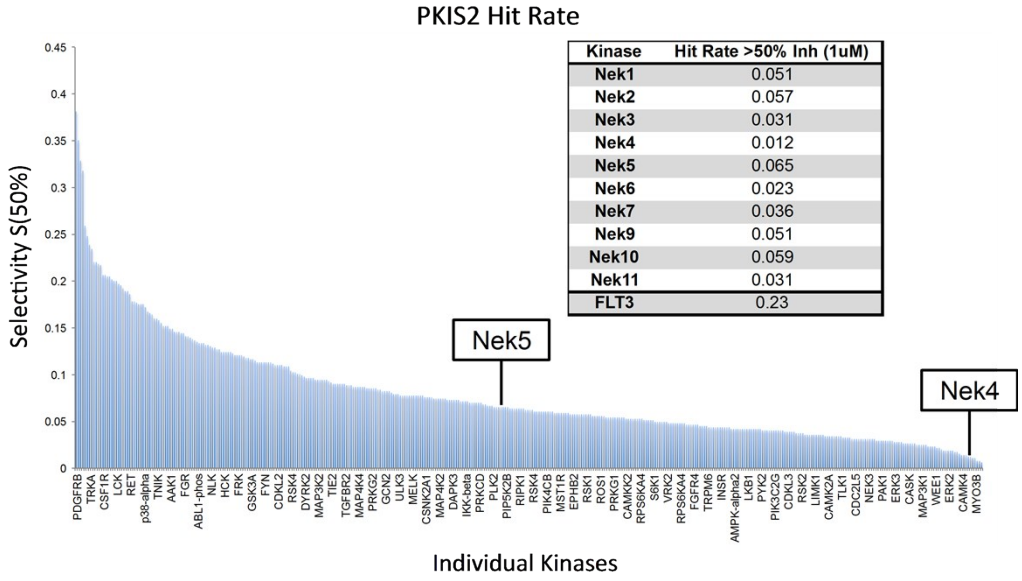
	Above outer lipophilic pocket	Turn in Gly-rich loop	Turn in Gly-rich loop	Conserved Val above purine	Conserved Ala above purine	Conserved Lys	Conserved Glu on C-helix	On C-helix	Back pocket on C-helix	Back pocket	Back pocket	Gatekeeper	Hinge inner H-bond acceptor	Hinge	Hinge donor & outer H-bond a	Outer hinge	Outer hinge	Solvent front	Solvent front & sugar pocket si	Surface helix	Surface helix	Gamma-phosphate region	Sugar pocket carbonyl	Mg binding	Outer hydrophobe below purir	Inner hydrophobe below purin	DFG Asp	DFG Phe
NEK1	I	S	F	A	V	K	E	V	L	V	I	M	D	Y	C	E	G	G	D	F	K	K	Q	N	F	G	D	F
NEK10	L	A	F	V	A	K	L	T	K	V	I	M	E	L	I	E	G	A	P	G	E	T	N	N	M	T	D	F
NEK11	L	S	F	V	V	K	E	A	L	V	I	T	E	Y	C	E	G	R	D	D	D	K	K	N	F	G	D	F
NEK2	I	S	Y	C	V	K	E	V	L	V	I	M	E	Y	C	E	G	G	D	A	S	K	A	N	F	G	D	F
NEK3	I	S	F	A	A	K	E	A	L	V	I	M	E	Y	C	D	G	G	D	M	Q	K	K	N	F	G	D	F
NEK4	V	S	Y	V	V	K	E	A	L	V	I	M	G	F	C	E	G	G	D	Y	R	K	Q	N	F	G	D	L
NEK5	I	A	F	A	V	K	E	V	L	V	I	M	E	Y	C	D	G	G	D	M	K	K	Q	N	F	G	D	F
NEK6	I	Q	F	V	A	K	E	I	L	I	I	L	E	L	A	D	A	G	D	S	Q	K	A	N	F	G	D	L
NEK7	I	Q	F	V	A	K	E	I	L	I	I	L	E	L	A	D	A	G	D	S	R	K	A	N	F	G	D	L
NEK8	V	A	F	V	I	K	E	C	L	I	I	M	E	Y	A	P	G	G	T	A	E	K	Q	N	L	G	D	F
NEK9	L	A	F	A	V	K	E	I	L	I	I	L	E	Y	C	N	G	G	N	Y	D	K	L	N	F	G	D	Y

Supplementary Table 1. Sequence alignment of 28 active site residues for Nek1-11. Residues named in the style of Bamborough et al., 2008. Sequence obtained from Kinase SARfari (https://www.ebi.ac.uk/chembl/sarfari/kinasesarfari/)



Supplementary Figure 2. Kinase selectivity.
A ranked bar chart of selectivity scores S(50%) at 1 μM for all tested kinases for PKIS2 compounds. All Nek family members are found in the right hand half of the X-axis which indicates that, at least for this compound set, the Nek family is in the bottom half of the kinome in terms of promiscuity. Nek5 has the highest hit rate in the family, and Nek4 has the lowest.

Currently, there are crystal structures available for three NEK family members - NEK1, NEK2 and NEK7. The PDB codes and references for deposited Nek structures are listed in this table (Supplemental Table 2).

Supplemental Table 2

Table . Crystal structures available with references

Kinase	PDB ID	Ligand	Reference	DFG	a-C	T-loop
NEK1	4B9D	CK7		out	out	n/a
NEK1	4APC	none		out	out	n/a
NEK2	5M57	Arylaminopurine 6	(2016) Oncotarget 8 19089-19124		out	
NEK2	5M51	Arylaminopurine 8	(2016) Oncotarget 8 19089-19124			
NEK2	5M53	Arylaminopurine 11	(2016) Oncotarget 8 19089-19124			
NEK2	5M55	Arylaminopurine 71	(2016) Oncotarget 8 19089-19124			
NEK2	4A4X	CCT248662	(2012) J Med Chem 55 3228			
NEK2	4AFE	Compound 21	(2012) J Med Chem 55 3228			
NEK2	2XNM	CCT	(2011) J Med Chem 54 1626-1639			
NEK2	2XNN	CCT242430	(2011) J Med Chem 54 1626-1639			
NEK2	2XNO	CCT243779	(2011) J Med Chem 54 1626-1639			
NEK2	2XNP	Aminopyrazine compound 5	(2010) J Med Chem 53 7682-7698			
NEK2	2XKE	Aminopyrazine compound 35	(2010) J Med Chem 53 7682-7698			
NEK2	2XK4	Aminopyrazine compound 17	(2010) J Med Chem 53 7682-7698			
NEK2	2XK6	Aminopyrazine compound 36	(2010) J Med Chem 53 7682-7698			
NEK2	2XK7	Aminopyrazine compound 23	(2010) J Med Chem 53 7682-7698			
NEK2	2XK8	Aminopyrazine compound 15	(2010) J Med Chem 53 7682-7698			
NEK2	2XKC	Aminopyrazine compound 14	(2010) J Med Chem 53 7682-7698			
NEK2	2XKD	Aminopyrazine compound 12	(2010) J Med Chem 53 7682-7698			
NEK2	2XKF	Aminopyrazine compound 2	(2010) J Med Chem 53 7682-7698			
NEK2	2WQO	Aminopyridine CCT241950	(2009) Mol Cell 36 560-570			
NEK2	2W5A	ADP	(2009) J Mol Biol 386 476-485			
NEK2	2W5B	ATP-gammaS	(2009) J Mol Biol 386 476-485			
NEK2	2W5H	None	(2009) J Mol Biol 386 476-485			
NEK2	2JAV	Pyrrole-indolinone	(2007) J Biol Chem 282 6833			
NEK7	2WQN	ADP	(2009) Mol.Cell 36: 560-570			
NEK7	5DE2	none	(2015) Nat Commun 6: 8771-8771			
NEK7	2WQM	none	(2009) Mol.Cell 36: 560-570			

Brief Methods for Nek1 docking

The originally deposited NEK1 structures (PDB ID 4APC and 4B9D) lacked six residues from the Gly-rich loop - including those at the turn. Before docking, these missing residues were modeled into the NEK1 structure using the coordinates from NEK2 (PDB ID 4AFE) as a template in Modeller. AutoDock Vina was used to dock the compounds onto NEK1 following the procedure published by Forli and colleagues (doi 10.1038/nprot.2016.051). To accommodate the extended conformation of the NEK1 activation segment, a large (35x26x32 **Å**) search box centered on the 'canonical' ATP-binding pocket was used. Selected side-chains were allowed to rotate freely during docking. An exhaustive search (96 iterations) was performed, and the best (lowest energy) nine poses were visually inspected.

Supplemental Table 3

Table S3 - Citation count, function, disease association and commercial assay availability of Nek family kinases

Kinase	# of Citations[a]	Prominent functions	Possible therapeutic significance	# of commercial assays[b]
Nek1	69	Formation and regulation of cilia	Cilliopathies - Polycystic Kidney Disease, nephronitis	8
Nek2	222	Centrosome separation,spindle assembly checkpoint, chromosome segregation	Overexpressed in several tumors - breast, colon, lung and gastric	9
Nek3	17	Regulates prolactin mediated motility in breast cancer cells	Overexpressed in breast cancer	5
Nek4	17	Regulates microtuble homeostasis	Overexpressed in lung cancer	6
Nek5	10	Regulation of centrosome integrity		3
Nek6	75	Required for the progression of mitosis	Overexpressed in several tumors - breast, colon, lung and gastric	9
Nek7	50	Required for spindle assembly and cytokinesis, mediates NLPR3 mediated inflammation	Novel target for inflammatory symptoms of gout, atherosclerosis and Type II diabetes	8
Nek8	45	Formation and regulation of cilia	Cilliopathies - Polycystic Kidney Disease, nephronitis	2
Nek9	39	Required for microtubule formation and spindle formation	Overexpressed in gliomas and kidney carcinoma cells	8
Nek10	10	Regulates cell cycle checkpoint in response to DNA damage	Anticancer target by inhibition of cell cycle checkpoint	1
Nek11	11	Regulates cell cycle checkpoint in response to DNA damage	Anticancer target by inhibition of cell cycle checkpoint	5
ERBB2[c]	5646	Involved in the regulation of a variety of vital functions, such as cell growth, differentiation, and apoptosis	Overexpressed in breast carcinomas and other types of malignancies	7

[a] - Based on keyword search in title/abstract in PubMed
[b] - Commercial assay availability based on 10 kinase vendors (Drewry et al. 2017)
[c] -Yu, D.; Hung, M. C., Overexpression of ErbB2 in cancer and ErbB2-targeting strategies. Oncogene 2000, 19 (53), 6115-21.