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Identification of new inhibitors of protein kinase R guided by statistical modeling

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ABSTRACT

We report the identification of new, structurally diverse inhibitors of interferon-induced, double-stranded RNA-activated protein kinase (PKR) using a combined experimental and computational approach. A training set with which to build a predictive statistical model was generated by screening a set of 80 known Ser/Thr kinase inhibitors against recombinant human PKR, resulting in the identification of 28 compounds from 18 chemical classes with <0.1 μM ⩽ IC_{50} ⩽ 20 μM. The model built with this data was used to screen a database of 5 million commercially available compounds in silico to identify candidate inhibitors. Testing of 128 structurally diverse candidates resulted in the confirmation of 20 new inhibitors from 11 chemical classes with 2 μM ⩽ IC_{50} ⩽ 20 μM. Testing of 34 analogs in the newly identified pyrimidin-2-amine active series provided initial SAR. One newly identified inhibitor, *N*-[2-(1*H*-indol-3-yl)ethyl]-4-(2-methyl-1*H*-indol-3-yl)pyrimidin-2-amine (compound **51**), inhibited intracellular PKR activation in a dose-dependent manner in primary mouse macrophages without evident toxicity at effective concentrations.

Interferon-induced, double-stranded RNA (dsRNA)-activated protein kinase (PKR) is a widely expressed serine/threonine (Ser/Thr) kinase that mediates diverse signaling pathways. PKR was discovered as a component of type I IFN signaling that is upregulated in response to IFN and phosphorylated upon exposure of cells to dsRNA[1] and viral infection.[2] PKR contains a C-terminal kinase domain and an N-terminal domain containing tandem dsRNA binding domains. In its latent form PKR exists predominantly as a monomer that dimerizes with low affinity.[3] Dimerization is sufficient to activate PKR in the absence of dsRNA. Recent data suggest that dsRNA brings PKR monomers in close proximity to enhance dimerization.[4] The dimer autophosphorylates on multiple Ser/Thr residues and becomes active in phosphorylating other protein substrates. The first identified substrate of PKR is eIF-2α. Phosphorylation of eIF-2α by PKR on Ser51 inhibits protein synthesis by preventing eIF-2α recycling via GDP/GTP exchange, mediating some of IFN's antiviral action.

Recently, additional PKR substrates have been identified, such as insulin receptor substrate.[5] It has emerged that PKR is activated by cytokines, growth factor withdrawal and stress signals and participates in pathways regulating stress response, cellular growth and proliferation, nutrient signaling and metabolism. Augmented PKR activity is associated with multiple pathological conditions and disease states, including neurodegeneration, obesity, diabetes, cancer, and infectious diseases caused by prions, influenza virus and other agents. For example, increased levels of phosphorylated PKR were detected in brains of patients with Parkinson's and Huntington's disease as compared with age-matched controls.[6] Phosphorylated PKR accumulated in senile plaques and the nuclei of degenerating hippocampal neurons in brains of patients with Alzheimer's disease.[7] An immunoblot-based survey of protein kinases in thoracic spinal cord from patients with amyotrophic lateral sclerosis identified phospho-PKR as the most highly elevated kinase compared to controls.[8] Activated PKR was also detected by immunostaining in neurons in the brains of patients with Creutzfeldt-Jakob disease[9] in proportion to the extent of neuronal apoptosis and the severity of disease. PKR activation

Abbreviations: dsRNA, double-stranded RNA; PKR, dsRNA-activated protein kinase; IFN, interferon; eIF-2α, α subunit of eukaryotic translation initiation factor 2.

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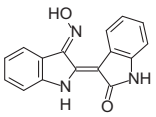
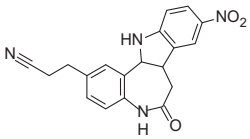
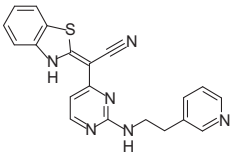
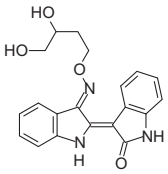
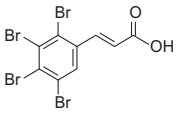
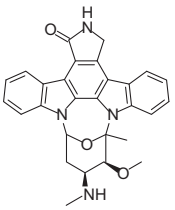
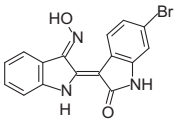
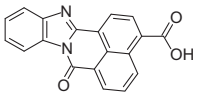
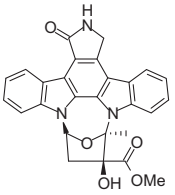
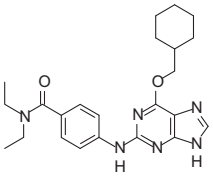
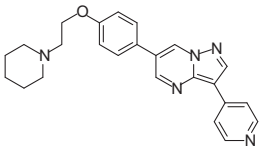
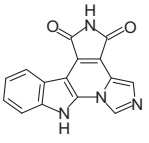
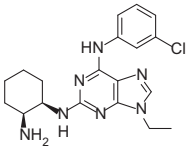
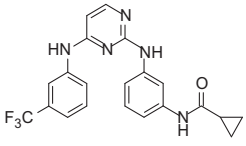
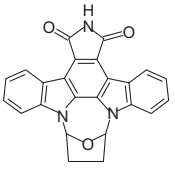
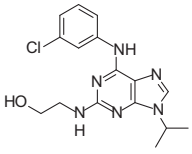
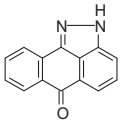
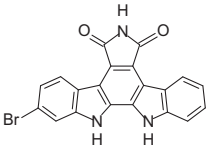
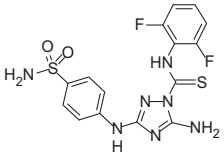
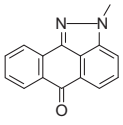
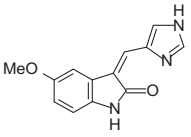
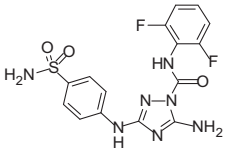
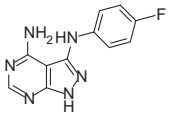
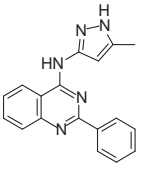
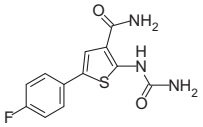
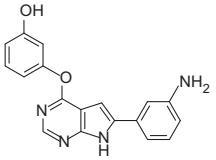
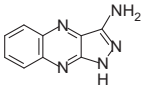
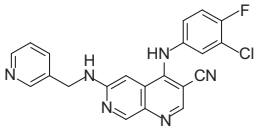
Table 1
New PKR inhibitors identified from a set of known Ser/Thr kinase inhibitors

No.	Structure	IC_{50} (μM)	No.	Structure	IC_{50} (μM)	No.	Structure	IC_{50} (μM)
1		<0.1	**11**		3.5	**21**		<0.1
2		<0.1	**12**		7	**22**		<0.1
3		0.6	**13**		<0.1	**23**		<0.1
4		2	**14**		16	**24**		<0.1
5		3	**15**		20	**25**		0.75
6		16	**16**		8	**26**		6
7		1.6	**17**		12	**27**		5
8		8	**18**		5	**28**		15
9		0.25	**19**		4			
10		6	**20**		0.25			