Novel cyano pyridopyrimidines as potent and selective inhibitors of CDK4 kinase

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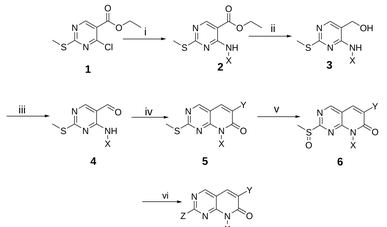
Introduction

Protein kinases (PKs) are an important class of intracellular enzymes involved in the regulation of a large variety of cellular processes. It is now well established that most solid tumors activate multiple signaling pathways and require that several of these pathways be inhibited for effective reduction of tumor burden. One of our goals is to modulate the paradigm of rational kinase inhibitor design to incorporate growth inhibition as an integral measurement to initially assess their ability to act as drug candidates. Instead of starting with target identification, we synthesized a large number (approximately 2000) of novel ATP mimetic kinase inhibitors and tested them for tumor growth inhibition. The use of this strategy has yielded a series of lead compounds, which allowed the identification of novel therapeutic agents that appear to target specific cellular kinases that seem to play a critical role in tumor cell growth. In this presentation, we describe the profile of ON123300, a small molecule kinase inhibitor, that induces cell cycle arrest of a wide range of tumor cells followed by their apoptotic death. This compound was found to be a potent inhibitor of CDK4, *In vivo*, this drug did not cause hematotoxicity, liver damage or any detectable neurotoxicity. In addition, this compound was found to be a potent inhibitor of tumor growth *in vivo*, and showed a high degree of synergism with several of the chemotherapeutic agents currently used in cancer therapy.

Chemistry

Synthetic route for the synthesis of pyrido[2,3-*d*]pyrimidines is shown in scheme. The reaction of alkyl amines with commercially available compound **1** in the presence of triethylamine to obtain a compound **2**. The ester group in compound **2** was reduced with $LiAlH_4$ and the resulting compound 3 was oxidized with MnO_2 to afford corresponding aldehyde **4**. The knoevenagel reaction of aldehyde **4** with active methylene compounds in the presence of benzylamine in acetic acid to give compound **5**. The compound **5** was treated with mCPBA to get corresponding sulfoxide **6**. The methylsulfoxide was replaced by treating with aryl or heteroaryl amines to get title compound pyrido[2,3-*d*]pyrimdidne (**ON 123160 – ON 1231820**)

Scheme: Synthesis of Pyrido[2,3-*d*]pyrimidines



ON 123160 to ON 1231820

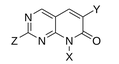
X = Methyl, Propyl, Cyclopentyl, Cyclohexyl

Y = CN, $PhSO_2$, CH_3SO_2

Z = Substituted aryl or heteroaryl amine

Reagents and Conditions. (i) X-NH_2, Et_3N, THF, RT, 3h. (ii) $LiAlH_4$, THF, -10 ^{0}C - RT, 3h. (iii) MnO_2, $CHCl_3$ RT, 36h (iv) Y-CH_2CO_2H, $BnNH_2$, ACOH, 100 ^{0}C, 5h (v) mCPBA, CH_2Cl_2, RT, 10h. (vi) Z, DMSO or Toluene, 100 ^{0}C, 3-10 h

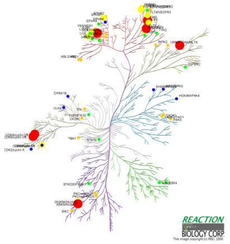
Table 1. *Invitro* Cytotoxicity



ON 123160 to ON 1231820

Compd. No.	X	Y	Z	IC_{50} (μ M)	
				K562	DU145
123460	C_3H_9	CN	NH-4-chlorophenyl	30	30
123610	C_3H_9	CN	NH-2-methoxyphenyl	15	30
123620	C_3H_9	CN	NH-benzyl	>100	75
123760	C_3H_9	CN	NH-3,4-dimethoxyphenyl	2	5
123770	C_3H_9	CN	NH-3,5-dimethoxyphenyl	15	15
123780	C_3H_9	CN	NH-2,4-dimethoxyphenyl	15	15
123790	C_3H_9	CN	NH-3,4,5-trimethoxyphenyl	2.5	5
123650	C_3H_9	CN	NH-4-indolyl	30	15
123660	C_3H_9	CN	NH-5-indolyl	5	5
123950	C_3H_9	CN	NH-2-pyridyl	0.5	3
123960	C_3H_9	CN	NH-2-methoxy-6-quinolino	0.25	3
123980	C_3H_9	CN	NH-4-cyano-2-pyridyl	5	30
1231480	C_3H_9	CN	NH-4-pyridinophenyl	10	10
1231250	C_3H_9	CN	NH-(4-morpholino)pyridyl	5	15
1231370	C_3H_9	CN	NH-(N-CH_3piperazino)pyridyl	5	15
123300	C_3H_9	CN	NH-(N-CH_3piperazino)phenyl	0.05	0.025
1231730	C_3H_9	CN	acetyl-N-(N-CH_3piperazino)phenyl	0.75	5
123350	C_3H_9	CN	NH-(N-morpholino)phenyl	5	15
123450	C_3H_9	CN	NH-piperazino(4-CF_3-2-pyridine)	75	>100
1231820	C_3H_9	CN	O-(N-CH_3piperazino)phenyl	5	15
1231000	C_6H_{11}	CN	NH-(N-CH_3piperazino)phenyl	40	1
123430	CH_3	CN	NH-(N-CH_3piperazino)phenyl	75	75
1231170	C_3H_7	CN	NH-(N-CH_3piperazino)phenyl	5	15
123220	C_3H_9	$PhSO_2$	NH-(N-CH_3piperazino)phenyl	5	5
123160	C_3H_9	CH_3SO_2	NH-(N-CH_3piperazino)phenyl	5	5

Kinome Activity Map



The kinases targeted by ON123300 are displayed on the Kinome activity Map. Red circles represent kinases targeted below 20nM. Yellow, Amber, Bright Green, Blue circles represent kinases targeted between 20-50nM, 50-100nM, 100-150nM and 150-250nm. The Human Kinome Map is adapted with permission from Reaction Biology Corp (http://www.reactionbiology.com/)

Schematic diagram of ON 123300 mechanism of action

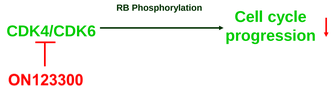
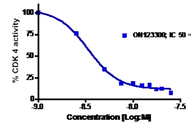


Table 2. ON 123300 GI50 against various tumor cell lines

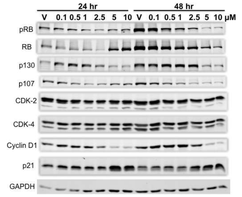
CELL LINE	Tumor Type	GI50 (μM)
K562	CML	0.5
DU145	PROSTATE	0.75
BT474	ErbB2 + Breast	0.25
SK-BR-3	ErbB2 + Breast	0.6
MCF-7	ER+ Breast	0.15
BT20	Breast	0.1
MDA-MB-468	BREAST (triple neg;RB neg)	1.5
Z138C	MCL	0.025
GRANTA-519	MCL	0.035
SK-OV-3	Ovarian	0.75
U87	Glioblastoma	0.1
MIA-PaCa-2	Pancreatic	0.25
HCT-15	Colon (MDR elevated)	0.4
COLO-205	Colon	0.2
HELA	Cervical	0.75
A549	NSCLC	0.2
N417	SCLC	0.25
N87	Gastric (ErbB2+)	0.9
SNU-5	Gastric	0.2
SNU-398	Gastric	0.5
SNU-449	Gastric	0.75
SNU-475	Gastric	0.3
U266	Multiple Myeloma	0.2
RAJI	B-Cell Lymphoma	0.25
JURKAT	T-Cell Lymphoma	0.15
DLD-1	COLORECTAL	0.1
SW480	COLORECTAL	0.1

ON123300 inhibits CDK4 / Cyc D1



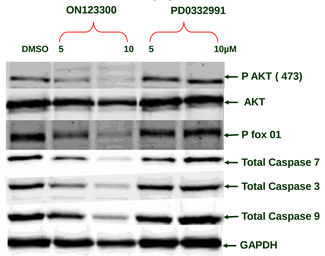
CDK4 kinase was incubated with the indicated concentrations of ON123300 for 30 minutes at room temperature. Kinase reactions were started by the addition of substrate mix (5μM ATP, 10 μCi γ32P-ATP, 1 μg substrate, 10 mM MgCl2) for 15 minutes at 30° C. Substrate phosphorylation is quantified and data is plotted (after background subtraction) using GraphPad Prism4 software as a non-linear regression plot with variable slope to obtain IC_{50} values.

ON123300 inhibits CDK4 and its effector molecules



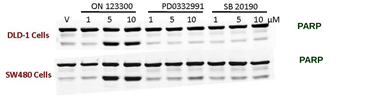
Sw480 cells are treated with specified concentration of ON123300 and lysates are prepared. 30-50 μg of sample was resolved on SDS page and processed for western blot. This blots are probed with corresponding antibodies. Treatment of ON12300 results in inhibition of CDK4/6 and its regulated pathway.

ON 123300 inhibits mTORC2, which inhibits the phosphorylation of down stream proteins like AKT and leads to apoptosis



Sw480 cells are treated with specified concentration of ON123300 and lysates are prepared. 30-50 μg of sample was resolved on SDS page and processed for western blot. This blots are probed with corresponding antibodies. Treatment of ON123300 inhibits mTORC2 and activation of caspases but not by PD0332991 a CDK4/6 inhibitor.

ON123300 induces apoptosis in SW480 and DLD1 cells



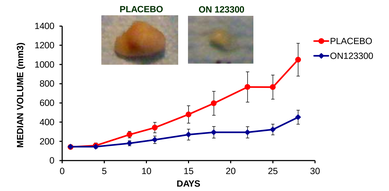
Sw480 and DLD1 cells are treated with specified concentration of ON123300 and lysates are prepared. 30μg of sample was resolved on SDS page and probed for apoptosis marker Cleaved PARP

Small Scale Toxicity Study

Group	Formulation	Route	Schedule	Survival
50 mg/kg	HCl-$H_2$0	IP	Single	5/5
100 mg/kg	HCl-$H_2$0	IP	Single	5/5
100 mg/kg	HCl-$H_2$0	IP	QDX5	3/3

Female CD-1 mice are injected with 50 or 100mg/kg of ON123300 in single and dose 5 every day and the mice were observed daily for survival and signs of toxicosis. No noticeable toxicity is observed in mice after treatment.

Invivo antitumor activity of ON123300



Human colon cancer Colo 205 fragments were implanted into female athymic nude mice and allowed to grow to an average of 150mm3. ON123300 was given daily IP at the 100 mg/kg of ON123300-HCL formulated in sterile water (N=10) or Placebo (N=10) by IP injections. Tumor volumes were determined and the median (+/- SEM) were plotted against day of treatment.

Summary and Conclusions

- A Series of Pyrido[2.3-*d*]pyrimdines were synthesized
- ON123300 inhibits CDK4 / CYC D1 kinase and its regulated pathway.
- ON 123300 Inhibit growth of various cancer cell lines at less than 1 μM.
- ON 123300 suppress growth of tumor in COLO 205 xenograft model.

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