Discovery of 6-arylsulfonyl pyridopyrimidinones as potent and selective PLK2 inhibitors

Muralidhar R. Mallireddigari [2], Vinaykumar Billa[1], Shashidhar S. Jatiani[1], Stephen C. Cosenza [1], E Premkumar Reddy [1] and M V Ramana Reddy [1]

1. Mount Sinai School of Medicine, Department of Oncological Sciences, New York, NY, 10029.

2. Onconova Therapeutics Inc, Department of Medicinal Chemistry, Newtown, PA , 18940.

Introduction

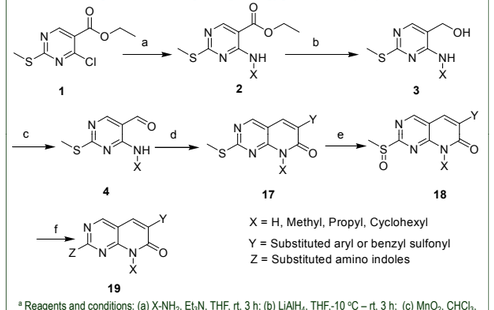
Several families of protein kinases have been shown to play a critical role in the regulation of cell cycle progression, especially progression through mitosis. These kinase families include the Aurora kinases, the Mps1 gene product and the Polo family of protein kinases. Polo like kinases play key roles in mitosis. While the up-regulation of PLK1 in cancers is well documented and PLK3 has been demonstrated to be a tumor suppressor, little is known about the oncogenic significance of PLK2. PLK2 kinase activity is essential for centriolar duplication and is also believed to play a regulatory role in the survival pathway by physically stabilizing the TSC1/2 complex in tumor cells under hypoxic conditions.

As a part of our research program, we have developed a library of novel ATP mimetic chemotypes that are cytotoxic against a panel of cancer cell lines. One of these chemotypes, 6-arylsulfonyl pyridopyrimidinones has shown cytotoxicity in nanomolar concentration in most of the cancer cells. The most potent of these compounds, ON 1231320 was found to be specific PLK2 inhibitor when profiled against a panel of 288 wild type, 55 mutant and 12 specific kinases. ON 1231320 exhibits an excellent safety profile with no overt signs of toxicity, no loss of body weight and 100% survival in mice given a single peritoneal dose of 200 mg/kg. Our ongoing efforts include efficacy studies in nude mouse models, identification of the structural determinants of the interaction between ON 1231320 and PLK2 by computational and crystallographic methods and the identification of novel PLK2 substrates to elucidate its role in cancer biology.

In this presentation, we describe the synthesis, structure activity relationship (SAR), in vitro kinase specificity and biotinylation of lead compound ON 1231360.

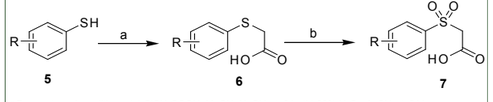
Chemistry

Scheme 1: Synthesis of Pyrido[2,3-d]pyrimidin-7(8H)-ones[a]



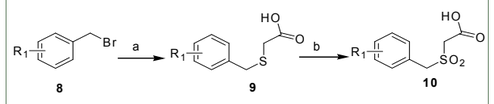
[a] Reagents and conditions: (a) X-NH_2, Et_3N, THF, rt, 3 h; (b) $LiAlH_4$, THF,-10 °C – rt, 3 h; (c) MnO_2, $CHCl_3$, rt, 36 h; (d) Substituted aryl or benzyl sulfonyl acetic acids, $BnNH_2$, AcOH, 100 °C, 5 h; (e) mCPBA, DMF/CH_2Cl_2, rt, 10 h; (f) DMSO or Toluene, 100 °C, 3-10 h.

Scheme 2: Synthesis of substituted phenylsulfonylacetic acids[a]



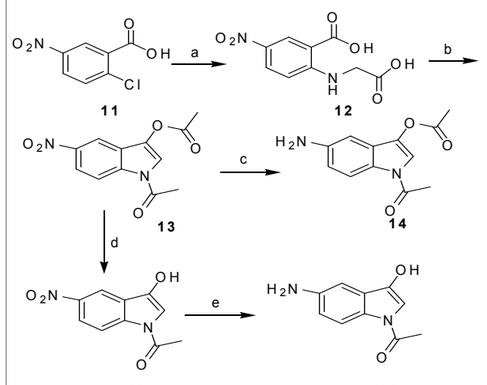
[a] Reagents and conditions: (a) $ClCH_2COOH$, MeOH, NaOH, rt, 3 h; (b) 30% H_2O_2, AcOH, rt, 24 h.

Scheme 3: Synthesis of substituted benzylsulfonylacetic acids[a]



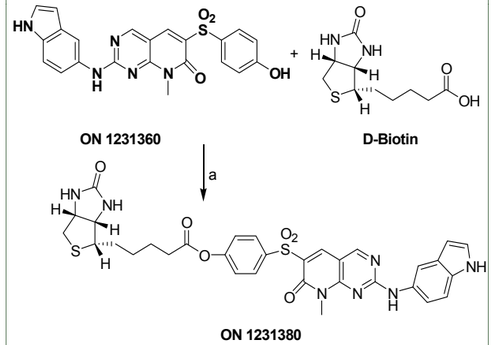
[a] Reagents and conditions: (a) $HSCH_2COOH$, MeOH, NaOH, rt, 3 h; (b) 30% H_2O_2, AcOH, rt, 24 h.

Scheme 4: Synthesis of substituted 5-amino indoles[a]



[a] Reagents and conditions: (a) Glycine, Na_2CO_3, copper powder, H_2O,reflux, 2 h; (b) AcONa, $(CH_3CO)_2O$, 110 °C, 2 h; (c) $Na_2S_2O_4$, acetone:water (2:1), 50 °C, 30 min; (d) EtOH, H_2O, Na_2SO_3, reflux, 20 min; (e) $Na_2S_2O_4$, acetone:water (2:1), 50 °C, 30 min.

Scheme 5: Synthesis of D-Biotin Ester of ON 1231360[a]



[a] Reagents and conditions: (a) EDCI, DMAP/DME, rt, 12 h.

In vivo effects of 19 on cells

Compd.No	X	Y	Z	IC_{50} (μM)	
				DU145	K562
19a	CH_3	$C_6H_5SO_2$	NH-5-Indolyl	5.0	0.7
19b	CH_3	$C_6H_5SO_2$	NH-6-Indolyl	20.0	20.0
19c	CH_3	4-$ClC_6H_4SO_2$	NH-4-Indolyl	5.0	5.0
19d	CH_3	4-$ClC_6H_4SO_2$	NH-5-Indolyl	0.6	0.7
19e	CH_3	4-$FC_6H_4SO_2$	NH-4-Indolyl	0.75	0.75
19f	CH_3	4-$FC_6H_4SO_2$	NH-5-Indolyl	0.2	0.075
19g	CH_3	4-$FC_6H_4SO_2$	NH-6-Indolyl	20.0	20.0
19h	CH_3	4-$BrC_6H_4SO_2$	NH-5-Indolyl	5.0	5.0
19i	CH_3	4-$CH_3C_6H_4SO_2$	NH-4-Indolyl	7.5	2.5
19j	CH_3	4-$CH_3C_6H_4SO_2$	NH-5-Indolyl	0.75	0.7
19k	CH_3	4-$CH_3C_6H_4SO_2$	NH-6-Indolyl	18.0	18.0
19l	CH_3	4-$OCH_3C_6H_4SO_2$	NH-4-Indolyl	5.0	5.0
19m	CH_3	4-$OCH_3C_6H_4SO_2$	NH-5-Indolyl	0.5	0.3
19n	CH_3	4-$COOCH_3C_6H_4SO_2$	NH-5-Indolyl	20.0	20.0
19o	CH_3	4-$FC_6H_3CH_2SO_2$	NH-4-Indolyl	17.0	17.0
19p	CH_3	4-$FC_6H_3CH_2SO_2$	NH-5-Indolyl	35.0	35.0
19q	CH_3	3-Cl,4-$FC_6H_3SO_2$	NH-4-Indolyl	75.0	75.0
19r	CH_3	3-Cl,4-$FC_6H_3SO_2$	NH-5-Indolyl	1.5	0.6
19s	H	3-Cl,4-$FC_6H_3SO_2$	NH-5-Indolyl	75.0	17.5
19t	C_3H_7	$C_6H_5SO_2$	NH-4-Indolyl	18.0	5.0
19u	C_3H_7	$C_6H_5SO_2$	NH-5-Indolyl	5.0	0.75
19v	C_3H_7	$C_6H_5SO_2$	NH-6-Indolyl	5.0	5.0
19w	C_5H_{11}	4-$ClC_6H_4SO_2$	NH-4-Indolyl	150	15.0
19x	C_5H_{11}	4-$ClC_6H_4SO_2$	NH-5-Indolyl	38.0	15.0
19y	CH_3	**2,4-$F_2C_6H_3SO_2$**	**NH-5-Indolyl**	**0.075**	**0.075**
19z	CH_3	3,4-$F_2C_6H_3SO_2$	NH-5-Indolyl	0.75	0.175
19aa	CH_3	3,4,5-$F_3C_6H_2SO_2$	NH-5-Indolyl	0.75	0.80
19ab	CH_3	2,4-$Cl_2C_6H_3SO_2$	NH-5-Indolyl	0.75	0.75
19ac	CH_3	4-$OHC_6H_4SO_2$	NH-4-Indolyl	5.0	1.5
19ad	CH_3	4-$OHC_6H_4SO_2$	NH-5-Indolyl	2.5	0.75
19ae	CH_3	4-$COOHC_6H_4SO_2$	NH-5-Indolyl	15.0	5.0
19af	CH_3	2,4-$F_2C_6H_3SO_2$	1,3-Diacetyl-NH-indolyl	5.0	75.0
19ag	CH_3	2,4-$F_2C_6H_3SO_2$	1-Acetyl-3-OH-5-NH-indolyl	5.0	5.0
19ah	CH_3	4-O-Biotin-$C_6H_4SO_2$	NH-5-Indolyl	0.15	0.6

Potent antitumor cytotoxicity of ON1231320 (19y)

CELL LINE	TUMOR TYPE	GI_{50} (μM)
K562	CML	0.075
DU145	PROSTATE	0.075
BT474	BREAST (ERBB2+)	0.1
MCF7	BREAST (ER+)	0.075
GRANTA-519	MCL	0.04
SK-OV-3	OVARIAN	0.075
U87	GLIOBLASTOMA	0.2
MIA-Pa-Ca-2	PANCREATIC	0.075
COLO-205	COLON	0.075
HELA	CERVICAL	0.05
A549	NSCLC	0.075
H1975	NSCLC	0.075
SK-MEL-28	MELANOMA	0.2
RAJI	B-CELL	0.05
U20S	OSTEOSARCOMA	0.05
JURKAT	T-CELL	0.025
HFL	NORMAL	>5.0

Inhibition of Tumor colony formation by ON 1231320 (19y)

HCT 116 Cells

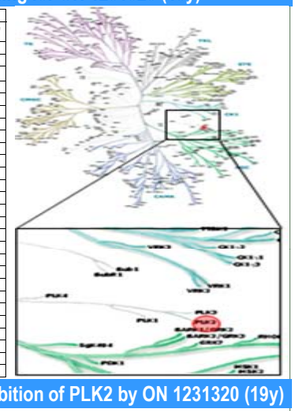
DMSO	0.075 μM ON1231320	0.10 μM ON1231320
0.125 μM ON1231320	0.15 μM ON1231320	0.20 μM ON1231320
0.30 μM ON1231320	0.40 μM ON1231320	0.50 μM ON1231320

Cells were treated for 96 hours with increasing concentrations of ON 1231320, washed and allowed to form colonies for 72 hours before fixing and staining with crystal violet. Colonies were counted by NIH Image J to calculate G150 va lues.

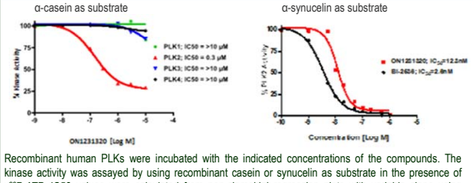
Cell Line	Tumor Type	GI_{50} Value (μM)
MDAMB-157	Breast (triple negative)	0.025
MDAMB-231	Breast (triple negative)	0.035
MCF-7	Breast (ER positive)	0.035
SKBR-3	Breast (PR positive)	0.035
BT-474	Breast (ERBB2 positive)	0.125
DLD-1	Colon	0.045
SW-480	Colon	0.075
Colo-205	Colon	0.100
HCT116	Colon	0.100
H460	Large Cell Lung	0.150
A549	NSCLC	0.150
H1299	NSCLC	0.200
DU145	Prostate	0.200
hMSC/hTERT	hTERT transformed	>1.00
hMSC	Normal	>1.00

Kinase profiling for ON 1231320 (19y)

Kinase	Compound IC_{50} (nM)	
	ON 1231320	Staurosporine
PLK2	287.00	813.17
ABL1	>10000	44.02
AKT1 (dPH,S437D)	>10000	2.73
ALK	>10000	1.34
Aurora A	>10000	<1.0
BLK1	>10000	7.40
c-Kit	>10000	57.05
c-Src	>10000	2.49
FGFR1	>10000	3.03
FGFR3	>10000	10.93
FLT3	>10000	<1.0
FYN	>10000	<1.0
HCK	>10000	1.33
IGF-1R	>10000	31.69
JAK1	>10000	<1.0
JAK2	>10000	<1.0
KDR/VEGFR2	>10000	10.77
LCK	>10000	2.51
LYN B	>10000	1.72
NLK	>10000	42.30
PDGFRb	>10000	1.45
PIM3	>10000	<1.0
PLK1	>10000	521.00
PLK3	>10000	3673.00
RSK1	>10000	<1.0
WNK2	>10000	719.80
YES	>10000	1.01

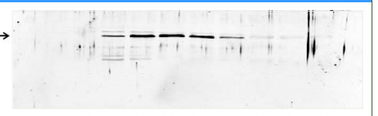


Selective *in vitro* inhibition of PLK2 by ON 1231320 (19y)

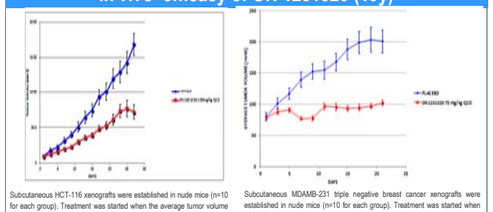


Recombinant human PLKs were incubated with the indicated concentrations of the compounds. The kinase activity was assayed by using recombinant casein or synucelin as substrate in the presence of γ^{32}P-ATP. IC50 values were calculated from non-sigmoidal regression plots with variable slope using Graph Pad Prism software.

Direct interaction between PLK2 and ON 1231320 (19y)



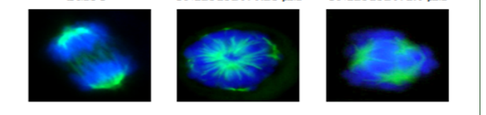
in vivo efficacy of ON 1231320 (19y)



Subcutaneous HCT-116 xenografts were established in nude mice (n=10 for each group). Treatment was started when the average tumor volume was 100mm^3.100 mg/kg ON 1231320 or vehicle was administered I.P every other day (Q2D) and tumor volume and body weights were measured.

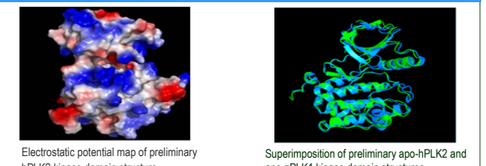
Subcutaneous MDAMB-231 triple negative breast cancer xenografts were established in nude mice (n=10 for each group). Treatment was started when the average tumor volume was 75 mm^3. ON1231320 at 75 mg/kg or vehicle was administered I.P. every other day (Q2D) and the tumor volumes and body weights were measured.

Multipolar spindles induced by ON 1231320 (19y)



HCT-116 cells were treated with DMSO, 0.25μM or 1 μM ON 1231320 or DMSO for 24 hours. DNA was stained with DAPI and microtubules with anti α-Tubulin antibody

Preliminary X-ray crystal structure of PLK2 kinase domain



Electrostatic potential map of preliminary hPLK2 kinase domain structure

Superimposition of preliminary apo-hPLK2 and apo-zPLK1 kinase domain structures

Summary and Conclusions

We have identified a novel chemo type, 6-arylsulfonyl pyridopyrimidinones, that exhibits anti-cancer activity.

- ON 1231320 is a potent inhibitor of tumor cell growth.
- ON 1231320 specifically targets PLK2, a mitotic kinase.
- ON 1231320 exhibits efficacy in a nude mouse xenograft cancer model

Acknowledgements

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