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Identification of novel, potent and selective inhibitors of Polo-like kinase 1

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ABSTRACT

A series of pyrimidodiazepines was identified as potent Polo-like kinase 1 (PLK1) inhibitors. The synthesis and SAR are discussed. The lead compound **7** (RO3280) has potent inhibitory activity against PLK1, good selectivity against other kinases, and excellent in vitro cellular potency. It showed strong antitumor activity in xenograft mouse models.

The Polo-like kinases (PLKs), members of the serine/threonine family of kinases, play a variety of roles in G2/M phase transition and have been identified as key regulators of cell mitosis.[1,2] PLKs are highly conserved from yeast to human and five Polo-like kinases, PLK1, PLK2 (Snk), PLK3 (Fnk), PLK4 and PLK5 have been identified.[1a,3] Among them, PLK1 is the best characterized. PLK1 is overexpressed in many solid tumors of various origin (breast, lung, colon, stomach, ovary, smooth muscle, and esophagus) and in non-Hodgkin lymphomas.[1a,4] The overexpression studies of PLK1 in NIH3T3 cell line showed these cells can form tumors in nude mice due to PLK1 overexpression.[5] Loss of PLK1 expression can induce pro-apoptotic pathways and inhibit growth.[6] An shRNA screen in mutant KRAS cells shows that PLK1 has a synthetic lethal interaction with this oncogene.[7] Inhibition of PLK1 kinase activity by a small molecule represents a novel approach to target mitosis and may be clearly differentiated from other mitosis-targeting agents on the market. The crystal structure of the kinase domain for PLK1 has been reported.[8] PLK1 is a validated target for cancer and several PLK1 inhibitors have shown promising results in phase I or II clinical trials.[9] Our own efforts to find inhibitors of PLK1 resulted in the identification of pyrimidodiazepines as a novel and potent class of ATP-competitive inhibitors of PLK1. This paper outlines the synthesis, structure–activity relationships, and in vitro and in vivo activity of this series of compounds.

Our interest in the diazepine ring system was to explore whether it offered improved physical and biological properties over the previously reported pteridines,[9a] as well as providing additional vectors for substitution.

The general synthetic approach for the preparation of this series is shown in Scheme 1.[10] Starting from β-amino ester **21**, regioselective substitution of the 4-chloro group of 2,4-dichloro-5-nitro-pyrimidine gave **22**. Reduction of the nitro group with in situ cyclization gave pyrimidodiazepinone **23**. Alkylation of the lactam nitrogen and substitution of the 2-chloro group gave carboxylic acid **25**, which was coupled with a series of amines to give the final products.

A key step in the synthesis of difluoro-analog **7** (RO3280) was the preparation of the intermediate α,α-difluoro-β-amino ester **27** (Scheme 2).[10] This was prepared by the reaction of cyclopentylamine with formaldehyde and benzotriazole[11] to give *N*-(α-aminoalkyl)-benzotriazole **26**. Reformatsky reaction[12] of ethyl bromodifluoroacetate with intermediate **26** provided the desired intermediate difluoro β-amino ester **27**. It was subsequently found that the β-amine ester could also be prepared by reductive amination of the aldehyde equivalent (ethyl hemiacetal form)[13] and sodium cyanoborohydride in the presence of chlorotrimethylsilane.

We explored the structure–activity relationships for substitutions around the ring system, as well as the portions of the

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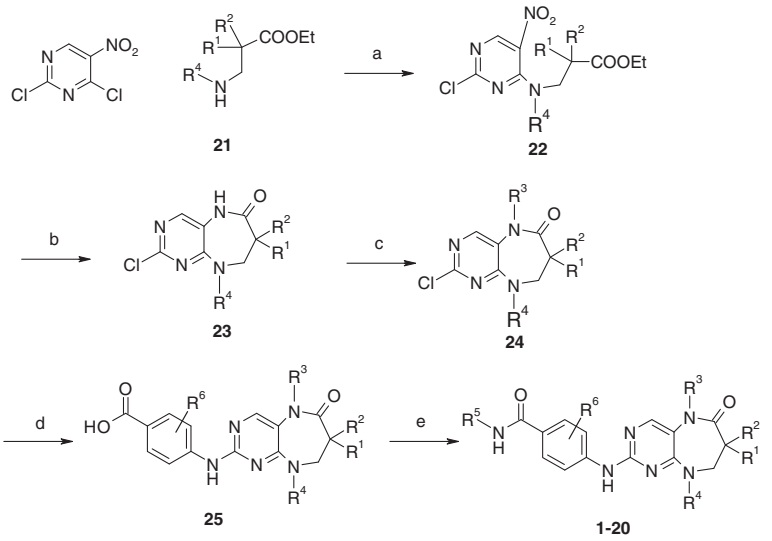
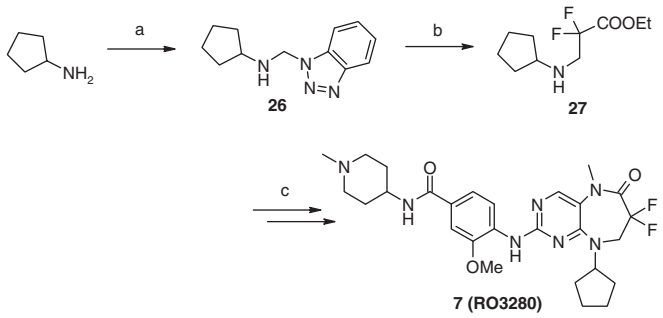


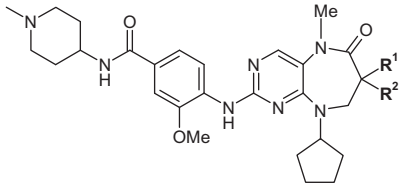
Scheme 1. Reagents and conditions: (a) $NaHCO_3$, EtOAc, 0 °C to rt, 18 h; (b) Fe, HOAc, 80 °C, 2 h; (c) R^3I, Cs_2CO_3, DMF, rt, 3 h; (d) 4-amino-3-methoxy-benzoic acid (for compounds **1**–**19**) or 4-amino-6-fluoro-3-methoxy-benzoic acid (for compound **20**), EtOH/H_2O/HCl, reflux, 18 h; (e) R^5-NH_2, HBPyU, DIEA, DMF, rt, 12 h.



Scheme 2. Reagents and conditions: (a) Benzotriazole, HCHO, ether, rt, 12 h; (b) $BrCF_2COOEt$, Zn, TMSCl, THF, −10 °C, 20 min, then compound **26**, −10 °C to rt, 3 h; (c) conditions (a) to (e) in Scheme 1.

template expected to extend out of the hinge-binding region. Small, di-substitutions, such as dimethyl, cyclopropyl, difluoro and dichloro for R^1 and R^2 gave analogs with good potency (Table 1). Because this initial dataset suggested that the difluoro substitution may be the more potent one, a series of matched pairs of difluoro and dimethyl analogs with substitutions elsewhere on the ring system were prepared and evaluated. In general, the difluoro compounds were 2- to 15-fold more potent (typically 3-fold) in the biochemical assay and 2- to 6-fold more potent (typically 3-fold) in the cell-based assay (data not shown). We found that difluoro-analog **7** (RO3280) gave the best results in both enzymatic (PLK1, IC_{50} = 3 nM) and cellular (lung cancer cell line H82, EC_{50} = 6 nM) assays. The increased potency for difluoro and

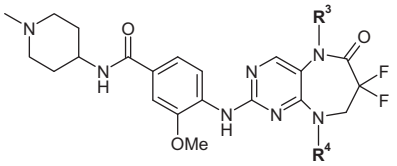
Table 1
Structure–activity relationship of pyrimidodiazepinone at R^1 and R^2 positions[a]



Compd	R^1, R^2	PLK1 IC_{50} (nM)	H82 cell EC_{50} (nM)
1	H,H	30	48
2	Me,H	34	53
3	Et,H	103	127
4	di-Me	15	23
5	*c*-propyl	10	13
6	di-Cl	4	10
7	di-F	3	6

[a] See Ref. 10 for assay details.

Table 2
Structure-activity relationship of pyrimidodiazepinone at R^3 and R^4 positions



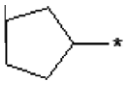
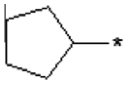
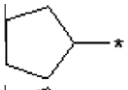
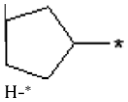
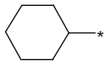
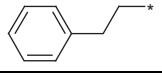
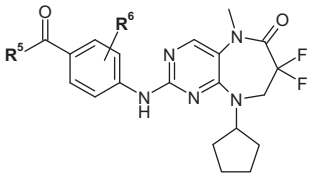
Compd	R^3	R^4	PLK1 IC_{50} (nM)	H82 cell EC_{50} (nM)
7	Me		3	6
8	H		234	NT
9	Et		138	NT
10	Propyl		355	NT
11	Me	H-*	123	NT
12	Me		6	15
13	Me		3	10
14	Me		7	8
15	Me		20	236

Table 3
Structure–activity relationship of pyrimidodiazepinone at R^5 and R^6 positions



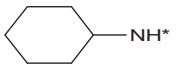
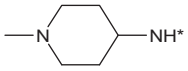
Compd	R^6	R^5	PLK1 IC_{50} (nM)	H82 cell EC_{50} (nM)
16	2-OMe	–OH	18	390
17	2-OMe	$-NH_2$	4	27
18	2-OMe	–NHMe	6	51
19	2-OMe		39	NT
20	2-OMe,5-F		6	12

Table 4
Selectivity of RO3280 against a panel of kinases[a]

Ambit gene symbol	K_d (nM)
ALK	230
CAMKK1	1100
CAMKK2	87
DAPK1	100
DAPK3	70
FER	53
GAK	87
MYLK	170
PTK2	84
PTK2B	130
RPS6KA6 (KinDom.2)	560
TTK	51
PLK1	**0.09**

[a] Ambit selectivity screen is a panel of 318 kinases (wild type + mutants). K_d values were determined for the kinases where the inhibition was >85% at 1 μM.

dichloro substitution at this position may be a consequence of additional interaction with nearby water molecules in the binding pocket according to computer modeling, that analysis will be deferred pending a crystallographic determination.

Further exploration of the structure activity relationships in our difluoro series ($R^1 = R^2 = F$) is summarized in Table 2 (R^3 and R^4 variations) and Table 3 (R^5 and R^6 variations). The optimal substitution for R^3 is methyl, with activity dropping off significantly either for no substitution ($R^3 = H$) or for larger groups. The substitution at R^4, which is expected to extend into a hydrophobic pocket, tolerates a much wider range of modification, including branched and cycloalkyl groups. Larger alkyl group at this position led to a loss in potency.

Table 3 shows the SAR in the R^5 and R^6 positions. Simple amide derivatives give good potency in the biochemical assay, but lose some potency in the cell-based assay. The best activities in the

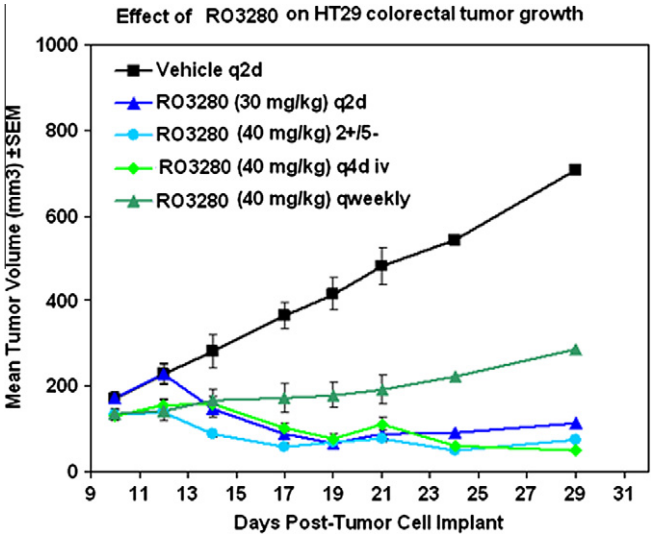


Figure 1. Tumor growth inhibition by intravenous (IV) administration of RO3280 in nude mouse implanted with HT-29 human colorectal tumors.

biochemical and cell-based assays were seen when the R^5 group contained a basic amine. Several substitutions on phenyl ring at R^6 position were well tolerated (e.g., compound **20**).

The most promising analogs were examined in both an in-house kinase selectivity panel and KINOMEscan™ from Ambit Biosciences.[14] Compound RO3280 was found to be a potent and selective PLK1 inhibitor. It shows no inhibitory activity against a panel of 48 in-house kinases and inhibited 13 out of 293 kinases in the Ambit panel more than 85% at 1 μM. Of the kinases read as a hit in the Ambit panel, K_d values were determined for the more active kinases, data summarized in the Table 4. From the K_d measurements, binding to PLK1 was more than 500-fold tighter than to any of the other kinases in the Ambit panel as shown in Table 4.

As shown in Table 1, RO3280 exhibits an EC_{50} value of 6 nM in a cell-based assay using the H82 lung cancer cell line. Table 5 shows the strong anti-proliferative activity of this compound in a panel of tumor cell lines including lung, colon cancer, breast cancer, prostate and skin cancer. An initial assessment of EC_{50} showed no correlation with MDR1 or MRP1 status, but does appear to correlate with p21 expression (Western blot, data not shown).[15] A more comprehensive description of the biological profiling of RO3280, as well as the development and characterization of resistant mutations (HT-29) will be the topic of a subsequent publication.

RO3280 was evaluated for its in vivo antitumor activity in a series of mouse xenograft models, of which the HT-29 colorectal cancer cell line is representative. As showed in Figure 1, RO3280 displayed robust antitumor activity ranging from 72% tumor growth inhibition when dosed once weekly at 40 mg/Kg, to complete tumor regression when dosed more frequently.

In summary, we report the discovery of a novel class of pyrimidodiazepinones as potent and selective ATP-competitive PLK1 inhibitors. RO3280 exhibits potent antiproliferative activity in a

Table 5
Inhibitory activity on various tumor cells for RO3280

Tumor cell line (EC_{50}, nM)												
Lung			Colon						Breast		Prostate	Skin
H82	H69	A549	HCT-116	RKO	SW480	Colo205	HT-29	SW620	MDA-MB-468	MTLn3	PC-3	A375
6	7	82	60	18	45	16	10	10	19	20	12	70

wide range of cancer cell lines, and shows strong anti-tumor activities in an in vivo HT-29 colorectal xenograft mouse model.

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References and notes

1. See reviews: (a) Chopra, P.; Sethi, G.; Dastidar, S. G.; Ray, A. *Expert Opin. Invest. Drugs* **2010**, *19*, 27; (b) Degenhardt, Y.; Lampkin, T. *Clin. Cancer Res.* **2010**, *16*, 384; (c) Strebhardt, K. *Nat. Rev. Drug Disc.* **2010**, *9*, 643; (d) Malumbres, M.; Barbacid, M. *Curr. Opin. Genet. Dev.* **2007**, *17*, 60; (e) Christoph, D. C.; Schuler, M. *Expert Rev. Anticancer Ther.* **2011**, *11*, 1117; (f) McInnes, C.; Wyatt, M. D. *Drug Discovery Today* **2011**, *16*, 619.
2. Lamazares, S.; Moreira, A.; Tavares, A.; Girdham, C.; Spruce, B. A.; Gonzalez, C.; Karess, R. E.; Glover, D. M.; Sunkel, C. E. *Gene Dev.* **1991**, *5*, 2153.
3. De Cárcer, G.; Escobar, B.; Higuero, A. M.; García, L.; Ansón, A.; Pérez, G.; Mollejo, M.; Manning, G.; Meléndez, B.; Abad-Rodríguez, J.; Malumbres, M. *Mol. Cell Biol.* **2011**, *31*, 1225.
4. Takai, N.; Hamanaka, R.; Yoshimatsu, J.; Miyakawa, I. *Oncogene* **2005**, *24*, 287.
5. Smith, M. R.; Wilson, M. L.; Hamanaka, R.; Chase, D.; Kung, H.; Longo, D. L.; Derris, D. K. *Biochem. Biophys. Res. Commun.* **1997**, *234*, 397.
6. Hu, K.; Lee, C.; Qiu, D.; Fotovati, A.; Davies, A.; Abu-Ali, S.; Wai, D.; Lawlor, E. R.; Triche, T. J.; Pallen, C. J.; Dunn, S. E. *Mol. Cancer Ther.* **2009**, *8*, 3024.
7. Luo, J.; Emanuele, M. J.; Li, D.; Creighton, C. J.; Schlabach, M. R.; Westbrook, T. F.; Wong, K. K.; Elledge, S. J. *Cell* **2009**, *137*, 835.
8. (a) Bandeiras, T. M.; Hillig, R. C.; Matias, P. M.; Eberspaecher, U.; Fanghanel, J.; Thomaz, M.; Miranda, S.; Crusius, K.; Putter, V.; Amstutz, P.; Gulotti-Georgieva, M.; Binz, H. K.; Holz, C.; Schmitz, A. A. P.; Lang, C.; Donner, P.; Egner, U.; Carrondo, M. A.; Muller-Tiemann, B. *Acta Crystallogr., Sect. D* **2008**, *D64*, 339; (b) Kothe, M.; Kohls, D.; Low, S.; Coli, R.; Cheng, A. C.; Jacques, S. L.; Johnson, T. L.; Lewis, C.; Loh, C.; Nonomiya, J.; Sheils, A. L.; Verdries, K. A.; Wynn, T. A.; Kuhn, C.; Ding, Y.-H. *Biochemistry* **2007**, *4*, 5960.
9. (a) Schöffski, P. *Oncologist* **2009**, *14*, 559; (b) Pandha, H. S.; Protheroe, A.; Wylie, J.; Parker, C.; Chambers, J.; Bell, S.; Munzert, G. *J. Clin. Oncol.* **2008**, *26*, 14547; (c) Schöffski, P.; Blay, J.-Y.; De Greve, J.; Brain, E.; Machiels, J.-P.; Soria, J.-C.; Sleijfer, S.; Wolter, P.; Ray-Coquard, I.; Fontaine, C.; Munzert, G.; Fritsch, H.; Hanft, G.; Aerts, C.; Rapion, J.; Allgeier, A.; Bogaerts, J.; Lacombe, D. *Eur. J. Cancer* **2010**, *46*, 2206; (d) Olmos, D.; Barker, D.; Sharma, R.; Brunetto, A. T.; Yap, T. A.; Taegtmeyer, A. B.; Barriuso, J.; Medani, H.; Degenhardt, Y. Y.; Allred, A. J.; Smith, D. A.; Murray, S. C.; Lampkin, T. A.; Dar, M. M.; Wilson, R.; de Bono, J. S.; Blagden, S. P. *Clin. Cancer Res.* **2011**, *17*, 3420; (e) Hofheinz, R.-D.; Al-Batran, S.-E.; Hochhaus, A.; Jaeger, E.; Reichardt, V. L.; Fritsch, H.; Trommeshauser, D.; Munzert, G. *Clin. Cancer Res.* **2010**, *16*, 4666.
10. (a) Chen, S. U.S. patent 7517873, 2008.; (b) Cai, J.; Chen, S.; Chu, X.-J.; Luk, K.-C.; Mischke, S. G.; Sun, H.; Wovkulich, P. M. U.S. patent app. 20090318408, 2009.
11. Katritzky, A. R.; Pilarski, B.; Urogdi, L. *J. Chem. Soc., Perkin Trans. 1* **1990**, 541.
12. Katritzky, A. R.; Nichols, D. A.; Qi, M. *Tetrahedron Lett.* **1998**, *39*, 7063.
13. Tsukamoto, T.; Kitazume, T. *J. Chem. Soc., Chem. Commun.* **1992**, 540.
14. Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. *Nat. Biotechnol.* **2008**, *26*, 127.
15. Degenhardt, Y.; Greshock, J.; Laquerre, S.; Gilmartin, A. G.; Jing, J.; Richter, M.; Zhang, X.; Bleam, M.; Halsey, W.; Hughes, A.; Moy, C.; Liu-Sullivan, N.; Powers, S.; Bachman, K.; Jackson, J.; Weber, B.; Wooster, R. *Mol. Cancer Ther.* **2010**, *9*, 2079.