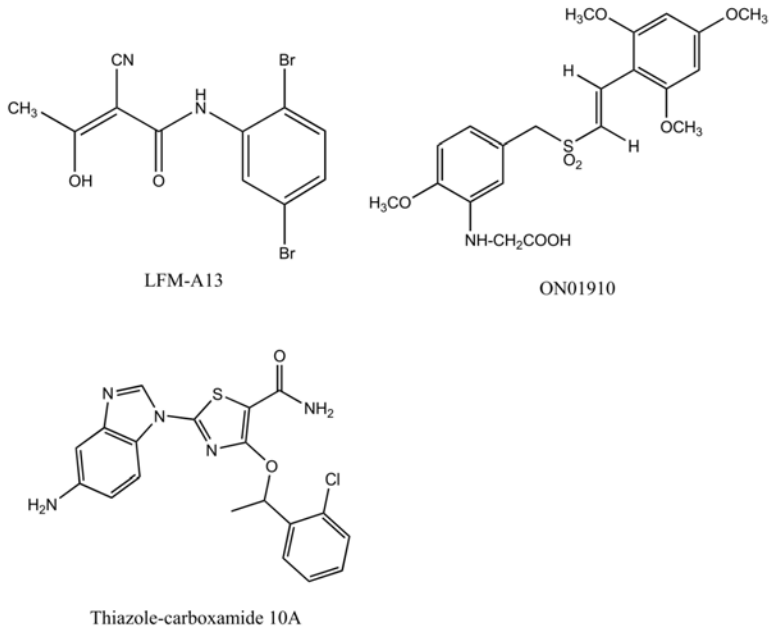


Figure S1. Molecular structures of three PLK1 inhibitors: LFM-A13, ON01910, and thiazole-carboxamide 10A.

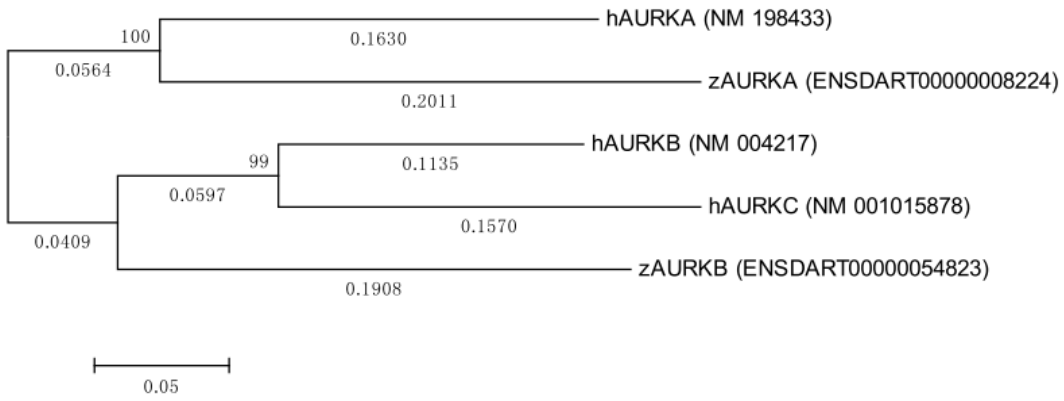


Figure S2. The phylogenetic tree of human and zebrafish *aurora* homologs. Amino acid sequences of the listed genes were used in multiple sequence alignment. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Bootstrap values in 1000 replicates are shown next to the branches. Phylogenetic analyses were conducted in MEGA4. The scale bar indicates 0.05 unit of branch length. Zebrafish AURKB (ENSDART00000054823) was an official symbol on zfin. Zebrafish AURKA was named according to the result of this multiple sequence alignment.

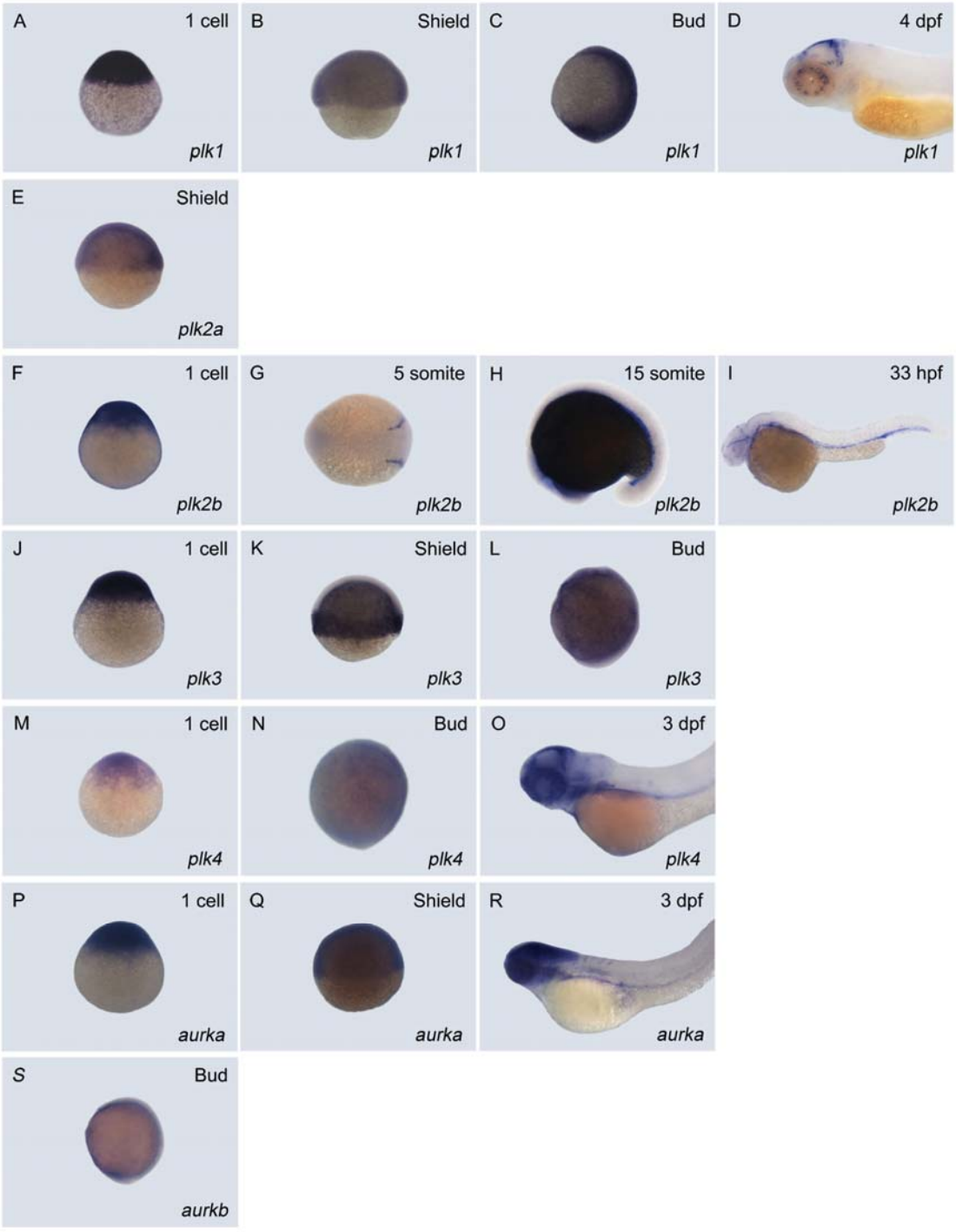


Figure S3. Expression patterns of zebrafish *plk* and *aurora* families at certain stages. Embryonic stages and genes are labeled in each panel. In A, B, E, F, J, K, M, P, and S, animal pole is to the top. In C, L, N, and S, embryos are anterior to the top and dorsal to the right. G, dorsal view. D, H, I, O, and R, embryos are anterior to the left and dorsal to the top.

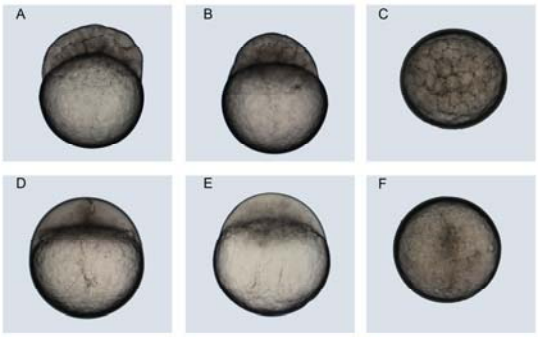


Figure S4. LFM-A13 disrupted the embryonic cell division when added at 2-cell stage. Panel A, B, and C are control embryos, treated with vehicle only. Panel D, E, and F are LFM-A13 treated embryos. Control embryos were at 64-cell stage.

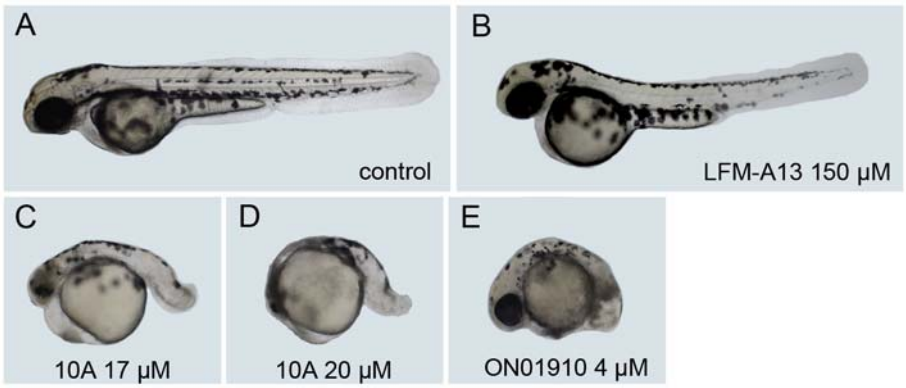


Figure S5. Three PLK1 inhibitors impaired embryogenesis when added at shield stage. Embryos were at 2 dpf. Panel A, control embryo, treated with vehicle only. Panel B to E, PLK1 inhibitors treated embryos.

Figure S6. Schematic map of MO1-EGFP fusion construct.

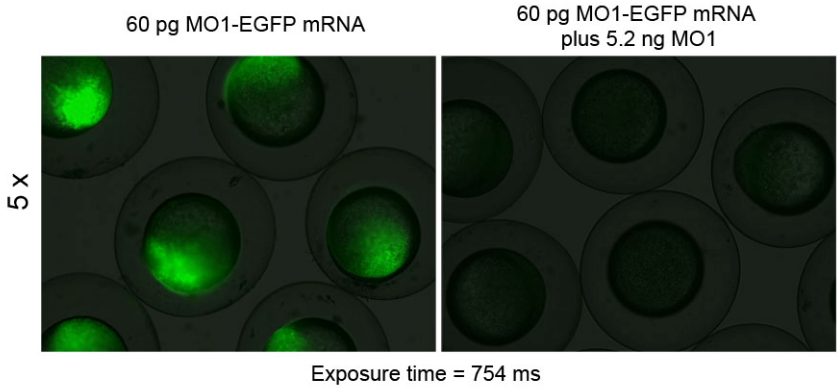


Figure S7. When MO1 and the MO1-EGFP fusion construct were injected together, the

fluorescence of EGFP decreased dramatically. Embryos were at 1k-cell stage.

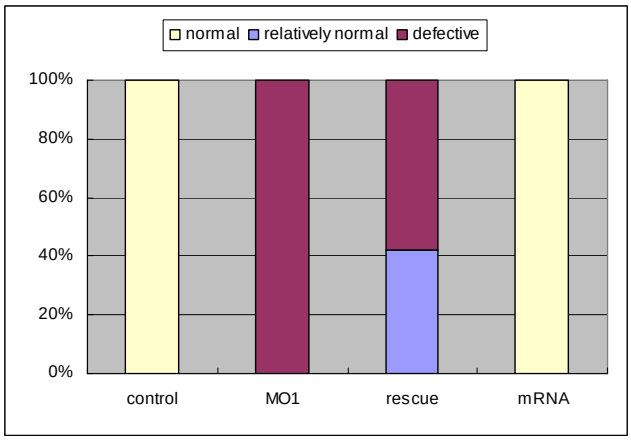


Figure S8. Quantitative analysis of *plk1* rescue assay. Of the MO1 injected embryos, 100% showed the defective phenotype (n=119). Of the rescue (MO1 and human *plk1* mRNA co-injection) embryos, 58% showed the defective phenotype and 42% showed a relatively normal phenotype (n=189). Of human *plk1* mRNA injected embryos, 100% was normal (n=92).

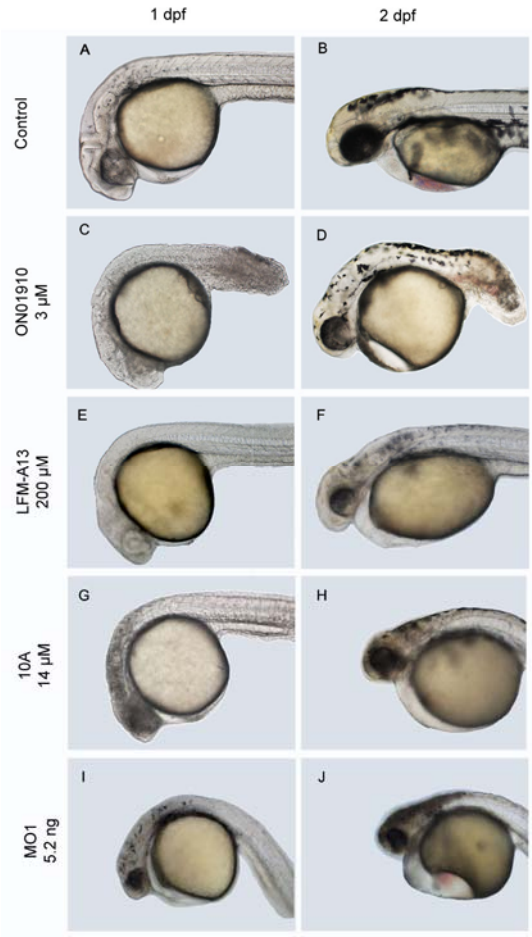


Figure S9. High magnification images of defects caused by *plk1* knockdown and three PLK1 inhibitors. Embryos are anterior to the left and dorsal to the top.

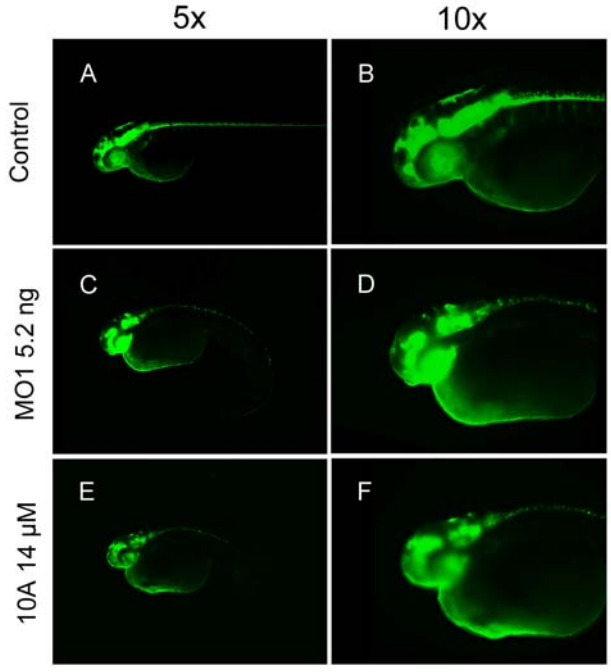


Figure S10. *Plk1* knockdown and 10A treatment in *Tg* (*gata2:GFP*) line. The numbers of neurons

decreased remarkably in C, D, E, and F.

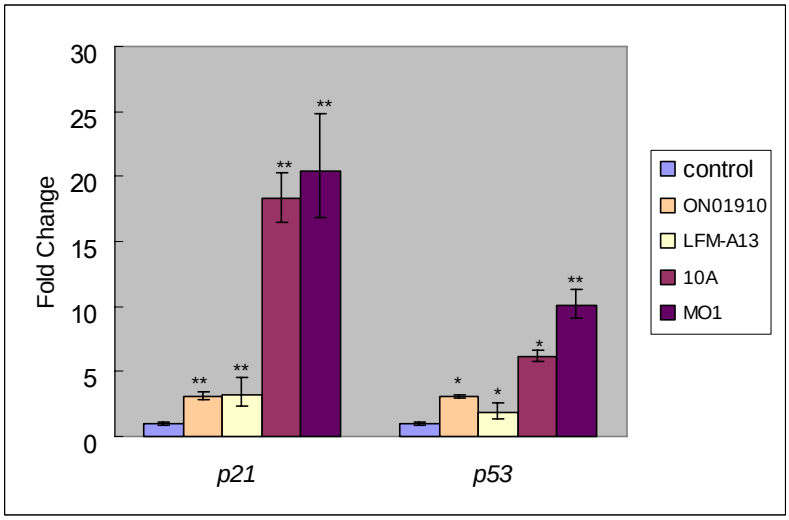


Figure S11. qPCR analysis of *p21* and *p53* transcripts of RNA samples from control, MO1 injected or PLK1 inhibitors treated embryos at 1 dpf. t-tests were used for statistical analysis. * $p<0.05$, * * $p<0.01$.

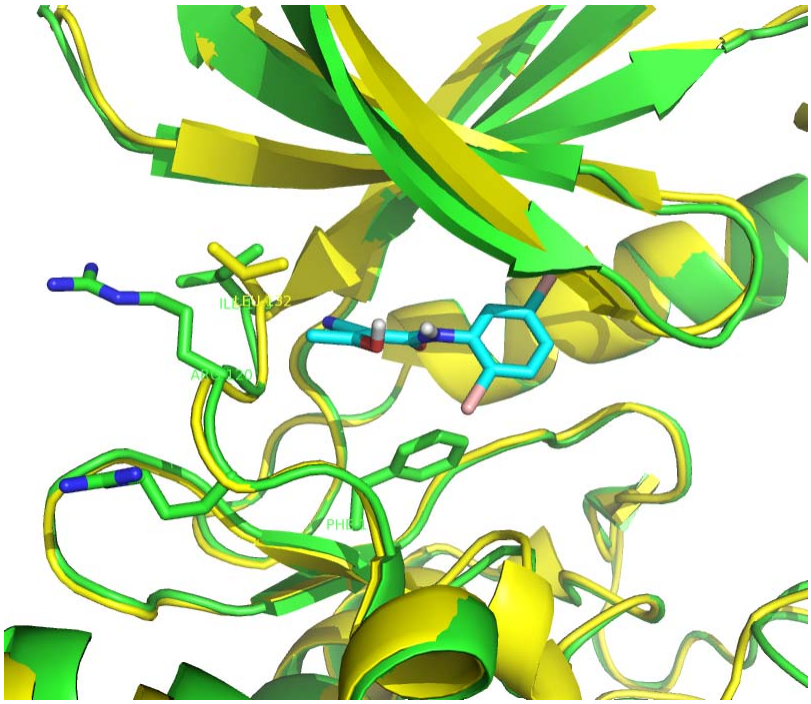


Figure S12. Docking of zebrafish PLK1 (green) and LFM-A13. The estimated binding free energy is -6.07 kcal/mol. The result shows that no effective hydrophobic and Van De Waal's interactions with the PLK1 KD domain was discovered. Human PLK1 (2rku, yellow) is shown also.

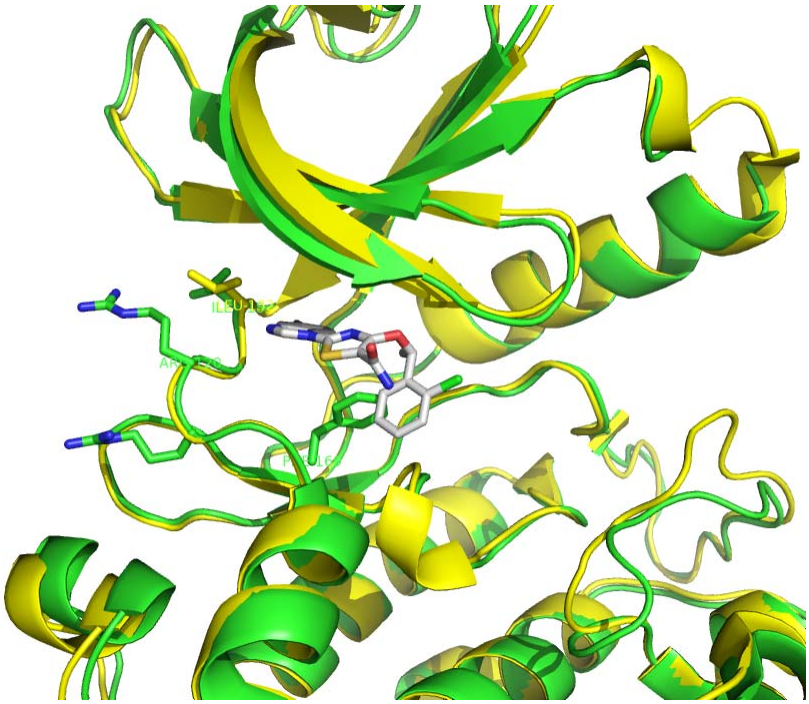
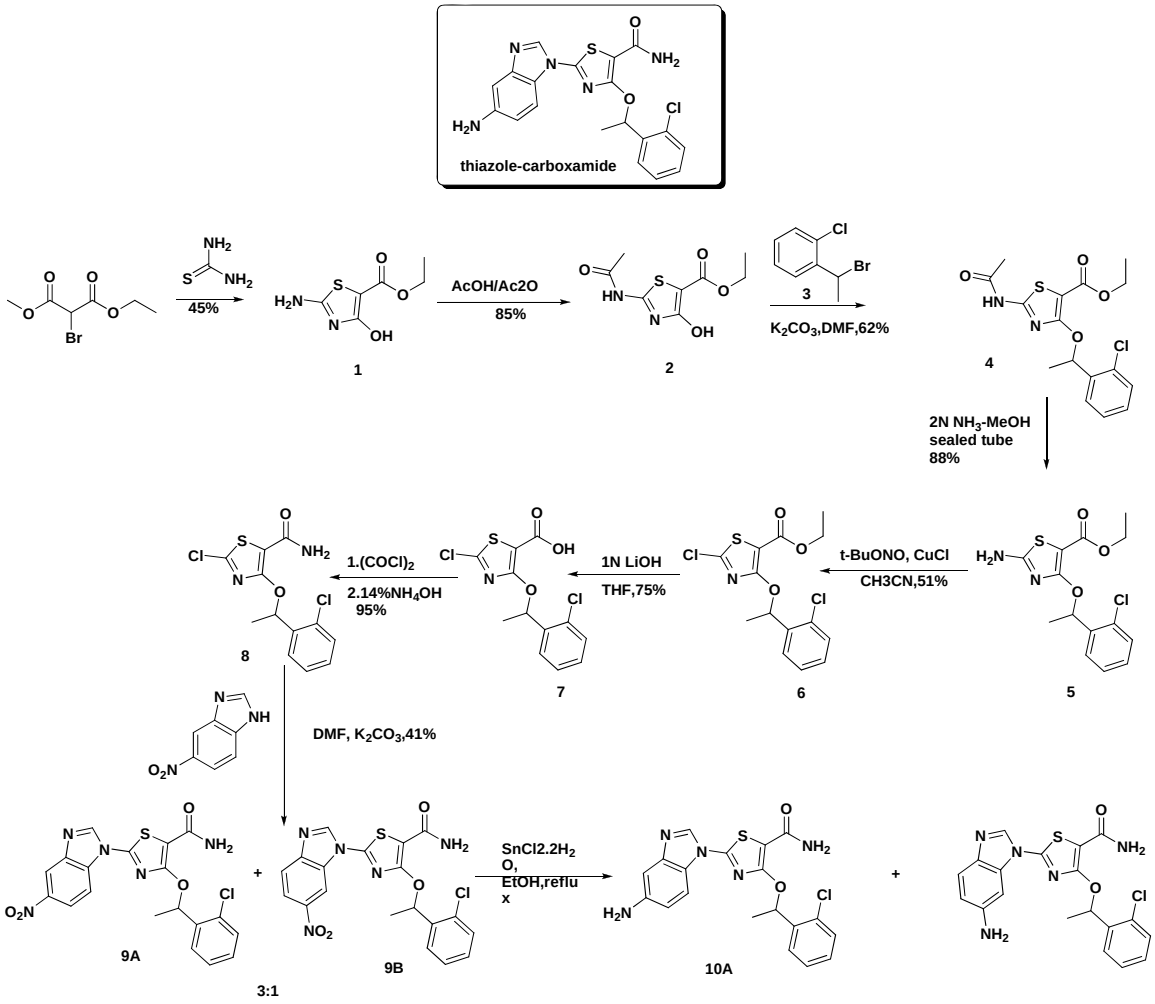


Figure S13. Docking of zebrafish PLK1 (green) and thiazole-carboxamide 10A. The estimated binding free energy is -8.44 kcal/mol. 10A forms two hydrogen bonds with Arg136 and Cys119 in the hinge region of zebrafish PLK1. The methyl of 10A is accommodated in the small pocket formed by Cys62, Lys82, Ala80, and Leu130. Human PLK1 (2rku, yellow) is shown also.



Scheme S1. The synthesis of the thiazole-carboxamide **10A.** The synthesis begins with Ethyl 2-amino-4-thiazole-5-carboxylate **1**, which was prepared according to literature procedure (Baldwin, *J. Med. Chem*. 23, 65) in 45% yield. After a few functional group transformations, compound **8** was formed. In the presence of K_2CO_3 in DMF at 75℃, compound **8** coupled with benzoimidazole to give two separable regioisomers 9A and 9B. Finally, reduction of the nitryl group of **9** to the amino group by $SnCl_2$ gave the thiazole-carboxamide **10A.**

Table S1. Concentrations of the three PLK1 inhibitors tested with zebrafish embryos.

Inhibitors	Concentrations (μM) when added at 2-cell stage	Concentrations (μM) when added at shield stage
Thiazole-carboxamide 10A	5, 7.5, 10	7.5, 14, 17, 20, 25
LFM-A13	50, 100, 200	50, 100, 200, 250
ON01910	1, 2, 3	1, 2, 3, 4, 5

Table S2. Defective phenotypes caused by low doses of the three PLK1 inhibitors when added at shield stage.

Thiazole-carboxamide 10A	7.5 μM	14 μM	17 μM	20 μM	25 μM
	Normal	Small and dark head, short trunk	Very small and dark head, short trunk	Diminished head structure, very short trunk	All dead
LFM-A13		50 μM	100 μM	200 μM	250 μM
		Normal	Almost normal	Relatively normal	All dead
ON01910	1 μM	2 μM	3 μM	4 μM	5 μM
	Normal	Short tail	Very short tail	Diminished tail	All dead

Experimental procedures

Quantitative PCR

Total RNA was isolated from 50 1 embryos at 1 dpf using RNA queous$^{@}$-4 PCR Kit (Ambion). And the equal quantities of cDNA were reverse transcribed from 0.5 μg RNA using the PrimeScript^{TM} RT reagent Kit (TaKaRa). The real-time PCR reactions comprised 1 μl cDNA, 12.5 μl 2×SYBR Premix ExTaq, 0.5μl ROX dye and 0.5 μl forward and reverse primers in a final volume of 25 μl. In the negative controls, templates were replaced by DEPC water. All reactions were carried out in triplicate using an ABI Prism 7300 sequence detection system (Applied Biosystems). Forty amplification cycles were performed, with each cycle consisting of 94 ºC for 5s followed by 60 ºC for 31s. For the analysis of real-time PCR reactions, the triplicate Ct values were averaged and the relative amount of RNA was determined by the $\Delta\Delta$ C_t method, with *β-actin* serving as the reference gene. And the t-tests were used for statistical analysis. The primers are (forward primer/reverse primer):

β-actin: 5'GCCGTGACCTGACTGACTACCT3'/5'CGCAAGATTCCATACCCAAGA3';
cyclinD1: 5'GGAGCACCAGTTGTTTTGCT3'/5'GGTGGGCTCCACAGATAAAA3';
p53: 5'CTGAAGTGGTCCGCAGATG3'/5'CGTTTGGTCCCAGTGGTGG3';
p21: 5'CGCCGCTGGAAAGGAAAA3'/5'TGGTAGAAATCTGTGATGTTGG3';
hoxb1b: 5'AAAGTTGGGTGTTTTCCTGTAG3'/5'TTTGTGCGTAAGATGTGCC3';
mixer: 5'GGAAACCTCGGGAATGGAC3'/5'TGCTGCGTGAAGTTTGTGC3';
tbx6: 5'TATCAGCCTTCACAAACCCC3'/5'GCAGAGCCGCACAGACACTA3';
sox17:5'GCGTTTATGGTGTGGGCG3'/5'TTTGTAGTTTGGGTGGTC3';
gata2a: 5'GACCTACCCAACCTACTCCC3'/5'TCACACATTCACGCCCCT3';
ntl: 5'ACTCGGTGGCTGGTTCCT3'/5'AGTGTTTGTGGTGTGGGC3';
c-myc: 5'TCCCTCTGTGGTCTTCCC3'/5'ATCCGTCTCGTGCCTTTT3';
c-jun: 5'TTGGATACAACCACAAGGCT3'/5'CGTCGGGAGAAGTGAGGATA3';
n-myc: 5'GGACCCTACCGTGGTTTTC3'/5'CACCGTGACGACATCAATCT3'.