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Investigation of biaryl heterocycles as inhibitors of Wee1 kinase

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Wee1 is a key tyrosine kinase that helps control the G2/M checkpoint by preventing entry into mitosis.[1,2] This is of particular interest for cancer therapy since many cancer cells depend more heavily than do normal cells on a functional G2/M checkpoint for DNA repair.[3-5] This suggests that inhibition of Wee1 could induce premature entry into mitosis of DNA damaged cancer cells leading to mitotic catastrophe and cell death.[6,7] Following on this insight many cancer programs have focused on the development of selective drug-like Wee1 inhibitors.[8–10] AZD1775 (aka MK-1775, adavosertib), the first selective Wee1 inhibitor reported, is currently in clinical trials for the treatment of solid tumors. Clinical results for AZD1775 showed on-target efficacy as a single agent and in combination thus providing additional support for targeting Wee1 in cancer.[11–13]

In a previous account,[14] we used a class of Wee1 inhibitors featuring an imino-dihydropyrimidone (**1**) that had emerged in the patent literature as a starting point. Then, aided by molecular modeling, a series of compounds bearing a pyrimidine-based tricyclic scaffold were design as exemplified by **2** and proved to be potent inhibitors of Wee1 (Figure 1). Our studies also showed that the fused imidazole ring of **2** occupies a key region within the ATP binding pocket in Wee1 adjacent to the bicyclic core that was not utilized by **1**. Encouraged by these results we sought to explore a variety of biaryl cores that would 1) possess the key elements for binding in the active site (i.e. proper hinge binder and pi interactions with phe433) and 2) accommodate a substituent next to the bicyclic bridge (position *C*-8 or *N*-8). This substituent could in turn be used as a vector for modulation of compound properties and to further explore this region of the binding pocket. Modeling studies suggested that biaryl system such as **3** or **4** could fulfill these requirements. Consequently, we sought to explore structure-

activity relationships for these biaryl cores bearing substituents at *C*-8 or *N*-8.[15] Building on the insights we gained during our exploration of tricyclic compounds **2**, we selected a bis-Cl aryl moiety at the *C*-6 or *N*-6 position and an arylpiperazine or cyclopropyl tetrahydroisoquinoline in the amino aryl region as preferred scaffolds to evaluate the various bicyclic cores. Our findings indicate that both 4-pyridones (**3**) and 2-pyridones (**4**) may serve as interesting lead series for the development of potent and selective Wee1 inhibitors with desirable pharmacokinetic properties as well as antitumor efficacy in a murine xenograft model.

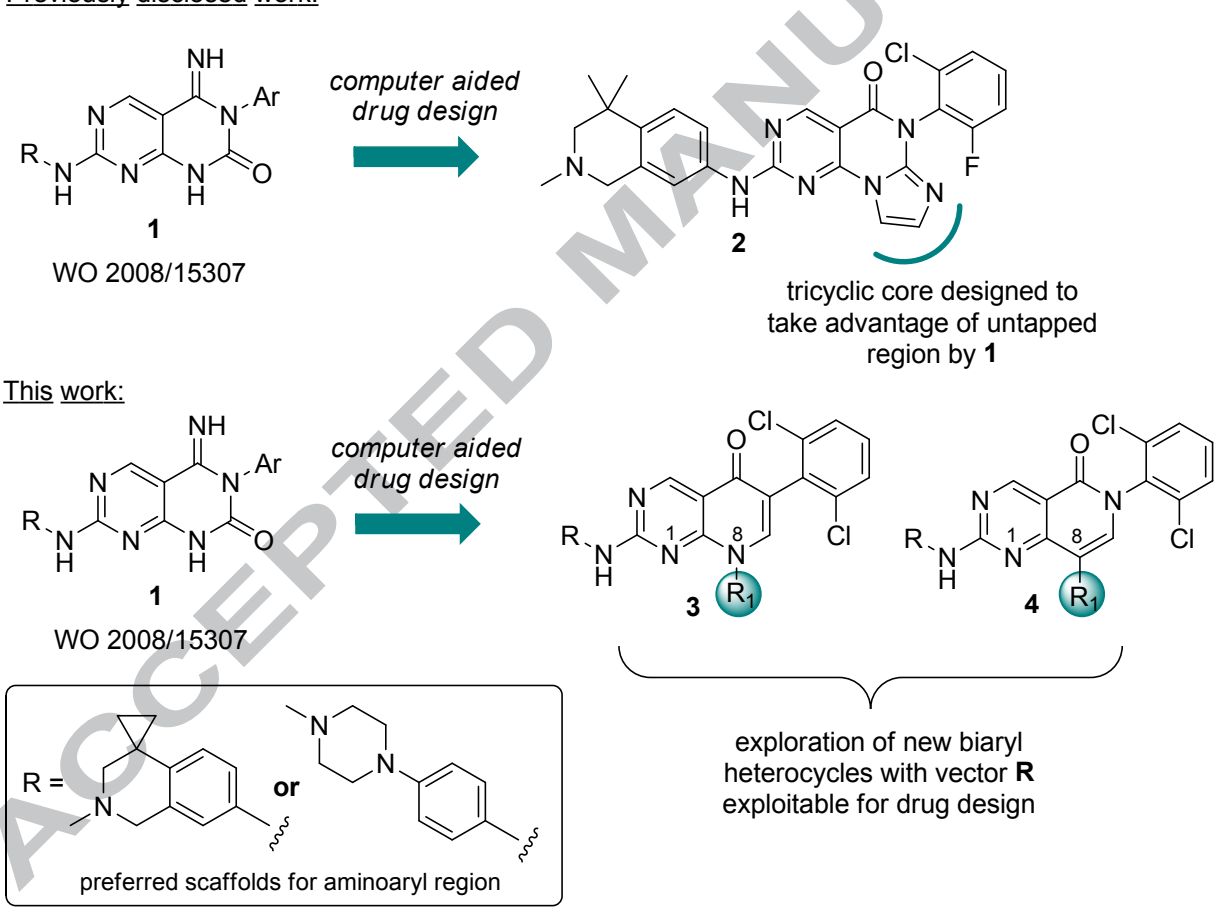
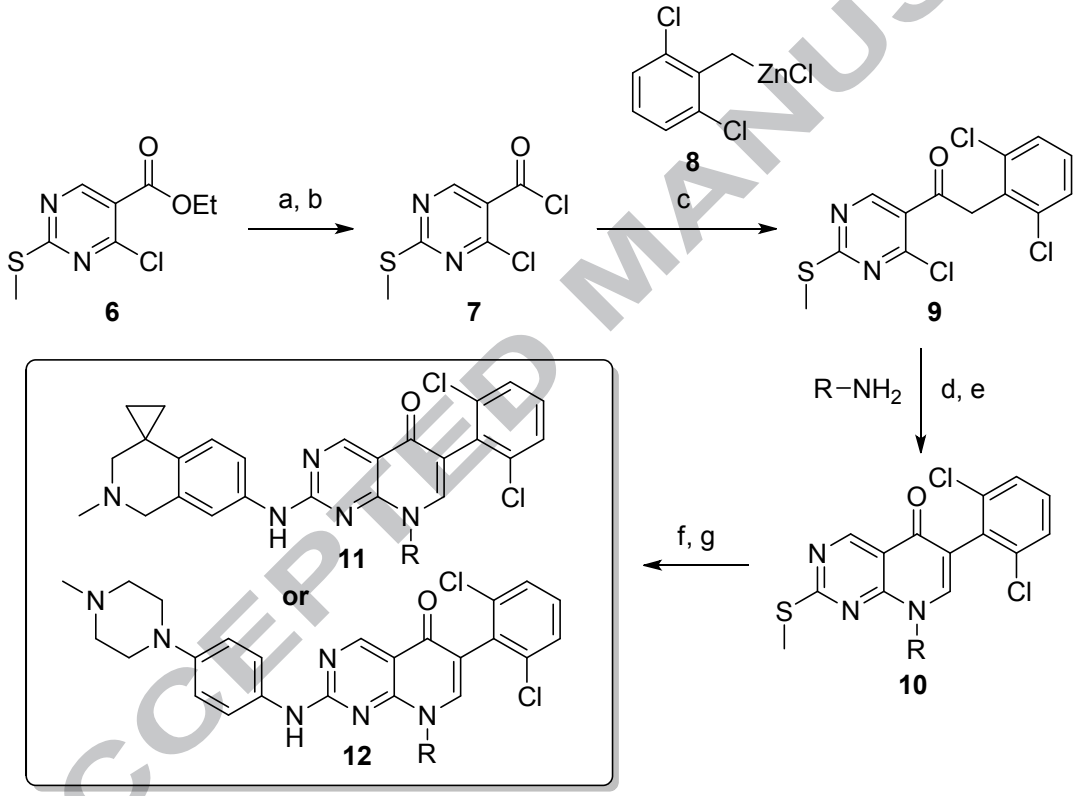


Figure 1. Modification strategies of novel and selective Wee1 inhibitors.

Schemes 1 & 2 outline the general synthesis of analogs related to biaryls **3** and **4**. As seen in Scheme 1, the known pyrimidine ester **5** was hydrolyzed under standard conditions to give the

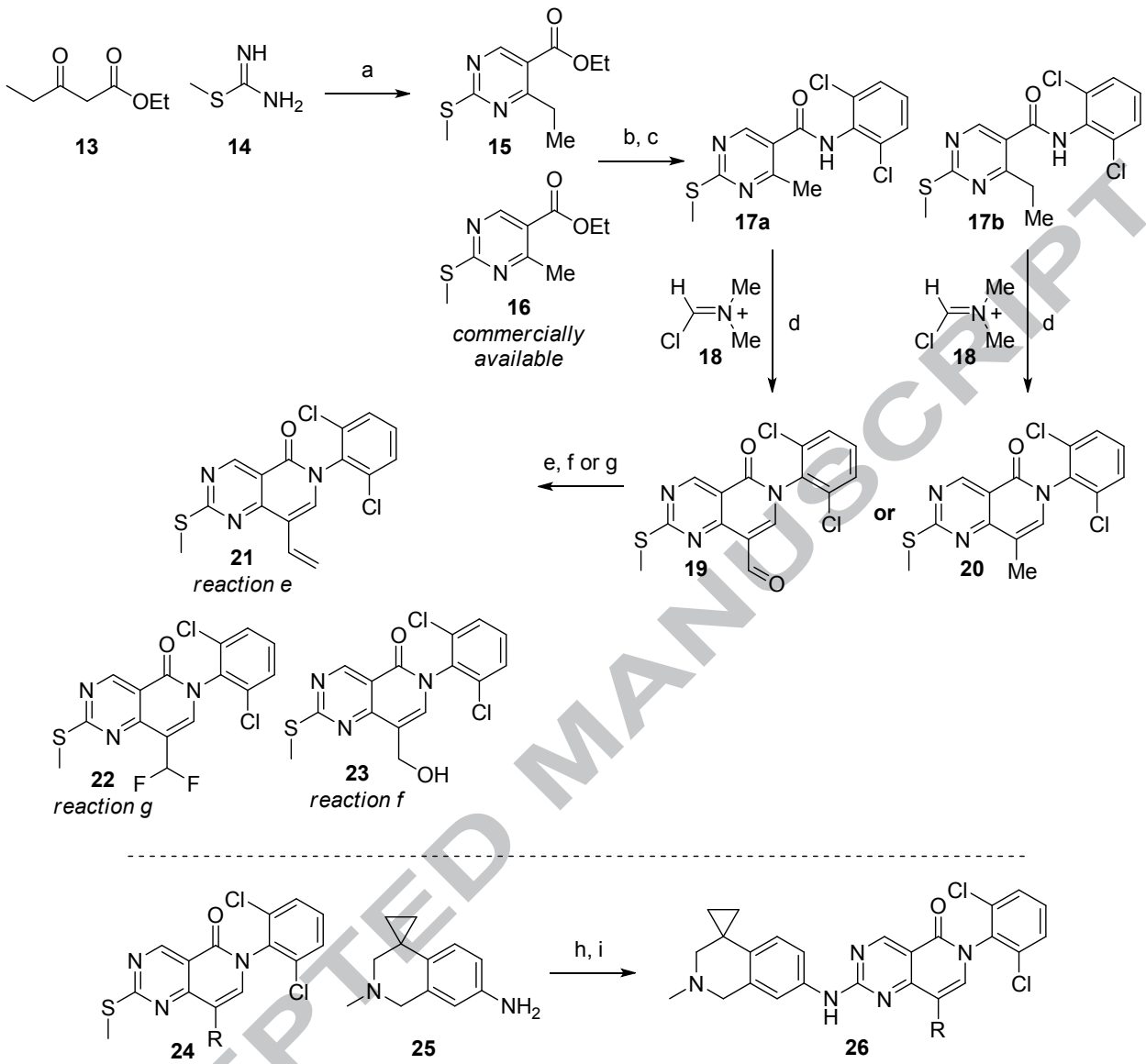
corresponding acid which in turn was treated with thionyl chloride to afford acyl chloride **6**. Next, an *in situ* generated organocuprate of compound **7** was added to yield ketone **8**. At this point, the desired substituent at R^1can be introduced by treatment of **8** with an appropriately substituted primary amine or aniline followed by condensation/cyclization with DMF−DMA at elevated temperature. The methyl thioether group of **10** was oxidized by mCPBA, setting the stage for the final S_NAr reaction with the desired aminoaryl substrate to provide analogs of 4-pyridone **3**.



Scheme 1. Synthesis of 4-pyridone series. Reagents and conditions: (a) 5 molar aqueous NaOH, THF, 60°C, 16 hrs, 89% yield; (b) $SOCl_2$, DMF (cat), 90°C, 3 hrs, 99% yield; (c) CuCN, LiCl, THF, –25°C to rt, 79% yield; (d) amine, 60°C, IPA, 16 hrs, (for R = Me, 91% yield): (e) DMF-DMA, 150°C,

90 minutes, (for R = Me, 76% yield); (f) *m*-CPBA, CH_2Cl_2, 30 minutes, rt; (g) Ar-NH_2, TFA, MeCN, 100^{o}C, 16 hrs (for R = Me and Ar = tetrahydroisoquinoline analog, two steps 65% yield).

2-Pyridone analogs of **4** were prepared as outlined in Scheme 2. The synthesis commences with either commercially available pyrimidine ester **15** or with pyrimidine ester **14**, the latter being obtained through the condensation of ethyl 3-oxopentanoate **12** with methyl carbamimidothioate **13** and DMF−DMA at elevated temperature. The esters were then converted to arylamides **16** *via* a three-step sequence involving saponification, acyl chloride formation then treatment with 2,6-dichloroaniline. The 2-pyridone ring can then be forged after treatment of compound **16** with Vilsmeier-Haack reagent **18** in DMF at 60°C. For **17b** a methyl group at *C*-8 was obtained but for **17a** an additional molecule of Vilsmeier-Haack reagent was added at *C*-8 to yield aldehyde **19**. This aldehyde can then act as an ideal functional handle to introduce various substituents for structure−activity studies near the bridged pyrimidine region as shown in Scheme 2. Finally the aminoaryl moiety was installed using the same synthetic sequence we described for the 4-pyridone series.

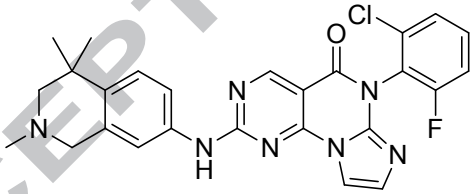
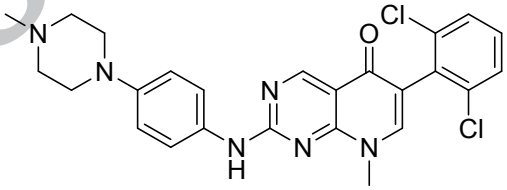
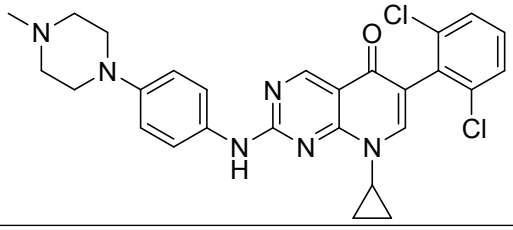


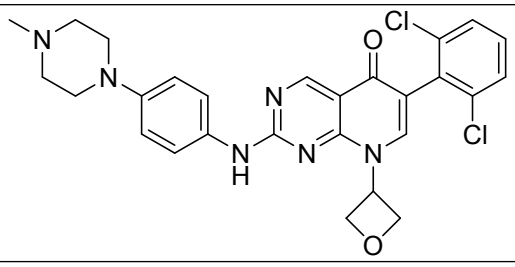
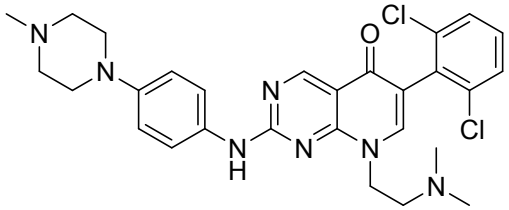
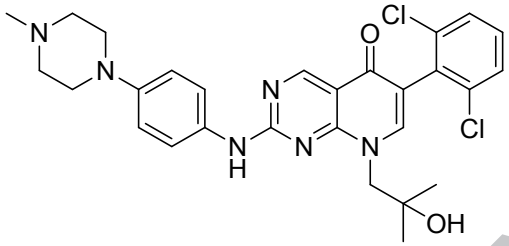
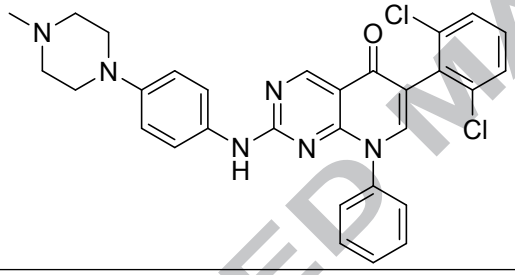
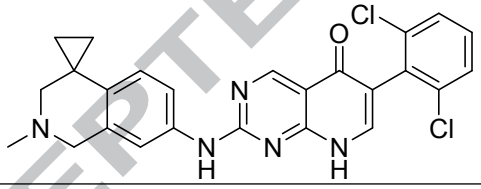
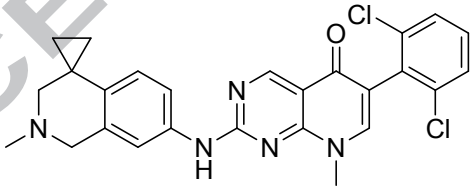
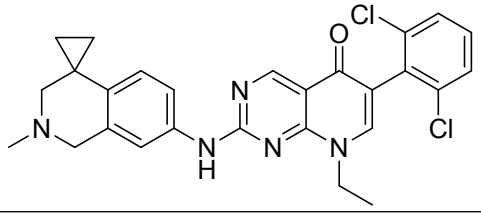
Scheme 2. Synthesis of 2-pyridone series. Reagents and conditions: (a) 13, DMF-DMA, 80°C, 10 minutes then methyl carbamimidothioate, reflux, 16 hrs, 52% yield; (b) 1 molar aqueous LiOH, MeOH, THF, rt, 2 hrs, (for **16**, 86% yield; for **15**, 74% yield); (c) $SOCl_2$, DMF (cat), dioxane, rt then 2,6-dichloroaniline, 100°C, 16 hrs, (for **17a**, 40% yield; for **17b**, 29% yield); (d) DMF, 60°C, 3 hrs, (for **19**, 29% yield, for **20**, 43%): (e) Ph_3PCH_3Br, KO*t*-Bu, THF, rt, 48 hrs, 62% yield; (f) $NaBH_4$, $CeCl_3$*$7H_2O$, CH_2Cl_2/MeOH (3:1), 0°C, 30 minutes, 70 % yield; (g) DAST, CH_2Cl_2, 3 hrs, rt, 61%

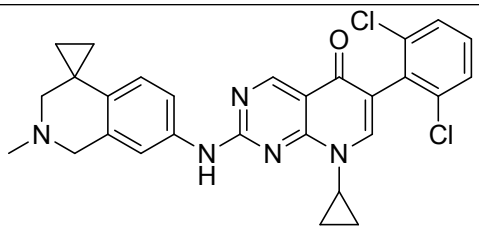
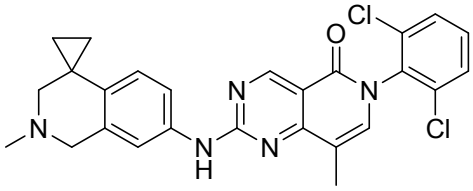
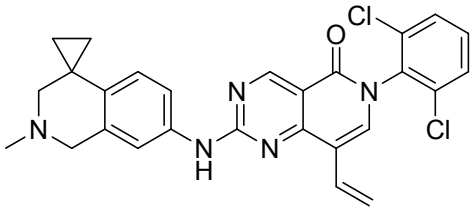
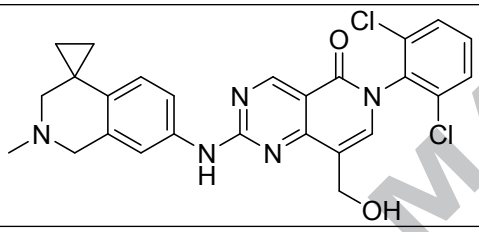
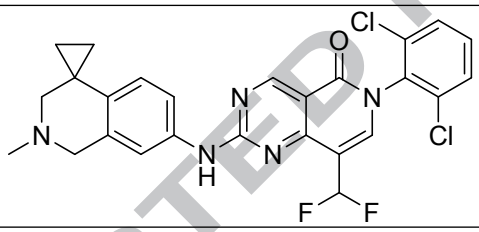
yield; (h) *m*-CPBA, CH_2Cl_2, 30 minutes, rt; (i) Ar-NH_2, TFA, MeCN, 100°C, 16 hrs. (for R = Me, two steps 81% yield)

Table 1 shows the structures of the compounds evaluated and their respective affinities presented as K_i values which was assessed in a routine 6-point TR-FRET binding assay. Activities in functional antiproliferative cell viability assays (H1299 cell line) and in a pCDK1 ELISA assay measuring the ability of Wee1 to phosphorylate CDK1 at Tyr 15, both utilizing the NCI-H1299 cell line (human non-small cell lung carcinoma), are also shown. In addition, mouse microsomal stability data (Cl(mic), L/hr/Kg) are also included in Table 1 for initial assessment of metabolic clearance.[16]

Table 1. Biaryl analogs.[17]

Compound	Structure	Ki (nM)	H1299 EC_{50} (μM)	pCDK1 EC_{50} (μM)	CL(mic) (L/hr/Kg) Mouse
2		1.1	0.31	0.060	38.1
26		0.3	0.52	0.079	16.2
27		1.0	0.46	0.022	34.9

28		1.3	1.72	0.324	34.9
29		24.4	7.65	2.85	118.0
30		0.2	>10	4.02	28.9
31		0.2	0.50	0.246	NA
32		0.6	0.33	0.029	31.9
33		0.2	0.10	0.011	24.8
34		0.3	0.21	0.019	NA

35		0.2	0.39	0.031	27.5
36		0.5	0.13	0.028	43.6
37		0.3	0.08	0.021	27.8
38		0.3	0.06	0.025	19.2
39		0.6	0.14	0.018	45.2

Most analogs yielded equal or better binding affinity than the lead molecule **2** suggesting that 1) the proposed cores can retain binding requirements to the protein similar to compound **1** and **2** and that 2) these biaryl systems are likely to participate in productive pi-pi interaction with Phe 433 as suggested by our docking studies.

Compounds **26-35** explored the effect of substituent variation at *N*-8 on binding affinity and cell antiproliferative activity for the 4-pyridone series. Computational modeling of compound **26**

predicted that a range of substitutions could be accommodated at *N*-8 since this vector is oriented towards an open area between Phe433 located at the base of the ATP binding site, and Val313 located in the Gly-rich loop (Figure 2).[18] The experimental results validated our computational model and showed that binding affinity remained largely unaffected when neutral, polar or hydrogen bond donor groups are present (compounds **26-28** & **30-35**). However, when a basic nitrogen was introduced (compound **29**) a significant reduction in binding affinity was observed. One hypothesis for the drop in affinity with amine **29** is that the amine substituent sits on a large hydrophobic patch with no polar residues to stabilize the net charge. Exploration of the *N*-8 substituent did confirm that this vector could be utilized for modulation of physicochemical properties as a range of cell permeability and microsomal clearance was obtained with variations of the stereoelectronic nature of the substituent. In general it was observed that small non-polar alkyl substituents offer the best balance of cell activity and compound stability. This can be seen with methyl analog **33** and ethyl analog **34** where both compounds compare well with first generation compound **2** in terms of binding affinity (K_i = *1*.1 nM vs 0.3 nM for **33**), cell viability (EC_{50} = 0.31 µM vs 0.10 µM for **33**) and in the PD assay pCDK1 ELISA (EC_{50} = 0.060 µM vs 0.011 µM for **33**). This establishes the 4-pyridone core as a suitable ring system for productive binding interaction in the active site of Wee1.

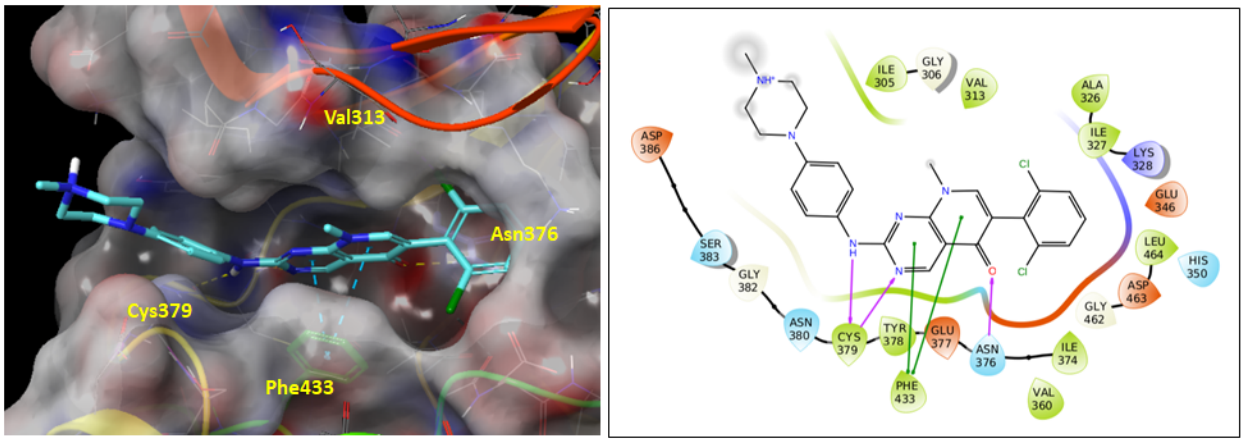


Figure 2. Structure and ligand interaction plot of compound **26** docked into the Wee1 kinase binding site. The model predicts two key hydrogen bond interactions with the backbone polar atoms of the hinge (Cys379) and an additional hydrogen bond interaction with the side chain of gate keeper residue Asn376 . The biaryl core is also predicted to engage in pi-pi interactions with Phe433.

Given the similar shape and electronic properties between 4-pyridones and 2-pyridones we decided to evaluate the latter to see if this new ring system could also be used as template for generation of potent Wee1 inhibitors. We determined that indeed the 2-pyridone analogs retain good enzymatic potency and cell potency. Both series offered a similar activity profile and the different chemical structures of the two could be utilized to introduce different yet complimentary substituents at position 8 of the bicyclic ring system.

Following these results, compound **33** was selected for advanced profiling. The compound showed desirable PK properties in mouse (*iv* $t_{1/2}$ = 2.7 h; *iv* CL = 1.5 L/h/kg; *po* AUC = 6.2 µM*h; *po* F = 44%) and was thus selected to study its ability to potentiate the efficacy of the DNA-

damaging agent irinotecan in BRCA1 deficient, p53 deficient MX-1 TNBC xenograft model. As shown in Figure 3, the combination of compound **33** with irinotecan achieved 88% tumor growth inhibition (TGI) when compared to vehicle and 57% TGI when compared to irinotecan alone on day 43. Moreover, the durability of the combination response was highlighted by 48% tumor growth delay when compared to irinotecan alone (using 1000 mm^3 as an endpoint) and therefore supports the enhanced efficacy of irinotecan by compound **33** *in vivo*. [19]

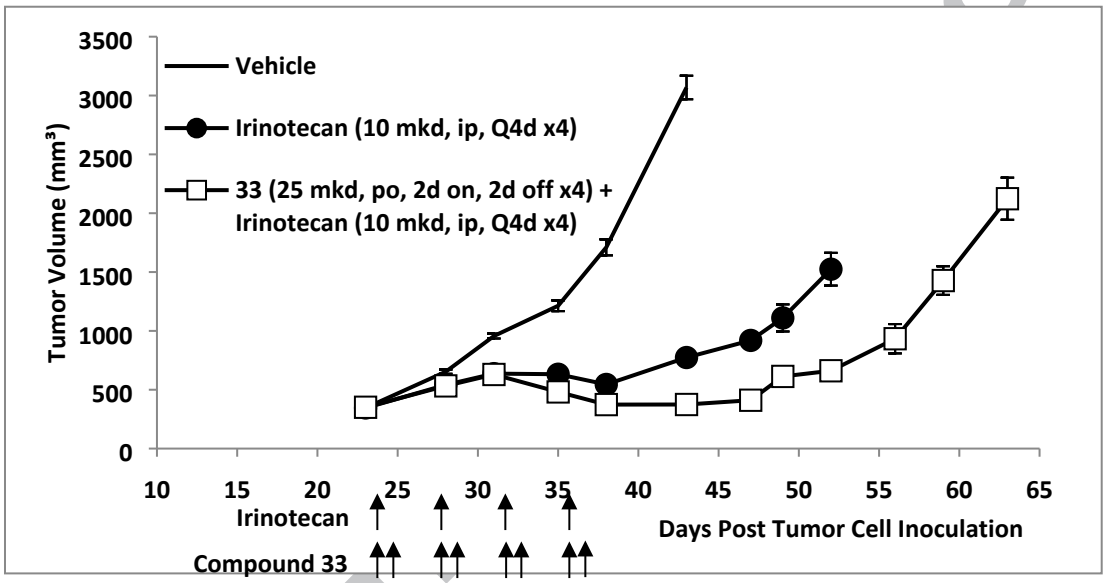


Figure 3. Tumor growth inhibition with compound **33** in combination with irinotecan.[20] MX-1 brei (homogenized tumor cell suspension) was sub-cutaneously injected into the flank of SCID mice. After the tumor volume reached ~350 mm^3, mice were treated with vehicle (black line), 10 mkd (mg/kg/day) irinotecan Q4Dx4 (closed circles), or 10 mkd irinotecan Q4Dx4 + 25 mkd compound **33** QD 2 days on 2 day off for 4 cycles (open squares). Dosing began on day 24. Tumor volumes were measured twice a week.

In summary, we designed and synthesized 3 new series of pyrimidine-based biaryl derivatives and evaluated their binding affinity and activity in cells. The data provided shows that both 4-pyridone and 2-pyridone cores are suitable replacements for the tricyclic core and allow for a new substitution pattern that can be used for evaluating substituents at position *C*-8 or *N*-8 of the bicyclic ring system. Our studies have also identified 4-pyridone **33** as a viable Wee1 inhibitor with improved *in vitro* activity in cell over benchmark compound **2**. This compound also exhibits good drug-like properties and potentiated the anti-proliferative activity of irinotecan *in vivo* when dosed orally in an MX-1 xenograft model. These findings suggest that pyridinone-based biaryl core such as **3** and **4** may serve as a suitable template for the development of potent and efficacious Wee1 inhibitors.

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Disclosures:

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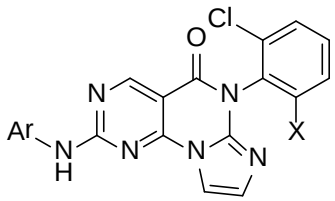
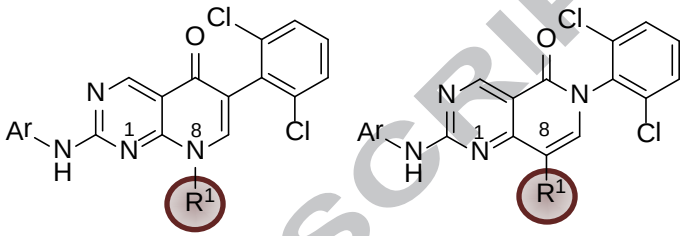
16. Mouse microsomal stability was used as a routine assay for translation of clearance into the efficacy models.
17. Note that the number of values for all data collected for each compounds were at least N=2.
18. The coordinates used for docking were obtained from the Protein Data Bank with the entry code 1X8B
19. For evidence of on-target activity see TR-FRET kinome profiling data of compound **33** in supporting information.
20. In house tumor efficacy studies showed that Wee1 inhibitor alone, in similar mouse xenograft tumor studies, shows only modest tumor growth inhibition thus supporting that Wee1 inhibition synergizes the action of Irinotecan. For an example of such studies see reference (14).

Proprietary benchmark series



X = H, F or Cl

New cores investigated



New cores for the development of potent and efficacious Wee1 inhibitors