Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 1931–1935
Bioorganic & Medicinal Chemistry Letters

Structure–activity relationships for 2-anilino-6-phenylpyrido[2,3-*d*]pyrimidin-7(8*H*)-ones as inhibitors of the cellular checkpoint kinase Wee1

Brian D. Palmer,[a,*] Jeff B. Smaill,[a] Gordon W. Rewcastle,[a] Ellen M. Dobrusin,[b] Alan Kraker,[b] Charles W. Moore,[b] Randall W. Steinkampf[b] and William A. Denny[a]

[a]*Auckland Cancer Society Research Centre, School of Medical Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand*

[b]*Pfizer Global Research and Development, Ann Arbor Laboratories, 2800 Plymouth Road, Ann Arbor, MI 48106-1047, USA*

Received 30 August 2004; revised 27 January 2005; accepted 28 January 2005

Abstract—A series of 2-anilino-6-phenylpyrido[2,3-*d*]pyrimidin-7(8*H*)-ones were synthesized and evaluated for their inhibitory properties against the non-receptor kinase c-Src and the G2/M checkpoint kinase Wee1. Overall, the compounds were 10–100-fold more potent inhibitors of c-Src than Wee1, and variation of substituents on the 6-phenyl ring did not markedly alter this preference. Solubilizing substituents off the 2-anilino ring in many cases increased Wee1 activity, thus lowering this preference to about 10-fold. 5-Alkyl substituted analogs were generally Wee1 selective, but at the expense of absolute potency.

The general class of 2-anilino-6-phenylpyrido[2,3-*d*]pyrimidin-7(8*H*)-ones are broad-spectrum inhibitors of a number of tyrosine kinase enzymes, including the receptor kinases EGFr (erbB1), PDGFr, FGFr, and non-receptor kinases such as c-Src. For example, compound **1** is reported[1] to have IC_{50} values for inhibition of substrate phosphorylation by isolated enzymes as follows; PDGFr = 79 nM, bFGFr = 43 nM, EGFr = 44 nM, and c-Src = 9 nM, with some other analogs being equally potent.[2] The breadth of this 'pan-kinase' activity is likely due to their action as competitive inhibitors at the ATP site,[3] where they are proposed[4] to form a key bidentate H-bond between the 3-aza and 2-NH atoms and a methionine residue (Met 341 in c-Src). Compound **1** also inhibited the formation of microcapillaries on Matrigel-coated plastic at 10 nM concentrations, and generated dose dependent inhibition of angiogenesis in vivo in mice when given orally at doses between 1 and 25 mg/kg.[5]

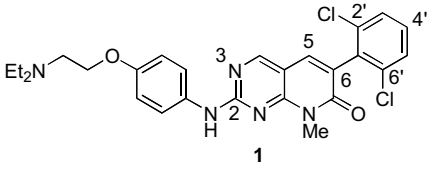
We identified **1** in a mass screen as a potent inhibitor (IC_{50} = 0.16 μM) of the Wee1 kinase.[6] This enzyme is involved in the maintenance of the G2/M checkpoint in the cell cycle, through its inhibitory phosphorylation of Cdc2 on Tyr-15. Normal cells arrest at both the G1/S and G2/M checkpoints in response to DNA damage caused by cytotoxic drugs or radiation, to allow time to either repair the DNA or to activate cell death pathways if this is not possible.[7,8] In contrast, many cancer cells lack a functional p53 gene, which means that the G1/S checkpoint is not functional.[8] Inhibitors of Wee1, via their abrogation of the G2/M checkpoint, were hypothesized to preferentially enhance the cytotoxic effects of DNA damaging agents on p53-negative cells. This was confirmed for **1**, which inhibited radiation-induced phosphorylation of Cdc2 at Tyr-15, and enhanced the effect of radiation selectively in p53-negative cells.[6,9]

In this paper we report the synthesis of a number of analogs of **1**, and the first SAR study of Wee1 inhibitors. A main aim of the study was to improve their selectivity for inhibition of Wee1 over c-Src, since inhibition of the latter has so many other cellular effects. Previous studies with related compounds showed that the selectivity for different enzymes can be significantly altered by the substituent pattern on the pendant 6-phenyl ring.

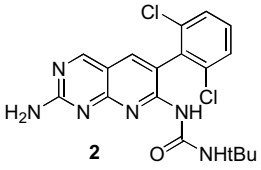
Keywords: Pyridopyrimidine; Checkpoint inhibitor; Wee1 kinase inhibitor.
* Corresponding author. Tel.: +64 9 373 7599; fax: +64 9 373 7502; e-mail: b.palmer@auckland.ac.nz

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.01.079

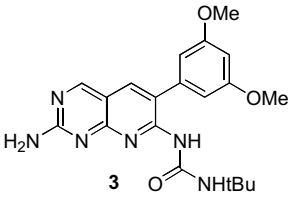
For example, in the related urea series, the 2′,6′-dichloro analog (**2**) is a potent inhibitor of both PDGFr (IC_{50} 1.1 μM) and FGFr (IC_{50} 0.13 μM), whereas the 3′,5′-diOMe analog (**3**) is selective for FGFr (IC_{50}s > 50 and 0.06 μM, respectively).[10] In the analogous napthyridine series,[11] unsubstituted 3-phenyl analogs were nonspecific inhibitors of c-Src, FGFr and PDGFr tyrosine kinases, whereas 2′,6′-dichloro analogs were most effective against c-Src and FGFr, and 3′,5′-dimethoxy derivatives showed high selectivity for FGFr alone (e.g., IC_{50}s 0.03 and >50 μM for **4** against FGFr and c-Src, respectively).



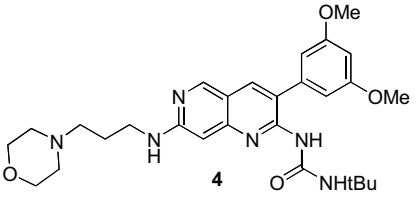
1



2



3

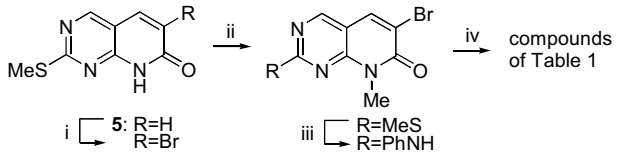


4

Most of the compounds were prepared from the known[12] 2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(1*H*)-one (**5**) (Method 1, Scheme 1), or from 4-(methylamino)-2-(methylsulfanyl)-5-pyrimidinecarbaldehyde (**6**) by reaction with various phenylacetonitriles, followed by oxidation of the methylsulfanyl group and subsequent displacement with anilines[13] (Method 2). In Method 1, different bases were used in the Suzuki coupling, depending on the reactivity of the arylboronic acid (i. K_2CO_3; ii. $Ba(OH)_2$; iii. CsF).

Further derivatization of some substituted 6-phenyl compounds, via standard synthetic methods, gave additional analogs (see footnotes to Table 1). Esters **48**–**50** were prepared from the acid **51** via activation with $SOCl_2$ and coupling with the appropriate alcohols (Method 3). The 5-methyl analogs (Table 3) were prepared as shown in Scheme 2 (Method 4). All compounds had satisfactory analyses, or MS/HPLC purity >95%. IC_{50} values for inhibition of Wee1[6] and c-Src[1] were determined by the published methods.

Table 1 gives data on the Wee1 and c-Src inhibitory properties of a wide range of phenyl-substituted compounds. The initial study was a comparison of various



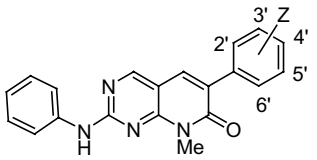
Scheme 1. Reagents: (i) NBS/DMF; (ii) Me_2SO_4/Cs_2CO_3; (iii) *m*-CPBA, then excess $PhNH_2$; (iv) $ArB(OH)_2/Pd(PPh_3)_4/AsPh_3$.

2′,6′-disubstituents against the 2′,6′-dichloro analog **7**, which is a moderate Wee1 inhibitor (IC_{50} 2.6 μM) but a much more potent c-Src inhibitor (IC_{50} 0.024 μM). A crystal structure of the related 2′,6′-dichloro urea analog **2**, which is substantially more potent than the unsubstituted compound, shows the phenyl ring essentially orthogonal to the chromophore.[10] Compounds **7**–**13** explore a range of symmetrically substituted compounds using substituents of varied electronic and steric properties. There were no consistent correlations between Wee1 or c-Src potencies, or Wee1/c-Src selectivity, and various measures of substituent electronic (σ), lipophilic (π) or steric (MR) properties. Extending this analysis to include the unsymmetrical 2′,6′-disubstituted compounds (**14**–**20**) did not change the result.

Using the 2′,6′-dichloro motif to anchor the phenyl ring orthogonal to the chromophore, compounds **21**–**41** explored SAR for a variety of additional substituents in the 3′- and 4′-positions. The 2′,6′-dichloro motif was chosen for ease of synthesis, and also because it was one of the most active. A few examples using the 2′,6′-diMe motif were also prepared, but these compounds were generally less active; compare **39**, **41** (Table 1) and **1**, **46** (Table 2). There were cases of improved comparative activity (**10**/**7** and **40**/**38**), but these pairs showed much lower absolute potencies. Calculations with the 3′- and 4′-substituted 2′,6′-dichloro analogs again showed no correlations between kinase potency or selectivity and measures of substituent lipophilic, electronic, or steric properties. In the 3′-substituted series (**21**–**30**), the 3-OH compound **27** stood out as being 35-fold more potent than the next most active compound against Wee1 (although it was also 10-fold more potent as a c-Src inhibitor as well). The remarkable contrast with the 3-CH_2OH and 3′-OMe analogs **23** and **28** suggests the likely existence of a geometrically constrained H-bond acceptor in this region of the protein. A similar but less striking pattern is shown by the 4′-OH and 4′-OMe analogs **32** and **34**. This suggests interaction with the same binding site, which is better positioned for the 3′- than the 4′-OH group.

Despite these exceptions, varying the substituents on the 6-phenyl ring did not generally significantly alter the selectivity of the compounds for Wee1 over c-Src. This

Table 1. 2-Anilino-6-phenylpyrido[2,3-*d*]pyrimidin-7(8*H*)-ones: variation of 6-phenyl substituents



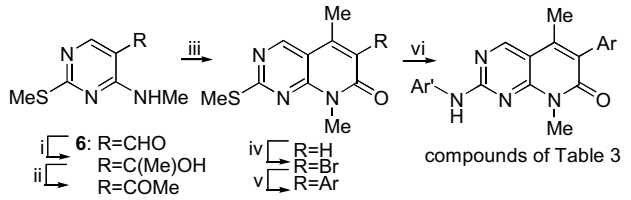
No.	Z	Mp (°C)	M[a]	IC_{50} (μM)[b]	
				Wee1	c-Src
7	2′,6′-DiCl	Ref. 1		2.6	0.024
8	2′,6′-DiF	267–269	1ii	9.7	0.066
9	2′,6′-DiBr	230–232	2	0.41	0.047
10	2′,6′-DiMe	207–209	1i	0.99	0.014
11	2′,6′-DiCF_3	246	2	41	8.1
12	2′,6′-DiOH	269–270	On **13**[c]	27	0.72
13	2′,6′-DiOMe	264–266	1iii	>50	0.98
14	2′-Cl, 6′-F	278–280	2	2.4	0.043
15	2′-Cl, 6′-Me	218–220	2	1.9	0.012
16	2′-Cl, 6′-CF_3	234–236	2	>50	0.21
17	2′-Cl, 6′-OMe	235–237	1ii	3.4	0.12
18	2′-Cl, 6′-OH	268–270	On **17**[c]	1.5	0.022
19	2′-Me, 6′-Br	224–228	On **9**[d]	4.5	0.058
20	2′-OMe, 6′-OH	251	On **13**[e]	11	0.05
21	2′,6′-DiCl, 3′-Cl	232	2	>50	0.27
22	2′,6′-DiCl, 3′-Me	243–244	2	50	2.8
23	2,6′-DiCl, 3′-CH_2OH	250–252	2	3.5	0.039
24	2′,6′-DiCl, 3′-CH_2NH_2	205–215	On **23**[f]	31	0.062
25	2,6′-DiCl, 3′-CO_2H	326–330	On **23**[g]	3.2	0.28
26	2′,6′-DiCl, 3′-$CONH_2$	180–190	On **25**[h]	8.6	0.13
27	2′,6′-DiCl, 3′-OH	300–304	On **28**[i]	0.074	0.009
28	2′,6′-DiCl, 3′-OMe	268–270	2	>50	0.54
29	2′,6′-DiCl, 3′-NH_2	284	On **30**[j]	2.6	0.09
30	2′,6′-DiCl, 3′-NHAc	160–163	2	>50	0.78
31	2′,6′-DiCl, 4′-Cl	239–241	2	8.6	0.33
32	2′,6′-DiCl, 4′-OH	318–321	On **34**[c]	0.22	0.073
33	2′,6′-DiMe, 4′-OH	286–288	On **35**[c]	0.58	0.017
34	2′,6′-DiCl, 4′-OMe	244–246	2	>50	6.5
35	2′,6′-DiMe, 4′-OMe	234	1i	>50	0.25
36	2′,6′-DiCl, 4′-NH_2	281–283	On **37**[j]	3.7	0.27
37	2′,6′-DiCl, 4-NHAc	313–315	2	36	1.5
38	2′,6′-DiCl, 3′,5′-diOMe	323–325	2	>50	>50
39	2′,6′-DiCl, 3′,5′-diOH	318–321	On **38**[k]	0.14	0.011
40	2′,6′-DiMe, 3′,5′-diOMe	255–258	2	33	39
41	2′,6′-DiMe, 3′,5′-diOH	288–291	On **40**[k]	1.0	0.11

[a] Method of synthesis; see text.
[b] IC_{50} values were determined for both Wee1[6] and c-Src[1] inhibition by the published methods cited. Values are the average of two or more independent determinations, with a variance of ±20%.
[c] 6 equiv BBr_3/DCM/reflux/16 h.
[d] $(CH_3)_4Sn/Pd(PPh_3)_4$/DMF/100 °C/3 h.
[e] 3 equiv BBr_3/DCM/rt/2 h.
[f] (i) PPh_3Cl_2/Et_3N/DCM/2 h, (ii) $NH_{3(g)}$/*i*-PrOH/110 °C/18 h.
[g] Jones reagent/acetone/2 h.
[h] (i) $SOCl_2$/cat. DMF/reflux/3 h, (ii) c. NH_3/THF.
[i] Pyridine·HCl/200 °C/1 h.
[j] Concd HCl/reflux/1 h.
[k] Pyridine·HCl/200 °C/10 min.

is evident from the overall relationship between these two activities for the compounds of Table 1 for which both IC_{50} values were available

$$\log IC_{50}\ (\text{Wee1}) = 0.75(\pm 0.14) \log IC_{50}\ (\text{Src}) + 1.27(\pm 0.14)$$
$$[n = 26, r = 0.74, F = 30].$$

Work in related series of compounds[10,11] has shown that solubilizing substituents off the 2-anilino ring in many cases provide substantial increases in potency, and this was explored in the 6-phenylpyrido[2,3-*d*]pyrimidin-7(8*H*)-ones with the compounds of Table 2 (*ortho*- and *meta*- X-substituted compounds were also evaluated, but were less active than the *para*-substituted compounds reported; data not shown). Compounds **42**–**45**



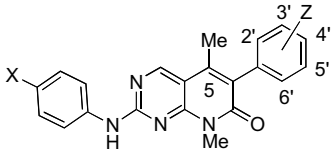
Scheme 2. Reagents: (i) MeMgBr/THF; (ii) MnO_2; (iii) $(EtO)_2P(O)CH_2CO_2Et$/NaH; (iv) NBS/$(PhCO_2)_2$; (v) $ArB(OH)_2$/$Pd(PPh_3)_4$/$AsPh_3$/$Ba(OH)_2$; (vi) *m*-CPBA, then $Ar'NH_2$.

use neutral amide side chains, and were about fivefold more potent than **7** against Wee1 with little change in c-Src potency, improving the ratio from about 100-fold to only about 10-fold in favor of c-Src. Compounds **1**, **46**–**50** explored cationic side chains of varying pK_a, but while in general these showed increased Wee1 potency, c-Src activity also increased. The anionic analogs **51**–**53** showed better ratios, with the butyric acid **51** being equally potent toward both Wee1 and c-Src in the enzyme inhibition assay. Compounds **54**–**57** incorporate a cationic or anionic side chain with the most potent of the 6-phenyl ring substitution patterns, but comparison of **54**, **55** with **27**, and **56**, **57** with **33**, suggests the motifs are not additive.

Finally, in Table 3 we explore a limited series of 5-methyl substituted compounds. Difficulties of synthesis precluded the preparation of all but a few analogs (2′,6′-dichloro compounds could not be obtained), but for the 2′-Cl series that could be prepared the 5-methyl group clearly distinguishes in favor of Wee1 over c-Src. This is less clear in the 2′,6′-diMe series, but even the cationic analog **62** has a Wee1/c-Src IC_{50} ratio of 1.5-fold, compared with 43-fold for the corresponding 5-H analog **46**.

The primary finding from this study is that variation of the substituents on the 6-phenyl ring does not significantly improve the selectivity of the compounds for Wee1 over c-Src, which was a primary goal. Solubilizing substituents off the 2-anilino ring in many cases did provide substantial increases in potency for Wee1, with an overall improvement of the Wee1/c-Src IC_{50} ratio to about 10-fold, but few compounds were better than this. 5-Alkyl substitution did improve the ratio, but at the ex-

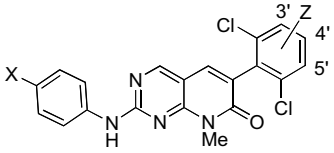
Table 3. 2-Anilino-5-methyl-6-phenylpyrido[2,3-*d*]pyrimidin-7(8*H*)-ones



No.	X	Z	Mp (°C)	M[a]	IC_{50} (μM)[b]	
					Wee1	c-Src
58	H	H	250–253	4	>50	>50
59	H	2′-Cl	258–262	4	0.41	>50
60	$O(CH_2)_2NEt_2$	2′-Cl	182–184	4	0.55	>50
61	H	2,6′-DiMe	215–217	4	1.2	15
62	$O(CH_2)_2NEt_2$	2′,6′-DiMe	155–159	4	0.54	0.36

[a,b] As for Table 1.

Table 2. 2-Anilino-6-phenylpyrido[2,3-*d*]pyrimidin-7(8*H*)-ones: solubilizing substituents



No.	X	Z	M[a]	Mp °C	IC_{50} (μM)[b]	
					Wee1	c-Src
42	CH_2CONH_2	H	2	268–270	0.12	0.013
43	$(CH_2)_2CONH_2$	H	2	141–144	0.19	0.030
44	$(CH_2)_4CONH_2$	H	2	188–193	0.26	0.028
45	OCH_2CONH_2	H	2	199–201	0.25	0.014
1	$O(CH_2)_2NEt_2$	H		Ref. 1	0.165	0.007
46[c]	$O(CH_2)_2NEt_2$	H[c]	2	122–125	0.99	0.023
47	$O(CH_2)_3CO_2H$	H	2	146–149	0.086	0.004
48	$(CH_2)_3CO_2(CH_2)_2$Nmorph	H	3	152	0.095	0.004
49	$(CH_2)_3CO_2(CH_2)_2NMe_2$	H	3	143–146	0.124	0.007
50	$(CH_2)_3CO_2(CH_2)_2$Npip	H	3	147–149	0.142	0.011
51	$(CH_2)_3CO_2H$	H	2	231–237	0.032	0.032
52	$CH_2CH(NH_2)CO_2H$	H	2	242	0.09	0.009
53	$(CH_2)_3$tetrazole	H	2	178	0.069	0.025
54	$O(CH_2)_2NEt_2$	3′-OH	2	180–186	0.15	0.006
55	$O(CH_2)_3CO_2H$	3′-OH	2	185–192	0.04	0.099
56	$O(CH_2)_2NEt_2$	4′-OH	2	220–228	0.08	0.008
57	$O(CH_2)_3CO_2H$	4′-OH	2	210–212	0.04	0.006

[a,b] As for Table 1.

[c] 2′,6′-Dimethyl derivative.

pense of overall lower potency. Selected representative analogs also showed potent inhibition of other kinases, including Chk1, PhosK, and PKC (data not shown).

References and notes

1. Klutchko, S. R.; Hamby, J. M.; Boschelli, D. H.; Wu, Z.; Kraker, A. J.; Amar, A. M.; Hartl, B. G.; Shen, C.; Klohs, W. D.; Steinkampf, R. W.; Driscoll, D. L.; Nelson, J. M.; Elliott, W. L.; Roberts, B. J.; Stoner, C. L.; Vincent, P. W.; Dykes, D. J.; Panek, R. L.; Lu, G. H.; Major, T. C.; Dahring, T. K.; Hallak, H.; Bradford, L. A.; Showalter, H. D. H.; Doherty, A. M. *J. Med. Chem.* **1998**, *41*, 3276.
2. Kraker, A. J.; Hartl, B. G.; Amar, A. M.; Barvian, M. R.; Showalter, H. D. H.; Moore, C. W. *Biochem. Pharmacol.* **2000**, *60*, 885.
3. Panek, R. L.; Lu, G. H.; Klutchko, S. R.; Batley, B. L.; Dahring, T. K.; Hamby, J. M.; Hallak, H.; Doherty, A. M.; Keiser, J. A. *J. Pharm. Exp. Ther.* **1997**, *283*, 1433.
4. Trumpp-Kallmeyer, S.; Rubin, J. R.; Humblet, C.; Hamby, J. M.; Showalter, H. D. H. *J. Med. Chem.* **1998**, *41*, 1752.
5. Dimitroff, C. J.; Klohs, W.; Sharma, A.; Pera, P.; Driscoll, D.; Veith, J.; Steinkampf, R.; Schroeder, M.; Klutchko, S.; Sumlin; Henderson, B.; Dougherty, T. J.; Bernacki, R. J. *Invest. New Drugs* **1999**, *17*, 121.
6. Wang, Y.; Li, J.; Booher, R. N.; Kraker, A.; Lawrence, T.; Leopold, W. R.; Sun, Y. *Cancer Res.* **2001**, *61*, 8211.
7. Hartwell, L. H.; Weinert, T. *Science* **1989**, *124*, 629.
8. Levine, A. J. *Cell* **1997**, *88*, 323.
9. Li, J.; Wang, Y.; Sun, Y.; Lawrence, T. S. *Radiat. Res.* **2002**, *157*, 322.
10. Hamby, J. M.; Connolly, C. J.; Schroeder, M. C.; Winters, R. T.; Showalter, H. D. H.; Panek, R. L.; Major, T. C.; Olsewski, B.; Ryan, M. J.; Dahring, T.; Lu, G. H.; Keiser, J.; Amar, A. M.; Shen, C.; Kraker, A. J.; Slintak, V.; Nelson, J. M.; Fry, D. W.; Bradford, L.; Hallak, H.; Doherty, A. M. *J. Med. Chem.* **1997**, *40*, 2296.
11. Thompson, A. M.; Connolly, C. J.; Hamby, J. M.; Boushelle, S.; Hartl, B. G.; Amar, A. M.; Kraker, A. J.; Driscoll, D. L.; Steinkampf, R. W.; Patmore, S. J.; Vincent, P. W.; Roberts, B. J.; Elliott, W. L.; Klohs, W.; Leopold, W. R.; Showalter, H. D. H.; Denny, W. A. *J. Med. Chem.* **2000**, *43*, 4200.
12. Barvian, M.; Boschelli, D.; Cossrow, J.; Dobrusin, E.; Fattaey, A.; Fritsch, A.; Fry, D.; Harvey, P.; Keller, P.; Garrett, M.; La, F.; Leopold, W.; McNamara, D.; Quin, M.; Trumpp-Kallmeyer, S.; Toogood, P.; Wu, Z.; Zhang, E. *J. Med. Chem.* **2000**, *43*, 4606.
13. Blankley, C. J.; Boschelli, D. H.; Doherty, A. M.; Hamby, J. M.; Klutchko, S.; Panek, R. L. U.S. Patent 5,620,981, 1998. *Chem. Abstr.* **1998**, *128*, 257440.