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2-Aminoquinazoline inhibitors of cyclin-dependent kinases

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Abstract—The inhibition of cyclin-dependent kinase 4 (Cdk4) causes cell cycle arrest and restores a checkpoint that is absent in the majority of tumor cells. Compounds that inhibit Cdk4 selectively are targeted for treating cancer. Appropriate substitution of 2-aminoquinazolines is demonstrated to produce high levels of selectivity for Cdk4 versus closely related serine-threonine kinases.

Cyclin-dependent kinases (cdks) are key regulatory components of the cell division cycle.[1–5] As such they represent potential molecular targets for new antiproliferative agents that may be used to treat cancer or other proliferative diseases. Of the cell cycle associated Cdks, Cdk4 especially has been targeted due to its fundamental role in the response to growth signals and the initiation of cell division.[6,7] Expression of the regulatory protein, cyclin D, is increased in response to growth factor signaling, resulting in the activation of Cdk4 kinase activity and sequestration of the Cdk inhibitor protein p27. The major substrate for phosphorylation by Cdk4 is the retinoblastoma protein (pRb). pRb is phosphorylated first by Cdk4/cyclin D and subsequently by Cdk2/cyclin E. Hyperphosphorylated pRb dissociates from the E2F/DP transcription factor complex, releasing it to transactivate the expression of genes essential for DNA synthesis including dihydrofolate reductase, thymidylate synthase, and RNA polymerase.[8,9] This pathway, the so-called Rb pathway, represents a key regulatory pathway controlling cell proliferation. The importance of this pathway is underscored by the observation that almost 100% of human tumors possess some defect in this regulatory function.[10] These defects manifest as mutation or deletion of pRb, overexpression of cyclin D, activating mutations in Cdk4, or loss of the Cdk4 inhibitor protein, p16. Biochemical experiments with dominant negative cyclin D, or cyclin D antisense, and experiments to replace p16 through adenoviral expression, all indicate that restoration of integrity to the Rb pathway can inhibit tumor cell proliferation.[6] On the basis of these results, inhibitors of Cdk4 are expected to be good candidates for antitumor therapy.[11–15]

Our interest in finding inhibitors of Cdk4/cyclin D led us to investigate a series of 2, 7, 8-substituted quinazolines. These compounds were modeled directly on our successful inhibitors from a series of pyrido[2,3-*d*]pyrimidin-7-ones that have been reported earlier.[16–21] Our anticipation was that the quinazolines would display a similar structure–activity relationship (SAR) profile to the pyrido[2,3-*d*]pyrimidin-7-ones, but might offer a different profile of physicochemical and pharmacokinetic properties.

A survey of published procedures for the preparation of appropriately substituted quinazolines rapidly led us to the conclusion that it would be necessary to develop a new route to the compounds that we were targeting. This route has been outlined in a previous communication.[22] The conversion of intermediate quinazolines **1**, wherein R^1 and R^2 may be hydrogen or methyl, and R^3 is a cyclopentyl or an isopropyl group, to final compounds containing appropriate side chains at the C-2

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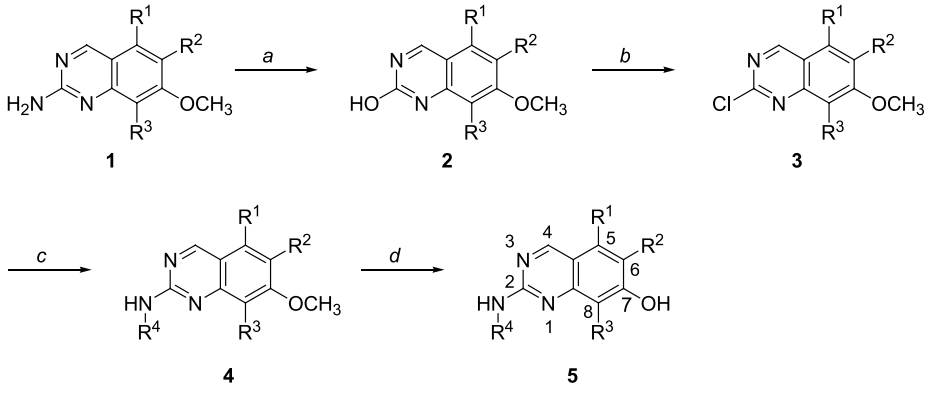
position was achieved as shown in Scheme 1. Diazotization and hydrolysis of the amine function at C-2 in compounds **1**, provided hydroxyquinazolines **2**. Chlorination with $POCl_3$ produced coupling partners **3** that allowed the introduction of a variety of amine groups at the C-2 position, albeit under relatively forcing conditions (heating to 110 °C in a sealed tube). Where necessary, the side chain protecting group in product **4** (typically a Boc group) was removed using standard procedures (e.g., TFA). Demethylation of the C-7 methyl ether was effected using sodium ethanethiolate to provide the phenols **5** (see Scheme 1).

Our initial expectation was that the quinazoline series of compounds would bind to the ATP site of Cdks in a similar manner to the pyrido[2,3-*d*]pyrimidin-7-ones we reported previously.[16] Therefore, our SAR studies began with an exploration of substituted aryl amine groups appended to the C-2 position (Table 1). These compounds were prepared with either a cyclopentyl group or an isopropyl group attached to C-8 since these two groups were anticipated to provide a good balance of potency and drug-like physical properties for this chemical series. Similarly, by extension from the related pyrido[2,3-*d*]pyrimidin-7-one series, electron-rich *para*-substituted aniline side chains at the C-2 position were anticipated to provide the most potent Cdk4 inhibitors. Thus, the initial choice of target compounds was very much grounded in experience obtained previously in a related chemical class bearing a similar substitution pattern, and was not influenced by SAR from previously disclosed 4-anilinoquinazoline Cdk inhibitors.[23]

As seen in Tables 1 and 2, the unsubstituted anilines themselves (compounds **6** and **15**) were quite potent inhibitors of Cdk4 with IC_{50} values of 0.125 and 0.166 μM, respectively.[24] Introduction of a pyrrolidine ring at the *para*-position of the aniline was detrimental to inhibition of Cdk4 with the potency of compound **16** dropping by an order of magnitude compared to compound **15**. In contrast, incorporation of a piperidine at the same position (**9**, **18**) led to an increase in potency against Cdk4 with the cyclopentyl derivative (**18**) displaying an $IC_{50} = 0.011$ μM. The difference between the potencies for the pyrrolidine and piperidine analogs may reflect better Van der Waals contacts between the six-membered ring and the protein at the mouth of the ATP binding site, and a steric clash between the protein and the pyrrolidine as a consequence of the lower conformational flexibility of this ring. The morpholine analogs (**10**, **19**), were comparable to the piperidines, however, a substantial further increase in potency against Cdk4 was observed in switching to the piperazine derivatives (**12**, **20**). This increase in potency does not appear to be associated with an ionic interaction between the protein and a positively charged piperazine nitrogen because acylation of the piperazine secondary amine with either acetate (**13** and **22**) or glycine (**14** and **23**) had no significant effect on the potency of Cdk4 inhibition (less than 2-fold difference in IC_{50} values). Similarly, fluorination of the aromatic ring had no detrimental effect on Cdk4 inhibition. The 3′-aminopyrrolidine analog that bears an isopropyl substituent at C-8 (compound **8**: $IC_{50} = 0.011$ μM) is equipotent with the isomeric piperazine (compound **11**: $IC_{50} = 0.016$



Scheme 1. Synthetic route used to produce quinazoline Cdk inhibitors. R^1, R^2, and R^3 are H or methyl. R^3 is isopropyl or cyclopentyl. R^4 substituents are illustrated in Figure 1. Reagents: (a) tetrafluoroboric acid, $NaNO_2$, (b) $POCl_3$, (c) R^4-NH_2, CH_3CN, sealed tube 110 °C, (d) EtSNa.

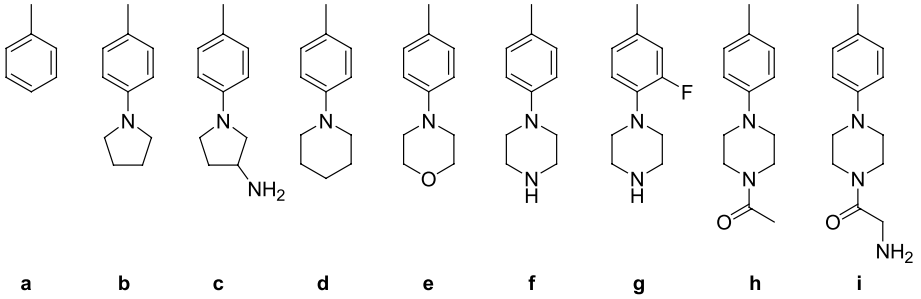
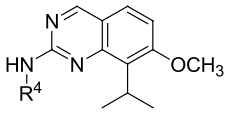


Figure 1. Side chains used at position R^4.

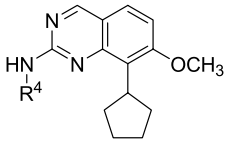
Table 1. SAR at the C-2 position when the N-8 substituent is isopropyl



	R^4	IC_{50} (μM)			
		CDK1/B	CDK2/A	CDK2/E	CDK4/D
6	**a**	1.04	0.253	1.08	0.125
7	**b**	>5	1.60	>5	0.560
8	**c**	1.30	0.296	2.55	0.011
9	**d**	>5	0.410	3.10	0.052
10	**e**	1.97	0.665	4.4	0.052
11	**f**	0.506	0.185	1.20	0.016
12	**g**	0.743	0.141	0.655	0.007
13	**h**	NA	NA	0.900	0.025
14	**i**	0.800	0.191	1.10	0.010

For an explanation of the R^4 substituents see Figure 1.
NA, not available.

Table 2. SAR at the C-2 position when the N-8 substituent is cyclopentyl



	R^4	IC_{50} (μM)			
		CDK1/B	CDK2/A	CDK2/E	CDK4/D
15	**a**	0.801	0.202	0.650	0.166
16	**b**	>5	>5	>5	1.75
17	**c**	0.398	0.203	0.775	0.115
18	**d**	>5	0.384	>5	0.011
19	**e**	1.59	0.384	0.650	0.018
20	**f**	0.132	0.028	0.250	0.001
21	**g**	0.214	0.062	0.300	0.004
22	**h**	>5	NA	0.265	0.002
23	**i**	0.270	0.095	0.450	0.002

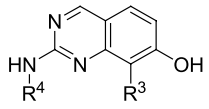
For an explanation of the R^4 substituents see Figure 1.
NA, not available.

μM). However, in the C-8 cyclopentyl series, the 3′-aminopyrrolidine **17** is significantly less potent than the isomeric piperazine **20**. In both the series the, aminopyrrolidine is more potent than the pyrrolidine. Compounds **16** and **17** are the only two C-8 cyclopentyl quinazolines that are less potent inhibitors of Cdk4 than the corresponding C-8 isopropylquinazoline analogs. The reason for this change in potency order is unclear. Overall, as in the pyrido[2,3-*d*]pyrimidin-7-one series, a C-2 side chain containing a *para*-piperazinyl aniline is preferred for potent inhibition of Cdk4. Consistent with these SAR trends, a crystal structure of compound **11** bound to Cdk2 showed the ligand located in the ATP-binding site in a similar orientation to the corresponding pyrido[2,3-*d*]pyrimidin-7-one.[16]

The methyl ether at C-7 in the compounds described here was considered a poor replacement for the amide carbonyl in the corresponding pyrido[2,3-*d*]pyrimidin-7-one series. Consequently, several analogs were prepared in which the methyl ether was cleaved to the parent phenol. A comparison of these phenols with their methyl ether counterparts revealed that the phenols were generally at least as potent as the corresponding ethers (Table 3). For example, compounds **25**, **26**, and **27**, within experimental error, were all as potent as the most potent methyl ether (Compound **20**, Table 2). In fact, these compounds may actually be more potent than they appear since their IC_{50} values approach the concentration of enzyme used in the assay. Thus, the compounds are essentially titrating the enzyme, which creates a minimum IC_{50} despite any further increase in affinity.

Besides potent inhibition of Cdk4, another goal of this program was to obtain compounds that are selective for Cdk4 versus other kinases, including other Cdks. Compounds **26** and **27** are both around 100-fold selective for Cdk4 versus Cdk2A, an enzyme against which

Table 3. SAR for C-7 hydroxyquinazolines



	R^3	R^4	IC_{50} (μM)			
			CDK1/B	CDK2/A	CDK2/E	CDK4/D
24	2-Propyl	**a**	0.813	0.250	0.550	0.325
25	Cyclopentyl	**e**	1.94	0.18	0.635	0.003
26	Cyclopentyl	**f**	0.030	0.082	0.320	0.001
27	Cyclopentyl	**g**	0.761	0.112	0.270	0.001

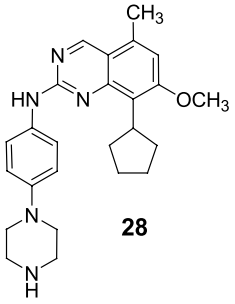
For an explanation of the R^4 substituents see Figure 1.

it is especially difficult to achieve selectivity with these ATP competitive inhibitors. Notably, the phenols appear to be slightly more selective for Cdk4 than the corresponding ethers by 3- to 8-fold.

To further improve the selectivity for Cdk4 versus other kinases, substitution at C-5 was examined. Once again the impetus for these changes was the increase in selectivity observed with C-5 methyl pyrido[2,3-*d*]pyrimidin-7-ones versus their unsubstituted homologs. C-5 substituted quinazolines were obtained following the chemistry outlined previously and in Scheme 1 above starting with dimethoxy toluene. The C-5 methyl derivatives **28** and **29** were both less potent than their C-5 H counterparts, however, compound **28** was still an effective inhibitor of Cdk4 and was quite selective for Cdk4 versus Cdk2A, Cdk2E, and Cdk1B. In contrast, compound **29** was a significantly less active inhibitor of Cdk4, possibly highlighting a size limitation in the ATP-binding site that restricts access to a compound with substitution both on the quinazoline ring and on the side chain piperazine. The C-7 phenol analog of **28**, compound **30**, was about 2-fold more potent an inhibitor of Cdk4 and similarly selective versus other cyclin-dependent kinases. Compound **30** displays similar potency and enzyme selectivity to the corresponding pyrido[2,3-*d*]pyrimidin-7-one analog, compound **31**. In addition, compound **30** displays antiproliferative activity in HCT-116 human carcinoma cells with IC_{50} = 100 nM. Notably, however, the quinazolines are generally more lipophilic and less soluble (in pH 6.5 phosphate buffer) than the corresponding pyrido[2,3-*d*]pyrimidin-7-one analogs as illustrated by the representative data provided in Table 4. Thus, while differing from the pyrido[2,3-*d*]pyrimidin-7-ones in physical properties, the quinazoline series does not offer any obvious advantages.

In conclusion, a series of 2, 7, 8-substituted quinazolines has been shown to inhibit cyclin-dependent kinases with a similar SAR to that displayed by a related series of pyrido[2,3-*d*]pyrimidin-7-ones. This observation enabled the rapid identification of potent and selective Cdk4 inhibitors with activity in cell-based assays.

Table 4. C-5 methylquinazolines



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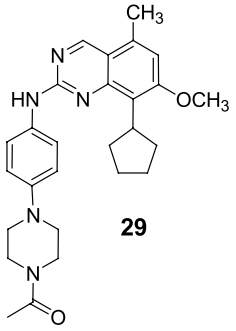

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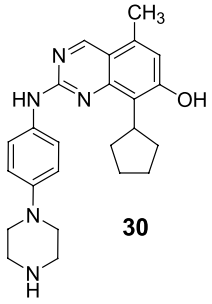

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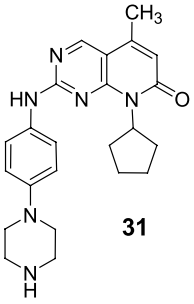

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	IC_{50} (μM)				c Log *P*	Log *D*(pH 7.4)	Solubility (μg/mL)
	CDK1/B	CDK2/A	CDK2/E	CDK4/D			
28	>5	>5	>5	0.055	5.86	2.32	2
29	>5	>5	>5	4.13	5.46	3.77	NA
30	>5	>5	>5	0.020	5.96	1.39	1
31	>5	>5	>5	0.014	3.52	0.63	13

NA, not available.

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