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Pyrazolo[1,5-*a*]pyrimidines as orally available inhibitors of cyclin-dependent kinase 2☆

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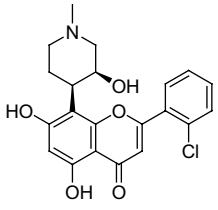
Abstract—Properly substituted pyrazolo[1,5-*a*]pyrimidines are potent and selective CDK2 inhibitors. Compound **15j** is orally available and showed efficacy in a mouse A2780 xenograft model.

One of the characteristics of cancer is uncontrolled cell growth and proliferation. Cyclin-dependent kinases (CDKs) are key regulators of the cell cycle[1] and the proper regulation of CDK activity is crucial for the ordered execution of the phases of the cycle. A large number of human neoplasias show overexpression of positive regulators of CDKs and/or decrease in negative regulators.[2] Abnormal expression of CDK2/cyclin E has been detected in colorectal, ovarian, breast, and prostate cancers.[3] CDK inhibitors have been shown to induce apoptosis in different tumor cell lines.[4] Therefore, CDK inhibitors have the potential to enlarge the group of anticancer agents.

A number of more or less selective CDK inhibitors have been described in the literature[5]; those undergoing clinical trials are flavopiridol (**1**),[6] roscovitine (**2**),[7] and BMS 387032 (**3**).[8] Recently, an article on a series of pyrazolo[1,5-*a*]pyrimidines (**4**) (with amines linked through NH or O at the 5-position and arylsulfones at the 7-position NH) possessing CDK2 inhibitory activity has been published.[9] Herein, pyrazolo[1,5-*a*]pyrimidines with benzylic substituents at the 7-position are described. The selectivity and pharmacokinetic profiles of these compounds are significantly different from those with *N*-aryl substitution at the 7-position.[9]



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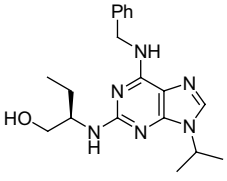

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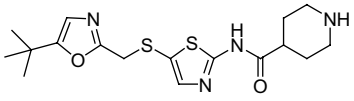

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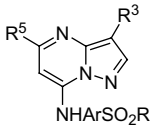

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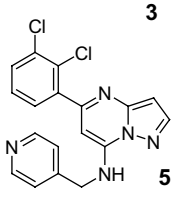

5

Keywords: CDK2; Kinase; Inhibitors; Pyrazolo[1,5-*a*]pyrimidine.
☆ The coordinates of compound **13** bound to CDK2 have been deposited in the Protein Databank pdb ID 2R3Q.
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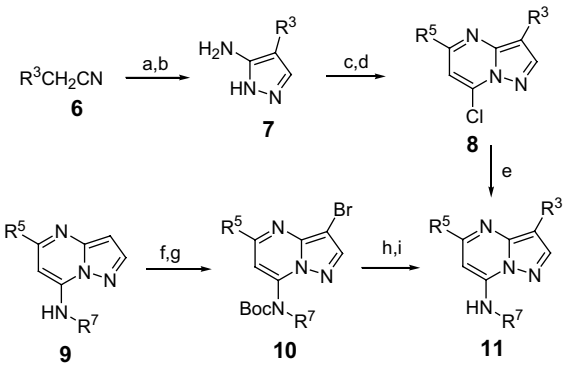


Scheme 1. Reagents: (a) HCO_2Et, *t*-BuOK, THF; (b) N_2H_4, AcOH, EtOH; (c) $R^5COCH_2CO_2Me$, $PhCH_3$; (d) $POCl_3$, *N*,*N*-dimethylaniline; (e) R^7NH_2, DIPEA, dioxane; (f) Boc_2O, DMAP, CH_2Cl_2; (g) NBS, CH_3CN; (h) $R^3B(OH)_2$, $Pd[PPh_3]_4$, Na_2CO_3, DME, H_2O or R^3SnBu_3, $Pd[PPh_3]_4$, dioxane; (i) TFA, CH_2Cl_2.

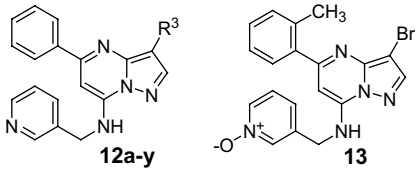
Our effort had started by identification of a relatively weak inhibitor **5** (CDK2/cyclinA IC_{50} = 500 nM). A number of pyrazolo[1,5-*a*]pyrimidines were synthesized from appropriately substituted acetonitriles and β-keto-esters as shown in Scheme 1. Desired substitution at the 3-position was achieved by choosing properly substituted acetonitriles **6** or via Pd-catalyzed coupling of intermediate **10**.

Incorporation of halogens and other small substituents at the 3-position resulted in significant improvement of potency (Table 1). Most active compounds were selective against GSK3β and MAPK kinases. **12b** exhibited activity in cells (measured by incorporation of radiolabeled thymidine) with IC_{50} = 350 nM. The X-ray crystal structure of **13** in CDK2 (without cyclin) given in Figure 1 is consistent with the observed SAR-only a relatively small cavity occupied by 3-substituents is available in the vicinity of Phe 80; (**13** CDK2/cyclinA IC_{50} = 49 nM). Thus, only small non-polar substituents (H, Br, Me, Et, *c*-Pr, and SCH_3) were tolerated; incorporation of large (Ph, Bn) or polar (NO_2, CH_2OH) motifs resulted in a sharp drop of activity.

Exploration of the 5-position led to a variety of inhibitors whose IC_{50}s were below 50 nM (Table 2). A somewhat greater differentiation was noted in the cell-based assay, where the compounds with relatively non-polar substituents showed best potency. Notable exceptions are piperidine-containing compounds **14l** and **14m**; the presence of the piperidine moiety, however, resulted in somewhat inconsistent SAR across the series. **14g**, **14h**, **14i**, and **14n** were prepared from 5,7-dichloro[1,5-*a*]pyrimidine by sequential displacements at the 7-position with 3-(aminomethyl)pyridine and at the 5-position with the corresponding nucleophiles followed by bromination with NBS.

A variety of substituents were tolerated at the 7-position (Table 3), which is close to the solvent-exposed part of the enzyme. The best cell activity (measured by radiolabeled thymidine uptake) was noted for the subclass containing pyridines and pyridine-*N*-oxides. In addition, unlike the aniline series,[9] those compounds exhibited good oral PK profile.

Table 1. CDK2 inhibitory activity of pyrazolo[1,5-*a*]pyrimidines **12a**–**12y**



Compound	R^3	CDK2/cyclin A IC_{50} (μM)	GSK3β IC_{50} (μM)	MAPK IC_{50} (μM)
12a	H	0.25	—	—
12b	Br	0.011	0.57	0.37
12c	Me	0.072	—	1.40
12d	Et	0.008	2.80	2.00
12e	Pr	0.890	—	—
12f	Bu	1.20	—	—
12g	Ethynyl	0.048	9.82	7.48
12h	Vinyl	0.090	—	—
12i	Propynyl	0.84	—	—
12j	*c*-Pr	0.071	—	—
12k	CF_3	0.095	—	—
12l	CH_2CF_3	0.71	—	—
12m	SCH_3	0.007	1.80	0.90
12n	OCH_3	1.10	—	—
12o	CH_2OH	2.17	—	—
12p	$CH(OH)CF_3$	3.40	—	—
12q	*i*-Pr	0.37	—	—
12r	Ph	>50	—	—
12s	CH_2Ph	16.0	—	—
12t	NO_2	>50	—	—
12u	$CH_2N(Me)_2$	34.0	—	—
12v	$COCH_3$	>50	—	—
12w	$S(CH_2)_2NHAc$	0.002	0.034	12.00
12x	$S(CH_2)_2OH$	0.030	—	—
12y	CH_2CN	0.049	1.10	1.33

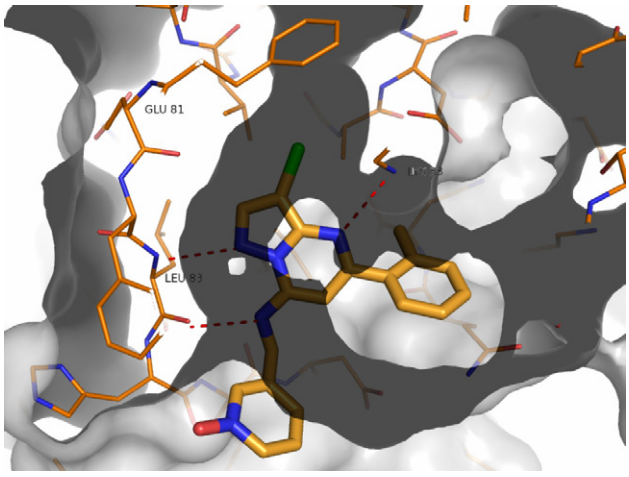
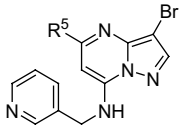


Figure 1. X-ray of crystal structure of **13** in CDK2.

Compound **15j** was profiled further: it was screened against a panel of 50 kinases (e.g. cSRC, JNK1, PDK1, PKB, ROCK-II) without observing any non-CDK cross-reactivity. The compound is moderately protein-bound (mouse: 90%, rat: 85%, monkey: 89%, dog: 93%, human: 95%). **15j** was active against a panel of 17 different tumor cell lines in the clonogenicity assay with IC_{50}s in the range of 120–390 nM. The compound is orally available and its PK parameters are summarized in Table 4.

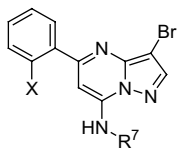
Table 2. CDK2 inhibitory activity of pyrazolo[1,5-*a*]pyrimidines **14a–14p**



14a-p

Compound	R^5	CDK2/cyclin A IC_{50} (μM)	GSK3β IC_{50} (μM)	Thym IC_{50} (μM)
14a	Me	0.060	3.53	1.80
14b	Et	0.018	0.92	0.52
14c	*i*-Pr	0.017	0.42	0.95
14d	CycloPr	0.045	1.13	1.50
14e	$CH(CH_3)OH$	0.038	0.50	1.40
14f	CO_2Et	0.089	0.79	2.90
14g	$NHCH_3$	0.043	4.6	1.20
14h	OCH_3	0.035	1.41	2.00
14i	SCH_3	0.038	1.18	0.99
14j	Ph-2-Cl	0.003	0.054	0.50
14k	Cyclohexyl	0.004	0.027	0.20
14l	3-Piperidinyl	0.008	0.18	0.06
14m	4-Piperidinyl	0.016	0.41	0.16
14n	Piperazine	0.210	—	—
14o	2-Furyl	0.008	0.31	15.19
14p	2-Thienyl	0.013	0.48	0.75

Table 3. CDK2 inhibitory activity of pyrazolo[1,5-*a*]pyrimidines **15a–15l**



15a-l

Compound	X	R^7	CDK2/cyclin A IC_{50} (μM)	GSK3β IC_{50} (μM)	Thym IC_{50} (μM)
15a	H	H	0.25	—	—
15b	Cl	Me	0.020	0.247	1.80
15c	Cl	Pr	0.163	—	—
15d	Cl	*c*-Pr	0.825	—	—
15e	F	Ph	0.408	13.31	
15f	Cl	Ph-4-SO_2CH_3	0.35	0.45	0.90
15g	F	Bn	0.300	15.2	—
15h	Cl	CH_2-3Pyr	0.004	0.054	0.50
15i	Cl	CH_2-3Pyr-O	0.011	0.035	0.17
15j	F	CH_2-3Pyr-O	0.013	0.13	0.21
15k	H	CH_2-3Pyr-O	0.034	0.60	0.14
15l	H	CH(Me)-3Pyr	3.000	—	—

Table 4. Pharmacokinetic parameters of **15j**

Species	Dose, mpk vehicle	AUC (μM h)	c_{max} (μM)	t_{max} (h)
Rat	10 0.4% MC	16.4	2.29	2.0
Mouse	40 20% HPBCD	17.9	6.81	2.0
Dog	5 20% HPBCD	2.44	2.58	
Monkey	10 0.4% HPMC	43.0	3.2	3.3

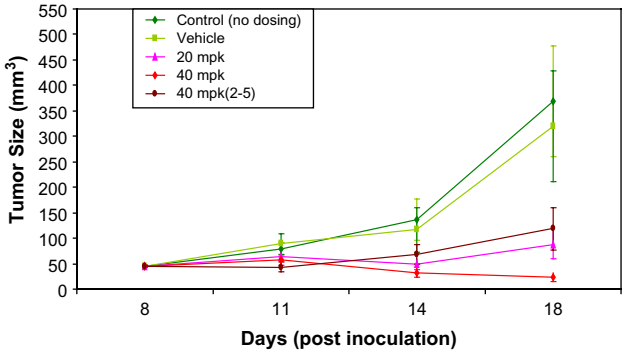


Figure 2. Efficacy of **15j** in A2780 xenograft model (mouse).

Compound **15j** demonstrated efficacy in a staged A2780 tumor xenograft model in the mouse (Fig. 2). The dose of 40 mpk, qd, PO for 10 days caused 96% tumor growth inhibition with observed tumor regression in 9 of 10 animals.

The compound was well tolerated and only a moderate and reversible decrease of white blood cells was observed.

In conclusion, we demonstrated that properly substituted pyrazolo[1,5-*a*]pyrimidines can serve as potent, selective, and efficacious orally available CDK2 inhibitors.

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