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SYNTHESIS AND ACTIVITY OF 2,6,9-TRISUBSTITUTED PURINES

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Abstract: The preparation of a series of 2,6,9-trisubstituted purines and the structure–activity data for the inhibition of cyclin dependent kinase, CDK2 are presented. © 1997 Elsevier Science Ltd.

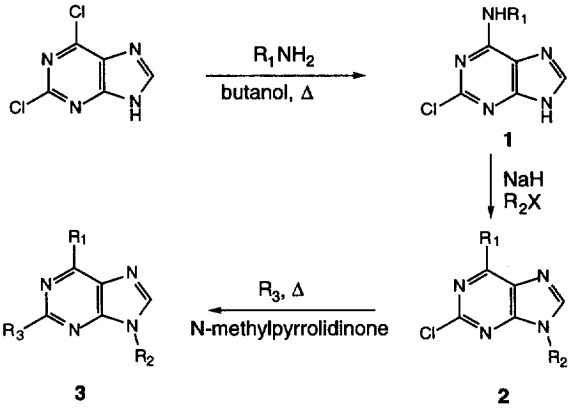
Recent advances in molecular and cellular biology have greatly contributed to our understanding of the mechanisms of cell proliferation and of specific steps of the cell cycle as cells progress through mitosis.[1] These studies have demonstrated that the cell cycle is tightly regulated by time dependent activation a family of serine/threonine kinases. These kinases are multiprotein complexes consisting of (a) a phosphorylated kinase called cyclin dependent kinase (CDK) and (b) a regulatory protein called cyclin. Different cyclin-CDK combinations control cell cycle steps such as growth, DNA replication, and cell division.[2] One key member of the CDK family of enzymes is CDK2. CDK2/cyclin A activity has been shown to be essential for mammalian cell cycle progression at the G1/S boundary. Olomoucine is a specific inhibitor of CDK2 with an IC_{50} of approximately 7 μM,[3] and in vivo studies using mammalian cells in culture demonstrate that olomoucine inhibits cell proliferation at an approximate concentration of 50 μg/mL. We report here the results of program to identify potent inhibitors of CDK2 to evaluate as potential therapeutic agents for controlling aberrant cell proliferation in a variety of diseases.[4]

Chemistry and Library Generation

There have been several recent reports on the preparation of combinatorial libraries of purine derivatives using solid-phase methodology.[5] Rather than trying to generate the most diverse set of structures available, we prepared a modest sized (ca 3000 compounds) biased library that appears to be a general resource for lead generation in kinase inhibition.[6] Solution-phase methodology was used for the preparation of the target compounds. The general reaction pathway is outlined in Scheme 1, and is based upon the differential reactivity of 2,6-dichloropurine.[7]

The 6-chloro group was displaced by refluxing with a primary amine or aniline in butanol for several hours. This reaction gave quantitative yields of analog **1**. Alkylation of the 9-position was achieved by treating **1** with NaH in DMF followed by addition of an alkyl halide to afford compound **2**. No N-7 alkylation was observed. As noted by Nugiel,[5b] the displacement of the 2-chloro proved to be the most difficult. The optimal conditions used a 1:1 mixture of N-methylpyrrolidinone and amine at 135 °C for 24 to 40 h. This allowed for the introduction of a variety of amines into 2-position. Even with these forcing conditions, anilines and certain sterically hindered amines would not displace the 2 chlorine.

Scheme 1



The library consisted mainly of single compounds that were characterized by NMR, TLC, or HPLC. However, some initial screening work was done using mixture chemistry. Because of the final displacement did not proceed well enough to maintain an even distribution of products, a single R3 amine was used, and the mixtures were kept small in numbers (5 to 25 compounds per pool) to aid in deconvolution. Thus, 1/5 equivalent each of five amines or anilines were added to 6-position followed by a alkylation of the N-9 position with 1/5 equivalent each of five alkyl halides to yield a mixture of twenty-five 2-chloropurines. This pool was then subjected to the final 2-chlorine displacement with a single amine to afford a mixture of twenty-five analogs. This mixture size proved to be quite adequate for screening and maximizing chemistry productivity.

Results and Discussion

The library was initially screened against CDK2.[4] Representative results in comparison to olomoucine are shown in Table 1. In general, 2-position substitution with ethanolamine or diethanolamine retained or increased activity relative to the 2-chloro substitution (Table 1, compounds **1** and **2**). Thus, compounds or mixtures were initially assayed at the 2-chloro substituted stage and if they did not show acceptable activity, compounds were not carried on to the final 2-chloro displacement. Substitution of the 9-position with an alkyl group was also necessary for good activity.

A wide variety of R1 analogs were surveyed, either as single compounds or in pools (**4**, **8**, **10**, **15**, and **20**). To obtain good activity, the R1 substituent must contain an aryl moiety which, based on the crystal structure of CDK2-olomoucine complex, sits between L89 and I10.[8] Neither the substrate nor ATP bind to this collateral lipophilic pocket. Binding interactions of a compound within this pocket may confer both CDK2 selectivity and binding potency on an inhibitor. Two sets of R1 aryl substituents, the anilines and the benzylamines, demonstrate significant potency. The phenethyl or phenpropylamine analogs (**18** and **19**) display no activity. The presence of R1 aryl group was highly preferable to a saturated ring system (**25**) indicating a significant aromatic electronic or steric binding preference within the R1 binding pocket. R1 arylheterocycles

yielded mixed results. For example, the quinoline isomers, **31** and **32** had different levels of activity. These differences may reflect the presence or absence of nitrogen-L89 interaction.

The most potent R1 analogs have substituted aryl rings. In general, substitution on the 4-position of the aryl group increased potency (**1**, **2**, **6**, and **7**). Clearly some very sterically demanding substituents (**34**, **45**, and **56**) can be accommodated within the R1 binding pocket. The biphenyl derivative, **45**, which presents its two phenyl rings in a near perpendicular orientation, shows high potency indicating that there is significant binding space beyond the L89/I10 domain. The 4-phenyl group may be replaced with an isosteric 2-thiophene moiety, compound **56**, without a loss of potency. It was surprising to note that the isomeric 3-thiophene derivative, compound **54**, was inactive. This loss of activity suggests that the binding interactions beyond the L89/Ile10 domain are not due solely to hydrophobic forces.

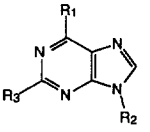
Aniline derivatives also showed modest to good potency. Similar to the substituted benzyl amines, the most potent analogs were substituted in the 4-position (**44**, and **52**). The large size of the substituents (biphenyl or bromo, respectively) reinforces the hypothesis that there is a sizable binding space beyond the L89/Ile10 domain. We are continuing to explore R1 substitutions to gain a better understanding of the binding nuances of the collateral pocket.

R2 substitutions is clearly restricted to small alkyl groups. Optimal activity is observed with an isopropyl substituent, although groups as large as cyclopentyl (**20**) are tolerated. Small polar groups, such as 2-hydroxyethyl (**12**), are also tolerated in this position. This feature may be useful for adjusting the water solubility of a drug candidate. Analysis of the CDK2/olomoucine crystal structure suggests that the pocket available for R2 groups will not accommodate groups larger than cyclopentyl. This was confirmed by our SAR. However, compound **43** (R2 is oleyl) is an exception to the observed SAR. The activity seen with **43** may reflect the fact that the purine binding site allows for a number of different binding modes for core heterocyclic ring,[8b] thus **43** may dock to the active site in one of these alternate binding orientations.

Although limited by the R3 amines that would undergo the displacement reaction at the 2-position, several diverse analogs were prepared. To obtain reasonable potency, one of the side chains must be hydroxyethyl functionality (**7**, **28** vs. **35**, **48**). In general, dihydroxy containing compounds (**7** and **13**) were found to have high potency. Examination of the CDK2-olomoucine crystal structure suggests that the second ethanolamine substituent may extend into the triphosphate binding domain and potentially generate additional hydrogen bonds between the protein and the inhibitor.

In summary, we have produced a directed purine library for optimizing a lead inhibitor for cyclin dependent kinase2. The synthetic scheme allows for the introduction of a wide variety of purine substituents at the 6- and 9-position, while the 2-position is restricted to nucleophilic amines. Exploration of the SAR of these analogs yielded several very potent CDK2 inhibitors for subsequent evaluation as antiproliferation agents.

Table 1



Compound	R_1	R_2	R_3	IC_{50} CDK2 (μM)
Olomoucine	Benzylamino	Me	ethanolamino	7
1	4-methoxybenzylamino	Me	Cl	4
2	4-methoxybenzylamino	Me	ethanolamino	2.5
3	4-chlorobenzylamino	Trifluoromethyl	Cl	1
4	benzylamino, 3-cyanopropylamino, 4-chlorobutylamino, methyl (4-carboxylate)benzylamino, 2-(phthalimido) ethylamino	H	Cl	>5
5	4-methoxybenzylamino	Isopropyl	ethanolamino	1.5
6	4-methoxybenzylamino	Me	Cl	0.7
7	4-methoxybenzylamino	Isopropyl	diethanolamino	0.5
8	3-methoxypropylamino, 2-methoxyethylamino, cyclopentylamino, 1-hydroxy-2-methylpropan-2-amino, N-benzylpiperidinyl-4-amino	Me	Cl	>5
9	4-methoxybenzylamino	Isopropyl	2-aminoethylamino	>5
10	3-pyridylmethylamino, 4-pyridylmethylamino, 1-hydroxy-6-hexylamino, phenethylamino, benzothiazolyl-2-amino	Me	Cl	>5
11	4-methoxybenzylamino	Isopropyl	pyrrolidino	5
12	4-methoxybenzylamino	2-hydroxyethyl	diethanolamino	1
13	4-methoxybenzylamino	Isopropyl	1-hydroxymethyl ethanolamino	0.5
14	4-methoxybenzylamino	Isopropyl	2-aminomethyl ethanolamino	1
15	3-pyridylmethylamino, 4-pyridylmethylamino, 1-hydroxy-6-hexylamino, phenethylamino, benzothiazolyl-2-amino	2-methylpropyl, cyclopentyl, propyl, ethyl, isopropyl	Cl	>5
16	4-methoxybenzylamino	Isopropyl	4-hydroxypiperidino	2
17	4-methoxybenzylamino	Isopropyl	N-(2-cyano propyl)-N-(3-pyridylmethyl)-amino	1
18	3-phenpropylamino	Isopropyl	diethanolamino	>5
19	2-indanylamino	Isopropyl	diethanolamino	>5
20	4-methoxybenzylamino	4-nitrobenzyl, 3-nitrobenzyl, cyclopentyl, 2-methylpropyl, 2-methylbutyl	diethanolamino	2
21	4-methoxybenzylamino	Isopropyl	3-hydroxy pyrrolidino	>5
22	4-methoxybenzylamino	Isopropyl	2-(3-indole) ethylamino	2

23	4-methoxybenzylamino	Isopropyl	2-hydroxy methylpiperidino	4
24	4-methoxybenzylamino	Isopropyl	2,3-dihydroxy propylamino	2
25	cyclopropylmethylamino	Isopropyl	diethanolamino	2
26	piperonylamino	Isopropyl	diethanolamino	0.8
27	4-methoxybenzylamino	Isopropyl	N-benzyl-N-2-hydroxy ethylamino	1
28	4-methoxybenzylamino	Isopropyl	2-hydroxy cyclohexylamino	1
29	4-methoxybenzylamino	Isopropyl	1-benzyl-2-hydroxyethylamino	2
30	4-methoxybenzylamino	Isopropyl	N-methyl-2-(3,4-dihydroxyphenyl)-2-hydroxy ethylamino	3
31	8-quinolinylamino	Isopropyl	diethanolamino	>5
32	3-quinolinylamino	Isopropyl	diethanolamino	2
33	anilino	Isopropyl	diethanolamino	2
34	4-butylbenzylamino	Isopropyl	diethanolamino	2
35	4-methoxybenzylamino	Isopropyl	diallylamino	>5
36	4-methoxybenzylamino	Isopropyl	N-methyl-2-phenyl-2-hydroxy ethylamino	>5
37	4-methoxybenzylamino	Isopropyl	2-((S)-2-anilino methyl) pyrrolidino	>5
38	4-methoxybenzylamino	Isopropyl	2-hydroxyethyl-3-hydroxy propylamino	
39	4-methoxybenzylamino	4-phenylbenzyl	Cl	>5
40	4-methoxybenzylamino	2,3-dihydroxypropyl	diethanolamino	>5
41	4-methoxybenzylamino	2-phenylbenzyl	diethanolamino	>5
42	4-methoxybenzylamino	2-naphthylmethyl	diethanolamino	5
43	4-methoxybenzylamino	oleyl	diethanolamino	3
44	4-phenylanilino	Isopropyl	diethanolamino	0.6
45	4-phenylbenzylamino	Isopropyl	diethanolamino	1
46	4-phenylbenzylamino	Isopropyl	2,3-dihydroxy propanamino	0.6
47	4-phenylbenzylamino	Isopropyl	bis-(2-methoxy ethyl)amino	5
48	4-phenylbenzylamino	Isopropyl	2-furanyl methyamino	>5
49	4-phenylbenzylamino	Isopropyl	ethanolamino	3
50	4-phenylbenzylamino	*t*-butyl ethanoate	diethanolamino	5
51	4-bromobenzylamino	Isopropyl	diethanolamino	2
52	4-bromoanilino	Isopropyl	diethanolamino	1
53	N-methyl-4-phenylbenzylamino	Isopropyl	Cl	>5
54	4-(3-thiophenyl) benzylamino	Isopropyl	diethanolamino	>5
55	4-bromoanilino	Isopropyl	4-bromoanilino	5
56	4-(2-thiophenyl) benzylamino	Isopropyl	diethanolamino	0.6

References and Note

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