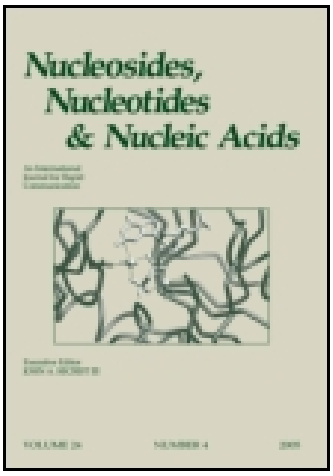

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THE DESIGN AND SYNTHESIS OF PURINE INHIBITORS OF CDK2. III

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THE DESIGN AND SYNTHESIS OF PURINE INHIBITORS OF CDK2. III

P. W. Shum,* N. P. Peet, P. M. Weintraub, T. B. Le, Z. Zhao, F. Barbone, B. Cashman, J. Tsay, S. Dwyer, P. C. Loos, E. A. Powers, K. Kropp, P. S. Wright, A. Bitonti, J. Dumont, and D. R. Borcherding

Aventis Pharmaceuticals Inc., Rt. 202-206, Bridgewater, New Jersey 08807

ABSTRACT

Cyclin-dependent kinases (CDKs) belong to a class of enzymes that control the ability of a cell to enter into and proceed through the cell division cycle. Using purine as a scaffold, we have synthesized a number of nanomolar inhibitors of CDK-2/cyclin E. In this report, the synthesis of a series of piperidine-substituted purine analogs will be presented, as well as some of their *in vitro* and *in vivo* biological effects.

Cyclin-dependent kinases (CDKs) belong to an important class of enzymes that are responsible for entry into and regulation of the cell division cycle (1,2). CDKs are activated by interaction with members of the cyclin family through the formation of heterodimeric complexes (1,2). Normal cell growth is regulated by changes in the balance between activators and endogenous inhibitors of CDKs. In cancer, the cells have lost this balance. Tumor cells exhibit mutations, aberrant expression levels, or altered activities of one or more of the regulatory proteins for the cell cycle checkpoints (within the G1, S, and G2/M phases). These regulatory components include the CDK inhibitors (such as p16, p21, and p27), the cyclin-CDK pairs (such as CDK-4/cyclin D1, CDK-2/cyclin E, and CDK-1/cyclin B), and tumor suppressors (such as p53 or pRb). Inhibitors of CDKs complexes like CDK-2/cyclin E, involved in the transition from G1 to S phase, may have therapeutic

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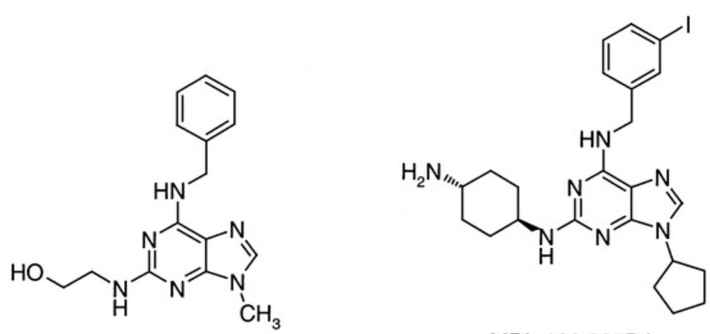
potential against tumor cell proliferation. Recent *in vitro* studies by Chen *et al.* (3) have suggested that selective killing of transformed cells over non-transformed cells may be accomplished with CDK2/cyclin E antagonists. Small molecule CDK inhibitors, including the purine analog olomoucine have antimitotic activity and been shown to inhibit cancer cell proliferation (4,5).

MDL 106,327DA was identified through database mining using olomoucine as the lead structure and found to inhibit CDK-2/cyclin E with an IC_{50} value of 50 nM (Fig. 1). This lead was then systematically modified to find the optimum inhibitory activity for CDK-2/cyclin E (6–8). One of the unique features of MDL 106,327DA is the *trans*-1,4-diaminocyclohexyl group in the 2-position of its purine ring. In an effort to optimize the affinity of this lead structure by replacing the substituent at the 2-position with a variety of other diamines, we found that the *trans*-1,4-diaminocyclohexyl group was the only substituent to give low nanomolar affinity for CDK-2/cyclin E (6). The 9-position generally accommodates relatively small alkyl groups (7). When the cyclopentyl group of MDL 106,327DA was replaced with an isopropyl group, the IC_{50} value was 180 nM (7). Small cycloalkyl groups, such as the cyclopentyl group, gave the best affinity of all substituents at 11 nM against CDK-2/cyclin E. The 6-position was very amenable to modification without significant loss of activity, because this position is exposed to the solvent when bound to human CDK-2 (6,8). Modifications of the imidazole in the purine ring also gave a loss of activity (9). When the N7 position was replaced with a CH group or the C8 position was replaced with a nitrogen atom, a reduction in activity was observed.



Olomoucine

MDL 106,327DA

CDK-2 IC_{50}: 5 μM
CDK-4 IC_{50}: >1000 μM

In vitro Proliferation
IC_{50} (μM)

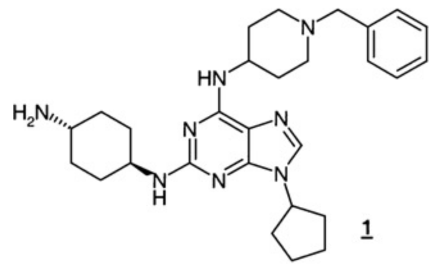
MCF-7	HT-29	HCT-15
35	53	62

CDK-2 IC_{50}: 50 nM
CDK-4 IC_{50}: 2.0 μM

In vitro Proliferation
IC_{50} (μM)

MCF-7	HT-29	HCT-15
0.7	0.9	1.9

Figure 1. Adenine scaffold lead from database mining around olomoucine.



1

MDL 108522

Enzyme Inhibition
CDK-2 IC_{50} = 190 nM
CDK-4 IC_{50} = 410 nM

Antiproliferation
HT-29 IC_{50} = 0.2 μM

Figure 2. MDL 108,522.

We have synthesized a number of very active inhibitors of CDK-2/cyclin E using rational drug design and parallel synthesis approaches. Another inhibitor with an extended substituent at C6 nitrogen MDL 108,522 (**1**) was shown to have good CDK-2/cyclin E activity, *in vitro* and *in vivo* antitumor activity (Fig. 2). Structure activity studies around this new structure using parallel synthesis was then initiated. In this report we will describe the synthesis, SAR, CDK-2/cyclin E activity, *in vitro* and *in vivo* antitumor activity for a number of compounds related to MDL 108,522.

Chemistry

MDL 108,522 and its analogs (**8**–**46**) were prepared by parallel synthesis. However, very few 1-substituted-4-aminopiperidines are commercially available. The 1-substituted-4-aminopiperidines were prepared according to Scheme 1 by alkylating piperidine-4-carboxyamide **2** using Cs_2CO_3 and potassium iodide in 3-pentanone to give **3** in yields ranging from 17 to 74%. The Hoffmann rearrangement (10) of compound **3** was accomplished by using [bis(trifluoroacetoxy)iodo]benzene in acetonitrile/water to afford **4** in 64% to quantitative yields. The final compounds, shown in Scheme 2, were prepared using parallel synthesis techniques. Compound **6** was prepared from **5** as previously described (6) and treated with the appropriately substituted piperidine **4** in EtOH at reflux to give >90% yields of the first intermediate (**7**) for **8**–**46**. The EtOH was removed by evaporation, *trans*-1,4-diaminocyclohexane was added neat and the mixture was heated to 150°C for 24 hours to yield **8**–**46** in 50 to 85% yields. The final compounds (**8**–**46**) were purified using 2 gram silica gel solid phase extractors and purities (>85%) were established by LC/MS.

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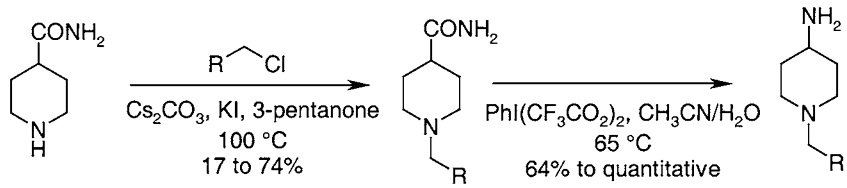
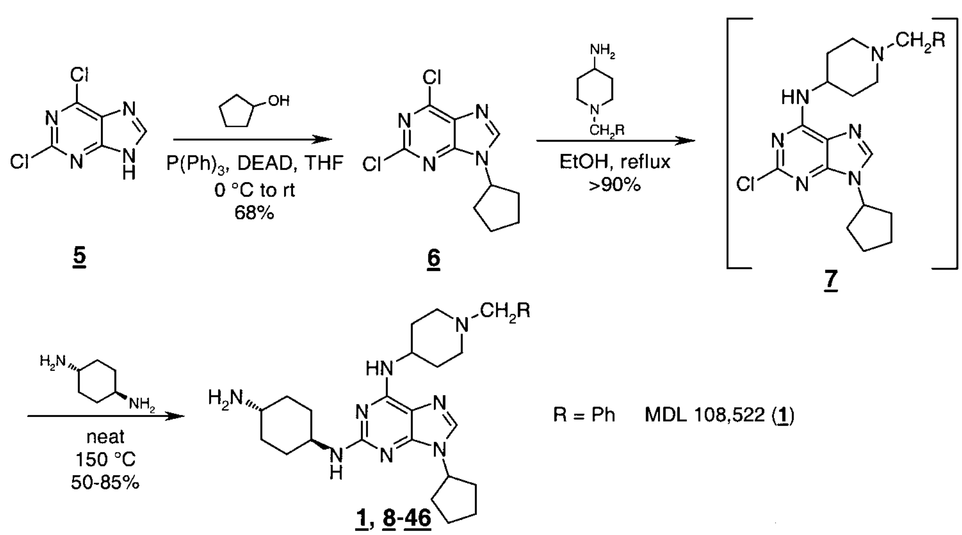


Scheme 1. Synthesis of 4-Amino-1-benzylpiperidines.

Biology

MDL 108,522 (**1**) was previously described as an inhibitor of CDK-2 and tumor cell proliferation (6). In an attempt to determine the SAR and the scope of the biological activity of MDL 108,522, a series of analogs were prepared. The compounds (**1** and **8**–**46**) were tested against human CDK-2 and CDK-4 obtained from SF9 cell lysates and IC_{50} values were measured (Table 1) (6). It was determined that substituted benzyl groups on the piperidine ring had minimal effects on biological activity with the IC_{50} values ranging from 0.071 to 2.1 μM. Therefore, these compounds only afforded a difference in activity of 30 fold. Compound **8** was the most potent against CDK-2, having an IC_{50} value of 0.071 μM, while compound **42** was the most selective, having a CDK-4/CDK-2 ratio of 89. Most compounds had an IC_{50} below 0.5 μM for CDK-2. This is not totally unexpected since known x-ray structures for this type of compound indicates that the piperidine group would be exposed to solvent when bound to CDK-2. Thus, substitution on the piperidine ring nitrogen may not have a large effect on the binding affinity of these compounds for CDK-2 (8).



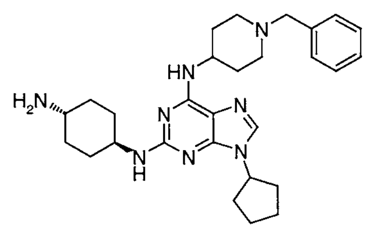
Scheme 2. Synthesis of MDL 108,522 and its analogs (via parallel synthesis).

Analysis of the data showed that 3,5-bis(trifluoromethyl) (**42**), 4-trifluoromethyl (**38**), 4-chloro (**23**) and 3,5-dichloro (**11**) substitution on the benzyl group gave the best CDK-4/CDK-2 selectivity ratios of 89, 42, 34 and 25, respectively. This indicates that substitution on the piperidine might effect CDK-4 binding more significantly, since CDK-4 binding affinities were more directly effected. Other 3,5-disubstituted compounds (**32**) and (**36**) which have difluoro or dimethoxy groups gave selectivity with CDK-4/CDK-2 ratios of 9 and 11, respectively. Compounds with electron withdrawing groups (**25** and **43**) in the 4-position which have fluoro and trifluoromethoxy groups gave selective compounds with CDK-4/CDK-2 ratios of 11 and 8, respectively. Compounds **13** and **20**, which have methoxy and methyl electron donating groups in the 4-position of the benzyl group, were determined to be almost equally potent against CDK-2 and CDK-4, with IC_{50} values below 0.5 μM. This small set of compounds would indicate that electron donating groups in the 4-position provide compounds with greater CDK-4 binding affinities. The 2,4-difluorobenzyl substituted compound **12** also showed little selectivity, but had IC_{50} values for both CDK-2 and CDK-4 below 0.5 μM.

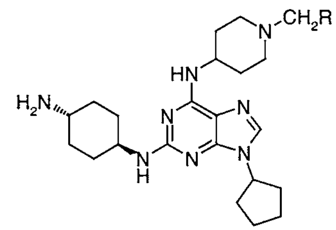
A SAR pattern emerged for the trifluoromethyl substituted series of compounds, where the 3-trifluoromethylbenzyl substituted compound **8** gave most potent IC_{50} value of 0.071 μM for CDK-2. Trifluoromethyl substitution at the 4- or 3,5-positions (**38** and **42**) gave decreased affinity for CDK-2 with IC_{50} values of 0.48 and 0.72 μM, respectively. A larger decrease in IC_{50} values for the CDK-4 activity was also observed for these compounds. When a trifluoromethyl group was placed in either the 2- or 3,4-position of the benzyl group as in compounds **45** and **46**, there resulted the least active compounds in this study having IC_{50} values of 1.1 and 2.1 μM, respectively. Compounds that had chloro or fluoro group substitution in general gave the best affinity for CDK-2, regardless of the substitution patterns. Monochloro- and dichloro-substitution on the benzyl group provided compounds with IC_{50} values of 0.34 μM or better, with the exception of compound **44** (CDK-2 $IC_{50} = 1.0$ μM), which had the 2,6-dichloro substitution pattern. Monofluoro- and difluoro-substituted benzyl groups all gave IC_{50} values for CDK-2 of less than 0.5 μM. When the phenyl group was replaced by napthyl as in compounds **31** and **35**, IC_{50} values for CDK-2 were 0.26 and 0.43 μM, indicating large groups were well-tolerated. Also, when the phenyl group was replaced by a cyclohexyl group (**9**) or a cyclopropyl group (**17**) good affinity for CDK-2 was maintained with IC_{50} values of 0.11 and 0.19 μM, respectively.

The compounds were then tested against seven tumor cell lines for antiproliferative effects. All compounds demonstrated potent activity against breast, colon, lung and prostrate tumor cells lines. The *in vitro* antiproliferation IC_{50} values generally correlated with the IC_{50} values generated for CDK-2 rather than CDK-4. The most selective compounds (**11**, **23**, **38** and **42**) showed submicromolar activity against the tumor cells, which correlated well with the CDK-2 IC_{50} values. These data would indicate that CDK-2, in this study, was responsible for the antiproliferative effects of these compounds since compounds like **38** and **42**, which have IC_{50} values for CDK-4 of 20 and 64 μM, respectively, still showed significant activity.

Table 1. Inhibition of Cyclin-Dependent Kinases and Effects of Compounds on Human Tumor Cell Proliferation *In Vitro*



MDL 108,522 **1**



MDL 108,522 analogs **8**-**46**

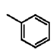
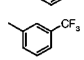
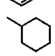
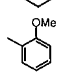
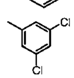
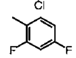
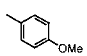
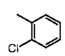
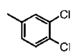
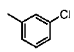
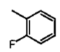
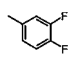
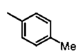
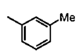
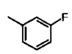
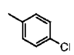
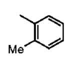
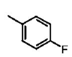
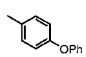
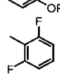
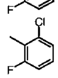
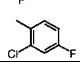
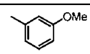
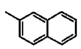
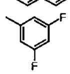
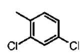
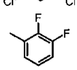
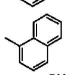
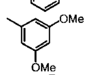
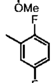
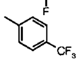
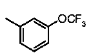
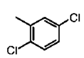
		In Vitro Inhibition IC_{50} (μM)		*In Vitro* Tumor Cell Proliferation IC50 (μM)						
				Breast		Colon		Lung	Prostate	
Compound	Structure	CDK2	CDK4	MDA-MB-231	MDA-MB-435	HT-29	Colo-205	A549	PC-3	DU-145
108522 **1**		0.19	0.14	0.19	0.27	0.20	0.18	0.12	0.19	0.15
8		0.071	0.37	0.33	0.49	0.35	0.18	0.20	0.38	0.34
9		0.11	1.3	0.32	0.43	0.34	0.26	0.21	0.34	0.28
10		0.14	0.69	0.22	0.35	0.35	0.18	0.19	0.36	0.31
11		0.16	4.0	0.38	0.54	0.38	0.32	0.21	0.45	0.37
12		0.17	0.23	0.47	0.63	0.44	0.28	0.19	0.46	0.35
13		0.18	0.29	0.25	0.29	0.27	0.23	0.16	0.32	0.28
14		0.18	0.91	0.40	0.45	0.73	0.26	0.24	0.42	0.31

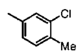
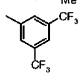
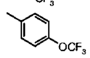
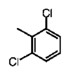
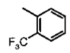
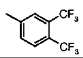
Table 1. Continued

15		0.18	1.8	0.84	1.0	0.80	0.54	0.43	0.78	0.73
16		0.18	1.0	0.27	0.41	0.27	0.19	0.15	0.28	0.23
17		0.19	0.74	1.1	2.3	1.8	1.3	0.63	0.19	1.5
18		0.19	2.9	0.32	0.39	0.33	0.21	0.23	0.35	0.23
19		0.19	2.8	0.35	0.48	0.37	0.25	0.23	0.39	0.29
20		0.20	0.33	0.24	0.32	0.28	0.17	0.17	0.27	0.23
21		0.21	0.61	0.32	0.38	0.32	0.26	0.18	0.32	0.22
22		0.21	1.3	0.21	0.29	0.26	0.17	0.15	0.22	0.21
23		0.22	7.4	0.36	0.37	0.35	0.29	0.25	0.30	0.32
24		0.23	2.3	0.21	0.26	0.22	0.17	0.12	0.19	0.16
25		0.24	2.7	0.31	0.46	0.39	0.23	0.23	0.36	0.30
26		0.24	3.0	0.64	0.94	0.75	0.56	0.41	0.67	0.58
27		0.24	2.0	0.92	1.2	0.80	0.68	0.57	0.98	0.55
28		0.25	1.6	0.77	0.95	0.68	0.38	0.36	0.83	0.60
29		0.25	2.2	0.37	0.55	0.45	0.34	0.29	0.41	0.76

(*continued*)

Table 1. Continued

Compound	Structure	*In Vitro* Inhibition IC_{50} (μM)		*In Vitro* Tumor Cell Proliferation IC50 (μM)						
				Breast		Colon		Lung	Prostate	
		CDK2	CDK4	MDA-MB-231	MDA-MB-435	HT-29	Colo-205	A549	PC-3	DU-145
30		0.26	1.1	0.31	0.36	0.32	0.23	0.19	0.35	0.26
31		0.26	3.1	0.59	0.64	0.72	0.47	0.37	0.56	0.54
32		0.31	2.8	0.30	0.36	0.30	0.15	0.17	0.30	0.24
33		0.32	3.8	0.76	0.84	0.66	0.61	0.45	0.60	0.52
34		0.34	3.7	0.44	0.61	0.45	0.33	0.33	0.41	0.38
35		0.43	2.5	0.35	0.41	0.32	0.26	0.17	0.28	0.28
36		0.43	4.6	0.25	0.34	0.31	0.20	0.21	0.28	0.26
37		0.47	1.6	0.40	0.50	0.37	0.28	0.26	0.41	0.30
38		0.48	20	0.50	0.62	0.49	0.45	0.31	0.43	0.43
39		0.57	3.6	0.66	0.88	0.68	0.44	0.36	0.69	0.60
40		0.57	7.2	0.40	0.63	0.48	0.35	0.33	0.47	0.43

41		0.59	5.1	0.35	0.51	0.39	0.34	0.23	0.41	0.34
42		0.72	64	0.45	0.82	0.39	0.45	0.24	0.66	0.76
43		0.86	6.8	0.36	0.54	0.44	0.42	0.24	0.39	0.32
44		1.0	9.9	0.80	0.86	0.63	0.51	0.39	0.69	0.49
45		1.1	3.5	0.69	0.97	0.63	0.55	0.41	0.71	0.54
46		2.1	9.2	2.8	0.54	2.60	3.5	3.9	4.2	4.4

Enzyme activities were measured in the presence of increasing drug concentrations. The IC_{50} values represent the 50% inhibitory concentration. Tumor cell monolayers were incubated with drug for 72 h. DNA content was measured by CyQuant staining and IC_{50} values were determined.

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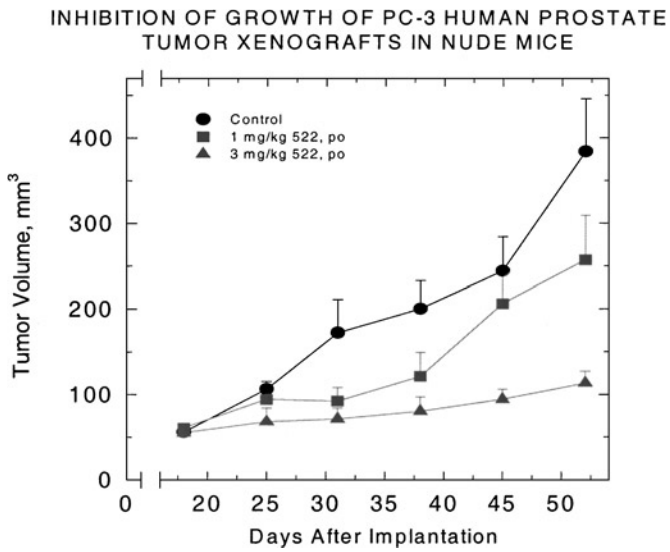

Figure 3.

It cannot be ruled out that CDK-1 or another kinase is responsible for part or all of the activity observed. However, CDK-4 activity does not appear to be a critical component for the activity of these compounds. It should pointed out that the least selective compounds (**12**, **13** and **20**) also showed good activity against these tumor cells as well. However, the compounds displayed IC_{50} values below 0.33 μM for both CDK-2 and CDK-4.

MDL 108,522 (**1**) was selected for *in vivo* testing in a xenograft tumor model in nude mice (6). Shown in Figure 3 are the *in vivo* effects of MDL 108,552 for the inhibition of PC-3 prostate tumor growth in nude mice. When dosed orally at 1 mg/kg, MDL 108,522 showed minimal activity; however, at 3 mg/kg the compound showed significant activity against PC-3 tumor cell growth.

In this report, we have shown that inhibition of CDK-2 with substituted purines can be correlated with the antiproliferative activity effects in the seven different tumor cell lines. These effects can translate into good *in vivo* activity, indicating that CDK-2 is a good target for the development of therapies where it is desirable to control unregulated cell growth such as that seen in cancers.

EXPERIMENTAL

General Procedure for the Preparation of 4-amino-1-alkylpiperidine 4-Amino-1-(2,4-dichlorobenzyl)piperidine

To a mixture of isonipecotamide (5.0 g, 39 mmol), cesium carbonate (8.0 g, 24 mmol), and potassium iodide (2 spatula tips, cat.) in 3-pentanone (25 mL) was added 2,4-dichlorobenzyl chloride (6.5 mL, 47 mmol). The mixture was heated at 100°C (oil bath) for 5 h. The mixture was filtered hot, and the filter cake was washed thoroughly with hot acetone. The filtrate was concentrated, and the resulting slurry was recrystallized from acetone to give 8.2 g (74%) of 4-[1-(2,4-dichlorobenzyl)piperidine]-carboxamide.

A solution of bis(trifluoroacetoxy)iodobenzene (3.6 g, 8.4 mmol) in acetonitrile (20 mL) and water (15 mL) was added to 4-[1-(2,4-dichlorobenzyl)piperidine] carboxamide (2.0 g, 7.0 mmol). This mixture was heated at 65°C (oil bath) overnight. Water (60 mL) was then added, and the mixture was cooled in an ice bath. Concentrated HCl (ca. 10 mL) was added, and the mixture was washed twice with diethyl ether. The aqueous layer was concentrated in vacuo, and the residue was dissolved in water (ca. 40 mL). The solution was saturated with solid potassium carbonate. This mixture was extracted with dichloromethane (2X). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to yield 1.35 g (74%) the title compound as a yellow oil. Other 4-amino-1-alkylpiperidines were prepared via the same procedure.

General Procedure for the Parallel Synthesis of Purine Scaffold Molecules

To a 4 or 20 mL vial was added 9-(cyclopentyl)-2,6-dichloropurine (0.20 mmol), TEA or DIEA (200 mg), reacting amine (0.2 mmol), ethanol or toluene (2 mL), and a stir bar. The vial was capped and the mixture was stirred at reflux for overnight, after which the solution was cooled to room temperature. The solution was concentrated, and *trans*-1,4-diaminocyclohexane (200 mg) was added. The vial was capped and heated at 150°C for 24 hours. After cooling the material was passed through a prepacked 1 gram or 2 gram silica gel column (Fischer or Alltech) pre-equilibrated with hexane, then the product was eluted with of CH_2Cl_2 (4 mL) then with of CH_2Cl_2:MeOH 4:1 (15 mL). The fractions which appeared to have the product based upon TLC analysis, were concentrated, and repurified by passing through another prepacked silica gel column pre-equilibrated with hexane, this time beginning with CH_2Cl_2, followed by CH_2Cl_2:MeOH 9:1. The concentrated fractions with pure product were dissolved in EtOH (2 mL). Six drops of 1N HCl were added, and the solutions were concentrated to give hydrochloride salts of the desired products. LC/MS was used to determine purity and mass. The R_f values were determined by an AQ 4 × 50 column (YMC) with a linear gradient from 100% C to 100% D in four minutes with a two minute hold at 100% D, where C is 5:95 acetonitrile:water with 0.1% TFA, and D is 95:5 acetonitrile:water with 0.085% TFA. Molecular ion determinations were made using a Finnigan MAT SSQ-710 mass spectrometer.

REFERENCES

1. Sielecki, T.M.; Boylan, J.F.; Benfield, P.A.; Trainor, G.L. *J. Med. Chem.* **2000**, *43*, 1–18.

2. Gray, N.; Detivaud, L.; Doerig, C.; Meijer, L. *Current Med. Chem.* **1999**, *6*, 859–875.
3. Chen, Y.-N.P.; Sharma, S.K.; Ramsey, T.M.; Jiang, L.; Martin, M.S.; Baker, K.; Adams, P.D.; Bair, K.W.; Kaelin, W.G., Jr. *Proc. Natl. Acad. Sci., USA* **1999**, *96*, 4325–4329.
4. De Azevedo, W.F.; Leclerc, S.; Meijer, L.; Havlicek L.; Strnad, M.; Kim, S.-H. *Eur. J. Biochem.* **1997**, *243*, 518–526.
5. Vesely, J.; Havlicek, L.; Strnad, M.; Blow, J.J.; Donella-Deana, A.; Pinna, L.; Letham, D.S.; Kato, J.; Detivaud, L.; Leclerc, S.; Meijer, L. *Eur. J. Biochem.* **1994**, *224*, 771–776.
6. Borcherding, D.R.; Shum, P.W.; Weintraub, P.M.; Le, T.B.; Zhao, Z.; Zhang, H.; Munson, R.; Shen, J.; Kropp, K.; Powers, E.; Zhang, S.; Barbone, F.; Cashman, B.; Tsay, J.; Loos, P.C.; Wright, P.S., Bitonti, A.; Dumont, J.; Peet, N.P. Bioorg Med. Chem. Lett. Manuscript in preparation.
7. Shum, P.W.; Weintraub, P.M.; Le, T.B.; Zhao, Z.; Zhang, H.; Munson, R.; Shen, J.; Kropp, K.; Powers, E.; Zhang, S.; Barbone, F.; Cashman, B.; Tsay, J.; Loos, P.C.; Wright, P.S., Bitonti, A.; Dumont, J.; Peet, N.P.; Borcherding, D.R. *Bioorg Med. Chem. Lett.* Manuscript in preparation.
8. Dreyer, M.K.; Borcherding, D.R.; Dumont, J.A.; Peet, N.P.; Tsay, J.; Wright, P.S.; Bitonti, A.J.; Shen, J.; Kim, S.H. *Proteins*, submitted for publication.
9. Shum, P.W.; Weintraub, P.M.; Le, T.B.; Zhao, Z.; Zhang, H.; Munson, R.; Shen, J.; Kropp, K.; Powers, E.; Zhang, S.; Barbone, F.; Cashman, B.; Tsay, J.; Loos, P.C.; Wright, P.S., Bitonti, A.; Dumont, J.; Peet, N.P.; Borcherding, D.R. *Bioorg Med. Chem. Lett.* Manuscript in preparation.
10. Boutin, R.H.; Loudon, G.H. *J. Org. Chem.* **1984**, *48*, 4277–4284.