Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 343–346

Synthesis and evaluation of indenopyrazoles as cyclin-dependent kinase inhibitors. Part 4: Heterocycles at C3

Eddy W. Yue,* Susan V. DiMeo, C. Anne Higley, Jay A. Markwalder, Catherine R. Burton, Pamela A. Benfield, Robert H. Grafstrom, Sarah Cox, Jodi K. Muckelbauer, Angela M. Smallwood, Haiying Chen, Chong-Hwan Chang, George L. Trainor and Steven P. Seitz

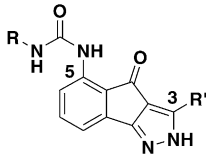
Bristol-Myers Squibb Company, Experimental Station, PO Box 80500, Wilmington, DE 19880-0500, USA†

Received 18 July 2003; accepted 5 November 2003

Abstract—New indeno[1,2-*c*]pyrazol-4-one cyclin dependent kinase inhibitors have been disclosed. The most promising compounds are nanomolar enzyme inhibitors with excellent activity against tumor cells. The most advanced compound retains cell culture activity even in the presence of human serum proteins. The most advanced compound did not kill the normal fibroblast line AG1523.
© 2003 Elsevier Ltd. All rights reserved.

With recent advances in biology, the focus of research has turned towards finding new targets for the treatment of cancer. One such area involves the cell cycle which functions as the vehicle for the crucial task of cell division and proliferation.[1] At the core of this machinery are the cyclin-dependent kinases (CDKs) which control the progression through the cycle.[2] CDK2/E and CDK4/D1 control the activity in G1 and entry into S. CDK2/A along with CDK2/E regulates passage through S and CDK1/B controls the G2 checkpoint and entry into mitosis. One of the better studied cell cycle processes is the phosphorylation of the retinoblastoma protein (pRb) by CDK4/D1. Once phosphorylated pRb releases the transcription factor E2F leading to the downstream activation of genes required for DNA synthesis. Dysregulation of the Rb pathway is a key event in transformation. Cyclins D, E and CDK4 have also shown aberrant expression in tumors. Inhibitors of CDKs have thus been suggested as a novel way to stop the proliferation of tumor cells.

We have been exploring the potential of the indenopyrazole class of CDK inhibitors (**1**).[3] Our previous studies have revealed that ureas and semicarbazides are generally preferred at C5 while a variety of groups are tolerated at C3 including phenyls, heterocycles, and alkyls.[4,5] In this paper, we report on a series of C3 heterocycles substituted at C5 with semicarbazides.

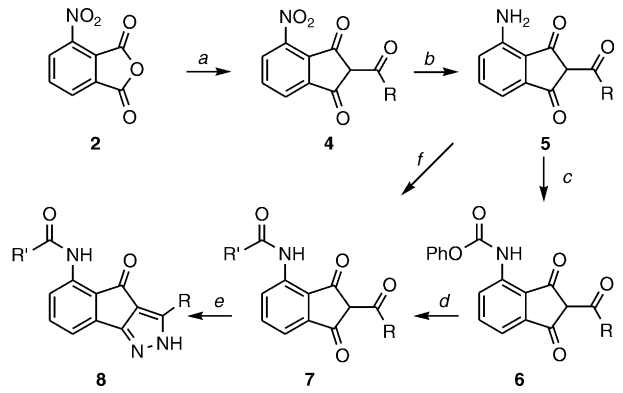


1

The synthesis of indenopyrazoles proceeded according to previously described chemistry.[5] Treatment of 3-nitrophthalic anhydride (**2**) with an appropriately substituted diketone (**3**) gave the triketone **4** (Scheme 1). This reaction allowed the installation of a variety of heterocyclic groups simply by varying the diketone (**3**, R = heterocycle). These diketones in turn were made from ethyl trifluoroacetate and the corresponding acetyl precursor of the heterocycle using Claisen condensation conditions.[5] Triketone **4** was reduced to the aniline **5**

* Corresponding author at present address: Incyte Corporation, Experimental Station, PO Box 80336, Wilmington, DC 19880-0336, USA. Tel.: +1-302-498-6902; fax: +1-302-425-2750; e-mail: eddyyue@incyte.com
† Present address: Bristol-Myers Squibb Company, Rt. 206 and Province Line Road, Lawrenceville, NJ 08543, USA.

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.11.008



Scheme 1. Reagents: (a) $RC(=O)CH_2C(=O)CF_3$ (**3**), Ac_2O, Et_3N, 25 °C; (b) Zn, $CaCl_2$, EtOH, H_2O, reflux; (c) $PhOC(=O)Cl$, Na_2CO_3, acetone, 50 °C; (d) R′ = hydrazines, DMSO, 90 °C; (e) H_2NNH_2, EtOH, reflux; (f) (**9**) = $R'CO_2Ph$, Et_3N, DMSO, 90 °C.

which allowed for functionalization at C5. Carbamate **6** was formed under standard conditions and upon treatment with a variety of hydrazines (R′) gave semicarbazide triketones (**7**). Cyclization of triketone **7** gave the desired indenopyrazole **8**. In certain cases, the synthesis could be reduced by one step when aniline **5** was treated directly with the preformed carbamate of the hydrazine to be introduced (**9**).

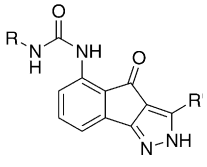
The first semicarbazides examined were those derived from 1,1-dimethylhydrazine (Table 1). As before, these compounds showed greater affinity for CDK2/E than CDK4/D1.[5] Substitution at the 5-position of 2-thienyl with small groups was preferred (**8b** and **8c**). For the 3-thienyl substitution the 5-chloro analogue proved to be one of the most potent inhibitors (**8f**). The 2,4-dimethylthiazol-5-yl analogue (**8i**) was also quite potent in CDK2/E and it exhibited good selectivity for CDK4/D1. Unfortunately, the utility of these compounds was limited by solubility.

In an attempt to improve solubility, semicarbazides were synthesized. Data for semicarbazides of 4-aminomorpholine are shown in Table 1. These compounds exhibited comparable or improved binding affinity for CDK2/E compared with the corresponding *N*,*N*-dimethyl semicarbazides. With the exception of **8n**, all of the compounds also had improved affinity for CDK4/D1. Because of this increase, compounds such as **8k** and **8l** had balanced profiles against CDK2/E and CDK4/D1. However, the analogue which again stood out was the 2,4-dimethylthiazol-5-yl (**8n**) which had good binding affinity for CDK2/E and maintained good selectivity against CDK4/D1. Unfortunately, the solubility of these semicarbazides was poor (**8n**: 4.4 μg/mL, 5% mannitol).

The next series of compounds were derived from 1-amino-4-methylpiperazine. Again, the most notable analogue contained 2,4-dimethylthiazol-5-yl (**8q**) which was still a potent inhibitor of CDK2/E but had lost some of its selectivity against CDK4/D1 compared with the other semicarbazides **8i** and **8n**. The switch of the semicarbazide from morpholino to piperazino had a dramatic effect on the solubility of **8q** (2.4 mg/mL, 5% mannitol).

With this data at hand, the focus shifted towards examining the C3 heterocycle binding site in more detail. Compound **8k** was chosen because the ethyl ester

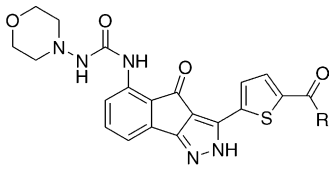
Table 1. C3 heterocyle, C5 semicarbazide SAR



Compd	R	R′	IC_{50} (nM)				
			CDK4/D1[a]	CDK2/E[a]	HCT-116[a]	HCT-116 (protein)[a,b]	AG1523[a]
8a	Dimethylamino	Thien-2-yl	340	36	75	NT	905
8b	Dimethylamino	5-(OMe)thien-2-yl	84	32	130	NT	1550
8c	Dimethylamino	5-(Me)thien-2-yl	81	25	48	NT	640
8d	Dimethylamino	5-(CO_2Et)thien-2-yl	185	28	380	NT	2800
8e	Dimethylamino	Thien-3-yl	107	24	68	NT	605
8f	Dimethylamino	5-(Cl)thien-3-yl	21	7	64	NT	615
8g	Dimethylamino	2,5-(di-Me)thien-3-yl	515	26	110	NT	1500
8h	Dimethylamino	Furan-2-yl	635	26	160	NT	NT
8i	Dimethylamino	2,4-(di-Me)thiazol-5-yl	150	4	NT	NT	NT
8j	Morpholin-4-yl	5-(Me)thien-2-yl	23	10	25	NT	285
8k	Morpholin-4-yl	5-(CO_2Et)thien-2-yl	30	31	110	3200	>17,000
8l	Morpholin-4-yl	5-(Cl)thien-3-yl	7	10	35	NT	385
8m	Morpholin-4-yl	2,5-(di-Me)thien-3-yl	125	<8	25	NT	345
8n	Morpholin-4-yl	2,4-(di-Me)thiazol-5-yl	>370	9	8	500	>19,000
8o	4-(Methyl)piperazin-1-yl	5-(CO_2Et)thien-2-yl	30	36	46	NT	NT
8p	4-(Methyl)piperazin-1-yl	2,5-(di-Me)thien-3-yl	90	12	24	NT	205
8q	4-(Methyl)piperazin-1-yl	2,4-(di-Me)thiazol-5-yl	46	8	14	260	>21,000

NT, not tested.
[a] Values correspond to $n = 2$.
[b] Values determined in the presence of human serum albumin and α-1-acidglycoprotein.

Table 2. C3 thienylamide, C5 morpholino semicarbazide SAR



Compd	R	IC_{50} (nM)				
		CDK4/D1[a]	CDK2/E[a]	HCT-116[a]	HCT-116 (protein)[a,b]	AG1523[a]
10a	2-(Dimethylamino)ethylamino	15	21	>800	NT	NT
10b	2-(Pyrrolidin-1-yl)ethylamino	23	25	475	NT	680
10c	2-(Piperidin-1-yl)ethylamino	22	31	20	NT	255
10d	2-(Morpholin-4-yl)ethylamino	86	51	860	NT	NT
10e	Piperidin-1-yl	22	25	52	16,750	NT
10f	3-(Dimethylamino)piperidin-1-yl	13	24	188	11,825	NT
10g	4-(Dimethylamino)piperidin-1-yl	8	23	210	>15,100	1000
10h	Piperazin-1-yl	4	38	700	15,400	NT
10i	4-(Methyl)piperazin-1-yl	10	32	110	9940	485
10j	4-(Ethyl)piperazin-1-yl	5	19	63	14,275	NT
10k	3-(Amino)pyrrolidin-1-yl	35	39	>800	NT	NT
10l	3-(Methylamino)pyrrolidin-1-yl	5	11	230	8500	NT
10m	3-(Dimethylamino)pyrrolidin-1-yl	12	44	64	2540	330
10n	Azepan-1-yl	17	23	53	8325	NT
10o	[1,4]Diazepan-1-yl	3	6	195	>19,700	NT
10p	4-(Methyl)-[1,4]diazepan-1-yl	4	15	19	870	63
10q	4-(Ethyl)-[1,4]diazepan-1-yl	7	14	33	7100	NT

NT, not tested.
[a] Values correspond to $n = 2$.
[b] Values determined in the presence of human serum albumin and α-1-acidglycoprotein.

at the 5-position of the thiophene ring provided a useful handle for derivatization. A large number of amines were coupled with **8k**, and some examples are shown in Table 2. It was apparent that both primary and secondary amides inhibited CDK4/D1 and CDK2/E similarly or better than the parent ester **8k**. Compound **10a** was an important analogue as it led to amides derived from cyclic amines (**10e–q**). By constraining the methylene units of the side chain of **10a**, it was hoped that an increase in binding affinity could be achieved. Indeed the results for these amides, ranging from five- to seven-membered rings showed an increase in binding affinity for CDK4/D1. Even more significant was that for the first time, CDK4/D1 selectivity was achieved and in the case of **10h** it was about 10-fold more selective than CDK2/E. This selectivity appears to be influenced by the presence of a distal basic nitrogen. For example, compound **10e** showed a relatively balanced inhibition profile for CDK4/D1 versus CDK2/E whereas both compounds **10h** and **10i** were more selective for CDK4/D1.

An X-ray crystal structure of **8q** complexed with CDK2 was obtained in order to determine the binding conformation. The conformation of **8q** is very similar to the C3-alkyl indenopyrazole previously determined[5] and the binding also occurs in the ATP pocket of the enzyme. The major interaction involves the pyrazole nitrogens of **8q** forming hydrogen bonds with Leu83 (Fig. 1). The amine of Leu83 is 2.9 Å from N1 and the carbonyl oxygen of Leu 83 is 2.4 Å from N2. The folding of the C5 semicarbazide toward the C3 region is not as pronounced as in the previous X-ray structure,[5] however,

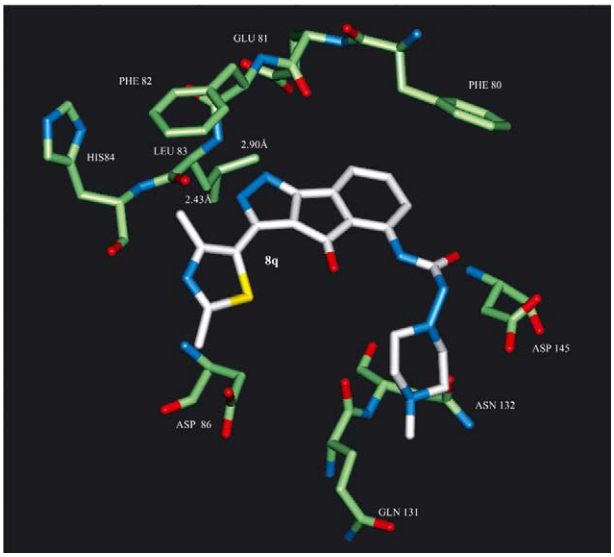


Figure 1. The structure of **8q** in the CDK2 ATP binding pocket. Protein carbons are colored light green and inhibitor carbons are colored white.

both the C5 and C3 groups are still partially solvent exposed. The X-ray crystal structure of **8q** is consistent with the observed activity of CDK inhibitors. The structure suggests there is ample room to accommodate a variety of groups at either C5 or C3 and is consistent with the fact that many such analogues are good inhibitors of CDK2/E.

Further profiling of these compounds was done in cell-based assays. A colon carcinoma-derived cell line,

Table 3. Enzymatic and cellular activity of **8q**

	IC_{50} (nM)[a]		IC_{50} (nM)[a]
CDK2/A	13	HT29	87
CDK1/B	6	HT1080	30
c-abl	17,600	L1210	105
PKA	>42,000	MCF7	23
PKC	<42,000	MiaPaCa2	26
AG1523 (arrested)	>21,000	NCI-H358	18
B16-F1	160	NCI-H460	104
H1299	12	PC3	125
HMEC	55	Skut 1A	25

[a] Values correspond to $n = 2$.

HCT116, was used to determine the effectiveness of these inhibitors in deterring cellular proliferation with and without plasma proteins (human serum albumin and α-1-acidglycoprotein). In addition, some of the compounds were tested in AG1523, a normal human fibroblast. AG1523 was chosen to give some indication of the potential therapeutic window these inhibitors possessed between proliferating cells versus arrested normal cells. Most of the compounds in Table 1 showed good translation from the enzymatic assays into the cellular assay. Most notable were the thiazolyl analogues **8n** and **8q**. Not only were they quite active in inhibiting HCT116, they retained their potency in the presence of plasma proteins.

For the thienylamides of Table 2, the results in HCT116 showed moderate to good enzymatic to cellular translation. Unfortunately most of these thienylamides were highly protein bound with IC_{50}'s greater than 2500 nM with the exception being **10p**.

Analysis of the AG1523 data revealed that most of the thienylamides tested had activity in AG1523 which was deemed unacceptable (Table 2). For example compound **10p** which had the best HCT116 protein-adjusted IC_{50} result was also quite potent against AG1523. Examination of the data in Table 1 revealed that semicarbazides derived from 1,1-dimethylhydrazine showed poor selectivity for HCT116 over AG1523. The results, however, improved with the cyclic semicarbazides. Compound **8k** had an IC_{50} in AG1523 greater than 17,000 nM. Although encouraging the HCT116 protein-adjusted IC_{50} was still quite high. Further evaluation revealed that both thiazolyl compounds, **8n** and **8q** were inactive in AG1523 yet had activity in HCT116 even in the presence of plasma proteins.

With its potent activity in HCT116 and large differential in AG1523, compound **8q** was selected for further evaluation. It displayed activity in CDK2/A and CDK1/B and good selectivity against other kinase targets such as the tyrosine kinase c-abl and protein kinases A and C (Table 3). Compound **8q** was also tested in a broad panel of human and murine tumor cell lines. Very good activity against these cell lines with several IC_{50} values less than 50 nM was seen.

The interesting profile of **8q** resulted in extensive evaluation of its effects on the cell cycle and profiling in vivo. The compound displayed good activity in HCT116 xenografts and in a MMTV *neu* transgenic model. These results will be reported in detail in due course.

The current SAR studies have led to several significant findings. Compound **8q** was found to be quite active in HCT116 even in the presence of plasma protein. Just as important was the fact that it was inactive in AG1523 which may be an indicator of an enhanced therapeutic window. In addition, **8q** displayed good selectivity against other kinase targets and very good activity in a large panel of tumor cell lines. These results will allow us to further refine our efforts in developing the optimal CDK inhibitor in the indenopyrazole series.

References and notes

1. (a) Lee, M.-H.; Yang, H.-Y. *Cell. Mol. Life Sci.* **2001**, *58*, 1907. (b) Owa, T.; Yoshino, H.; Yoshimatsu, K.; Nagasu, T. *Curr. Med. Chem.* **2001**, *8*, 1487. (c) Sausville, E. A.; Johnson, J.; Alley, M.; Zaharevitz, D.; Senderowicz, A. M. *Annals of the New York Academy of Sciences* **2000**, *910*, 207. (d) McDonald, E. R., III; El-Deiry, W. S. *Intl. J. Oncol.* **2000**, *16*, 871. (e) Fry, D.; Garrett, M. D. *Cur. Opin. Oncol. Endocrine Metab. Investig. Drugs* **2000**, *2*, 40. (f) Sielecki, T. M.; Boylan, J. F.; Benfield, P. A.; Trainor, G. L. *J. Med. Chem.* **2000**, *43*, 1.
2. (a) Morgan, D. O. *Annu. Rev. Cell Dev. Biol.* **1997**, *13*, 261. (b) Pines, J. *Semin. Cell Biol.* **1994**, *5*, 399. (c) Hartwell, L. H.; Kastan, M. B. *Science* **1994**, *266*, 1821. (d) Sherr, C. J. *Cell* **1993**, *73*, 1059.
3. Nugiel, D. A.; Etzkorn, A.-M.; Vidwans, A.; Benfield, P. A.; Boisclair, M.; Burton, C. R.; Cox, S.; Czerniak, P. M.; Doleniak, D.; Seitz, S. P. *J. Med. Chem.* **2001**, *44*, 1334.
4. Nugiel, D. A.; Vidwans, A.; Etzkorn, A.-M.; Rossi, K. A.; Benfield, P. A.; Burton, C. R.; Cox, S.; Doleniak, D.; Seitz, S. P. *J. Med. Chem.* **2002**, *45*, 5224.
5. Yue, E. W.; Higley, C. A.; DiMeo, S. V.; Carini, D. J.; Nugiel, D. A.; Benware, C.; Benfield, P. A.; Burton, C. R.; Cox, S.; Grafstrom, R. H.; Sharp, D. M.; Sisk, L. M.; Boylan, J. F.; Muckelbauer, J. K.; Smallwood, A. M.; Chen, H.; Chang, C.-H.; Seitz, S. P.; Trainor, G. L. *J. Med. Chem.* **2002**, *45*, 5233.