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Parallel synthesis of acylsemicarbazide libraries: preparation of potent cyclin dependent kinase (cdk) inhibitors

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Abstract—Potent cyclin dependent kinase inhibitors were prepared using parallel synthesis methodology. Treating advanced intermediate **2** with a variety of hydrazides in DMSO at 80 °C for 30 min gave the desired acylsemicarbazides in good to excellent yield. Several compounds were active against cdk4/D1 and cdk2/E in the low nanomolar range. The SAR indicates a wide variety of substituents are tolerated at the acylsemicarbazide moiety.

1. Introduction

A fundamental process in biology is the division of cells mediated by the cell cycle.[1] Over expression of the tumor promoting components or the loss of tumor suppressing products can lead to unregulated cellular proliferation and the generation of tumors.[2] Cyclin dependent kinases (cdks) play a key role in regulating the cell cycle machinery.[3] An increasing body of evidence has shown a link between tumor development and cdk-related malfunctions.[4] This evidence has led to an intense search for small molecule inhibitors of the cdk family as an approach to cancer chemotherapy.[5] We recently disclosed a novel class of potent cdk inhibitors based on the indenopyrazole core (Fig. 1).[6] This report describes a new class of inhibitors prepared in an efficient manner using parallel synthesis.

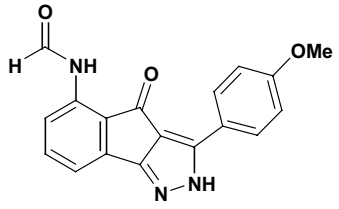

Figure 1. Potent indenopyrazole-based cyclin dependent kinase inhibitor. Cdk2/E IC_{50} = 0.24 μM; cdk4/D1 IC_{50} = 0.4 μM.

2. Chemistry

Scheme 1 details the preparation of acylsemicarbazide containing indenopyrazoles using parallel synthesis. The preparation of intermediate **1** was described previously.[6] This aniline was selectively protected on the pyrazole NH using SEMCl and triethylamine in refluxing $CHCl_3$ in 78% yield. The aniline was converted to the corresponding phenylcarbamate using phenylchloroformate and K_2CO_3 in 75% yield. Intermediate **2** was our key building block from which acylsemicarbazide libraries were generated. Treating carbamate **2** with assorted carbazates in DMSO at 80 °C gave good to excellent yields of the desired acylsemicarbazides. The DMSO was removed at reduced pressure and the SEM protecting group was removed by dissolving the residue in a 1:1 mixture of EtOH and 4 N HCl in dioxane. Heating this mixture for 30 min at 80 °C and subsequent purification by LC/MS gave the desired products in 70–93% yield for the two steps. ^{1}H NMR, MS, and HPLC, characterized each compound.

3. Results and discussion

Our library consisted of 84 compounds that were screened against cdk2/E and cdk4/D1 at an initial inhibitor concentration of 0.03 μM to determine preliminary

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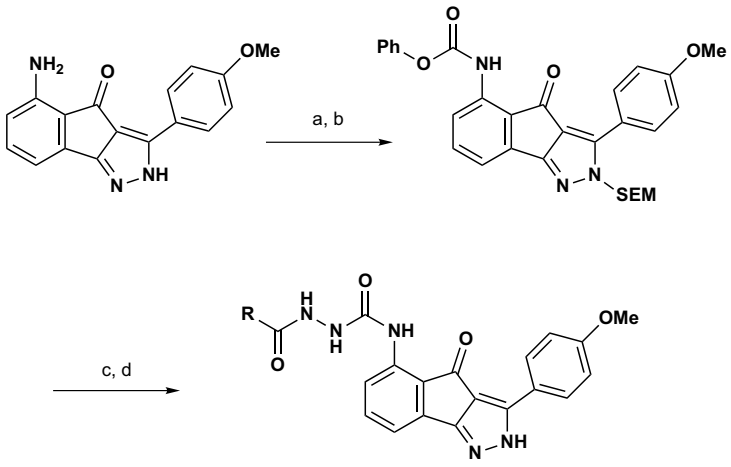


Scheme 1. Reagents and conditions: (a) SEMCl, Et_3N, $CHCl_3$, reflux 78%; (b) PhOC(O)Cl, K_2CO_3, acetone, 50 °C, 75%; (c) $RC(O)NHNH_2$, DMSO, 80 °C, 70–93%; (d) 4 N HCl, dioxane, 80 °C, 91%.

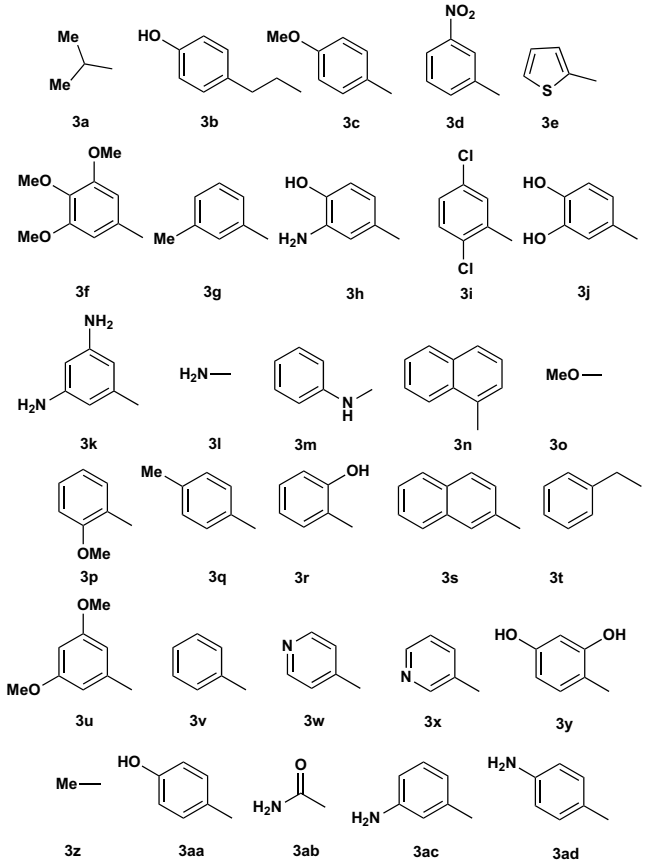
activity. Analogs showing a percent inhibition of >50% had standard six-point IC_{50} curves determined. The results of those measurements are presented in Table 2.

In general, acylsemicarbazides provide an excellent scaffold for generating inhibitors of both cdk2 and cdk4. A wide variety of substituents are tolerated in the SAR. There is a clear preference for substituted aromatic functionality. Branched alkyl groups are tolerated (**3a**) but a simple methyl group looses around 5-fold in potency especially against cdk4. The binding pocket accommodates both electron donating (**3f**,**k**,**u**) and electron withdrawing groups (**3d**,**i**,**w**) in a similar fashion. These substituents can be displayed in a variety of patterns without a significant impact on the compound's potency (Table 1).

This compound class is slightly more active against cdk4 than cdk2. The margin of selectivity is not large, at best 3-fold in favor of cdk4 inhibition (**3h**). In one example the activity profile is reversed and cdk2 inhibition is greater than cdk4 inhibition (**3ab**). This compound is also 10-fold less potent than the other examples in the table. This observation may have more to do with the desolvation penalty associated with binding this highly solvated substituent rather than an unfavorable interaction with the residues lining the binding pocket.

Table 2 contains cell-based data for compounds that inhibit the growth of a transformed human colon cancer cell line (HCT116). The compounds are active in the 0.03–1 μM range. Compounds containing hydrophobic substituents at the acylsemicarbazide moiety give better results than compounds with polar substituents. This observation seems to reflect the compound's ability to cross the cell membrane and influence cell growth rather than a measure of the compound's ability to inhibit enzyme activity. Most of the compounds taken into the cell-based assay are quite potent against both cdks measured (<0.03 μM) with little variance between the strongest and weakest inhibitors.

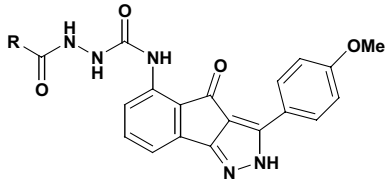
Table 1. List of substituents used to prepare acylsemicarbazide library



4. Conclusions

We have presented a facile method for preparing a novel class of cdk inhibitors using parallel synthesis. These acylsemicarbazides are highly potent against both cdk2/E and cdk4/D1. The compounds also inhibit the growth of a transformed human colon cancer cell line.

Table 2. Enzymatic and cellular activity for selected compounds



Compounds	Cdk2/E IC_{50}, μM[a]	Cdk4/D1 IC_{50}, μM[a]	HCT116 IC_{50}, μM[a]
3a	0.019	0.0065	NT
3b	0.013	0.009	NT
3c	0.015	0.011	NT
3d	0.024	0.010	NT
3f	0.020	0.007	NT
3g	0.013	0.007	NT
3h	0.024	0.007	NT
3i	0.036	0.028	NT
3j	0.010	0.005	NT
3k	0.039	0.016	NT
3l	0.13	NT	NT
3m	0.019	NT	0.28
3n	0.053	NT	NT
3o	0.016	0.008	0.05
3p	0.014	NT	0.04
3q	0.024	NT	0.14
3r	0.009	0.06	0.08
3s	0.035	0.013	0.12
3t	0.033	0.027	NT
3u	0.041	0.024	NT
3v	0.012	0.007	0.03
3w	0.012	0.009	0.19
3x	0.016	0.009	0.07
3y	0.016	0.012	0.25
3z	0.037	0.057	NT
3aa	0.009	0.005	1.1
3ab	0.097	0.24	1.2
3ac	0.013	0.007	0.08
3ad	0.01	0.01	0.07

[a] Values are means of two experiments, NT = not tested. For in vitro assay conditions see Ref. 7. For cell-based assay conditions see Ref. 8.

A wide variety of substituents are tolerated at the point of diversity indicating the binding pocket that accommodates the acylsemicarbazide moiety is rather large and somewhat lipophilic. We can take advantage of the promiscuous nature of this binding pocket to improve the physical properties of these compounds in an attempt to improve biologically relevant properties.

Acknowledgements

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7. The in vitro assays employ cell lysates from insect cells expressing either of the kinases and subsequently their corresponding regulatory units. The cdk/cyclin lysate is combined in a microtiter-type plate along with a kinase compatible buffer, ^{32}P-labeled ATP at a concentration of 50 mM, a GST–Rb fusion protein and the test compound at varying concentrations. The kinase reaction is allowed to proceeded with the radiolabeled ATP, then effectively stopped by the addition of a large excess of EDTA and unlabeled ATP. The GST–Rb labeled protein is sequestered on a GSH–Sepharose bead suspension, washed, resuspended in scintillant, and the ^{32}P activity detected in a scintillation counter. The compound concentration, which inhibits 50% of the kinase activity, was calculated for each compound.
8. We examined the effect of these compounds on cultured HCT116 cells and determined their effect on cell-cycle progression by the colorimetric cytotoxicity test using sulforhodamine B (Skehan et al. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112). HCT116 cells are cultured in the presence of test compounds at increasing concentrations. At selected time points, groups of cells are fixed with trichloroacetic acid and stained with sulforhodamine B (SRB). Unbound dye was removed by washing and protein-bound dye was extracted for determination of optical density.