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FOREWORD

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## **(5) Introduction**

The subject of this application was to develop novel methods to synthesize by combinatorial methods libraries of small molecules and to test small molecules for their ability to inhibit protein phosphatases thought to be important for the development of breast cancer. The scope of the research activity demanded that we develop robotic-based high throughput assays to determine inhibitory activity. The potential inhibitors are to be inspected for their phosphatase selectivity and for their kinetic interactions with the dual specific phosphatase Cdc25A, Cdc25B and/or Cdc25C. The cellular activity of selected inhibitors was to be examined and antitumor actions determined.

## **(6) Body**

### **Technical Objective #1. Development of Combinatorial Chemical Libraries**

The development of the methodologies for the synthesis of 3,000 novel compounds proposed in the original SOW has proven to be more challenging for us than expected. This was due to the complexities of the natural product structures, novel and unexpected aspects of the synthetic schemes, difficulties in maintaining compound stability and scale-up, and the use of new combinatorial-based equipment. Nonetheless, we have expanded both our pharmacophore platforms and the modular moieties extensively, especially during the funding period. Thus, we proposed to synthesize 1,000 per year for Technical Objective #1 during this last funding period and we synthesized 500 new compounds this last year. During the entire funding period we synthesized more than 1,200 compounds, which is slightly less than half of the number we outlined in our original SOW. Additionally, we have supplemented our evaluation efforts with compounds from the National Cancer Institute's Compound Library, which contains more than 130,000 compounds. Thus, we have also evaluated 10,000 compounds in the NCI's chemical library, including 2,000 compounds in their Diverset, which is family of compounds selected to represent the chemical diversity found in the NCI Library. Thus, we have been able to make and acquire sufficient compounds to achieved most of our SOW goals in Technical Objectives #2-4 of the application. Moreover, we have developed the methodology and instrumentation to further expand the concept of combinatorial-derived inhibitors of oncogenic phosphatases. Most importantly, our thoughts on the scientific process of finding novel inhibitors of phosphatase have evolved enormously. We have focussed considerable effort on understanding the mechanism of activity of our lead compounds because we believe this will facilitate the identification of the most desirable compound for the future.

For the entire funding period, we consider most significant accomplishments of Technical Objective #1 are the generation of new approaches to the synthesis of small molecule libraries and the synthesis of more than 1,200 new chemical entities. Because the progress of the application has been record extensively in the previous reports, I will outline the progress here of the last year in more detail. Specifically, during the last year we extensively modified the structure of the most active compound in our original library, 4-(benzyl-(2-[2,5-diphenyl-oxazole-4-carbonyl]-amino)-ethyl)-carbamoyl)-2-decanoylamino butyric acid (or SC- $\alpha\alpha\delta 9$ ), to improve its inhibitory activity. We have used a combinatorial/parallel synthetic approach to rigidify the variable core region and modify the side chains of SC- $\alpha\alpha\delta 9$ . We increased the complexity of the terminal R4 position to reduce hydrophobicity (Ducruet et al., 2000); (Figures 1 and 2). Approximately 50 carefully selected analogs of SC- $\alpha\alpha\delta 9$  were made by rigidifying the ethyldiamine central core (Scheme 1 and Figure 1), the most interesting of which was FY21- $\alpha\alpha 09$ . We also synthesized FY21- $\alpha\alpha\delta 9$ , with the expectation that it would combine the best properties of the rigid structure with the highly active variable side-chain groups. As second-generation platforms, we have designed new pharmacophores (Figures 3 and 4) with fewer amide bonds and a reduced degree of lipophilicity that would follow the structural guidelines of the most active compounds in previous libraries. This includes the use of a difunctionalized lysine core and a sulfonlated aminothiazole core (Scheme 2). We have also generated a family of novel quinones that have been extensively evaluated; especially notable is Compound 5 (Cpd 5) (Tamura et al., 2000) (Figure 5). The details of our overall synthetic approaches are found in the appended manuscripts.

## Technical Objective #2. AntiDSPase activity Assays

We have evaluated the ability of more than 11,000 compounds to inhibit Cdc25B *in vitro* using a newly developed high throughput assay; this greatly exceeds the proposed number outlined in our original SOW (3,000 compounds). This was due to the purchase of a Biomek 2000 laboratory robot, which permitted us to assay compounds provided both by the Combinatorial Chemistry Center and from the NCI. We have also assayed the selectivity of most of these compounds by measuring their ability to inhibit Cdc25A, Cdc25C, VHR (vaccinia human related phosphatase), and PTP1B (protein tyrosine phosphatase 1B) *in vitro*. Thus, we have conducted almost 10,000 different assays during the past year alone and have greatly exceeded our SOW aims for technical Objective #2. As mentioned above the accomplishments of the previous years or outline in the annual reports and the main emphasis here will be on results of the last year. The highlights of our assay during the last year have been the identification of several analogues that are effect inhibitors of PTP1B, VHR and Cdc25B<sub>2</sub> (Ducruet et al., 2000). Two compounds, FY3- $\alpha\alpha$ 09 and FY21- $\alpha\alpha$ 09, were partial competitive inhibitors of Cdc25B<sub>2</sub> with K<sub>i</sub> values of  $7.6 \pm 0.5$  and  $1.6 \pm 0.2$   $\mu$ M, respectively. At the time this was the most potent inhibitor of Cdc25B reported. Moreover, in contrast the parent compound SC- $\alpha\alpha$ 89, these two compounds were less active against PTP1B (Ducruet et al., 2000; see Table 2 within that reference) (see below; Technical Objective #3). We have assayed all of the lysine and sulfonylated aminothiazole derivatives and have found several inhibitors with IC<sub>50</sub> (median inhibitory concentration) values of approximately 10  $\mu$ M. None, however, were more potent or selective than previously described compounds. We have also examined the NCI Diverset and found at least 8 compounds that inhibit Cdc25B<sub>2</sub> with an IC<sub>50</sub> of <1  $\mu$ M. Several of these compounds are quinones and, thus, we were interested in making and examining additional compound of this chemical class. Thus, we assayed a small (<50 member) library of quinones that were synthesized based on the structure of vitamin K (Tamura et al., 2000). One analog, 2-(2-mercaptoethanol)-3-methyl-1,4-nathoquinone or Compound 5 (Cpd 5), was found to be a potent, selective, and partial competitive inhibitor of the Cdc25A, Cdc25B and Cdc25C. The K<sub>i</sub> values for Cdc25A, B<sub>2</sub> and C were 15, 1.7 and 1.3  $\mu$ M, respectively. Furthermore, the inhibition of Cdc25B<sub>2</sub> was dependent on the length of enzyme exposure to Cpd5; a 30 min pre-incubation with 2  $\mu$ M Cpd 5 caused almost 50% more inhibition in enzyme activity than in samples that were exposed to Cpd 5 at the time of substrate addition (Tamura et al., 2000). Pre-incubation longer than 30 min did not produce greater inhibition, possibly because Cpd 5 became inactivated. No reduction in enzyme activity was seen when Cdc25B<sub>2</sub> was preincubated with 0.5% DMSO for 90 min or less. The time-dependent inhibition was irreversible: a 90 min incubation in Cpd 5-free buffer did not restore the lost enzyme activity.

## Technical Objective #3. Inhibitor Specificity Assays

An important but often overlooked aspect of drug discovery is the identification of compounds that are highly selective for the molecular target. Thus, we proposed and used several counter assays that should decrease significantly the concern about nonspecific inhibition of protein phosphatases. In our original proposal, we suggested that CL-100, PTP1B and PP1 Ser/Thr phosphatases would be suitable. We found, however, that CL-100 is extremely difficult to express and isolate. Consequently, we used VHR, which is more attractive since it was discovered by Denu and colleagues to be the DSPase that dephosphorylates the important mitogen activated kinase (MAPK). VHR is easy to generate and quite stable. We have also stopped using PP1 as a counter assay, because we have found no inhibitors of this enzyme. In retrospect this is not surprising, because of the extreme structural differences between the Ser/Thr phosphatases and the DSPases. As mentioned above the Biomek 2000 allows us to quickly determine the effects of our compound on the counter phosphatases. Thus, we have evaluated more than 3,000 compounds for their activity against one or more of these other phosphatases during the period of this proposal. We have also obtained Pyst1 and 2, which are DSPases that may dephosphorylate MAPK, but we have not yet been successful in expressing large quantities of these phosphatases to use in our analyses.

#### Technical Objective #4. Breast Cancer Growth Inhibition/Apoptosis Studies

We have examined the cellular activity of all compounds that have biochemical activity as well as many of the other members of the libraries. Our original SOW for Technical Objective #4 was to assay 3,000 compounds with human MCF-7 and MDA-MB-231 cells. We have found, however, that the usage of compounds for the biochemical assays has sometimes required more of the compound than we anticipated. Therefore, the relatively large amount of compound needed for the cellular assays were not always available. Nevertheless, we examined all compounds that were available in sufficient amounts, which were approximately 500, and probed active compounds deeply. This later activity required considerable research time. For example during the last year, we examined most of the difunctionalized lysine core analogs, the sulfonylated aminothiazole core analogs and the modified quinones. The FY- series was also examined with both cell lines (Ducruet et al., 2000) (Figure 6). Several of these compounds inhibited human breast cancer growth. For example, in the FY series, FY21- $\alpha\alpha$ 09 caused greater than 90% inhibition of MDA-MB-231 or MCF-7 cell growth at 100  $\mu$ M (Figure 6).

Active compounds were examined in greater detail. Therefore, we probed the cellular effects of SC- $\alpha\alpha$ 89, FY21- $\alpha\alpha$ 09 and Cpd 5 with the temperature sensitive tsFT210 cells, which provide a very convenient reagent for examining effects on cyclin-dependent kinase (cdk) phosphorylation status. This has been mentioned for SC- $\alpha\alpha$ 89 in previous Progress Reports. A 6 h exposure of tsFT210 cell to 100  $\mu$ M FY21- $\alpha\alpha$ 09 partially blocked cell cycle progression at the G2/M checkpoint while the biochemically inactive congener SC- $\alpha\alpha$ 09 did not cause any block (Figure 7). FY21- $\alpha\alpha$ 09 at 100  $\mu$ M was as effective inhibiting the G2/M transition as the tubulin binder nocodazole at 1  $\mu$ M. The G2/M block observed with FY21- $\alpha\alpha$ 09 is consistent with intracellular inhibition of Cdc25B. FY21- $\alpha\alpha$ 09 also blocked in G1/S consistent with inhibition of Cdc25A (Figure 8). We also found that 10  $\mu$ M Cpd 5 cause a prominent inhibition of G2/M transition in the tsFT210 cell that was comparable to that seen with higher concentrations of either FY21- $\alpha\alpha$ 09 or SC- $\alpha\alpha$ 89 (Figure 9). Cpd 5 also caused a prominent block in G1/S checkpoint transition in the tsFT210 cells (Figure 10) (Tamura et al., 2000).

Because one of the putative, endogenous, cellular, substrates for both Cdc25B and Cdc25C is the mitotic inhibitor Cdk1, which must be dephosphorylated to allow entry into mitosis. Thus, we reasoned that an effective Cdc25 inhibitor would not only cause a G2/M cell cycle block but would also prevent Cdk1 dephosphorylation. We, therefore, performed Western blotting on tsFT210 cell extracts to determine the Cdk1 (a/k/a Cdc2) phosphorylation levels in the presence or absence of SC- $\alpha\alpha$ 89, FY- $\alpha\alpha$ 09 or Cpd 5. Because the results with SC- $\alpha\alpha$ 89 were described previously, the results with Cpd5 will be mentioned here in greater detail. Protein lysates of tsFT210 cells arrested at the G2/M boundary were harvested and analyzed by SDS-PAGE. Approximately 50% of Cdk1 was in the mitotic-inactive hyper-phosphorylated form as reflected by a slower migrating Cdk1 (Figure 11). The phosphorylation of Cdk1 decreased gradually after cells were released from G2/M block, and most of the Cdk1 was dephosphorylated 6 h after G2/M release, even in the presence of the DMSO vehicle (Figure 11A). When we incubated cells with 1  $\mu$ M nocodazole, which caused a G2/M arrest, no hyper-phosphorylation of Cdk1 was seen (Figure 11B), consistent with its proposed inhibitory activity after Cdk1 activation. In contrast, Cdk1 dephosphorylation was partially blocked with 10  $\mu$ M Cpd 5 and completely blocked with 15-20  $\mu$ M (Figure 11C). SC- $\alpha\alpha$ 89 at 50  $\mu$ M also caused hyperphosphorylation of Cdk1 (Figure 11B). Because the phosphorylation status of Cdk1 determines its enzymatic activity, we examined the kinase activity of immunoprecipitated Cdk1 by measuring histone H1 phosphorylation *in vitro*. We found that the Cdk1 kinase activity in cells treated with 1  $\mu$ M nocodazole was significantly increased, which was consistent with a previous study using tsFT210 cells. The Cdk1 kinase activity in cells treated with 10-20  $\mu$ M Cpd 5 was markedly reduced (Figure 11D). Inactive congeners, however, did not block this kinase activity as expected by their lack of effect on Cdc25 activity.

Cdk4 plays a central role in regulating the G1 transition by its association with cyclin D1. This complex remains inactive until Cdc25A dephosphorylates it. Cdk2 is also involved in regulating the G1/S transition by its association with cyclin E or cyclin A. The Cdk2/cyclin E complex has been shown to be dephosphorylated at Thr14 and Tyr15 and, thereby, activated by Cdc25A treatment *in vitro*.

To clarify the mechanism of G1 cell cycle block by Cpd 5, we treated tsFT210 cells with  $\leq 20 \mu\text{M}$  Cpd 5 for 6 h, immunoprecipitated Cdk2 or Cdk4 from the cell lysates, and then determined tyrosine phosphorylation by Western blotting, using anti-phosphotyrosine monoclonal antibody. As illustrated in Tamura et al. (Tamura et al., 2000), the phosphorylation of both Cdk2 and Cdk4 increased after Cpd 5 treatment. We confirmed that there was equivalent loading of Cdk2 or Cdk4 with anti-Cdk2 or anti-

Cdk4 antibody, respectively. To quantify the phosphorylation level of Cdk2 or Cdk4, we determined the intensity of the bands by densitometer, and calculated a phosphorylation level. Both Cdk2 and Cdk4 tyrosine phosphorylation increased in a concentration-dependent manner after Cpd 5 treatment with a >5-fold increase being seen after exposure to  $20 \mu\text{M}$  Cpd 5.

Cdk requires noncovalent interactions with cyclins to be functional. To exclude the possibility that Cpd 5 simply blocked such an intracellular interaction, we treated tsFT210 cells with Cpd 5 for 6 h, immunoprecipitated Cdk2 from cell lysates with an anti-Cdk2 antibody, and then examined the immunoprecipitate for cyclin A and E content by Western blotting. We also immunoprecipitated Cdk4 from cell lysates with anti-Cdk4 and determined cyclin D1 protein levels. Cyclin A or E association with Cdk2 was unchanged after Cpd 5 treatment. Similarly, Cdk4 association with cyclin D1 was unaffected by the Cpd 5 treatment (Tamura et al., 2000).

The phosphorylation of Rb, which is a critical regulator of the G1 checkpoint, is controlled in part by Cdk2. Thus, we examined the phosphorylation status of Rb in synchronous tsFT210 cells at various times after addition of  $20 \mu\text{M}$  Cpd 5. As expected, the Rb phosphorylation increased with passage into G1 phase. Within 1.5 h after exposure of cells to Cpd 5, however, there was a marked inhibition of Rb phosphorylation with no alteration of Rb protein levels. Thus, our results support the hypothesis that Cpd 5 blocked cell cycle progression through the G1 checkpoint by disruption of functional cyclin dependent protein kinase activity through inhibition of Cdc25A activity. To ensure that the inhibition of Cdk4 kinase activity and cell cycle arrest were not secondary to p53 induction or increased Cdk inhibitors, we measured p53, p21 and p16 levels in tsFT210 cells after Cpd 5 treatment. tsFT210 cells, which had been treated with an equitoxic etoposide concentration, displayed elevated p53 levels while Cpd 5 produced no increase. We also saw no increase in p21 or p16 with Cpd 5 suggesting that the dual cell cycle phase arrest was not due simply to nonspecific cell stress or DNA damage. These results can be found elsewhere (Tamura et al., 2000)

#### **Technical Objective #5. Cell Culture Cdc25A/B Specificity**

We developed the SV-40 large T antigen mouse embryonic fibroblast (MEF) system in the first year of the proposal as proposed. We have tested at least ten of the most promising compounds for specificity with the MEF system, most notably SC- $\alpha\alpha\delta 9$ , FY21- $\alpha\alpha 09$  and Cpd5. Results with SC- $\alpha\alpha\delta 9$  were reported previously. FY21- $\alpha\alpha 09$  showed selective inhibition of growth for MEF expressing higher levels of Cdc25 (namely the SV40 transformed cells). Cpd 5 had no selective effect on MEF cell growth. Neither compound had a selective effect when we examined them with the Cdc25B null cells. We are still evaluating the Cdc25B null cells for their phenotype and the possibility that there is altered Cdc25A, Cdc25C, Cdk1, Cdk2 or Cdk4 expression, however. Our current interpretation is that potential Cdc25 inhibitors could affect several Cdc25 isoforms and selective resistance to an inhibitor of Cdc25B might not be detected with this cell type. Thus, it may be necessary to develop a double (namely Cdc25B and Cdc25C-/-) or triple (namely, Cdc25A, Cdc25B and Cdc25C-/-) knockout cell. These may not be viable, however. We also examined SC- $\alpha\alpha\delta 9$  in a tumor model for antitumor activity and it was found to be active. These results have been reported in the previous Progress Report.

#### **(7) Key Research Accomplishments**

- We developed new methods to synthesize compounds by combinatorial methods.
- We synthesized more than 1,200 new chemical entities.

- We have evaluated more than 10,000 compounds from the National Cancer Institute's Compound Library, which contains more than 130,000 compounds.
- We have established the first robotic-based high throughput assay system in our University for studying compound activity.
- We have discovered three of the most potent inhibitors of Cdc25 reported to date.
- We have documented that these inhibitors, SC- $\alpha\alpha\delta 9$ , FY21- $\alpha\alpha 09$  and Cpd5, block cells at the predicted cell cycle checkpoints associated with Cdc25 activity.
- We have documented that these inhibitors, SC- $\alpha\alpha\delta 9$ , FY21- $\alpha\alpha 09$  and Cpd5, inhibit Cdc25 within cells.
- We have shown that SC- $\alpha\alpha\delta 9$  has antitumor activity in an animal model.
- We have provide additional evidence that CDc25 is an excellent target for future drug discovery activities for breast cancer-directed therapies.

## **(8) Reportable Outcomes**

### Bibliography of All Publications:

Wipf P, Cunningham A, Rice RL and Lazo JS (1997) Combinatorial synthesis and biological evaluation of library of small-molecule Ser/Thr-protein phosphatase inhibitors. *Bioorg. Med. Chem.* 5:165-177.

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Vogt A, Rice RL, Settineri CE, Yokokawa F, Yokokawa S, Wife P and Lazo JS. (1998) Disruption of IGF-1 signaling and downregulation of Cdc2 by SC-  $\alpha\alpha\delta 9$ , a novel small molecule antisignaling agent identified in a targeted array library. *J. Pharmacol. Exper. Therap.* 287:806-813.

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Vogt A, Wang AS, Johnson CS, Fabisiak JP, Wipf P and Lazo, JS. (2000) In vivo antitumor activity and induction of insulin-like growth factor-1-resistant apoptosis by SC - $\alpha\alpha\delta 9$ . *J. Pharmacol. Exper. Therap.* 292:530-537.

Tamura K, Southwick EC, Kerns J, Rosi, K, Carr B, Wilcox C and Lazo JS. (2000) Cdc25 inhibition and cell cycle arrest by a synthetic thioalkyl vitamin K analogue. *Cancer Res.* 60:1317-1325.

Lazo JS and Wipf P. (2000) Combinatorial chemistry and contemporary pharmacology. *J. Pharmacol. Exp. Therap.* 293:705-708.

Ducruet AP, Rice RL, Tamura K, Yokokawa F, Yokokawa S, Wipf P and Lazo JS. (2000) Identification of new Cdc25 dual specificity phosphatase inhibitors in a targeted small molecule array. *Bioorg. Med. Chem.* 8:1451-1466.

### Abstracts:

Pestell K, Southwick EC, Wilcox C and Lazo JS. (2000) Vitamin K analogs inhibit Cdc25B and disrupt Cdc25B2 subcellular distribution. *Proc. Amer. Assoc. Cancer Res.* 41:371.

Ducruet AP, Rice RL, Tamura K, Yokokawa F, Yokokawa S, Wipf P and Lazo JS. (2000) Identification of Cdc25 dual specificity phosphatase inhibitors in a targeted small molecule array. Proc. Amer. Assoc. Cancer Res. 41:2998.

Patent:

Inhibitors of protein phosphatase. US provisional application in the process of being submitted by the University of Pittsburgh.

Degrees awarded:

Robert Rice, M.D., Ph.D.  
Alexander P. Ducruet, Ph.D. student

Pending Funding:

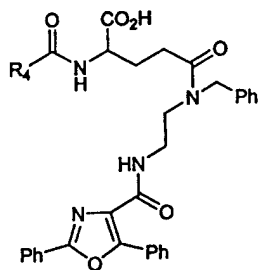
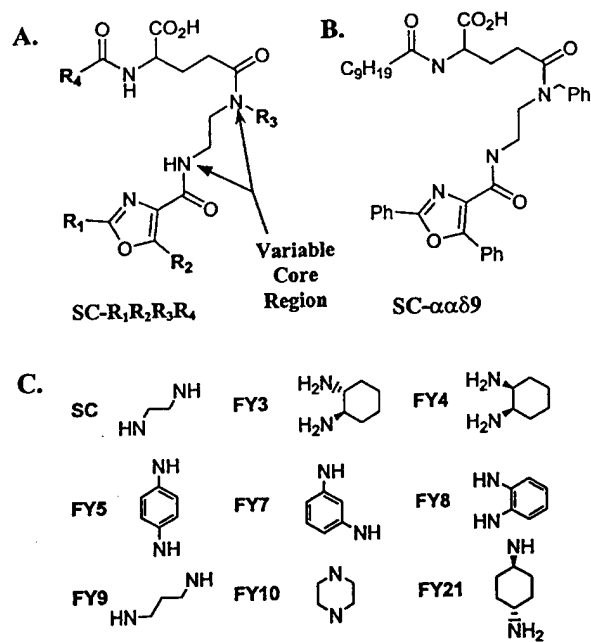
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**(9) Conclusions**

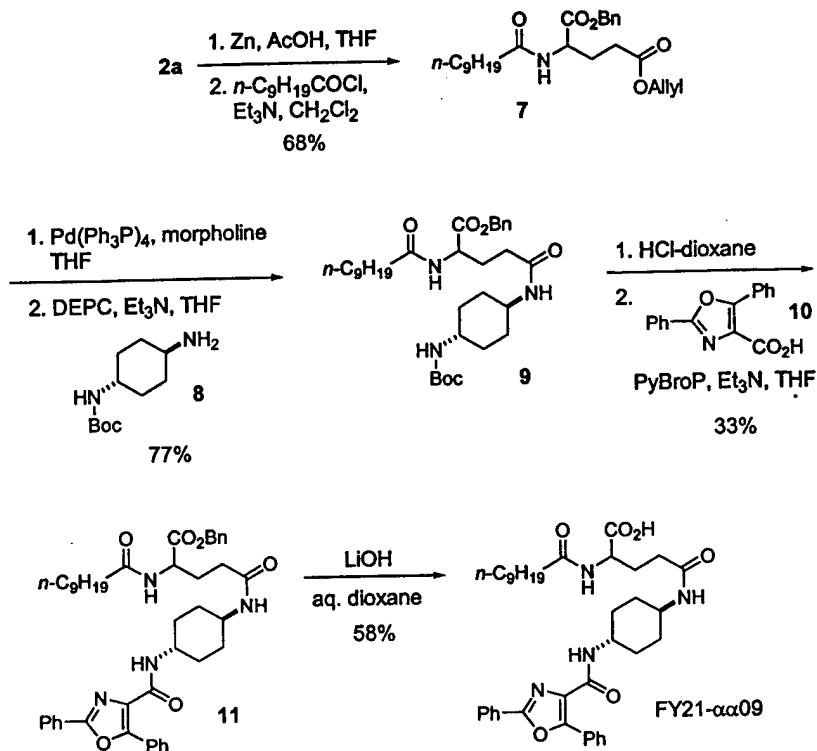
The results of this research have established a robotics-based high throughput system for compound analysis in an academic setting. This is a model for others within our University and elsewhere. The compound libraries that are now available for us to use for a variety of exploratory purposes also is very unique and valuable. This work has also provided important new reagents that allow us and others to probe the role Cdc25 and other protein phosphatases have in biological processes. Compounds like SC- $\alpha\alpha$ 89, FY21-- $\alpha\alpha$ 09 and Cpd5 are the most potent and selective inhibitors of Cdc25 reported to date and they provide a useful platform for future analog development. Importantly, our work validates the use of Natural Products as a starting point for compound identification and has provided additional evidence that specific inhibitors of Cdc25 and other protein phosphatases can be uncovered. Moreover, we hope that these compounds or analogs may be useful as future agents to treat human breast cancer.



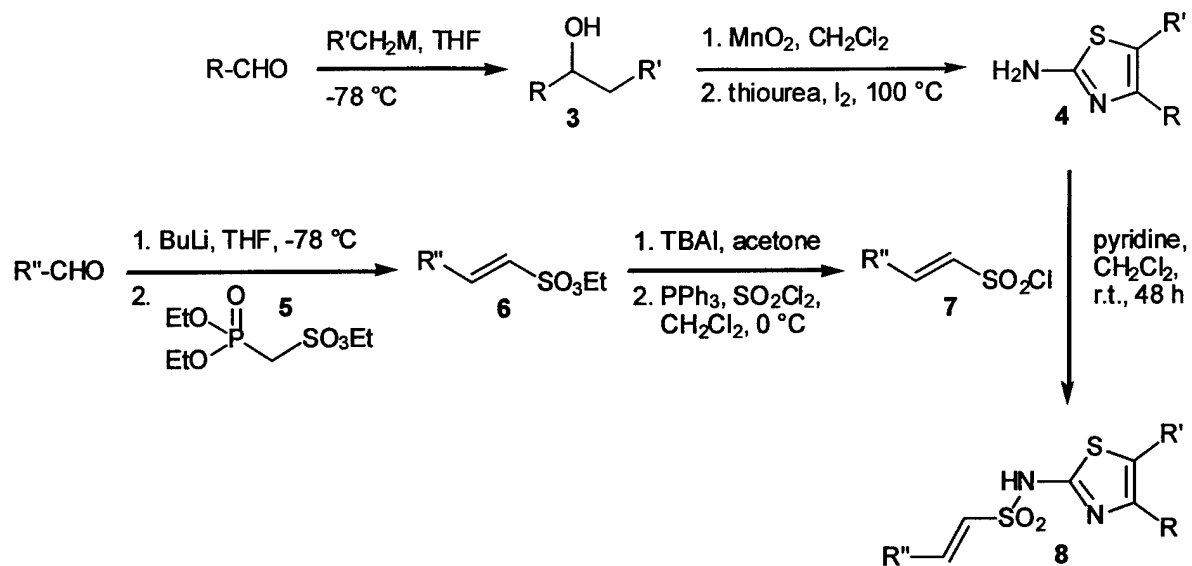
**Figure 1.** SC pharmacophore and variable core region substituents. General chemical structure for the founding SC pharmacophore series.  $-R_n$  indicate the combinatorial sites on the pharmacophore and are sequentially listed after the core prefix: (A) the variable core region; (B) chemical structure of SC- $\alpha\alpha\delta 9$ ; (C) structure of variable core diamine linker substitutions.

Compound	$R_4$
SC- $\alpha\alpha\delta 9$	$\text{Me}(\text{CH}_2)_8$
SC- $\alpha\alpha\delta 15$	$\text{Me}(\text{CH}_2)_{14}$
SC- $\alpha\alpha\delta 17\text{A}$	$\text{Me}(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7$
SC- $\alpha\alpha\delta 17\text{B}$	$\text{Me}(\text{CH}_2)_4\text{CH}(\text{CH}_2)_7$
SC- $\alpha\alpha\delta\text{A}$	
SC- $\alpha\alpha\delta 6\text{III}$	$\text{Me}(\text{OCH}_2)_3\text{OCH}_2$
SC- $\alpha\alpha\delta 4\text{II}$	$\text{Me}(\text{OCH}_2)_2\text{OCH}_2$

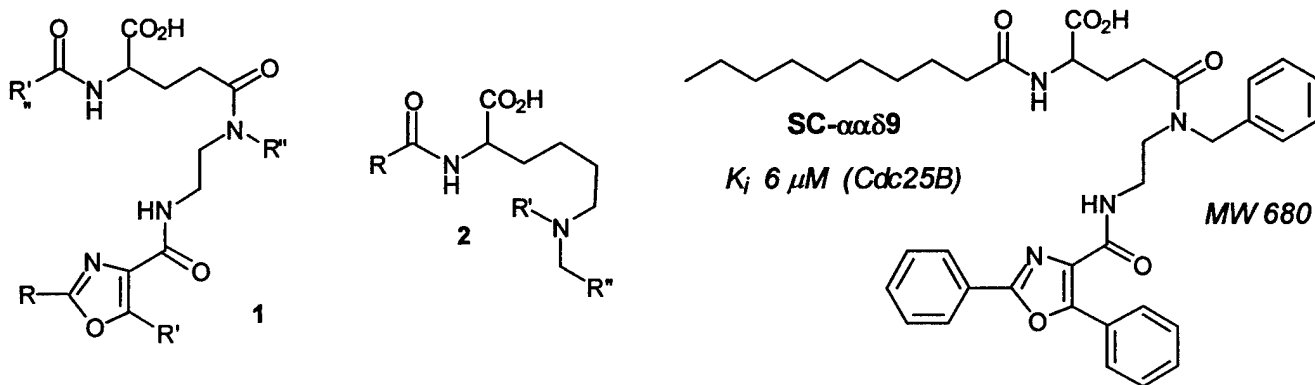
**Figure 2.** Chemical structures of SC- $\alpha\alpha\delta 9$   $R_4$  analogues.



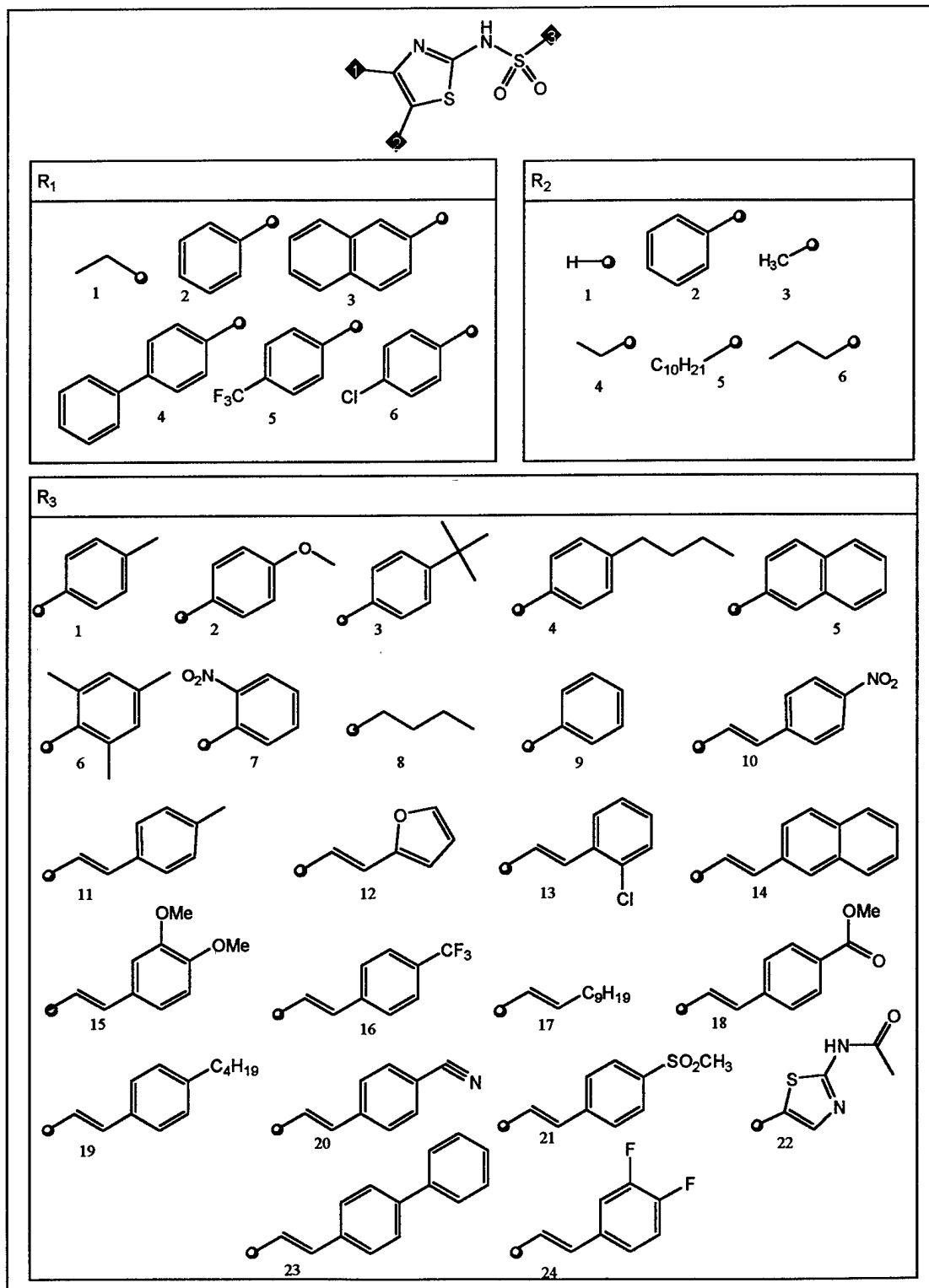
## Scheme 1



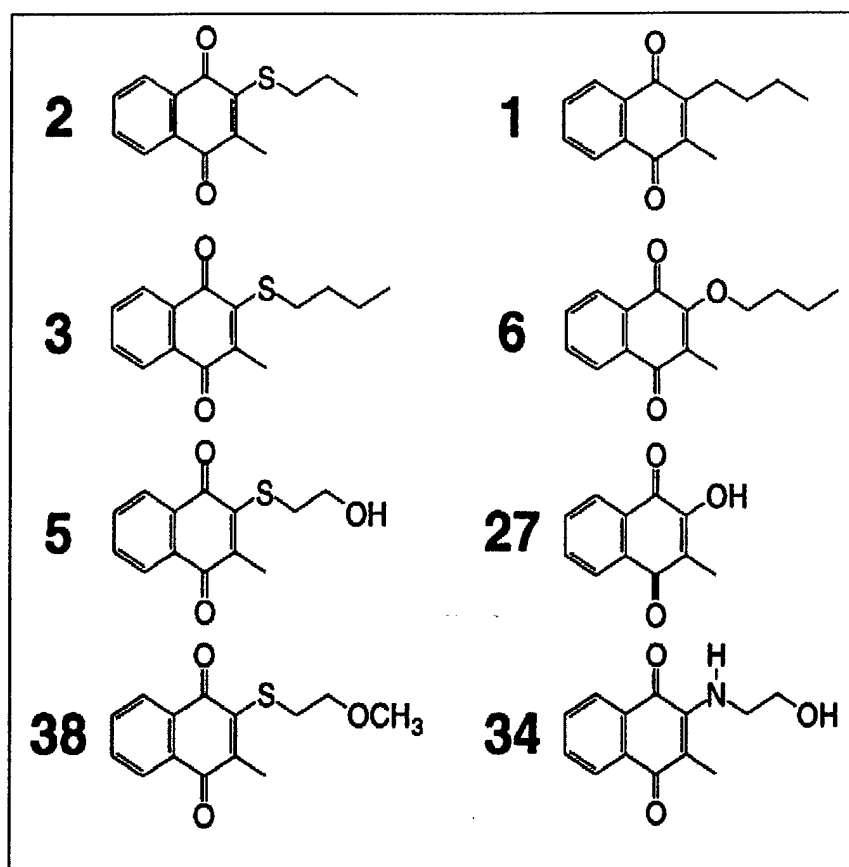
**Scheme 2**



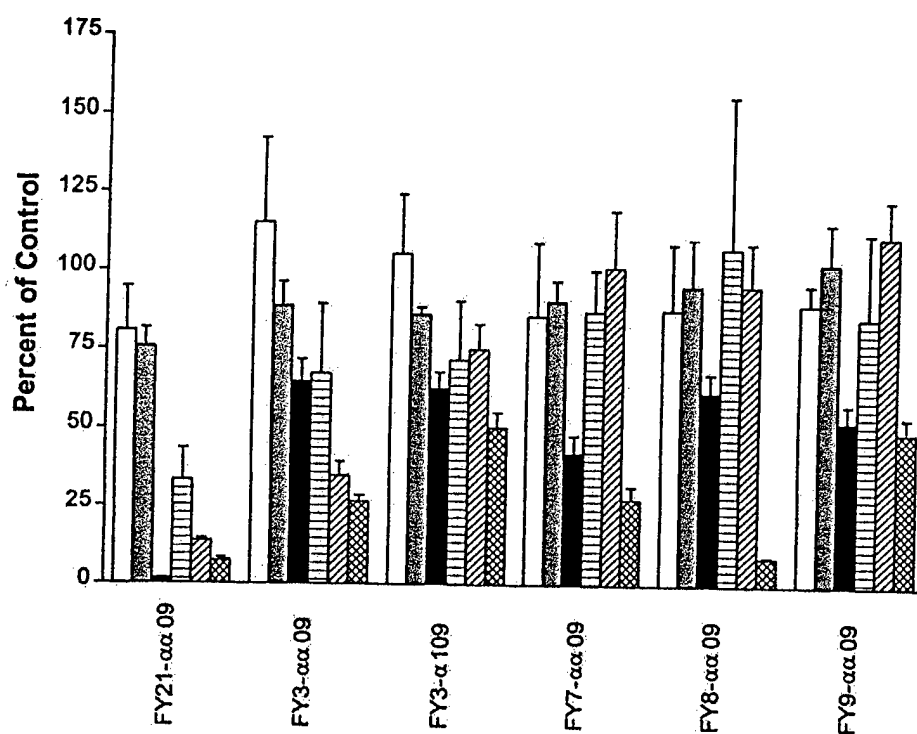
**Figure 3**



**Figure 4**



**Figure 5**



**Figure 6.** Antiproliferative effects of selected compounds on MDA-MB-231 and MCF-7 human breast cancer cells. Antiproliferative effect of compounds. MDA-MB-231 cells: 10  $\mu\text{M}$  ( $\square$ ) 30  $\mu\text{M}$  ( $\blacksquare$ ) and 100  $\mu\text{M}$  ( $\blacksquare$ ), MCF-7 cells: 10  $\mu\text{M}$  ( $\square$ ), 30  $\mu\text{M}$  ( $\boxtimes$ ) and 100  $\mu\text{M}$  ( $\boxtimes$ ). The antiproliferative effect of compounds was determined as described in Experimental procedures and results were normalized to vehicle treated controls. Error bars represent standard deviations ( $n=6$ ).

Figure 7. Inhibition of cell cycle progression at G2/M by FY21- $\alpha\alpha$ 09 and SC- $\alpha\alpha$ 09 in tsFT210 cells.

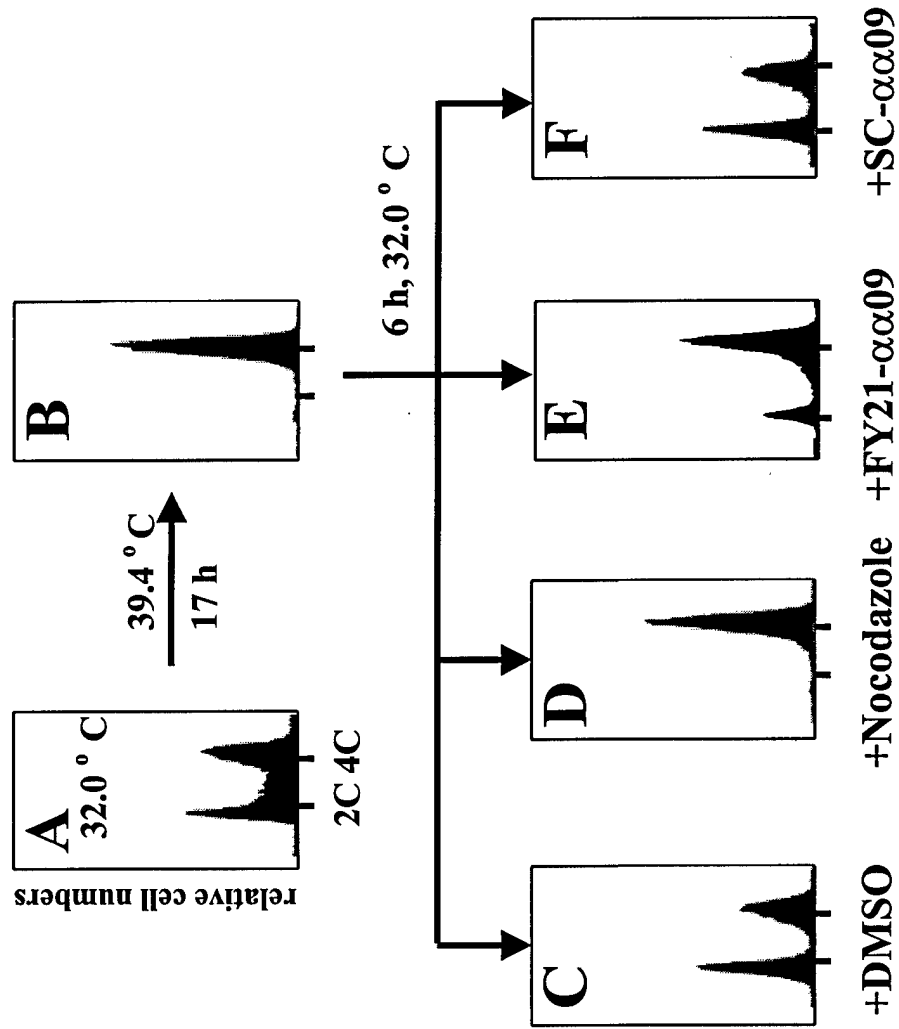


Figure 8. Inhibition of cell cycle progression at G1 by FY21- $\alpha\alpha 09$  and SC- $\alpha\alpha\delta 9$  in tsFT210 cells.

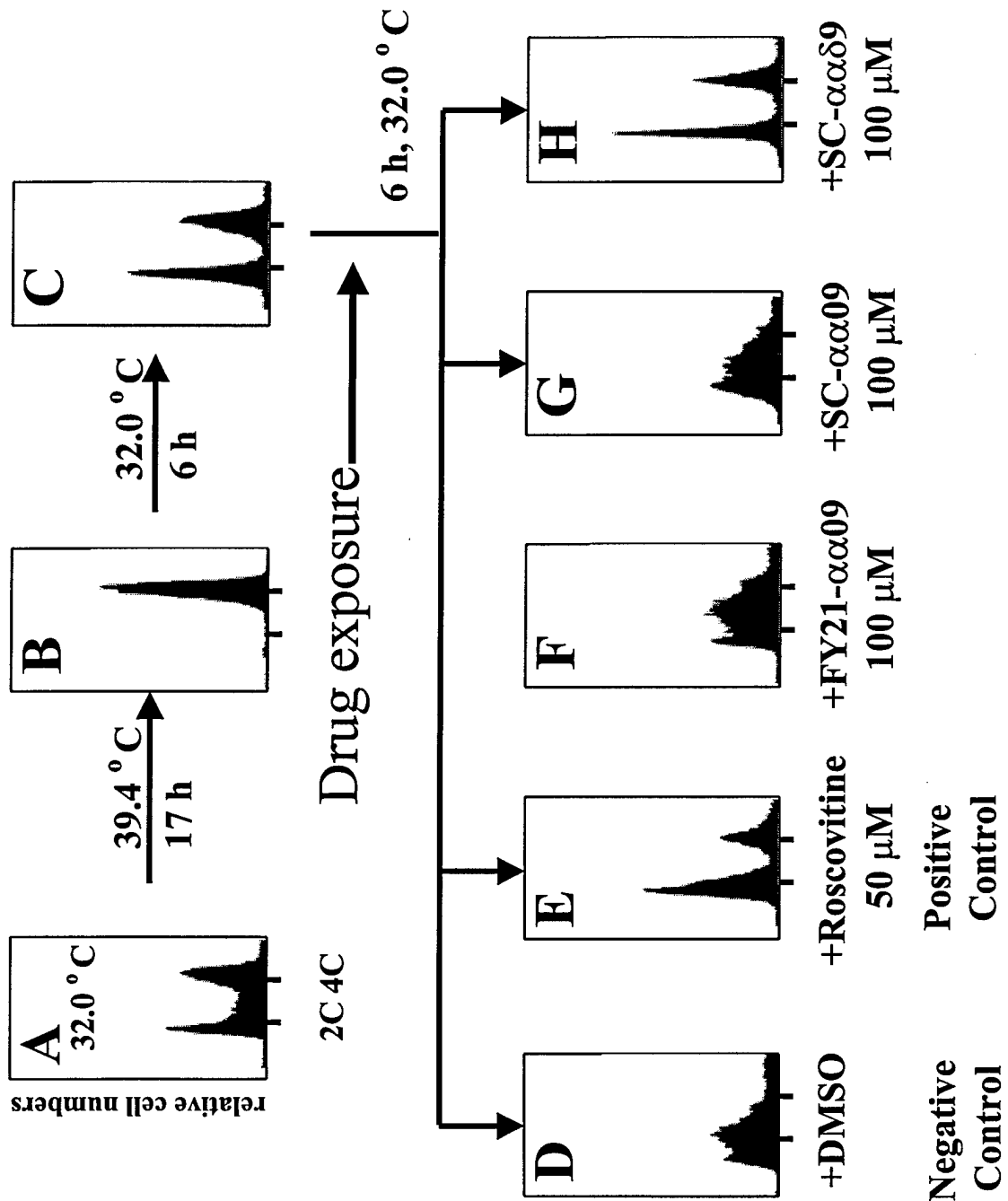


Figure 9. Inhibition of cell cycle progression at G1 by Cpd 5 and SC- $\alpha\alpha\delta 9$  in tsFT210 cells.  
 Cpd 16 and Cpd 22 are inactive compounds.

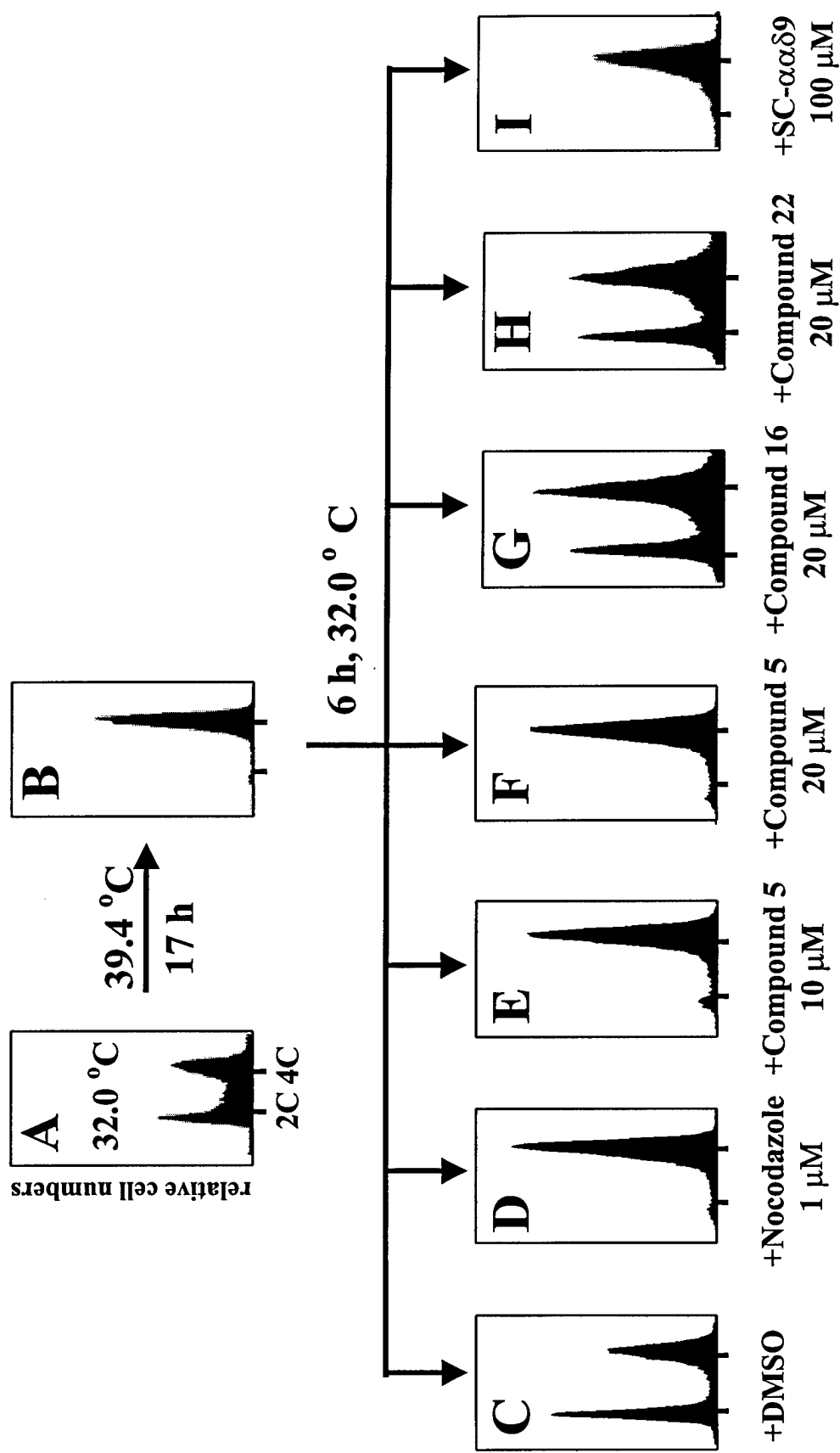


Figure 10. Inhibition of cell cycle progression at G1 by Cpd 5 and SC- $\alpha\alpha\delta 9$  in tsFT210 cells.

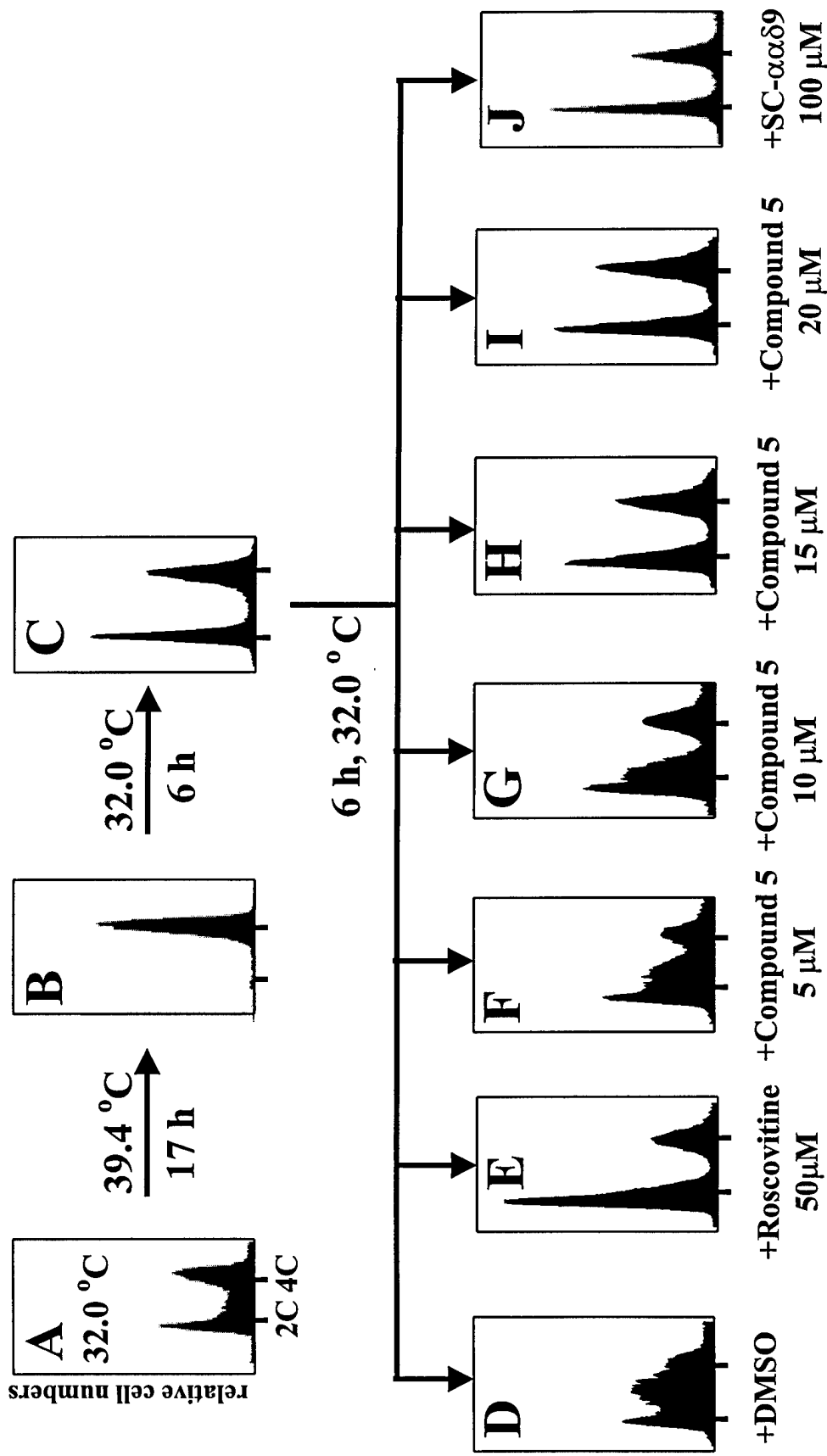


Figure 11. Inhibition of Cdk1 dephosphorylation by Cpd 5 and SC- $\alpha\alpha\delta 9$  in tsFT210 cells.

