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# A Catch-and-Release Strategy for the Combinatorial Synthesis of 4-Acylamino-1,3-thiazoles as Potential CDK5 Inhibitors

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**Abstract**—Two-dimensional libraries of 4-acylamino-1,3-thiazoles **9** were prepared via Curtius rearrangement of 1,3-thiazole-4-carbonyl azides **6**, trapping of the intermediate isocyanates with oxime resin, and thermal regeneration of the isocyanates from the washed resin in the presence of nucleophiles. Several compounds proved to be selective inhibitors of CDK5/p25 versus the closely homologous CDK2/cyclin A enzyme, with the best analogue (**43**) possessing over 100-fold selectivity.


One of the pathological hallmarks of Alzheimer's Disease is cytoplasmic filamentous material known as neurofibrillary tangles (NFT) that accumulates in the neuronal cell soma.[1,2] The main component of NFT is an aggregate of the microtubule-stabilizing protein tau in a hyperphosphorylated state. NFT are associated with microtubule destabilization, synapse dysfunction and dementia. Cyclin-dependent kinase 5 (CDK5) is a serine/threonine kinase that is required for normal neuronal development.[3] Association of CDK5 with its regulatory subunit p35 is necessary for enzyme activation, and this complex phosphorylates a variety of substrates. CDK5/p35 (also known at Tau Protein Kinase II or TPKII) is believed to be one of the major kinases that phosphorylates tau in AD brains.[4] CDK5 is constitutively activated in brain tissue of Alzheimer's patients due to the displacement of the normal regulatory subunit p35 by a truncated form p25.[5] In addition, the enzyme complex has been shown to co-localize with NFT, suggesting that inhibitors of CDK5 may have therapeutic efficacy for the treatment and/or prevention of Alzheimer's disease.[6]

Broad screening of the Pharmacia compound collection for inhibitors of TPK II led to the identification of **4** ($K_i = 0.5–2.0$ μM). Of particular interest was the modest degree of specificity it exhibited for CDK5 versus the closely homologous cell cycle regulatory kinase complex CDK2/cyclin A ($K_i = 10–20$ μM). For obvious reasons, specific inhibition of a particular target kinase relative to the plethora of other crucial regulatory kinases is of paramount importance.

Preliminary medicinal chemistry work (not shown) on **4** quickly established that simple urea or hydrazide analogues were not active, indicating that the aminourea moiety was important. The 1,3-thiazole nucleus of **4** suggested a straightforward synthesis of two-dimensional combinatorial libraries (varying the 2-substituent and the 4-aminourea) commencing with the known preparation of thiazole-4-carboxylates from thioamides (Scheme 1, steps a,b).[7] This route was designed to avoid the intermediacy of 4-amino-1,3-thiazoles, which have been reported[8a] to be unstable, by directly generating the 4-amino substituent in an acylated form (isocyanate) ready for nucleophile introduction.[8b] Although **4** could be successfully prepared from thioamide **1** by this route, the final product was contaminated by numerous impurities, from which the desired product had to be chromatographically isolated in poor yield. The major byproducts were the symmetrical urea derived from the aminothiazole and compounds resulting from insertion of the intermediate nitrene into solvent.

To facilitate the production of libraries free of these impurities, we developed a catch-and-release approach,[9]

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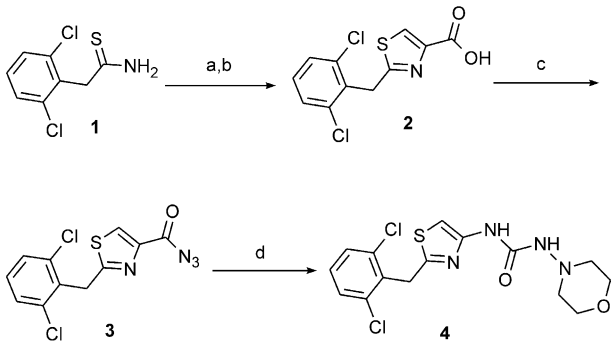
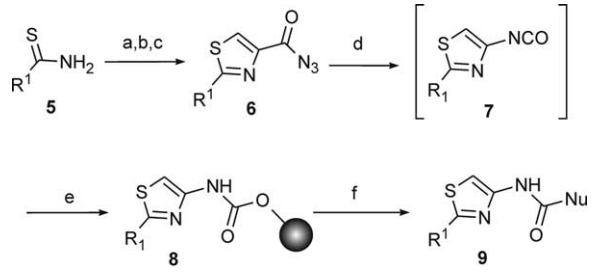


**Scheme 1.** (a) $BrCH_2COCO_2Et$, 100 °C (30%); (b) aq KOH, THF (93%); (c) oxalyl chloride, $CHCl_3$; $NaN_3$, acetone (66%); (d) toluene, 80 °C; 4-aminomorpholine (34%).

that is outlined in Scheme 2. Thioamides **5** were converted to acyl azides **6** via a protocol similar to that described in Scheme 1 (76% avg yield). Addition of oxime resin [polystyrene-C(4–$NO_2$–Ph)=NOH] to the intermediate isocyanates **7**, generated from thermolysis of azides **6**, afforded resins **8**, which could be rinsed free of impurities by repeated washing with $CH_2Cl_2$ and MeOH. The presence of the resin-bound carbamate was confirmed by FTIR (1751–1759 $cm^{-1}$).[10] Heating the washed resins **8** in a mixture of the desired nucleophile (NuH), triethylamine and either toluene or 1,2-dichloroethane afforded the desired acylamino thiazoles **9**. *The addition of triethylamine base was found to greatly facilitate the elimination of the isocyanate from the oxime resin, giving improved yields and purities*. Purification of the crude products was accomplished by treating the reaction mixtures with isocyanate resin (to remove excess NuH) prior to filtration, followed by SLE to remove excess triethylamine. To illustrate the superiority of this catch and release protocol to the solution phase route described in Scheme 1, lead compound **4** could be reproducibly obtained from the corresponding azide **3** in nearly quantitative yield with a purity of 93–94% using the method in Scheme 2.[11]

The route depicted in Scheme 2 proved useful for the production of both aminourea analogues ($Nu=R^2R^3NNR^4$) and alkoxyurea analogues (Nu=R-ONH), using commercially available hydrazines and alkoxyamines. Thioamides **5** were either commercially available, prepared from the corresponding carboxamides (Lawesson's reagent, toluene, 65 °C) or prepared from the corresponding nitriles (i) 30% $H_2O_2$, 3M NaOH, $Bu_4NHSO_4$; (ii) Lawesson's reagent, toluene, 65 °C). A combination of 22 thioamides, 56 hydrazines and nine alkoxyamines were employed in the course of preparing several two-dimensional libraries (50–100 compounds each- not all possible combinations were prepared), from which products could be obtained in satisfactory purity (>80% by HPLC/MS) from 20 thioamides, 41 hydrazines and all nine alkxoyamines. Yields for the various libraries averaged about 70% from the precursor acyl azides. Representative examples are included in Table 1.



**Scheme 2.** (a) $BrCH_2COCO_2Et$, EtOH, 75 °C; (b) 12 M KOH, 50 °C; 5 M HCl; (c) Oxalyl chloride, cat. DMF, $CH_2Cl_2$, rt; $NaN_3$, THF, 40 °C; (d) toluene, 80 °C; (e) oxime resin, $CH_2Cl_2$, rt; washes; (f) toluene or 1,2-dichloroethane, triethylamine, NuH, 80 °C.

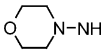
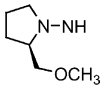
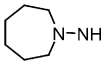
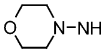
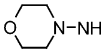
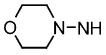
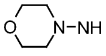
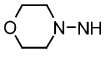
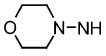
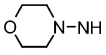
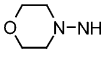
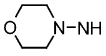
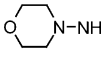
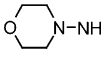
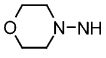
All new compounds were assayed for their ability to inhibit CDK5/p25 (TPK II). Selectivity was determined by evaluation in an assay for inhibition of CDK2/cyclin A. Results for selected analogues are compiled in Table 1.[12]

The first SAR facet to emerge from this work was the absolute requirement that the hydrazines be 1,1-disubstituted. This is strikingly apparent when comparing the compounds **15** and **16** as well as the compounds **17**, **19** and **20**. In each closely related set, only the terminal disubstituted isomers are active. In fact, of the numerous monosubstituted and 1,2-disubstituted hydrazine diversity elements incorporated into these libraries, not a single one afforded an active analogue (most data not shown in Table 1). Several 1,1-dialkylhydrazines and related cyclic analogues possessed significant activity, although none were superior to the lead **4**. Evidently some steric constraints are operative, as substituting the pyrrolidine ring of the active analogue **10** abolished activity (**11**), and the dibenzyl analogue **14** was virtually inactive.

Modification of the thiazole moiety was obviously more labor intensive than altering the hydrazine component, thereby limiting diversity input. Nevertheless, 20 thioamides were successfully incorporated into thiazole analogues, 16 of which are represented in Table 1. The 2,6-dichlorobenzyl moiety unfortunately proved to be optimum for both activity and selectivity versus CDK2/cyclin A. Removal of a single chlorine atom (**21**) or both (**22**) from the benzyl moiety of the lead attenuated activity over 20-fold, suggesting that the conformation of the sidechain is important. Interestingly, when the phenyl ring was attached directly to the thiazole ring, activity could be maintained without aromatic substitution (**30**), but kinase inhibition was non-selective. A comparison of 2-versus 4-chloro and 3-versus 4-methyl phenyl analogues (**33** vs **34** and **31** vs **32**) indicates that 4-substitution is not tolerated, suggesting a steric interaction at that point that might be avoidable with the more flexible benzyl analogues. In fact, the even larger sidechains present in **24**, **26** and **27** were tolerated, presumably due to flexibility. A requirement for an aromatic ring is suggested by the inferior activity of methylsulfonyl analogue **28** versus arylsulfonyl analogue **27**, as well as the inactive ethyl analogue **29**.

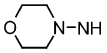
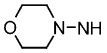
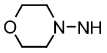
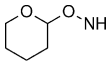
Of particular interest were the results obtained by replacing the hydrazine moiety of **4** with alkoxyamines. Despite the fact that the oxygen can only be monosubstituted, these compounds exhibited remarkably

**Table 1.** CDK5 versus CDK2 inhibition for selected analogues **9**

| Compd | $R^1$ | Nu | CDK5/p25[a] $IC_{50}$ (C.I.)[c] | CDK2/A[a] $IC_{50}$ (C.I.)[c] |
|---|---|---|---|---|
| **4** | 2,6-diCl–$PhCH_2$ |  | 1 | 10 |
| **10** | 2,6-diCl–$PhCH_2$ |  | 3 (1.5–5) | 20 (18–26) |
| **11** | 2,6-diCl–$PhCH_2$ |  | >100 | |
| **12** | 2,6-diCl–$PhCH_2$ |  | 6 (4–9) | 30 (20–42) |
| **13** | 2,6-diCl–$PhCH_2$ |  | 10 (8–19) | 70 (42–117) |
| **14** | 2,6-diCl–$PhCH_2$ | $(PhCH_2)_2NNH$– | >100 | |
| **15** | 2,6-diCl–$PhCH_2$ | PhNHNH– | >100 | |
| **16** | 2,6-diCl–$PhCH_2$ | PhN(Me)NH– | 10 (5–29) | 60 (45–77) |
| **17** | 2,6-diCl–$PhCH_2$ | MeNHNH– | >100 | |
| **18** | 2,6-diCl–$PhCH_2$ | $NH_2NH$– | >100 | |
| **19** | 2,6-diCl–$PhCH_2$ | $Me_2NNH$– | 4 (3.5–5) | 10 (5–28) |
| **20** | 2,6-diCl–$PhCH_2$ | MeNHNHMe | >100 | |
| **21** | 2-Cl–$PhCH_2$ |  | 30%[b] | |
| **22** | $PhCH_2$ |  | 55 (36–78) | >100 |
| **23** | 4-Br-$PhCH_2$ |  | 26%[b] | |
| **24** | $PhSCH_2$ |  | 30 (20–35) | 30 (13–86) |
| **25** | $PhOCH_2$ |  | 80 (59–102) | >100 |
| **26** | 4-MeO-$PhOCH_2$ |  | 30 (10–77) | 20 (6–31) |
| **27** | 2-pyr-$SO_2CH_2$ |  | 10 | 5 |
| **28** | $MeSO_2CH_2$ |  | 35%[b] | |
| **29** | $CH_3CH_2$ |  | >100 | |
| **30** | Ph |  | 9 | 6 |
| **31** | 3-Me-Ph |  | 30 | 20 |
| **32** | 4-Me-Ph |  | >100 | |

*(continued)*

Table 1 (*continued*)

| Compd | $R^1$ | Nu | CDK5/p25[a] $IC_{50}$ (C.I.)[c] | CDK2/A[a] $IC_{50}$ (C.I.)[c] |
|---|---|---|---|---|
| **33** | 2-Cl–Ph |  | 30 | 10 |
| **34** | 4-Cl–Ph |  | >100 | |
| **35** | Thiophen-2-yl |  | 30 | 10 |
| **36** | 2,6-diCl–$PhCH_2$ | MeONH | 9 (6–17) | >100 |
| **37** | 2,6-diCl–$PhCH_2$ | EtONH | 5 (3–7) | 20 (13–35) |
| **38** | 2,6-diCl–$PhCH_2$ | Allyl-ONH | 6 (4–10) | 40 (32–60) |
| **39** | 2,6-diCl–$PhCH_2$ | *t*BuONH | 1 (0.5–3) | 15 (11–20) |
| **40** | 2,6-diCl–$PhCH_2$ | $PhCH_2ONH$ | 40%[b] | |
| **41** | 2,6-diCl–$PhCH_2$ | $C_6F_5CH_2ONH$ | >100 | |
| **42** | 2,6-diCl–$PhCH_2$ |  | 3 (2–6) | 90 (55–130) |
| **43** | 2,6-diCl–$PhCH_2$ | PhONH | **0.9** (0.5–1.8) | >**100** |
| **44** | 3-MePh | PhONH | 16 (4–70) | >100 |
| **45** | $MeSO_2CH_2$ | PhONH | 6 (5–11) | 90 (50–150) |
| **46** | Ph | PhONH | 8 (7–15) | 35 (25–54) |
| **47** | $PhCH_2$ | PhONH | 20 (9–43) | >100 |

[a]Ref 12.
[b]% Inhibition at 20 μM.
[c]$IC_{50}$ and 95% confidence intervals (C.I.) expressed in μM.

good activity, in striking contrast to the corresponding terminal monosubstituted hydrazine analogues. This is dramatically illustrated by comparing methyl analogues **36** versus **17** and phenyl analogues **43** versus **15**. *In fact, phenoxyamine proved to be superior to aminomorpholine with regard to both activity and selectivity in several examples* (**4** vs **43**, **31** vs **44**, **28** vs **45** and **30** vs **46**). Even the non-aromatic THP analogue **42** possessed selectivity (>30-fold) surpassing that of the lead **4**. The lack of activity of the benzyloxyamino analogues **40** and **41** is puzzling in light of the otherwise forgiving SAR in this series. One can only speculate that the benzyl groups are too large to be accommodated by the binding site. The unique ability of the 2,6-dichlorobenzyl substituent on the thiazole to confer both potency and selectivity was also observed in the alkoxyamine series, as illustrated by a comparison of compounds **43**–**47**. The optimum compound from this series proved to be **43**, exhibiting submicromolar activity for CDK5/p25 and over 100-fold selectivity versus CDK2/cyclin A.

In an attempt to rationalize the fairly consistent activity of the *N*,*N*-dialkylhydrazine and alkoxyamine analogues versus the uniformly inactive *N*-monosubstituted hydrazine analogues, molecular modeling[13] was undertaken on the three closely related analogues **17**, **19** and **36**. It was found that **19** has two low-energy conformations available, differing only by inversion of the terminal $sp^3$ nitrogen (1.3 kcal). Compounds **17** and **36** have only one low energy conformation each, with the next higher ones being 4.8 and 6.7 kcal greater in energy, respectively. Common to all three is a co-planar arrangement of the thiazole ring and the urea moiety, with the thiazole nitrogen situated anti to the carbonyl (Fig. 1, only **19** shown). Also common is the observation that the urea NH bond distal to the thiazole is slightly out of plane with the thiazole/urea system (Fig. 2). Of particular interest is the observation that the two active analogues (**19**, **36**) each have a pair of electrons on the terminal heteroatom projecting at an acute dihedral angle to the carbonyl. The inactive analogue **17**, on the other hand, is steered into a conformation that prefers to have the hydrogen of the terminal nitrogen projecting toward the carbonyl in a *syn*-periplanar orientation (dihedral angle for CNNH system is −32.1°), thereby forcing the nitrogen's lone pair to point in a different direction than in **19** and **36**. This conformational difference makes

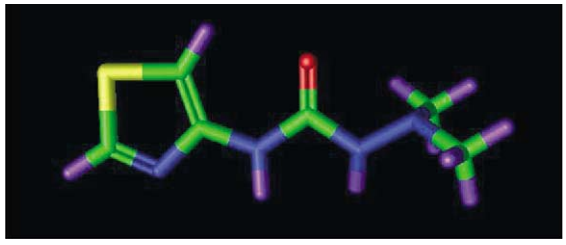


**Figure 1.** One of the low-energy conformations of **19**.

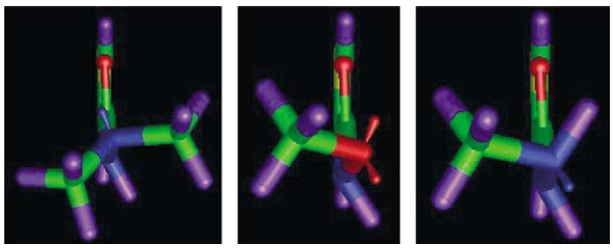


**Figure 2.** End view of low-energy conformations of **19**, **36**, and **17**, respectively. Lone pairs are shown for illustrative purposes only.

the electrostatics about the terminal nitrogen for **19** and **36** quite different than **17**, which could conceivably adversely affect the electrostatic complementarity of the enzyme binding site with **17** and other terminal NH analogues.

In summary, a catch-and-release protocol was developed for the synthesis of 2-dimensional libraries of 2-substituted-4-acylamino-1,3-thiazoles that entailed trapping of intermediate thiazole isocyanates onto oxime resin and subsequent release under basic catalysis following washing. The protocol was successful at avoiding contamination of the final analogues with byproducts arising from the intermediate Curtius rearrangement. Assay of the resulting libraries for selective inhibition of CDK5/p25 versus the closely homologous enzyme CDK2/cyclin A revealed that only analogues derived from 1,1-dialkylhydrazines and alkoxyamines retained activity. Molecular modeling indicated that the only apparent conformational difference between the active analogues and the inactive ones devrived from monoalkyl hydrazines was the *syn*-periplanar orientation of the terminal NH to the urea carbonyl, which changes the position of the nitrogen lone pair, altering the electrostatic contribution about the terminal heteroatom.

## Acknowledgements

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11. Representative $^1$H NMR spectra (300 MHz, DMSO-$d_6$) δ: Compound **2**: 4.62 (s, 2H), 7.41 (t, $J=6$ Hz, 1H), 7.56 (d, $J=6$ Hz, 2H), 8.31 (s, 1H), 12.9 (s, 1H). Compound **3**: 4.75 (s, 2H), 7.25 (t, $J=6$ Hz, 1H), 7.40 (d, $J=6$ Hz, 2H), 8.15 (s, 1H). Compound **4**: 2.74 (s, broad, 4H), 3.65 (s, broad, 4H), 4.52 (s, 2H), 7.12 (s, 1H), 7.40 (t, $J=6$ Hz, 1H), 7.55 (d, $J=6$ Hz, 2H), 7.93 (s, 1H), 8.86 (s, 1H).
12. **Kinase assays.** CDK2/cyclin A and CDK5/p25 kinase assays were performed using the scintillation proximity assay (SPA) format with the following conditions. The assays contained 50 mM HEPES, pH 7.5, 20 μM $Na_3VO_4$, 15 mM $MgCl_2$, 1 mM DTT, 0.1 mg/mL BSA, 0.2 mg/mL BGG, 0.01% Triton X-100, 1 μM biotinylated peptide (PKTPKKAKKL), and 0.2 μCi [$\gamma^{33}$P]-ATP. CDK5/GST-p25 was used at 0.5 nM and CDK2/GST-cyclin A was used at 4 nM. Reactions were incubated for 30 min at 37 °C and then terminated with the addition of 500 μg streptavidin SPA beads (Amersham) in 50 μM ATP, 5 mM EDTA, and 0.1% (v/v) Triton X-100 in PBS without calcium and magnesium. CPM for each well were determined using a Packard TopCount scintillation counter. Percent inhibition compared to 100% control was calculated using the formula $100\times$(1-(unknown−bkgd)/(control−bkgd)). $IC_{50}$ values were calculated using the sigmoidal dose-response equation in Prism (Graphpad). When tested more than once, the % inhibition or $IC_{50}$ values were averaged.
13. Several starting geometries were chosen for each compound and then subsequently each geometry was optimized using ab initio quantum mechanics (HF/6-31G*) in the GAMESS[14] suite of programs. Each compound led to two or three low energy conformations, and Hessians were obtained for the two lowest energy conformations for each compound to confirm the nature of the stationary points–that is minima on the potential energy surface. Results were visualized with MacMolPlt[15] and conformations were overlaid using Mosaic2.[16]
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16. Mosaic2 is a Pharmacia-developed modeling suite of programs.