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Discovery and SAR of 2-aminothiazole inhibitors of cyclin-dependent kinase 5/p25 as a potential treatment for Alzheimer's disease

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Abstract—High-throughput screening with cyclin-dependent kinase 5 (cdk5)/p25 led to the discovery of *N*-(5-isopropyl-thiazol-2-yl)isobutyramide (**1**). This compound is an equipotent inhibitor of cdk5 and cyclin-dependent kinase 2 (cdk2)/cyclin E (IC_{50} = ca. 320 nM). Parallel and directed synthesis techniques were utilized to explore the SAR of this series. Up to 60-fold improvements in potency at cdk5 and 12-fold selectivity over cdk2 were achieved.

Cyclin dependent kinases (cdk) play important roles in cell cycling, and cdk inhibitors have been intensively researched as therapeutic agents for the treatment of cancer.[1] In addition, cdk5 and its noncyclin cofactor p35 phosphorylate the cytoskeletal stabilizing protein tau, a critical component of cell structure regulation.[2] The discovery that the short-lived, membrane-bound p35 can be proteolytically cleaved by calpain to the more stable, cytosolic form p25 led to the hypothesis that cdk5/p25 plays an important role in Alzheimer's disease etiology. The longer-lived cdk5/p25 complex is thought to hyperphosphorylate tau, leading to the formation of paired helical filaments and deposition of cytotoxic neurofibrillary tangles.[3] Inhibition of the anomalous cdk5/p25 complex is, therefore, a viable target for treating Alzheimer's disease by preventing tau hyperphosphorylation and neurofibrillary tangle formation. We have developed a series of potent and selective 2-aminothiazole inhibitors of cdk5/p25 as potential therapeutic agents for the treatment of Alzheimer's disease and other neurodegenerative disorders.[4,5]

High-throughput screening with the cdk5/p25 complex uncovered *N*-(5-isopropyl-thiazol-2-yl)isobutyramide (**1**), as one of the most interesting hits due to its good potency versus cdk5/p25 (IC_{50} = 321 nM), low molecular weight (MW = 212), and relatively straightforward chemical modification (Fig. 1). In the presence of increasing concentrations of ATP, **1** became less effective at inhibiting cdk5/p25, suggesting that this compound is competitive at the ATP binding site. Screening of **1** versus a panel of 24 diverse protein kinases showed only weak inhibitory activity.[6] Further testing against other cyclin dependent kinases revealed that **1** was essentially equipotent at inhibiting cdk2/cyclin E (IC_{50} = 318 nM), a cancer target.[1] Consequently, our objectives were to improve cdk5 potency and minimize cdk2 activity.

We focused our synthetic efforts on varying the amide side chain and the 5-isopropyl group. Amides were prepared in a high-speed parallel fashion by reacting the 2-amino-5-substituted thiazole with acid chlorides in

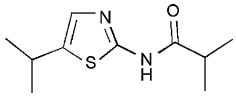

Figure 1. High-throughput screening lead **1**.

Keywords: 2-Aminothiazole; Cyclin-dependent kinase 5; p25; Cyclin-dependent kinase 2; Cyclin E; Alzheimer's disease.
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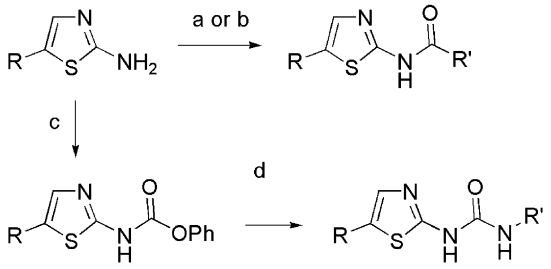
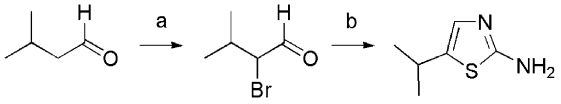


Scheme 1. (a) (i) R′C(O)Cl (1.2 equiv), morpholinemethyl resin (4.5 equiv), 1,2-dichloroethane, (ii) aminomethyl resin (3.75 equiv); (b) $R'CO_2H$ (1.2 equiv), Et_3N, (n-PrP(O)O)$_3$ (1.2 equiv), ethyl acetate, 23 °C, 40–85% yield; (c) PhOC(O)Cl (1 equiv), (i-Pr)$_2$NEt, CH_2Cl_2, −78 to 0 °C, 80% yield; (d) $R'NH_2$ (1.2 equiv), 1,4-dioxane, 100 °C, 30–85% yield.



Scheme 2. (a) 5,5-dibromobarbituric acid (0.5 equiv), Et_2O, 23 °C, 18 h, filter, concentrate in vacuo, add pentane, filter, concentrate in vacuo, 46% yield; (b) thiourea (1 equiv), ethanol, reflux, 18 h, 58% yield.

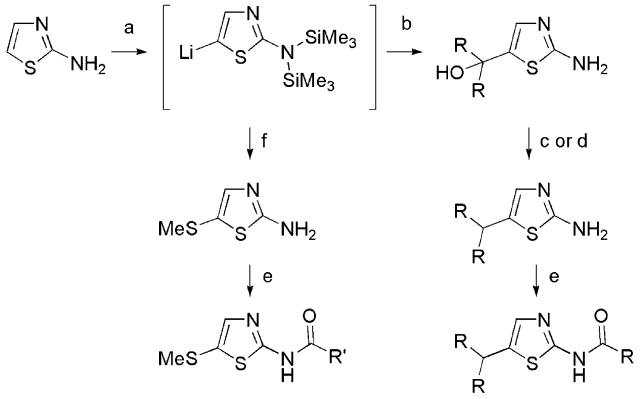
the presence of a resin-bound morpholine base followed by addition of an aminomethyl resin to scavenge excess acid chloride (Scheme 1). Amides were also prepared utilizing the tripropylphosphonic anhydride mediated coupling of carboxylic acids with the 2-aminothiazole core. Ureas were readily synthesized in a high-speed parallel format by conversion of a 2-aminothiazole to the corresponding phenyl carbamate, which was reacted with an amine in 1,4-dioxane at reflux.

2-Amino-5-substituted thiazoles were prepared by three methods. For simple 5-alkyl analogs, commercially available aldehydes were converted into α-bromoaldehydes and condensed with thiourea (Scheme 2).

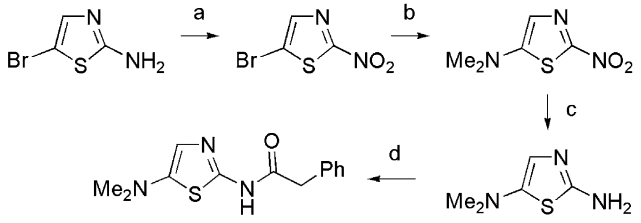
The second method utilized Katritzky's in situ silylation/metallation of 2-aminothiazole followed by trapping with an electrophile (Scheme 3).[7] When trapping with an aldehyde or ketone, the resultant carbinol was eliminated to give the olefin using trifluoroacetic acid. The carbinol was reduced via ionic hydrogenolysis (trifluoroacetic acid, triethylsilane) or using acid-catalyzed elimination/hydrogenation (trifluoroacetic acid, palladium hydroxide on carbon, 50 psi hydrogen).

For the synthesis of a 5-dimethylamino analog, 5-bromo-2-aminothiazole was first converted to 5-bromo-2-nitrothiazole under Sandmeyer conditions. Nucleophilic aromatic substitution of bromide with dimethylamine followed by catalytic hydrogenation and acylation afforded the desired compound (Scheme 4).

Kinase inhibition was measured by the use of scintillation proximity assays (SPA).[8] The ATP final concentration used was the same in both the cdk5/p25 and cdk2/cyclin E assay. The K_m of ATP was measured to be 12 μM for cdk5/p25 and 19 μM for cdk2/cyclin E. For



Scheme 3. (a) (i) n-BuLi (2 equiv), THF, −78 °C, 1 h, (ii) Me_3SiCl (2 equiv), −78 to −20 to −78 °C, (iii) n-BuLi (1 equiv), −78 °C, 10 min; (b) RRC=O, 33–71% yield; (c) Et_3SiH, CF_3CO_2H, 63–91% yield; (d) H_2 (50 psi), $Pd(OH)_2$ carbon, CF_3CO_2H, 62–91% yield; (e) $R'CO_2H$ (1.2 equiv), Et_3N, (n-PrP(O)O)$_3$ (1.2 equiv), ethyl acetate, 23 °C, 40–85% yield; (f) $(MeS)_2$, 74% yield.

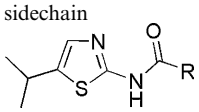


Scheme 4. (a) HBF_4 (5 equiv), SM, H_2O, rt, Cu(s) (1.6 equiv), $NaNO_2$, (15 equiv), H_2O, 0 °C 7%; (b) Me_2NH_2Cl, Et_3N, DMSO, 60 °C, 60%; (c) H_2, Pd/C, EtOAc, 77%; (d) $PhCH_2CO_2H$, (n-PrP(O)O)$_3$, Et_3N, EtOAc, 23 °C, 40%.

compounds with $IC_{50} < 1$ μM, the IC_{50}s reported are the mean of at least two independent measurements with standard error calculated. Each IC_{50} was determined from a six-point dose–response curve run in triplicate.

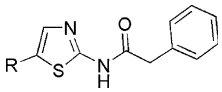
Variation of the amide side chain of **1** with high speed and traditional chemistries allowed us to rapidly explore the first arm of the pharmacophore (Table 1). Relative to the parent isopropyl (**1**), benzamide (**2**), phenyl carbamate (**3**), and phenyloxylate substitution (**4**) all resulted in decreased potency. However, phenyl urea (**5**) and phenylacetamide (**6**) provided the first analogs that demonstrated improved activity. We observed little to no change in the selectivity of these analogs versus cdk2.

Table 1. SAR of amide sidechain



Compd	R	Cdk5 IC_{50} (nM)	Cdk2 IC_{50} (nM)	Select (k2/k5)
1	i-Pr	321 ± 18	318 ± 18	1
2	Ph	1150	1020	0.9
3	OPh	1600	980	0.6
4	C(=O)Ph	4580	9770	2.1
5	NHPh	102 ± 19	265 ± 46	2.6
6	CH_2Ph	64 ± 12	98 ± 16	1.5

Table 2. SAR of 5-position



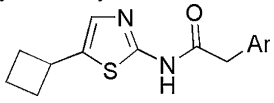
Compd	R	Cdk5 IC_{50} (nM)	Cdk2 IC_{50} (nM)	Select (k2/k5)
6	*i*-Pr	64 ± 12	98 ± 16	1.5
7	Et	621 ± 212	412 ± 140	0.7
8	*c*-Butyl	25 ± 1	74 ± 11	3.0
9	*c*-Pentyl	64 ± 2	155 ± 18	2.4
10	*c*-Hexyl	390 ± 133	997 ± 275	2.6
11		6530	6510	1.0
12	*n*-Pentyl	3270	4120	1.2
13	Ph	1570	2000	1.3
14	Acetyl	7670	4600	0.6
15	MeS	91 ± 4	105 ± 3	1.2
16	Br	900	480	0.5
17	Me_2N	3990	4810	1.2

Investigation of the 5-position SAR was carried out with the 2-phenylacetamide as a constant (Table 2). The ethyl group (**7**) was 10-fold less potent than the parent isopropyl (**6**). The cyclobutyl (**8**) improved potency by ca. 3-fold relative to isopropyl and cyclopentyl (**9**) was equipotent with isopropyl. Cyclohexyl (**10**), however, dropped off noticeably in potency, as did other groups such as cyclopentenyl (**11**), *n*-pentyl (**12**), phenyl (**13**), and acetyl (**14**). Direct heteroatom attachment afforded analogs with a range of activities. Thiomethyl (**15**) provided potent inhibitory activity, whereas bromo was considerably weaker (**16**). Dimethylamino (**17**), which is sterically similar to isopropyl but more polar, also lost considerable potency.

Attention was then turned to optimization of the arylacetamide. The incorporation of fused heteroaryl groups led to improved potency (Table 3). The 5-benzimidazole substituent (**18**) showed excellent cdk5 activity and provided the first signs of selectivity versus cdk2 (fivefold). Other fused heteroaryl analogs with β-type substitution such as 6-quinolyl (**19**) and 6-benzthiazole (**20**) were more potent than the parent phenyl compound but did not display the desired cdk2 selectivity. Relative to β-type substitution, fused heteroaryl analogs with α-type substitution were somewhat less potent (**21**–**24**). The 5-isoquinolyl derivative (**21**), however, did show 5-fold selectivity versus cdk2.

The improved potency and selectivity observed in the fused heteroarylacetamide analogs led us to study these same SAR trends in the corresponding ureas (Table 4). The phenyl urea (**25**) was ca. 7-fold less potent than the corresponding phenylacetamide (**8**) with little cdk2 selectivity. The incorporation of quinolines and isoquinolines with β-type substitution (**26**,**27**) afforded improved potency relative to the parent phenyl urea and improved cdk2 selectivity (ca. 4-fold). Compounds with α-type substitution such as 5-quinolyl (**28**) gave even better cdk5 activity. Changing the position of the quinolyl nitrogen (**29**) improved cdk5 potency and for the first time afforded >10-fold selectivity versus cdk2. The 8-isoquinolyl derivative (**30**) was equally selective versus cdk2 and had slightly improved cdk5 IC_{50}. A number of other fused heteroaryl derivatives were synthesized (e.g., **31**,**32**), but all had noticeably decreased cdk5 activity.[9]

Table 3. SAR of aryl/heteroaryl acetamides



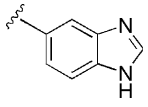
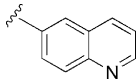
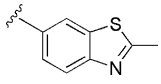
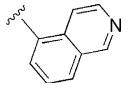
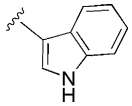
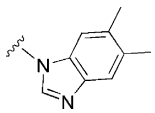
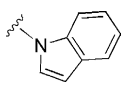
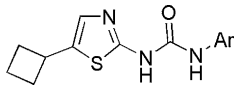
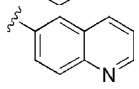
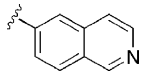
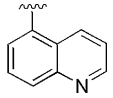
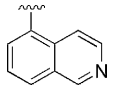
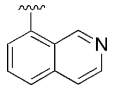
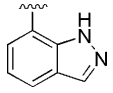
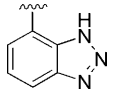
Compd	Ar	cdk5 IC_{50} (nM)	cdk2 IC_{50} (nM)	Select (k2/k5)
8		25 ± 1	74 ± 11	3.0
18		7 ± 1	33 ± 6	4.7
19		10 ± 1	26 ± 2	2.6
20		12 ± 4	16 ± 1	1.3
21		13 ± 4	70 ± 11	5.4
22		16 ± 1	40 ± 6	2.5
23		13 ± 4	26 ± 4	2.2
24		48 ± 5	100 ± 11	2.1

Table 4. SAR of aryl/heteroaryl ureas



Compd	Ar	cdk5 IC_{50} (nM)	cdk2 IC_{50} (nM)	Select (k2/k5)
25		183 ± 89	290 ± 50	1.6
26		44 ± 11	167 ± 27	3.8
27		44 ± 1	176 ± 37	4.0
28		13 ± 1	43 ± 4	3.3
29		8 ± 1	96 ± 12	12.0
30		5 ± 2	55 ± 5	11.0
31		190 ± 8	650 ± 72	3.4
32		41 ± 5	106 ± 38	2.6

Modeling of these analogs in the ATP binding region of cdk5/p25 suggests hydrogen bonding between the aminothiazole N–H and heterocyclic N with the carbonyl and N–H of Cys83, respectively.[10] Substituents at the aminothiazole 5-position appear to make a hydrophobic interaction with Phe80, which is in line with SAR observed (Table 2). Aromatic amides (Table 3) and ureas (Table 4) may be interacting with IleA10, a possible reason for increased potency with these groups.

The challenge in gaining selectivity over cdk2 is clear, considering that only two out of the 29 ATP binding pocket residues are different. The two that are different, Cys83 and Asp84 in cdk5 versus Leu83 and His84 in cdk2, are located in the backbone hydrogen-bonding region, but the side chains do not point into the ATP binding pocket, reducing their ability to influence selective binding of a substrate. The observed selectivity most likely is the result of subtle differences in conformations of conserved residues near the bound inhibitor. For example, modeling of the selective analog **30** in cdk5/p25, Lys89 is disposed to make a hydrogen bond with the isoquinolyl nitrogen, whereas that same residue is further away in cdk2 and cannot make the same interaction, possibly explaining the observed selectivity.

In summary, modifications to HTS hit **1** successfully decreased the cdk5 IC_{50} to <10 nM and improved cdk2 selectivity to >10-fold while keeping the molecular weight of the target compounds below 350 amu, exemplified by **32**. SAR patterns that emerged are (1) potency and selectivity are increased by the emplacement of fused heteroaryl rings in both acetamides and ureas off of the 2-amino position and (2) a small lipophilic pocket appears to exist in cdk5 near the thiazole 5-position, with cyclobutyl providing optimal enzyme interactions. These insights will be useful in further chemistry efforts.

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4. For recent articles describing 2-aminothiazole inhibitors of cdk2 see: (a) Kim, K. S.; Kimball, S. D.; Misra, R. N.; Rawlins, D. B.; Hunt, J. T.; Xiao, H.-Y.; Lu, S.; Qian, L.; Han, W.-C.; Shan, W.; Mitt, T.; Cai, Z.-W.; Poss, M. A.; Zhu, H.; Sack, J. S.; Tokarski, J. S.; Chang, C.-Y.; Pavletich, N.; Kamath, A.; Humphreys, W. G.; Marathe, P.; Bursuker, I.; Kellar, K. A.; Roongta, U.; Batorsky, R.; Mulheron, J. G.; Bol, D.; Fairchild, C.; Lee, F. Y.; Webster, K. R. *J. Med. Chem.* **2002**, *45*, 3905; (b) Misra, R. N.; Xiao, H.-Y.; Kim, K. S.; Lu, S.; Han, W.-C.; Barbosa, S. A.; Hunt, J. T.; Rawlins, D. B.; Shan, W.; Ahmed, S. Z.; Qian, L.; Chen, B.-C.; Zhao, R.; Bednarz, M. S.; Kellar, K. A.; Mulheron, J. G.; Batorsky, R.; Roongta, U.; Kamath, A.; Marathe, P.; Ranadive, S. A.; Sack, J. S.; Tokarski, J. S.; Pavletich, N. P.; Lee, F. Y. F.; Webster, K. R.; Kimball, S. D. *J. Med. Chem.* **2004**, *47*, 1719; (c) Misra, R. N.; Xiao, H.; Williams, D. K.; Kim, K. S.; Lu, S.; Keller, K. A.; Mulheron, J. G.; Batorsky, R.; Tokarski, J. S.; Sack, J. S.; Kimball, S. D.; Lee, F. Y.; Webster, K. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2973.
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6. The kinase selectivity panel consisted of Akt-1, PKCα, phosphorylase kinase, lck, p70 S6K, p38δ, p38γ, p38β, p38, PRAK, ROKα, SGK, CKII, ChK1, PDK1, JNK, GSK3β, MAPK, RSK2, MSK1, PKA, MAPKAP kinase-2, AMPK, and MEK1. All assays were conducted in the presence of 100 μM ATP. Compound **1** showed less than 50% inhibition versus the kinases tested.
7. Katritzky, A. R.; Laurenzo, K. S.; Relyea, D. I. *Can. J. Chem.* **1988**, *66*, 1617–1624.
8. See Ref. 5a for detailed cdk5/p25 and cdk2/cyclin E assay methods.
9. ^{1}H NMR and mass spectral data for representative compounds:
   Compound **1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 (s, 1H), 3.13 (m, 1H), 2.71 (m, 1H), 1.31 (d, J = 6.8 Hz, 6H), 1.27 (d, J = 6.8 Hz, 6H); MS (AP/CI) 213.3 $(M+H)^+$, 100%.
   Compound **5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, J = 7.7 Hz, 1H), 7.3 (m, 3H), 7.09 (m, 1H), 7.02 (s, 1H), 3.1 (m, 1H), 1.32 (d, J = 6.8 Hz, 6H); MS (AP/CI) 262.2 $(M+H)^+$, 100%.

Compound **6**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3 (m, 5H), 7.02 (s, 1H), 3.79 (s, 2H), 3.08 (m, 1H), 1.29 (dd, J = 1.4, 6.8 Hz, 6H); MS (AP/CI) 261.3 $(M+H)^+$, 100%.

Compound **8**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3 (m, 5H), 7.02 (s, 1H), 3.81 (s, 2H), 2.37 (m, 2H), 2.13 (m, 2H), 2.0 (m, 2H); MS (AP/CI) 273.1 $(M+H)^+$, 100%.

Compound **18**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.67 (s, 1H), 7.58 (m, 2H), 7.26 (dd, J = 1.4, 8.3 Hz, 1H), 7.02 (s, 1H), 3.87 (s, 2H), 3.6 (m, 1H), 2.35 (m, 2H), 2.1 (m, 2H), 1.95 (m, 1H), 1.87 (m, 1H); MS (AP/CI) 313.0 $(M+H)^+$, 100%.

Compound **30**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.54 (s, 1H), 8.50 (d, J = 5.4 Hz, 1H), 8.27 (d, J = 6.6 Hz), 7.61 (t, J = 7.9 Hz, 2H), 7.56 (d, J = 5.6 Hz, 1H), 7.01, (s, 1H), 3.54 (m, 1H), 2.36 (m, 2H), 2.09 (m, 2H), 1.97 (m, 1H), 1.89 (m, 1H); MS (AP/CI) 325.0 $(M+H)^+$, 30%; 323.2 $(M-H)^-$, 100%.

10. cdk5/p25 crystal structure ID code is 1h4L in the Protein Data Bank.