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Potent and cellularly active 4-aminoimidazole inhibitors of cyclin-dependent kinase 5/p25 for the treatment of Alzheimer's disease

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ABSTRACT

Utilizing structure-based drug design, a 4-aminoimidazole heterocyclic core was synthesized as a replacement for a 2-aminothiazole due to potential metabolically mediated toxicity. The synthetic route utilized allowed for ready synthesis of 1-substituted-4-aminoimidazoles. SAR exploration resulted in the identification of a novel *cis*-substituted cyclobutyl group that gave improved enzyme and cellular potency against cdk5/p25 with up to 30-fold selectivity over cdk2/cyclin E.

Cyclin dependent kinases (cdk) carry out critical roles in cell cycling, and cdk inhibitors have been extensively researched as therapeutic agents for the treatment of cancer.[1] Binding of cdk5 by the protein p35 results in kinase activation and the ability to phosphorylate the cytoskeletal stabilizing protein tau, a critical component of cell structure regulation.[2] The membrane-bound p35 can be proteolytically cleaved by calpain to the more stable, cytosolic p25 which has led to the hypothesis that cdk5/p25 may play an integral role in Alzheimer's disease development. The longer-lived cdk5/p25 complex is believed to over-phosphorylate tau, resulting in the formation of paired helical filaments and deposition of cytotoxic neurofibrillary tangles.[3] Thus, inhibition of the aberrant cdk5/p25 complex is a viable target for treating Alzheimer's disease by preventing tau hyperphosphorylation and subsequent neurofibrillary tangle formation.[4] In addition to being a target for Alzheimer's disease, recent evidence suggests that inhibition of cdk5 could also be relevant for the treatment of type-II diabetes, pain, and stroke, furthering interest in this enzymatic target.[5,6]

We reported on the optimization of potency and cdk2/cyclin E selectivity in a series of 2-aminothiazole cdk5/p25 inhibitors that bind in the ATP binding pocket.[7] Selectivity over cdk2 is desired due to its role in modulating the cell cycle and potential side effects. This is a challenging task, considering 93% (27/29) of residues are conserved in the respective ATP pockets of cdk5 and cdk2 and that the two differing amino acid residues (Cys83 and Asp84 in cdk5; Leu83 and His84 in cdk2) have sidechains that project away from the ATP pocket, thereby reducing their impact on inhibitor binding.

In furthering our exploration of this series, alternative heterocyclic cores to the 2-aminothiazole (**1**) were sought, based upon the metabolism-induced toxicity potential of this class of heterocycles[8] (Fig. 1). Considering the binding mode of inhibitors to cdk5, as observed in computer modeling in addition to X-ray crystal structures in cdk2, key H-bond acceptor and donor interactions with Cys 83 in the hinge region (Leu 83 in cdk2) were to be maintained along with necessary lipophilic groups. The 4-aminoimidazole core (**2**) was appropriately disposed to make the requisite hydrogen bonding and hydrophobic interactions and was thus deemed a suitable target for biological evaluation.

The synthesis of 4-aminoimidazole analogs was carried out as shown in Scheme 1.[9] The treatment of 1,4-dinitroimidazole[10] (**3**) with a primary amine afforded 1-substituted 4-nitroimidazoles **4**. CAUTION: *thermodynamic testing showed that **3** was a highly energetic substance with the potential for explosion.*[11] Catalytic hydroge-

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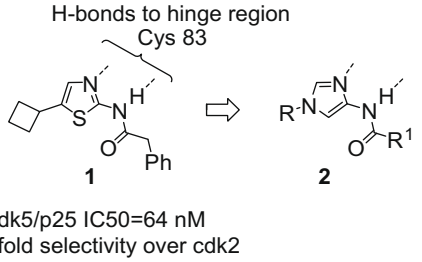
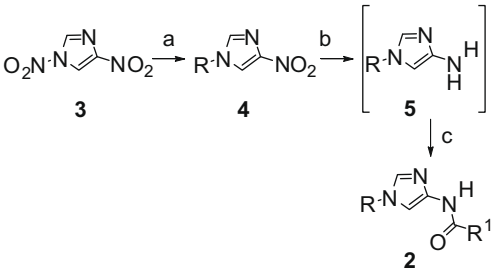


Figure 1.



Scheme 1. Reagents and conditions: (a) RNH_2, MeOH, 23 °C, 42–75% yield; (b) H_2 (50 psi), Pd/C, EtOAc; (c). $R'CO_2H$, tri-*n*-propylphosphonic anhydride, Et_3N, CH_2Cl_2, −10 °C, 32–72% yield.

nation gave an unstable 4-aminoimidazole **5** that, following filtration through Celite, was immediately acylated with an activated carboxylic acid to afford compounds of formula **2**.

Extensive SAR of the amide side chain (not shown) revealed that the aryl acetamides were the most potent substituents. As shown in Table 1, phenylacetamide **6** was ca. eightfold less potent than the corresponding aminothiazole **1**.[12] Addition of a 4-methoxy group increased potency ca. fourfold (**7**). The 1-napthyl acetamide **8** afforded the best potency with IC_{50} = 46 nM. Activity against cdk2 was essentially equivalent to cdk5 activity.

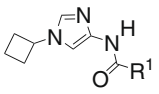
The ability to readily synthesize *N*-1 group analogs as shown in Scheme 1 allowed for a rapid and broad exploration of SAR at this position(Table 2). The role of the *N*-1 group is critical as methyl **9** was inactive. Isopropyl analog **10** and cyclopropyl analog **11** showed improved activity, whereas cyclobutyl **7** and cyclopentyl **12** were preferred groups with IC_{50} <200 nM. Cyclohexyl **13** lost activity as compared to **7** and **12**, as did benzyl **14**. Compound **7** was subsequently profiled for selectivity against 20 diverse kinases at 10 μM and showed >30% inhibition against three (GSK3β, 34%; AMPK, 48%; PHK, 32%).[13]

At this point, modeling of the cyclobutyl derivative **7** in a cdk5 homology model based upon a cdk2 X-ray crystal structure suggested that placement of polar functionality on the 3-position of the cyclobutyl of **7** could possibly lead to interactions with polar amino acid side chains, Lys33 and Asp144 (Asp145 in cdk2), that interact with the phosphates of bound ATP.[14] These analogs were synthesized via methods that have been previously described in detail.[15]

The initial polar group employed was hydroxyl (Table 3, **15** and **16**). A significant effect of stereochemistry was observed with *cis*-derivative **15** being ca. 10-fold more potent than *trans* **16**, but with similar potency to the unsubstituted cyclobutyl analog **7**. A similar *cis*/*trans* preference was observed with the methyl esters **17** and **18**. An X-ray crystal structure of **15** bound to cdk2 was obtained (Fig. 2).[16] Key observations were that the cyclobutyl appears to align with Phe80, resulting in a hydrophobic interaction that explains the observed SAR. Additionally, the hydroxyl group is in position to make a hydrogen bond with Asp145 (2.7 A) (Asp144 in cdk5), and is in proximity (3.4 A) to Lys33. Equivalent cdk5 potency of **15** as the unsubstituted cyclobutyl analog **7** suggests that the hydroxyl interaction is energetically neutral, potentially due to desolvation penalties as a result of removing waters associated with the polar hydroxyl group, the Lys33 and/or Asp144, or with energetic loss associated with disrupting a favorable salt bridge between Lys33 and Asp144.

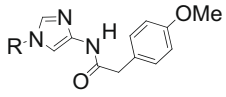
We thus sought to identify other possible polar groups to forge interactions with Lys33 and/or Asp144 that would afford increased potency through enthalpic contributions. The *N*-acetyl analog **19** gave a 10-fold potency increase (IC_{50} = 9 nM) relative to **15** and also showed sevenfold greater potency for cdk5 than cdk2, demonstrating that productive interactions were in fact being made. *N*-methylsulfonyl derivative **20** maintained a similar potency preference for cdk5 over cdk2 (sixfold), but cdk5 potency dropped off considerably (IC_{50} = 178 nM) compared to **19**. With the 1-naphthyl acetamide on the 4-aminoimidazole, *N*-acetyl analog **21** had similar potency (IC_{50} = 8 nM) to **19** and was more selective for cdk5 over cdk2 (17-fold). The placement of polar oxygen and nitrogen atoms was demonstrated to be critical as the reversed amide **22** lost significant cdk5 potency (IC_{50} = 391 nM) and selectivity over cdk2 (twofold). Interestingly, the *N*-methyl-*N*-acetyl analog **23** lost cdk5 potency (IC_{50} = 107 nM), but maintained a preference for cdk5 potency over cdk2 (15-fold). Importantly, this indicates that the amide carbonyl was a primary contributor to selectivity and the amide *N*–H contributed more to potency. Compound **19** was profiled against a panel of 49 kinases and was found to inhibit four >30% at 10 μM. Subsequent profiling against these four kinases was carried out at 1 μM to ascertain potency at lower doses; GSK3β (100% @ 10 μM, 94% @ 1 μM), MAPK1/ERK2 (89%, 47%), CK1-δ (39%, 7%), CDK2/Cyclin A (101%, 94%), and CLK1 (92%, 55%).[17] In particular, the significant increase in potency for GSK3β activity is striking with the addition of the acetyl group in **19** as compared to the unsubstituted cyclobutyl analog **7** (94% @ 1 μM vs 34% @

Table 1
SAR of 4-*N*-acyl group



	R^1	cdk5 IC_{50} nM (std dev)
6		500
7		145 (8)
8		46 (18)

Table 2
SAR of *N*-1 substituent



	R	cdk5 IC_{50} nM (std dev)
9	Me	>10,000
10	*i*-Pr	425
11	*c*-Pr	814 (161)
7	*c*-Bu	145 (8)
12	*c*-Pentyl	154 (27)
13	*c*-Hexyl	748 (192)
14	Benzyl	8640 (1490)