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Design and synthesis of 6-oxo-1,6-dihydropyridines as CDK5 inhibitors

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ABSTRACT

Cyclin-dependent kinase 5 (CDK5) is a serine-threonine protein kinase that plays a significant role in neuronal development. In association with p25, CDK5 abnormally phosphorylates a number of cellular targets involved in neurodegenerative disorders. Using active site homology and previous structure–activity relationships, a new series of potent CDK5 inhibitors was designed. This report describes the optimization of 6-oxo-1,6-dihydropyridines as CDK5 inhibitors.

A number of studies have demonstrated the essential role of cyclin-dependent kinase 5 (CDK5) in the early development of the central nervous system.[1] CDK5 is ubiquitously expressed in all tissues, but its highest expression and associated kinase activity are observed in the CNS. Deregulation of CDK5 is implicated in several neurodegenerative disorders.[1,2] As a result, CDK5 inhibitors have potential therapeutic use for diseases such as Alzheimer's disease,[3] amyotrophic lateral sclerosis,[4] and ischemic stroke.[5]

Our initial effort began with a high throughput screening campaign that led to the identification of a series of acyclic thiazole-ureas as potent CDK2 and CDK5 inhibitors.[6] After obtaining a co-crystal structure of CDK2 and acyclic urea **1**, we developed an active site homology model of CDK5 to guide the design and synthesis of conformationally constrained inhibitors (Fig. 1). Recently we reported the investigations of 3,4-dihydro-1*H*-quinazolin-2-ones[7] (**2**, CDK5 IC_{50} = 79 nM) and quinilon-2*H*-ones[8] (**3**, CDK5 IC_{50} = 54 nM) as potent CDK5 inhibitors. To further explore this line of strategy, we envisioned keeping the donor-acceptor binding portion of the molecule intact while reducing molecular weight. Additionally, we sought to improve the solubility of the molecules. Towards this end, we developed a series of potent inhibitors based on the 6-oxo-1,6-dihydropyridine core (**4**, Fig. 1). This report will profile the design, synthesis, and biological activity of 6-oxo-1,6-dihydropyridines as CDK5 inhibitors.

The general synthesis of 6-oxo-1,6-dihydropyridines analogues began with the appropriately substituted ethyl acetoacetates **5**. Typically, **5** was heated with dimethylamino dimethylacetal (DMF-DMA) to give substituted ethyl 2-dimethylaminomethylene-3-oxoalkonates **6** in good yields. Treatment of **6** with sodium acetoacetamide in THF at 60 °C gave the desired 6-oxo-1,6-dihydropyridine **7**, also in good yield.[9] Subsequent bromination and thiazole formation with the appropriate thioamides in EtOH at 80 °C furnished the final products **4**, **11–19**.[8] This synthetic scheme proved to be highly flexible and tolerated a large variety of substituents.

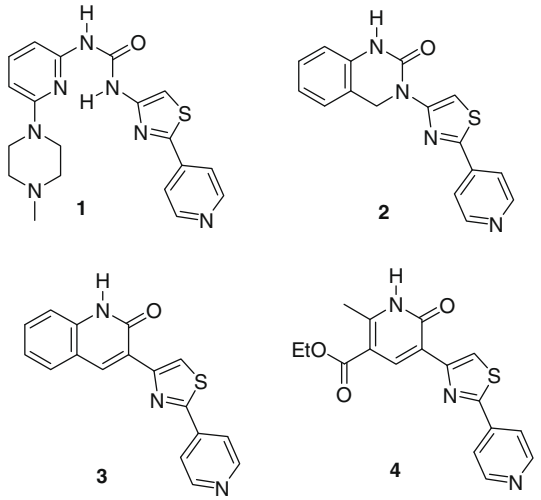

Figure 1. CDK5 inhibitors.

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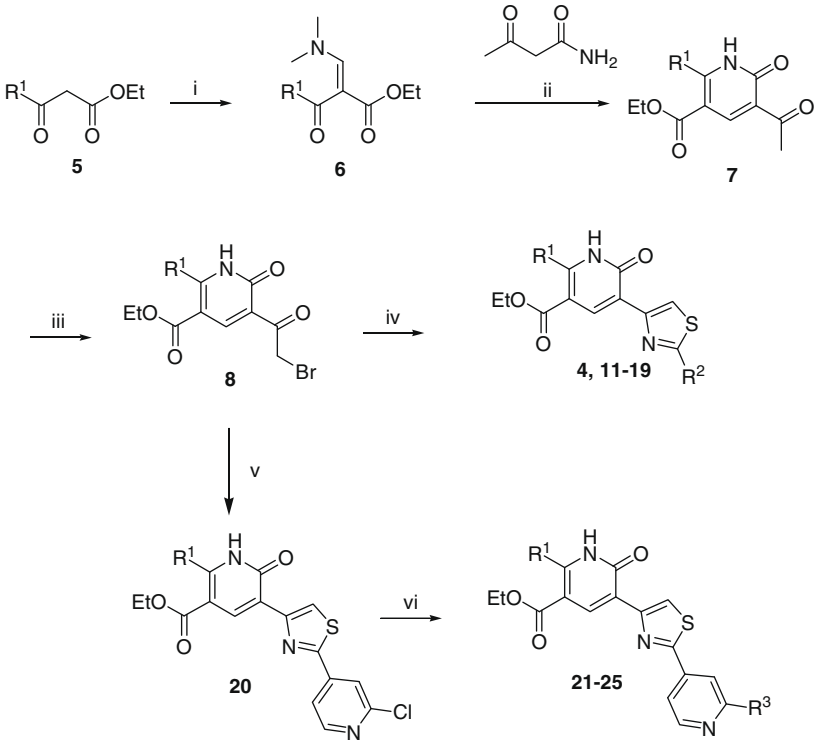
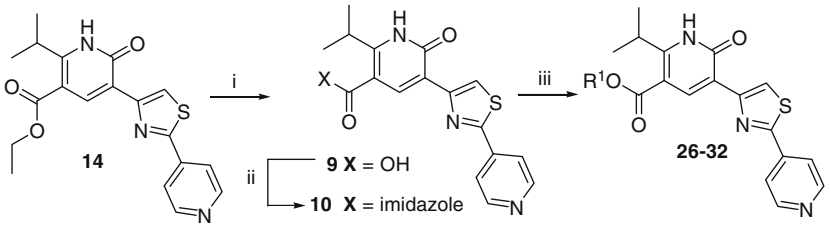


Scheme 1. Reagents and conditions: (i) $(CH_3)_2NCH(OCH_3)_2$, neat, 100 °C, 2–4 h; (ii) NaH, THF, 60 °C, 12 h; (iii) 5,5-dibromobarbituric acid (0.55 equiv), THF, reflux, 2–4 h; (iv) thioisonicotinamide, EtOH, 80 °C, 12 h; (v) 2-chloropyridine-4-carbothioamide, EtOH, 80 °C, 12 h; (vi) RNH_2, 120 °C or NaOMe in refluxing MeOH.



Scheme 2. Reagents and conditions: (i) KOH (s), EtOH/water, 120 °C, 10 min, microwave; (ii) CDI, DIPEA, CH_2Cl_2/DMF, 16 h; (iii) excess alcohol, 150 °C, neat, 10 min, microwave.

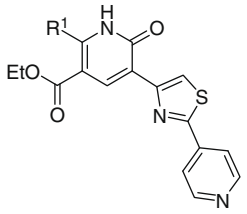
Compounds with substituents on the pyridyl ring (**21–25**) were made from the common intermediate **20** (Scheme 1). 2-Chloroisonicotinamide was treated with Lawesson's reagent to give 2-chloropyridine-4-carbothioamide[11] which was used to prepare **20**. The chloropyridine **20** was then heated with the appropriate amines or sodium methoxide to give the desired pyridyl amines **21–24** and methoxy pyridine **25** in moderate yield.[12] Compound **25** was prepared by reacting **20** with excess sodium methoxide in refluxing MeOH.

Outlined in Scheme 2 is the general synthesis of esters **26–32** from intermediate **14**. Treatment of **14** with KOH (5 equiv) in aqueous EtOH for 10 min at 120 °C in the microwave synthesizer effected hydrolysis[9] and yielded acid **9**, which was converted to the corresponding acyl imidazole **10** with carbonyl diimidazole in the presence of *N*,*N*-diisopropylethylamine in good yield. The acyl imidazole **10** was heated with excess commercially available alcohols at 150 °C for 10 min in a microwave synthesizer to provide the corresponding esters **26–32**.[10]

The analogues prepared for this study were tested for their ability to inhibit purified human CDK5/p25 in a homogenous time-resolved fluorescence assay run in the presence of 25 μM ATP using histone-H1 as the phosphorylation substrate.[13] The IC_{50} values were determined from dose–response curves and are reported in Tables 1–4 as the average of at least three replications. In addition to CDK5, the compounds were screened in an HTRF human CDK2/E2 assay that was run in the presence of 50 μM ATP and 0.5 μM histone-H1 as the substrate. Most analogues displayed only modest selectivity over CDK2, with the aryl sulfone series showing the best selectivity (up to 70-fold).

Our SAR exploration began with the finding that compound **4** was a modest CDK5 inhibitor (IC_{50} = 131 nM). To follow up with this encouraging result, we initially focused on expanding the SAR of substituents at the 2 position of the 6-oxo-1,6-dihydropyridine core (Table 1). It was quickly determined that a wide range of alkyl substituents were tolerated. For example, an isopropyl group or a cyclopropyl group at this position gave inhibitors **14** and **15** with comparable potency to **4**. Interestingly, inhibitors **12** and **13** with an ethyl group and an *n*-propyl group, respectively, were found to be more potent than **4**, with the former showing >30-fold improvement. The introduction of electron withdrawing groups or sterically bulky groups at the position led to reduction in activity as demonstrated by compounds **11** and **16**. Selected compounds from this subset of analogs were evaluated in rats for in vivo PK properties. In general, the compounds displayed moderate to high in vivo

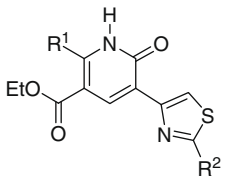
Table 1
SAR of the 2 position of substituted 6-oxo-1,6-dihydropyridines

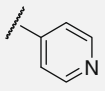
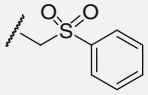
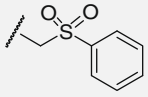
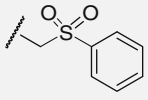


Compound	R^1	CDK5/p25 (IC_{50}, nM)[a]
4	CH_3	131 ± 66
11	CF_3	11489 ± 3429.9
12	C_2H_5	4.1[b]
13	*n*-C_3H_7	55 ± 14
14	*i*-C_3H_7	157 ± 131
15	*c*-C_3H_4	198 ± 55
16	$BnOCH_2$	779 ± 234

[a] At least three independent experiments were performed for each compound to determine the IC_{50} values.
[b] One experiment was performed to determine the IC_{50} value.

Table 2
SAR of pyridin-4-yl replaced 6-oxo-1,6-dihydropyridines



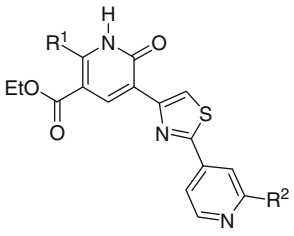
Compound	R^1	R^2	CDK5/p25 (IC_{50}, nM)[a]
4	CH_3		131 ± 66
17	CH_3		3.3 ± 1.1
18	*n*-C_3H_7		1.4 ± 0.2
19	$BnOCH_2$		5.3 ± 3.7

[a] At least three independent experiments were performed for each compound to determine the IC_{50} values.

clearance and poor to moderate brain exposure. Among these inhibitors, a notable exception, **14** showed a measured total brain concentration of 4640 ng/g one half hour after a 2 mg/kg IV dose in rats, an 8–10-fold improvement in brain exposure over other measured compounds.

Previous studies in the quinilon-2*H*-one series of CDK5 inhibitors have shown that the replacement of the 4-pyridyl group with

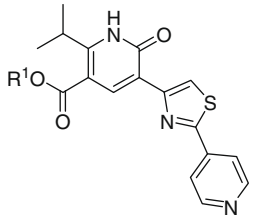
Table 3
SAR at the 3 position of the pyridine-4-yl moiety

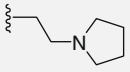
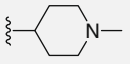
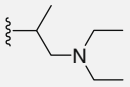
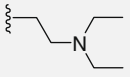
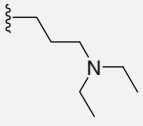


Compound	R^1	R^2	CDK5/p25 (IC_{50}, nM)[a]
4	CH_3	H	131 ± 66
20	CH_3	Cl	160[b]
21	CH_3	NH_2	20[b]
22	CH_3	$NHCH_2CH(Me)_2$	14 ± 10
23	CH_3	$NH(CH_2)_2N(Et)_2$	139 ± 37
24	*i*-C_3H_7	$NHCH_3$	28 ± 14
25	*i*-C_3H_7	OCH_3	672 ± 37

[a] At least three independent experiments were performed for each compound to determine the IC_{50} values.
[b] One experiment was performed to determine the IC_{50} value.

Table 4
SAR of different esters at the 3 position of 6-oxo-1,6-dihydropyridines



Compound	R^1	CDK5/p25 (IC_{50}, nM)[a]
14	C_2H_5	157 ± 131
26	CH_2CH_2Ph	119[b]
27		112 ± 20
28		647 ± 410
29		73 ± 30
30		36 ± 16
31		53 ± 34
32		47 ± 6

[a] At least three independent experiments were performed for each compound to determine the IC_{50} values.
[b] One experiment was performed to determine the IC_{50} value.

arylsulfones led to significant improvements in potency.[8] This SAR trend was also observed in the current series (Table 2). In general, arylsulfones provided potent inhibitors in the low nanomolar

range. For example, phenylsulfone derived compounds **17** and **18** displayed IC_{50} values of 3.3 nM and 1.4 nM, respectively. It is worth noting that the arylsulfone replacements greatly improved the potency of molecules with less than ideal groups at the 2 position. This was illustrated by compound **19** which displayed >150-fold improvement in potency than the corresponding 4-pyridyl inhibitor **16**.

The arylsulfone derived inhibitors were very potent; however, they gave lower brain exposure (**17**, 44 ng/g at 0.5 h, 2 mg/kg IV dose in rats) in comparison to the 4-pyridyl substituted molecules. This is not that surprising considering the higher polar surface area for these compounds along with lower permeability (typically 2–6×10^{-6} cm/s) these compounds demonstrated in comparison to the 4-pyridyl substituted molecules. Therefore, we focused our further exploration on the pyridyl compounds. Table 3 shows selected examples where the 2 position of the 4-pyridyl was substituted. Initially, this position was explored to mitigate possible CYP 450 inhibition due to the 4-pyridine; however it was soon evident that this additional substitution could also offer other benefits. The chloro derivative **20** had comparable potency to parent molecule **4**, while the aminopyridine compound **21** gave a sixfold improvement in activity. Although inhibitor **23** showed comparable activity to **4**, the other two alkylaminopyridine compounds **22** and **24** demonstrated a 10- and 5-fold increase in potency, respectively. In contrast to the aminopyridines, alkoxypyridines, such as **25**, proved to be less potent.

Table 4 summarizes the effects of replacing the ethyl ester. It should be noted here that these esters can be viewed as vinylogous carbamates and therefore are more stable than typical esters. This is consistent with the observation that the hydrolysis of these esters required very forcing conditions during synthesis (KOH, 5 equiv, in aqueous EtOH for 10 min at 120 °C in the microwave synthesizer). Based on this observation, we reasoned that it was worth exploring a variety of ester analogs. We quickly found that the hydrophobic alkyl ester analogs, exemplified by the phenethyl ester **26**, provided no improvement in potency in comparison to the ethyl ester. Drawing analogy to the SAR in the acyclic thiazole urea series for improving potency and solubility, we incorporated alkylamino groups into the ester sidechain. We found that a large number of substituted or ring constrained aminoethyl esters were potent CDK5 inhibitors (**27** and **29–31**, Table 4). Interestingly, substituted aminopropyl ester **32** was found to be a potent inhibitor, while its ring constrained variant **28** was 13-fold less potent.

Based on previous crystal structures of **1** and molecular modeling of **2** we propose that the 6-oxo-1,6-dihydropyridine series binds in a similar U-shape configuration where in the 6-oxo-1,6-dihydropyridine is involved in a donor-acceptor hydrogen bonding network with Leu83 (Fig. 2). In this model, the thiazole-pyridine extends down to make an interaction with the Lys33-Asp145 salt bridge of the ATP binding site. In addition, Lys89 also makes a hydrogen bond with the carbonyl of the ester. These binding interactions reconcile well with the observed SAR in this series.

In summary, guided by an active site homology model and SAR from our early studies in the 3,4-dihydro-1*H*-quinazolin-2-one and quinilon-2*H*-ones series,[7,8] we were able to develop a new series of 6-oxo-1,6-dihydropyridines as potent CDK5 inhibitors. We explored the 2 position of the 6-oxo-1,6-dihydropyridine core and determined that small alkyl substituents were optimal. Furthermore, we found that the 4-pyridyl group on the thiazole ring was a good compromise for maximizing potency and maintaining adequate brain exposure. The initial ethyl ester could be replaced with esters that also provided modestly improved potency and improved solubility.

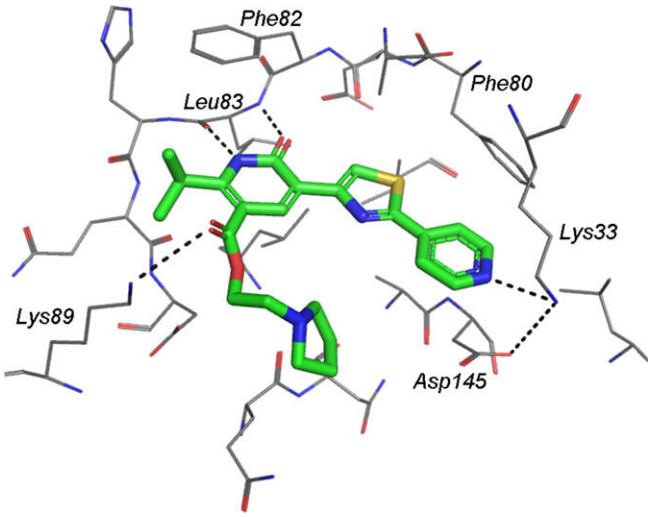


Figure 2. A proposed binding mode for 6-oxo-1,6-dihydropyridine (**27**)–CDK2 complex. Nitrogen atoms are shown in blue, oxygen atoms are shown in red, sulfur atoms are shown in yellow, and carbon atoms of active site residues are shown in gray. Carbon atoms of compounds **27** are shown in green.

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