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Pyridyl aminothiazoles as potent Chk1 inhibitors: Optimization of cellular activity

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ABSTRACT

Translation of significant biochemical activity of pyridyl aminothiazole class of Chk1 inhibitors into functional CEA potency required analysis and adjustment of both physical properties and kinase selectivity profile of the series. The steps toward optimization of cellular potency included elimination of CDK7 activity, reduction of molecular weight and polar surface area and increase in lipophilicity of the molecules in the series.

DNA-damaging agents have achieved widespread use as chemotherapy drugs and still constitute a basic tool set in the treatment of cancer. Despite making a major impact on the survival of cancer patients, this class of medicines has significant shortcomings, including toxicity associated with lack of selectivity for tumors over normal proliferating cells, as well as efficacy limitations. Therefore, widening the therapeutic window of DNA-damaging chemotherapies would lead to significant improvement in cancer care.[1] For maximal efficacy to be achieved with such chemotherapy, a dividing tumor cell should progress into mitosis after sustaining DNA damage and subsequently undergo mitotic catastrophe and apoptosis. However, cells have the ability to halt cell cycling in G1, G2, or S phases to allow for DNA repair.[2,3] This survival mechanism can normally be mediated through both p53 and Checkpoint Kinase 1 (Chk1) pathway activation.[4] The p53 pathway is dysfunctional in a large percentage of tumors.[5] These p53- impaired tumors must rely on Chk1-mediated G2/S arrest as a dominant defense mechanism from DNA-damaging chemotherapy.[2] Inhibition of Chk1 in such p53-deficient cancer cells with damaged DNA would abrogate the cell-cycle arrest and force the progression into mitosis resulting in cell death, thus selectively sensitizing these tumor types to chemotherapy. Accordingly, combination therapy comprising a DNA-damaging agent with Chk1 inhibitor will potentially have significantly higher therapeutic index than chemotherapy alone.[6–8] The pyridyl aminothiazole class of Chk1 inhibitors[9] originated from lead structure **1** (Table 1), that emerged from a previous effort directed at KDR kinase.[10,11] Compounds belonging to pyridyl aminothiazole series have demonstrated potential for high levels of Chk1 enzyme inhibition in vitro.[12] Here, we describe our efforts aimed at translating the biochemical potency of aminothiazoles into functional cellular activity in a Checkpoint Escape Assay (CEA). CEA measures release of H1299 tumor cells from DNA-damage induced cell-cycle arrest and progression into mitosis following Chk1 inhibition.[13]

Investigation of CEA potency of the series initiated with lead **1** and its analogs bearing substitutions at C6 position of the aminopyridine ring (**2**, **3**, **4**). To our surprise, none of the compounds **1**–**4** demonstrated measurable cellular activity and only flat or bell-shaped titration curves were observed in CEA experiment. However, **5** and **6**, analogs with piperazine substitution at C4 of the pyridine ring, were significantly active in checkpoint abrogation with intrinsic potency levels equivalent to **1**–**4**.

Especially puzzling was the comparison of isomers **4** and **5**, differing only in the placement of piperazine moiety. While an equally potent Chk1 inhibitor, **4** was inactive in CEA, **5** had an EC_{50} of 1370 nM and a normal sigmoidal curve. Such close structural similarity suggested that differences in functional activity could not be explained on the basis of cellular permeability, but rather by some kind of off-target activity. A similar lack of cellular potency was observed with several Chk1 inhibitors from the indolyl indazole class.[14] In this case, concomitant Cdk7 inhibition was determined to be the culprit behind large shifts between functional and biochemical potency levels.[14] Cdk7 is a member of the cyclin-dependent kinase family and plays a central role in regulating progression of the cell cycle. Because Cdk7 activity is critical for maintaining cell cycle transitions, it was proposed that Cdk7

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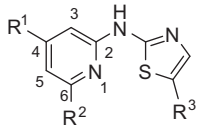
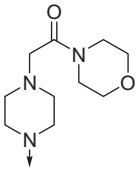
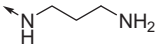
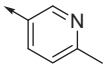
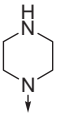
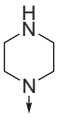
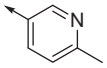
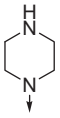
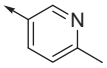
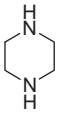
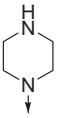
Table 1
Inhibitory profile and CEA activities of **1**–**7**



Compd	R^1	R^2	R^3	Chk1 IC_{50}	Cdk IC_{50}	CEA EC_{50}
1		H		47	1760	>50,000
2	H			27	870	>16,670
3	H			33	26	>16,670
4	H			38	1000	>16,670
5		H		37	>20,000	1370
6		H		29	11,000	1530
7	H			160	410	—

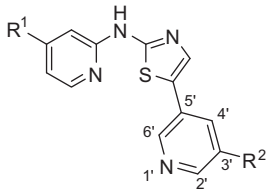
inhibition resulted in cellular arrest that precluded checkpoint abrogation event mediated by Chk1 inhibition. This hypothesis was further supported by an experiment where Cdk7 siRNA inhibited CEA effect of functionally active Chk1 inhibitor.[14] Therefore, counterscreening of **1**–**6** for Cdk7 inhibition was warranted. In the event, **1** and all C6 substituted analogs **2**, **3**, **4**, **7** demonstrated similar levels of Cdk7 and Chk1 inhibition (Table 1). As expected, functionally active C4 substituted compounds were virtually devoid of Cdk7 activity.

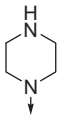
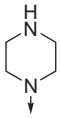
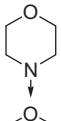
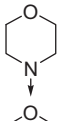
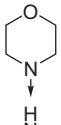
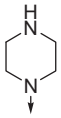
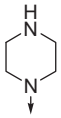
This discovery has prompted us to discontinue development of compounds modified at C6 of the western pyridine ring and implement Cdk7 assay as a routine counterscreen for the series. Having fixed the substitution on western pyridine at C4, we then turned our attention to investigation of cellular activity of analogs bearing eastern pyridine ring modified at C3′ (Table 2). A series of compounds with a biaromatic motif attached to the thiazole ring was prepared by Suzuki couplings with corresponding bromides, for example, **8**.

While bromide **8** itself is a Cdk7 inhibitor with no CEA activity, the majority of compounds carrying an aromatic ring at this position did not show detectable potency against Cdk7. However, even in the in the absence of Cdk7 inhibition shifts between potency in CEA and Chk1 enzymatic assay remained very large, for example, **9**, **10**, and **11** showed little to no functional activity. Some improvement was observed with triazole **12** and pyrazole **13** modified with piperazine as R^1, however only micromolar CEA levels were achieved even with Chk1 potency now in the single nM range. The best cellular potency in this series was demonstrated by bipyridine **14** with CEA EC_{50} = 570 nM and a 5 nM IC_{50} in the Chk1 enzyme assay. A major issue for this subseries emerged when Chk1 biochemical activity in the presence of 30% human serum was tested. Many triaryl compounds demonstrated significant drop in potency in this experiment (data not shown), probably due to high amount of nonspecific protein binding. The findings helped explain some of the lackluster CEA results, since CEA assay is also performed in the presence of serum (10%). Thus, while some gains in functional activity with heteroaryl derivatives at C3′ were achieved; a superior replacement needed to be identified.

After some experimentation, amides on the C3′ of the eastern pyridyl ring were found to provide significant gain in biochemical potency for this class (Table 3). Both the precursor carboxylic acid **15** and primary amide **16**, despite being relatively potent Chk1 inhibitors at 16 and 10 nM, respectively, showed no CEA activity. We were therefore pleased to find that simple methylation of the amide afforded a compound (**18**) with noticeable improvement in CEA potency (EC_{50} = 296 nM). Even higher levels of cellular activity were expected from replacement of the methyl amide with ethylenediamine amide, since it afforded compounds with pM activity levels in Chk1 assay. For example, with R^1 = morpholine (**19**) 50 pM IC_{50} was achieved, and both sulfonamide **20** and *N*-acetylpiperazine **21** showed 30 pM IC_{50}s.

Table 2
Chk1 and CEA activity of triaromatic compounds **8**–**14**



Compd	R^1	R^2	Chk1 IC_{50}	Cdk7 IC_{50}	CEA EC_{50}
8		Br	21	480	>50,000
9			8	260	5530
10			19	>10,000	>10,000
11			37	>10,000	>10,000
12			9	>10,000	1390
13			6	>2000	1330
14			5	>10,000	570

Unfortunately, this remarkable boost in intrinsic potency did not translate into comparable levels of cellular activity, even though high selectivity against Cdk7 was maintained with C3′ amides. We postulated that one reason for the disappointing increase in the CEA/Chk1 shift[15] could be that gains in intrinsic potency resulting from ethylenediamine introduction came at the expense of dramatically increased polar surface area (PSA).[16] Indeed, PSA values for **19**–**21** were now in the range of 130–160 Å, compared to ca. 100 Å for methyl amide **18**. Elevated PSA and multiple basic functionalities in Chk1 inhibitors have been previously correlated with decreased cellular permeability and high CEA/Chk1 shift ratios, and PSA of less than 100 Å was suggested as a prerequisite for achieving cellular potency.[17,18] Accordingly, both **19** and **20** only achieved ca. 500 nM CEA IC_{50}s, while **21** showed worse than 1000 nM activity in this assay.

Further improvements in amide series could therefore only be realized through enhancements in physical properties of the compounds necessary for better cell membrane permeability. To this end, we designed a strategy to address the issue in three ways: decreasing molecular weight, removing one or more nitrogen atoms from the core, and introducing fluorination to increase general lipophilicity of the compounds.

The first approach was driven by realization that significant Chk1 activity could be maintained through incorporation of ethylenediamine amide even in the absence of solvent front engaging polar groups at the western pyridine ring, as exemplified by compound **22** (Table 4). Thus, notable decrease in PSA and number of basic nitrogens was achieved leading to a more reasonable CEA/Chk1 shift of 12×. Analysis of X-ray structures of aminothiazoles[12,17] and pyrazoloquinolinones[19] suggested an improvement in potency could be obtained by relocating the Cl atom in **22** one position closer to the aminogroup. Gratifyingly, the *o*-substituted **23** was 20 times more potent Chk1 inhibitor than *m*-substituted **22** with identical PSA and Cdk7 inhibitory values.

However, only a modest 2.8-fold improvement in cellular activity (942–329 nM) was observed as CEA/Chk1 shift has perplexingly increased 10 fold, driven solely by the change in the Cl placement. An analog with Me substitution at the same position (**24**) was shown to have virtually identical profile to **23** in terms of Chk1, Cdk7 inhibition and PSA, and a similar CEA potency of 431 nM.

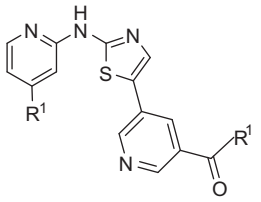
Our second strategy was to study the effect of removal of nitrogen atoms from the pyridyl aminothiazol system on Chk1 binding, PSA and CEA shift parameters. Representative compounds stemming from this work are shown in Table 5.

A significant synthetic effort was expended in preparation of these analogs resulting in a decrease in PSA to ca. 100 Å range for most compounds. However, Chk1 biochemical potency was also dramatically affected by all such manipulations. For example, substitution of eastern (**25**) and western (**27**) side pyridine nitrogens with CH caused 150 and 110 fold drop in potency, respectively, and displacement of carboxamide moiety afforded CH_2-linked compound **26** that was 240 times less active than the corresponding amide **19**. Accordingly, most similarly 'denitrogenated' molecules had little measurable CEA activity. Replacement of terminal amino group with hydroxyl (e.g., **28**) was also not successful and led to 300 fold potency loss. These derivatives showed no cellular potency either.

The third approach commenced with screening of possible fluorinated replacements for the ethylenediamine group while the western pyridine C4 substitution was fixed as morpholine (Table 6). Of these, trifluoroethylamine amides proved to be the best derivatives in terms of cellular activity as we were encouraged to find that some decrease in Chk1 inhibitory activity for **29** was compensated by low Chk1/CEA shift, resulting in CEA EC_{50} = 200 nM. With trifluoroethylamine in place, the screening of western C4 derivatives was next carried out and provided sulfonamide **30** with even higher CEA activity of 106 nM. Introduction of fluorine in this part of the molecule was also explored. We were happy to discover that exposure of fluorinated piperidines to the solvated area of the binding cleft did not result in the loss of intrinsic activity as compared to morpholine and piperazine. For example, monofluoro- and difluoropiperidine derivatives **31** and **32** were highly potent Chk1 inhibitors with IC_{50} of 70 and 50 pM, respectively, and displayed over 6000 fold selectivity over Cdk7. Once again, fluorination helped significantly improve CEA/Chk1 shift, with difluoro derivative **32** being significantly more potent than monofluoro **31** with EC_{50} = 135 nM. Overall, the fluorination strategy proved most successful in translating biochemical potency of pyridyl aminothiazole Chk1 inhibitors into functional cellular activity.

The synthesis of C3′ amides subset of aminothiazole series is shown on Scheme 1.[20] A bromonicotinamide **33** can be coupled with protected aminothiazole **34** in a Heck-type reaction catalyzed by $Pd(PPh_3)_4$ to give biaromatic compound **35** in modest to good yields and complete regioselectivity. Removal of pivaloyl group was then performed in acidic conditions and was accompanied by concomitant hydrolysis of the amide affording amino-acid **36** as a hydrochloride. The amine in **36** was then converted into chloride through the intermediate diazonium salt formed in the presence of copper(II) chloride.

Table 3
Cellular and biochemical potency of C3′ amides **15–21**



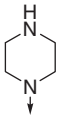
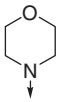
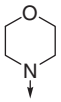
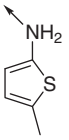
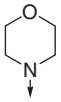
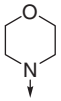
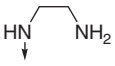
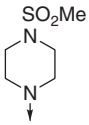
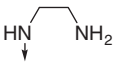
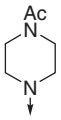
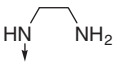
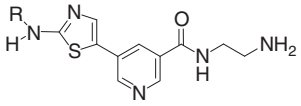
Compd	R^1	R^2	Chk1 IC_{50} (nM)	Cdk7 IC_{50} (nM)	PSA	CEA EC_{50} (nM)
15		OH	16	>10,000	117	>10,000
16		NH_2	10	>3700	119	>10,000
17			12	>10,000	114	920
18		NHMe	7	>10,000	104	300
19			0.05	340	130	460
20			0.03	170	162	500
21			0.03	210	140	1210

Table 4
Potency and selectivity profile of **22–24**



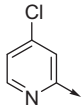
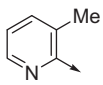
Compd	R	Chk1 IC_{50} (nM)	Cdk7 IC_{50} (nM)	PSA	CEA EC_{50} (nM)
22		16	940	111	940
23		0.3	960	110	330
24		0.4	910	110	430

Table 5
Profiles of aminothiazoles with N replacements **25**–**28**

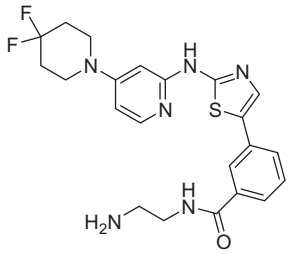
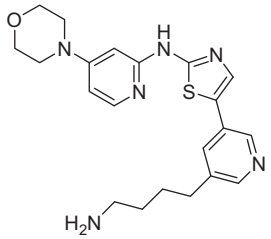
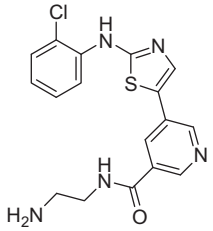
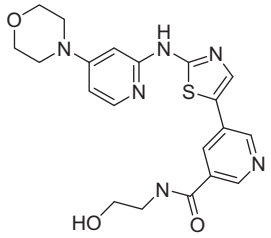
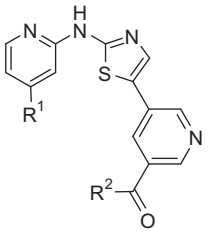
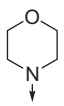
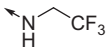
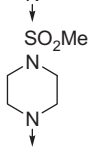
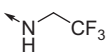
Compd	Structure	Chk1 IC_{50} (nM)	Cdk7 IC_{50} (nM)	PSA	CEA EC_{50} (nM)
25		8	160	103	>10,000
26		12	>10,000	102	—
27		33	>10,000	97	>10,000
28		15	>10,000	125	>10,000

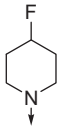
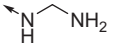
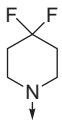
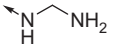
Table 6
Examples of fluorinated aminothiazoles **29**–**32**

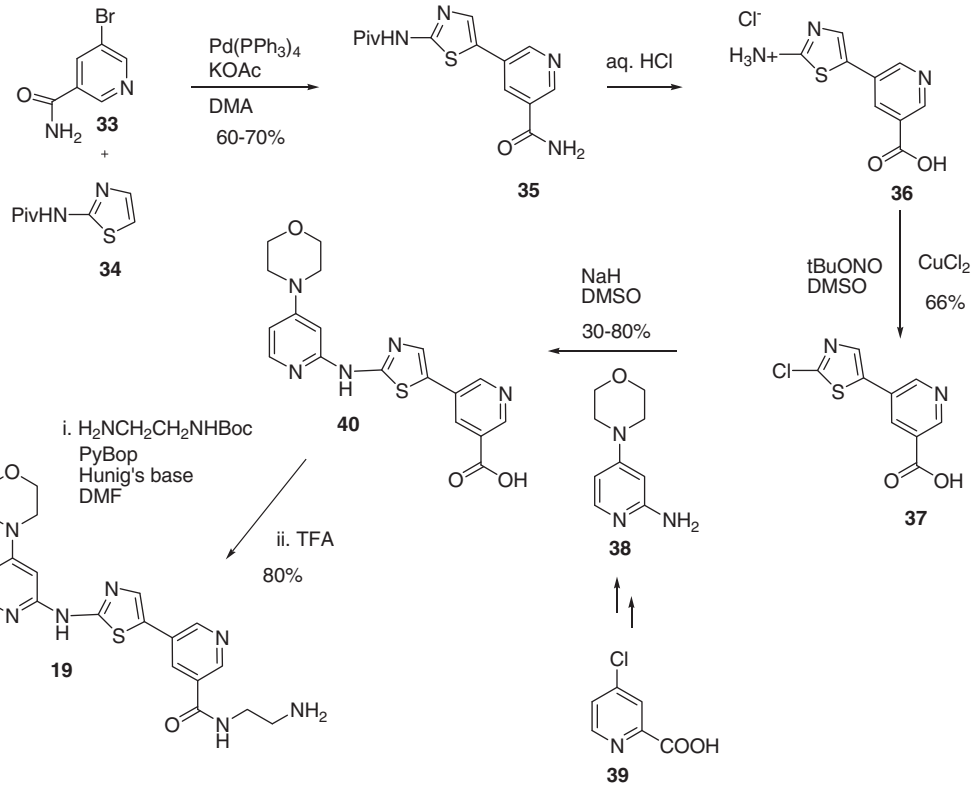


Compd	R^1	R^2	Chk1 IC_{50} (nM)	Cdk7 IC_{50} (nM)	PSA	CEA EC_{50} (nM)
29			1.6	705	101	198
30			1.7	2100	131	106

(*continued on next page*)

Table 6 (*continued*)

Compd	R^1	R^2	Chk1 IC_{50} (nM)	Cdk7 IC_{50} (nM)	PSA	CEA EC_{50} (nM)
31			0.07	350	116	484
32			0.05	317	116	134



Scheme 1. Synthesis of ethylenediamine amide **19**.

The resulting chlorothiazole **37** was then coupled in the presence of sodium hydride with substituted aminopyridine **38**, itself prepared from pyridinecarboxylic acid **39**. Ethylenediamine moiety was finally introduced through amide bond formation followed by Boc deprotection. Alternatively, when screening of pyridine substituents is desired, **37** can be efficiently coupled with a 4- or 6-chloro-2-aminopyridine, followed by S_NAr displacement of the chloride with an appropriate amine.

In summary, translation of the intrinsic potency of the pyridyl aminothiazole series of Chk1 inhibitors into functional cellular activity required investigation and tuning of parameters governing both selectivity and cell membrane permeability. In particular, Cdk7 inhibition was again identified as a factor impeding checkpoint abrogation activity. Elevated PSA values can be generally considered as a potential indicator of low cellular permeability and therefore a predictor of high shifts between cellular and biochemical potency. In case of pyridyl aminothiazoles, fluorination proved to be the most effective strategy to combat this problem. Through this approach, several fluorinated derivatives were discovered with optimized cellular activity profile.

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