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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3121–3124

4-(Aminoalkylamino)-3-benzimidazole-quinolinones as potent CHK-1 inhibitors

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Received 25 January 2006; revised 9 March 2006; accepted 20 March 2006
Available online 5 April 2006

Abstract—CHK-1 is one of the key enzymes regulating checkpoints in cellular growth cycles. Novel 4-(aminoalkylamino)-3-benzimidazole-quinolinones were prepared and assayed for their ability to inhibit CHK-1. These compounds are potent cell permeable CHK-1 inhibitors and showed synergistic effect with a DNA-damaging agent, camptothecin.

Upon DNA damage by ultraviolet light, radiation or cytotoxic drugs, the cellular response is to arrest the cell cycle at one of three checkpoints (G1/S, intra-S or G2/M) to either permit DNA repair or to initiate apoptosis. The serine/threonine checkpoint kinase, (CHK-1), regulates both the G2/M and intra-S checkpoints, and plays an important role in cell-cycle progression,[1–4] especially for *p53*-defective cancer cells. Since cell-cycle arrest is a mechanism by which tumor cells can overcome the damage induced by cytotoxic agents, abrogation of the G2/M checkpoint with novel small molecule compounds may increase the sensitivity of *p53*-deficient tumors to chemotherapy.[5,6] Importantly, in contrast to many current therapies for cancer, this mechanism potentially carries with it only a low risk of toxicity against non-malignant cells, as CHK-1 inhibition is most effective in *p53*-defective cancer cells. Thus, a major advantage of CHK-1 inhibitors as a treatment for cancer is their selective activity in conjunction with cytotoxics, such as DNA-damaging reagents.

Several CHK-1 inhibitors have been reported in the literature. Of these, UCN-01 (7-hydroxystaurosporine) is a potent inhibitor of CHK-1 (K_i = 5.6 nM)[7–9] and has modest selectivity among other kinases.[7] UCN-01 is currently in clinical trials.[10] Other CHK-1 inhibitors reported in the literature include indazole–imidazole analogs,[11] and more selective UCN-01 analogs, such as ICP-1.[12] Recently a series of novel CHK-1 inhibitors were reported, including *N*-aryl-*N*′-pyrazinylurea,[13] 1,3-dihydro-indolone,[14] and furanopyrimidine.[15] Here, we report 4-(aminoalkylamino)-3-benzimidazole-quinolinone derivatives as potent CHK-1 inhibitors with synergistic effects with DNA-damaging agents in tumor cells.

The synthesis of 4-(aminoalkylamino)-3-benzimidazole-quinolinone is shown in Scheme 1. Reaction of ethyl 2-benzimidazole-acetate with 1-*N*-*p*-methoxy-benzyl (PMB) protected isatoic anhydride[16] in the presence of a base in THF yielded 4-hydroxy-3-benzimidazole-quinolinone **A** in good yields. Conversion of the 4-hydroxy compound to the corresponding bis-triflate **B** was accomplished with triflic anhydride in the presence of pyridine at −5 °C. Reaction of the bis-triflate with alkylamines via a S_NAr reaction was carried out in acetonitrile with Hünig's base at 80 °C. Deprotection of the PMB group and removal of the triflate group on the benzimidazole of the intermediate **C** occurred in one pot under acidic conditions (trifluoroacetic acid and concentrated HCl; v/v, 7:1) at 90 °C to yield 4-alkylamino-3-benzimidazole-quinolinone (4-alkyl ABIQ) compounds in good yields.[17]

Unsubstituted 4-aminobenzimidazolequinolone **1** has moderate activity (IC_{50} = 0.73 μM) against CHK-1. A

Keywords: CHK-1 kinase inhibitor; Kinase; Synergy.
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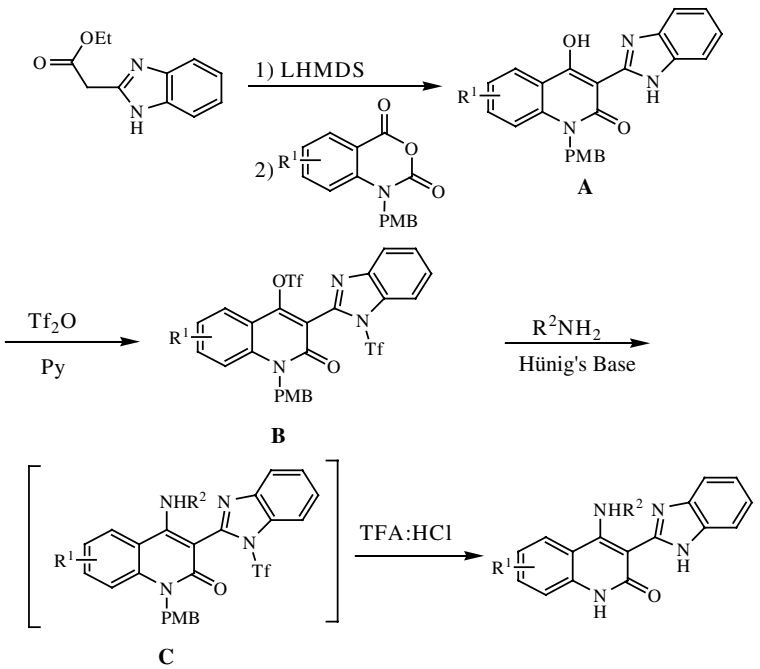
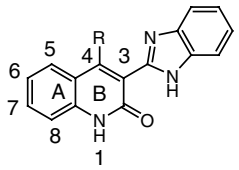
doi:10.1016/j.bmcl.2006.03.059



Scheme 1. Preparation of CHK-1 inhibitors.

NH group at C-4 is favorable for the potency. Presumably, a hydrogen-bond donor at C-4 or simply a small size hydrogen is important for keeping the two bicycles in a co-planar configuration. Replacement of the C-4 amine group with a hydroxy group reduced the potency by 4-fold, while the 4-diethylamino analog **3** was inactive.



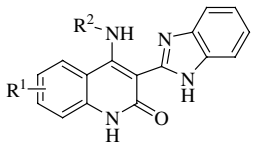
1 R = NH_2, IC_{50} = 0.731 μM
2 R = OH, IC_{50} = 2.95 μM
3 R = NEt_2, IC_{50} > 30 μM

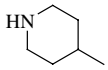
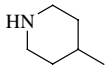
The affinity of ABIQ analogs was sensitive to substitutions. The hydrogen bond donor–acceptor–donor (DAD) motif of the quinolinone and benzimidazole interacts with the lower hinge of the ATP-binding pocket. Methylation at N-1 eliminated the hydrogen-bond interaction with the protein and abolished activity. Simple 4-alkylamino groups were tolerated with moderate success (data not shown). Furthermore, an aminoalkylamine, e.g., (*S*)-3-aminoquinuclidine, introduced at the 4-position of product **4**, led to a dramatic increase in potency. To further explore the SAR of the C-4 alkylamine group and the effect of substituents on the A-ring, a set of amino-containing ABIQs was prepared. Table 1 highlights the inhibitory properties of representative amine-containing alkyl ABIQs. The inhibition of CHK-1 activity was dependent on the nature of aminoalkyl group, especially its stereochemistry. For example, compound **5** was 60-fold less potent than its enantiomer **4** although the effect was less striking for the enantiomeric pair **9** and **10**.

To better understand the interactions of these inhibitors with the CHK-1 protein, a co-crystal structure of compound **11** with CHK-1 was obtained.[18] The co-crystal structure showed that the inhibitor formed hydrogen bonds with Glu85 and Cys87 in the hinge region, explaining the general kinase affinity of this ABIQ scaffold. Significantly improved potency for the amino groups at 4-position was derived from the strong interactions of the tertiary-amine of **11** with an acidic patch formed by Glu91 and Glu134, as shown in Figure 1.[19] This charge–charge interaction also greatly improved selectivity of this series of inhibitors against many other kinases (cdk and cdc data are shown below).[20] Several inhibitors in the literature occupied the same ATP-binding pocket. However, none of the known inhibitors has fully utilized the acidic patch.[14,15]

The ability of the aminoalkyl ABIQ to inhibit CHK-1 activity was also sensitive to the substitution on the A-ring of the quinolinone. Thus, modification of the substitutions on the A-ring provided a series of potent ABIQ-containing CHK-1 inhibitors (IC_{50} < 1 nM). X-ray crystal structure shows a hydrophobic space near C-6. Thus, substitutions at position C-6 are well tolerated. Medium size groups, such as methyl and chloro, enhanced the activity more than 10-fold (compounds **6**–**9** and **11**–**13**). Multiple substitutions on the A-ring diminished the affinity (compound **14**), while any group at position C-8 (methyl in compound **15**) was detrimental to the activity.

Table 1. IC_{50} of CHK-1 inhibitors



Compound	R^1	R^2	IC_{50} (nM)
4	H		7.6
5	H		482
6	6-Me		0.35
7	6-Me		1.4
8	6-Me		3.3
9	6-Cl		0.50
10	6-Cl		7.2
11	6-Cl		0.32
12	6-Cl		1.04
13	6-Cl		3.3
14	5,6-di-Cl		21.7
15	8-Me		138

An ideal CHK-1 inhibitor would be minimally cytotoxic, while enhancing the anti-tumor effect of a real cytotoxic agent that would be used in combination with the inhibitor. Our CHK-1 inhibitors have shown excellent selectivity over a panel of kinases including those regulating the cell cycle, but these compounds have also shown cytotoxicity to tumor cell lines. Compound **11** had an IC_{50} of 0.32 nM for CHK-1 with an EC_{50} of 80 nM (breast cell line MDA-MB-435), while its selectivity ratio for cdk2/cyclin A, cdk4/cyclin D, and cdc2/cyclin B was over 500-, 5000-, and 1500-fold, respectively.[21] More importantly, this class of compounds was synergistic with known cytotoxic agents. When MDA-MB-435 cells were treated with compound **11** and camptothecin (CPT), an inhibitor of topoisomerase 1, compound **11** significantly accelerated cell death (Fig. 2).[22,23] This represents a new example of small molecule CHK-1 inhibitors having synergistic activity with traditional cytotoxic agents.

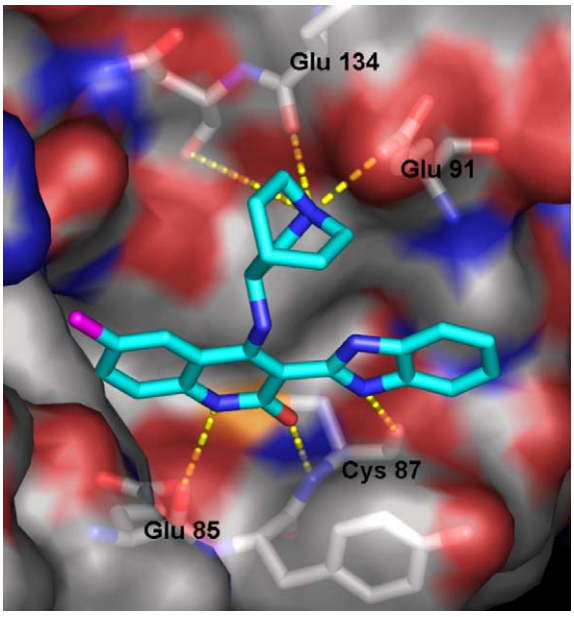


Figure 1. X-ray co-crystal structure of ABIQ **11** with CHK-1.

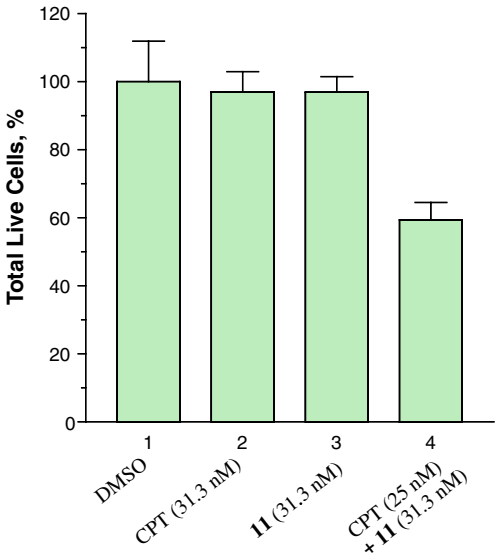


Figure 2. Synergestic effect of compound **11** with CPT.

In summary, we have identified a novel class of small molecule CHK-1 inhibitors. The current synthetic approach provided us with easy access to structurally diverse 4-(aminoalkylamino)-3-benzimidazole-quinolinones. These inhibitors were potent, selective, and able to enhance anti-tumor effect in conjunction with cytotoxic agents. Their PK properties in rodents and in vivo efficacy data will be reported in due course.

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17. General procedure for the preparation of 4-[((3*S*)-quinuclidin-3-yl)amino]-3-benzimidazol-2-yl-6-chloro-hydroquinolin-2- one (**11**): 3-(1H-benzimidazole-2yl)-4-hydroxyl-1-(4-methoxybenzyl)-6-chloro-1H- quinolinone (1 equiv) was suspended in dichloromethane (DCM) in the presence of pyridine (20 equiv). The mixture was warmed to dissolve the solid. The mixture was cooled to −5 °C and triflic anhydride (8 equiv) in DCM was added dropwise. After the reaction was stirred at −5 °C for 4 h, saturated aqueous sodium bicarbonate was added. The aqueous layer was extracted with DCM twice. The combined organic layers were washed with 1 M citric acid, 1 M sodium bicarbonate, water, and brine, dried over sodium sulfate, and concentrated to give 1-[(4-methoxyphenyl)methyl]-2-oxo-3-{1-[(trifluoromethyl)-sulfonyl]benzimidazol-2-yl}-6-chloro-4-hydroquinolyl(trifluoromethyl)sulfonate, which was used for the next reaction without further purification. A solution of the bis-triflate prepared above, (*S*)-3-aminoquinuclidine (1.2 equiv), and Hünig's base (4 equiv) in acetonitrile was heated at 80 °C for 20 h. After cooling down to room temperature, the reaction mixture was diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate, water, and brine, dried over sodium sulfate, and concentrated to give a solid. The solid was dissolved in a mixture of trifluoroacetic acid and concentrated HCl (7:1), and heated at 90 °C for 20 h. The reaction was diluted with water and ethyl acetate, and slowly basified to pH 9 with saturated aqueous sodium bicarbonate. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with water and brine, and dried over sodium sulfate, and concentrated. The product was purified with preparative HPLC to give the desired product **11**.
18. The CHK1 complex with compound **11** has been assigned the PDB ID code: 2GDO.
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22. Cell Synergy Assay: MDA-MB-435 cells (5× 103) were plated onto a 96-well plate in 100 μL cell culture medium and incubated for 4 h at 37 °C under 5% CO_2. DMSO (vehicle control), CPT alone, compound **11** alone, and compound **11** + CPT, all in 100 μL cell culture medium, were added, resulting in the final concentrations indicated. The cells were incubated further for 48 h. MTS was added to the cultures, permitted to develop for 4 h at 37 °C under 5% CO_2, then read on a microplate reader at 490 nm. The ODs were corrected for background absorbance by subtracting the values obtained for wells containing tissue culture medium alone. Percent viable cells were calculated by: $\{[A_{490}$ (DMSO-treated cells) − A_{490} (test sample)]/A_{490} (DMSO-treated cells)$\} \times 100\%$. Synergy was defined as the effect observed by the combination of the two compounds being greater than the sum of the effect of each compound alone.
23. Detailed isobologram analysis of the synergistic effect will be published elsewhere (see Ref. 21).