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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6467–6471
Bioorganic & Medicinal Chemistry Letters

# 2-Aryl benzimidazoles featuring alkyl-linked pendant alcohols and amines as inhibitors of checkpoint kinase Chk2

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Received 24 July 2007; revised 27 September 2007; accepted 28 September 2007
Available online 4 October 2007

**Abstract**—A series of benzimidazole compounds containing pendant alcohol and amine moieties was found to be active against checkpoint kinase Chk2. These compounds were prepared to examine a potential hydrogen bond interaction with an active site residue and to investigate replacement of a biaryl linker with an aliphatic system in an effort to improve solubility.


The checkpoint kinase Chk2 is activated by ATM (ataxia-telangiectasia mutated) in response to DNA damage and controls the downstream p53-dependent apoptosis machinery.[1] Deletion of Chk2 in mice blocks apoptosis after lethal radiation in several cell types and increases cell survival.[2,3] Since tumor tissue is generally more apoptosis resistant than normal cells, it is hypothesized that inhibition of Chk2 would not protect these tissues. Small molecule inhibitors of Chk2 may therefore have utility in clinical situations, such as radio- and chemotherapy, where p53-dependent apoptosis triggered by DNA insult leads to tissue toxicity.[4–7]

Lead compounds from a series of benzimidazoles that were identified as potent inhibitors of Chk2 are shown in Figure 1.[8,9] Molecular modeling suggested that the high affinity of compound **1** is potentially due to a hydrogen bonding interaction between an active site residue (histamine 157) and the hydroxyl group of the terminal phenol (Fig. 2). In an effort to exploit this possible interaction toward increasing activity and to improve overall solubility, analogs with pendant hydroxyl and amine groups were desired.

Previous reports from this laboratory described the synthesis of benzimidazole compounds with ether or thioether-linked biaryl moieties (Fig. 1).[8,9] The role of the distal aryl ring in binding was unknown in this system and was thought to be a strong contributor to the poor

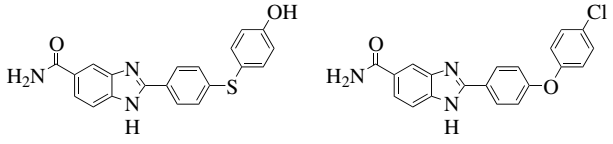


**Figure 1.** Lead biaryl benzimidazole compounds.

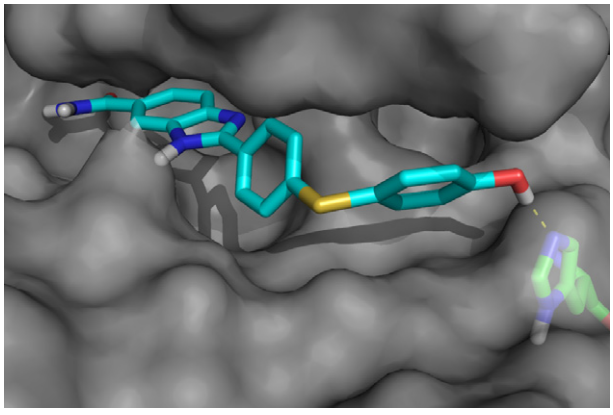

**Figure 2.** Compound 1 docked into an active site model of Chk2 showing hydrogen-bonding to Hist-157.

*Keywords*: Chk2; Cds1; Solubility; Aminoalcohol; Mitsunobu.
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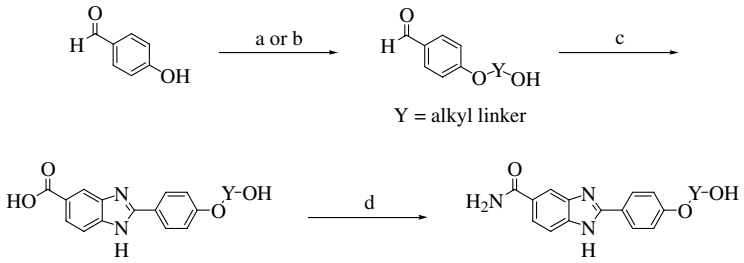
doi:10.1016/j.bmcl.2007.09.098




**Scheme 1.** Synthesis of alkyl-linked pendant alcohols. Reagents and conditions: (a) Bromo alkyl alcohol, $K_2CO_3$, DMF, rt; (b) Diol, DIAD, $PPh_3$, $CH_2Cl_2$, rt; (c) 3,4-Diaminobenzoic acid, $Na_2S_2O_5$, DMF, 90 °C (d) i—HOBt, DICI, DMF; ii–Rink amide resin, $CH_2Cl_2$; iii—20% TFA/$CH_2Cl_2$.

solubility of these compounds. To exemplify this issue, the solubility of **2** in simulated intestinal fluid (SIF, pH 7) is 0.007 mM and in simulated gastric fluid (SGF, pH 2) is 0.031 mM.[10] It was hoped that incorporation of more flexible alkyl linkers might increase solubility; such changes would also allow for the placement of hydrogen bond donors and acceptors in different areas of the active site. As a result, benzimidazoles containing alkyl-linked alcohols, amines, and amides were envisioned. The synthesis of such alkyl-linked pendant alcohol analogs is shown in Scheme 1.[11]

The necessary aldehyde precursors were obtained through alkylation of 4-hydroxybenzaldehyde with desired bromoalcohols or coupling of appropriate diols under Mitsunobu conditions. Subsequent benzimidazole formation was carried out using the resultant aldehydes with 3,4-diaminobenzoic acid in the presence of sodium metabisulfite. These intermediates were coupled onto Rink amide resin, allowing impurities to be removed by thorough rinsing before acidic cleavage from the resin to provide the desired amides.

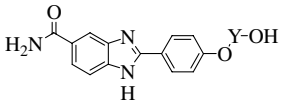
The resulting compounds (Table 1) retained reasonable activity against Chk2.[11] Though these compounds were less potent than compound **1**, the inhibition shown indicated that a biaryl system was not necessary to retain nanomolar activity against the target. Interestingly, the 100-fold difference in activity between the *cis* versus *trans* configurations of the methyl cyclohexane analogs (entries **7** and **8**) implied that not only the presence but also the position of the pendant alcohol was important for kinase inhibition. Reducing the molecular weight of the compounds and incorporating a hydroxyl group yielded increased solubility for compounds **3** and **5** at neutral pH and increased solubility for compounds **3**–**5** at low pH, when compared to compound **2**.

The synthesis of alkyl-linked pendant amines is shown in Scheme 2.

Aldehyde intermediates were accessed by combining 4-hydroxybenzaldehyde and various aminoalcohols under Mitsunobu conditions. These aldehydes were cyclized to benzimidazoles as previously described. The resulting carboxylic acid was readily converted to the primary amide by reaction with carbonyldiimidazole in DMF followed by addition of ammonium carbonate.

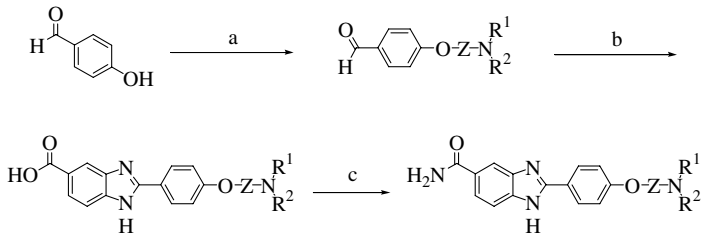
Though the resulting amine analogs (Table 2) lacked notable SAR trends with regard to the pendant amine, all compounds tested for solubility reached the maximum measured level at both neutral and low pH levels. Secondary amines (entries **12**, **15**, **17**, **18**) were not significantly more potent than tertiary amines (entries **9**–**11**, **13**, **14**, **16**). Additionally, changes in linker length between the ether and amine, from two to four carbons, did not drastically affect activity. Increasing the size and lipophilicity of the molecule by substituting the amine moiety with methyl and benzyl groups resulted in an increase in activity (entries **9** and **10**). Changing substitution on the amine from a dimethyl to a cyclohexyl group (entries **13** and **14** consecutively), which also increased the size of the molecule, yielded no change in potency.

**Table 1.** Pendant alcohols



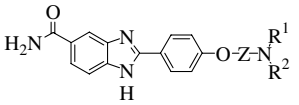
| Compound | Chk2 $IC_{50}$[a] | Y[b] | SIF[c] (mM) | SGF[c] (mM) |
|---|---|---|---|---|
| **3** | 52 ± 4 | Ethane | >0.400 | >0.400 |
| **4** | 73 ± 7 | 1,3-Propyl | 0.007 | 0.185 |
| **5** | 72 ± 23 | 1,3-Cyclopentane (*cis*/*trans*) | 0.139 | >0.400 |
| **6** | 80 ± 8 | 1,4-Cyclohexane (*cis*/*trans*) | ND | ND |
| **7** | 35 ± 2 | 1,4-Methyl-cyclohexane (*cis*) | ND | ND |
| **8** | 400 ± 20 | 1,4-Methyl-cyclohexane (*trans*) | ND | ND |

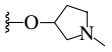
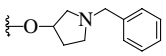
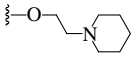
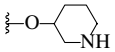
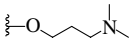
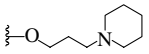
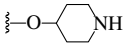
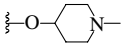
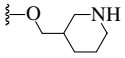
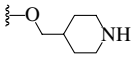
[a] $IC_{50}$ values expressed in nM ± SEM, all values are means of at least three replicated experiments.
[b] As described in Scheme 1.
[c] Compounds with solubility >0.4 mM are soluble above the upper limit of the assay.



**Scheme 2.** Synthesis of alkyl-linked pendant amines. Reagents and conditions: (a) Aminoalcohol, DIAD, $PPh_3$, $CH_2Cl_2$, rt; (b) 3,4-Diaminobenzoic acid, $Na_2S_2O_5$, DMF, 90 °C; (c) i—CDI, DMF, rt; ii—$(NH_4)_2CO_3$.

**Table 2.** Pendant amines

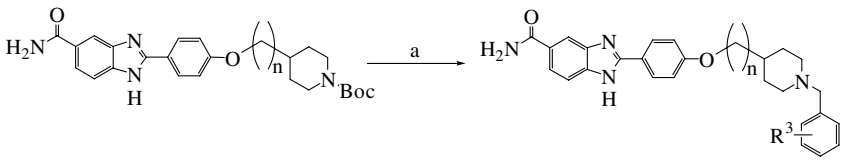


| Compound | Chk2 $IC_{50}$[a] | -O-Z-$NR^1R^2$ | Linker length[b] | SIF[c] (mM) | SGF[c] (mM) |
|---|---|---|---|---|---|
| **9** | 710 ± 120 |  | 2 | ND | ND |
| **10** | 109 ± 2 |  | 2 | ND | ND |
| **11** | 174 ± 7 |  | 2 | >0.400 | >0.400 |
| **12** | 180 ± 15 |  | 2 | >0.400 | >0.400 |
| **13** | 176 ± 16 |  | 3 | >0.400 | >0.400 |
| **14** | 110 ± 3 |  | 3 | >0.400 | >0.400 |
| **15** | 710 ± 340 |  | 3 | ND | ND |
| **16** | 233 ± 50 |  | 3 | >0.400 | >0.400 |
| **17** | 158 ± 11 |  | 3 | ND | ND |
| **18** | 231 + 15 |  | 4 | >0.400 | >0.400 |

[a] $IC_{50}$ values expressed in nM ± SEM, all values are means of at least three replicated experiments.
[b] Number of carbons present between ether linkage and basic amine.
[c] Compounds with solubility >0.4 mM are soluble above the upper limit of the assay.

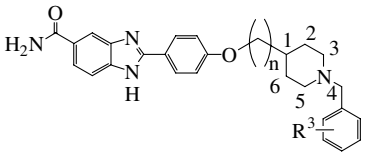
The promising activity of the benzyl substituted tertiary amine (entry **10**), along with the encouraging solubility data for related compounds, led to the exploration of a set of benzyl-substituted piperidine compounds synthesized by the route shown in Scheme 3. *N*-Boc-aminoalcohol intermediates were deprotected with trifluoroacetic acid in methylene chloride, after which they were submitted to reductive amination conditions to yield the desired tertiary amine products.

Varying linker length and moving the nitrogen around the piperidine ring did not reveal a preferred location for the



**Scheme 3.** Synthesis of benzylated piperidine analogs. Reagents and conditions: (a) i—20% TFA, $CH_2Cl_2$; ii—$NaBH(OAc)_3$, aryl aldehyde, 4 Å MS, DMF.

**Table 3.** Substituted piperidine analogs



| Compound | Chk2 $IC_{50}$[a] | $R^{3b}$[b] | n[b] | N location[b] | SIF (mM) | SGF (mM) |
|---|---|---|---|---|---|---|
| **19** | 37 ± 3 | H | 1 | 3 | 0.267 | >0.400 |
| **20** | 57 ± 10 | 4-$CH_3$ | 1 | 3 | ND | ND |
| **21** | 55 ± 2 | 4-OMe | 1 | 3 | ND | ND |
| **22** | 14 ± 8 | 4-Cl | 1 | 3 | ND | ND |
| **23** | 180 ± 150 | 3,4-*di*-Cl | 1 | 3 | ND | ND |
| **24** | 70 ± 3 | H | 1 | 4 | >0.400 | >0.400 |
| **25** | 70 ± 16 | 4-$CH_3$ | 1 | 4 | ND | ND |
| **26** | 67 ± 20 | 4-OMe | 1 | 4 | ND | ND |
| **27** | 60 ± 8 | 4-Cl | 1 | 4 | ND | ND |
| **28** | 176 ± 148 | 3,4-*di*-Cl | 1 | 4 | ND | ND |
| **29** | 45 ± 5 | H | 2 | 2 | 0.155 | >0.400 |
| **30** | 61 ± 25 | 4-$CH_3$ | 2 | 2 | 0.097 | >0.400 |
| **31** | 74 ± 47 | 4-OMe | 2 | 2 | 0.084 | >0.400 |
| **32** | 53 ± 42 | 4-Cl | 2 | 2 | 0.115 | >0.400 |
| **33** | 114 ± 50 | 3,4-*di*-Cl | 2 | 2 | 0.229 | 0.039 |
| **34** | 90 ± 40 | H | 2 | 4 | 0.005 | 0.110 |
| **35** | 90 ± 5 | 4-$CH_3$ | 2 | 4 | 0.003 | 0.084 |
| **36** | 95 ± 14 | 4-OMe | 2 | 4 | <0.001 | >0.400 |
| **37** | 82 ± 22 | 4-Cl | 2 | 4 | 0.002 | 0.179 |
| **38** | 157 ± 33 | 3,4-*di*-Cl | 2 | 4 | 0.015 | 0.013 |

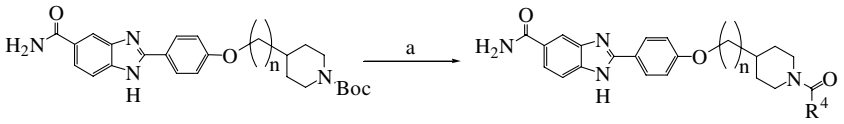
[a] $IC_{50}$ values expressed in nM ± SEM, all values are means of at least three replicated experiments.
[b] As described in Scheme 3.

nitrogen. The results shown in Table 3 demonstrate that substitution on the benzyl group was well tolerated: 4-chloro substitution was generally best, while 3,4-dichloro substitution yielded the lowest activity. Though almost all of the compounds tested for solubility in Table 3 exceeded the solubility of the biaryl series (represented by compound **2** [SIF 0.007 mM, SGF 0.031 mM]), these compounds did not retain the solubility of the smaller, more simple pendant amines shown in Table 2.

In order to gain further knowledge about the ability of these benzimidazole analogs to participate in hydrogen bonding, acetylated and benzoylated analogs were synthesized by the route shown in Scheme 4. Again, *N*-Boc-aminoalcohol intermediates were deprotected under acidic conditions. The resulting secondary amines were coupled with various anhydrides using DMAP in DMF to form the desired amide products.

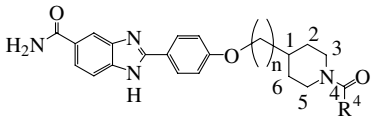
The results shown in Table 4 illustrate that hydrogen bond donating ability was not necessary to retain activity, as the benzamide analogs were equipotent to the pendant alcohol compounds shown in Table 1. Notably, the benzamide analogs were more active than their acetylated counterparts. Unfortunately the removal of the basic amine resulted in reduced solubility, especially evident in compounds **40** and **41**, which have no basic amine and an additional aryl group.

In summary, efforts to achieve greater solubility for benzimidazole Chk2 inhibitors were successful. The addition of pendant alcohols and amines as well as reduction in compound size seem to play a role in increasing the overall solubility of these molecules. Exploration of a potentially beneficial hydrogen bond interaction through the display of these pendant alcohols and amines resulted in relatively flat SAR with respect to the Chk2 kinase; moreover, complete removal of hydrogen bonding capability was not detrimental to activity, as evidenced through the preparation of a series of amide analogs. Furthermore, compounds with aliphatic linkers revealed that the biaryl system in previously described Chk2 inhibitors is not required to achieve enzyme inhibition below 100 nM.



**Scheme 4.** Synthesis of benzoylated piperidine analogs. Reagents and conditions: (a) i—20% TFA, $CH_2Cl_2$; ii—$(R^4CO)_2O$, DMAP, DMF.

**Table 4.** Amide analogs



| Compound | Chk2 $IC_{50}$[a] | $R^{4b}$ | n[b] | N location[b] | SIF (mM) | SGF (mM) |
|---|---|---|---|---|---|---|
| **39** | 39 ± 18 | Ph | 1 | 4 | ND | ND |
| **40** | 52 ± 51 | Ph | 2 | 2 | 0.018 | 0.028 |
| **41** | 23 ± 3 | Ph | 2 | 4 | 0.003 | 0.003 |
| **42** | 100 ± 20 | $CH_3$ | 0 | 3 | ND | ND |
| **43** | 140 ± 31 | $CH_3$ | 1 | 3 | ND | ND |
| **44** | 78 ± 16 | $CH_3$ | 1 | 4 | ND | ND |
| **45** | 292 ± 40 | $CH_3$ | 2 | 2 | 0.193 | >0.400 |
| **46** | 86 ± 10 | $CH_3$ | 2 | 4 | 0.002 | 0.087 |

[a] $IC_{50}$ values expressed in nM ± SEM, all values are means of at least three replicated experiments.
[b] As described in Scheme 4.

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9. McClure, K. J.; Huang, L.; Arienti, K. L.; Axe, F. U.; Brunmark, A.; Blevitt, J.; Breitenbucher, G. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1924.
10. Equilibrium solubility: Solubility samples were prepared by mixing 400 μmol of compounds (solids dissolved in DMSO) with either 1 mL of SGF (0.1 N HCl, 0.2% NaCl, pH 1.2) or FasSIF (Galia et al. Pharm. Res. 15(5), 698–705, 1998) for at least 3 days at room temperature. The resulting mixtures were then filtered and the supernatants were analyzed by UV-HPLC against external standards.
11. Activity of inhibitors was determined by incubating inhibitory compounds with recombinant full-length Chk2: 5 nM recombinant human Chk2, 50 mM Hepes (pH 7.4), 100 mM NaCl, 10 mM $MgCl_2$, 25 μM synthetic peptide substrate (Biotin-SGLYRSPSMPENLNRPR, 1 μM ATP, 50 μCi/mL [g-33P]) ATP, and a protease inhibitor mixture. The reaction mixtures were incubated at 37 °C for 3 h, and the peptide substrate was captured on streptavidin conjugated to agarose beads. The agarose beads were washed repeatedly with a 0.1% solution of Tween-20 in phosphate-buffered saline, pH 7.4. Enzyme activity at different Chk2 inhibitory compound concentrations was determined by measuring the amount of radioactive phosphate bound to the substrate peptide by scintillation counting.