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Bioorganic & Medicinal Chemistry Letters 16 (2006) 1924–1928
Bioorganic & Medicinal Chemistry Letters

Novel non-benzimidazole chk2 kinase inhibitors

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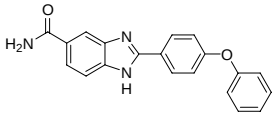
Received 28 October 2005; revised 19 December 2005; accepted 22 December 2005
Available online 25 January 2006

Abstract—In a recent paper, [Arienti, K. L.; Brunmark, A.; Axe, F. U.; McClure, K. M.; Lee, A.; Blevitt, J.; Neff, D. K.; Huang, L.; Crawford, S.; Chennagiri, R. P.; Karlsson, L.; Brietenbucher, J. G. *J. Med. Chem.* **2005**, *48*, 1873], we described the discovery of a class of benzimidazole chk2 kinase inhibitors, exemplified by compound **1**, which had radio-protective effects in human T-cells subjected to ionizing radiation. Here, a series of non-benzimidazole analogs intended to define the scope of the SAR about this new series of inhibitor, and allow for refinement of the binding model of these compounds to the chk2 kinase is described.

Radiotherapy is still the most commonly used treatment for cancer patients, with >50% of all cancer patients receiving some sort of radiotherapy. Side effects from radiation therapy represent a major clinical problem, which seriously affects both quality-of-life and clinical outcome. Despite major improvements in the use of focused and fractionated dosing of radiation side effects are still generally dose limiting. Thus, compounds that act to increase the therapeutic window of radiotherapy would have great clinical utility in the treatment of cancer. Additionally, such an agent might find utility in protecting humans from occupational exposure to ionizing radiation.

Chk2 is a serine/threonine kinase that was first described in 1998.[2] Chk2 is involved in the cellular response to DNA strand breaks that occur upon exposure to γ-radiation.[3] Chk2 knockout mice demonstrate increased survival to radiation. These animals also show suppressed apoptosis in thymus, hippocampus, and skin tissues after exposure to γ-radiation.[4,5] As a result, inhibition of chk2 would be expected to be radio protective and potentially useful as an adjunct to radiotherapy.

(4-Aryloxy-phenyl)benzimidazoles such as compound **1** (also compounds **1a**, **1b** and **1c** in Table 1) were discovered to be potent inhibitors of the chk2 kinase.[1] In this paper, a series of benzimidazole replacement analogues, which were designed to further define the SAR of this series of inhibitors as well as refine the existing binding model, is described. To probe the SAR of the benzimidazole-containing analogues, we replaced the benzimidazole core with closely related 5,6-fused heterocycles. Maintaining a constant geometric arrangement of pendant groups allowed for the precise determination of the critical binding elements of the benzimidazole group. The routes utilized to arrive at these analogues as well as their corresponding biological data are reported below (Table 1).



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Simple methyl substitution at either nitrogen of the imidazole, as represented in compounds **4** and **7** of Table 1, was accessed utilizing the route shown in Scheme 1.

In the first step, the fluoro group of 4-fluoro-3-nitrobenzoic acid (**2**) was displaced with methylamine followed by conversion of the acid to a primary amide, and nitro reduction giving the diamine intermediate **3**. The diamine was then oxidatively condensed under standard conditions with 4-aryloxybenzaldehyde giving compound **4** in good yield. Compound **7** was prepared in a similar fashion starting from 3-fluoro-4-nitrobenzoic acid.

The benzoxazoles **9** and **11** were prepared via the route shown in Scheme 2.

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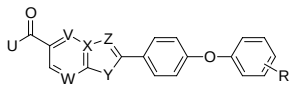
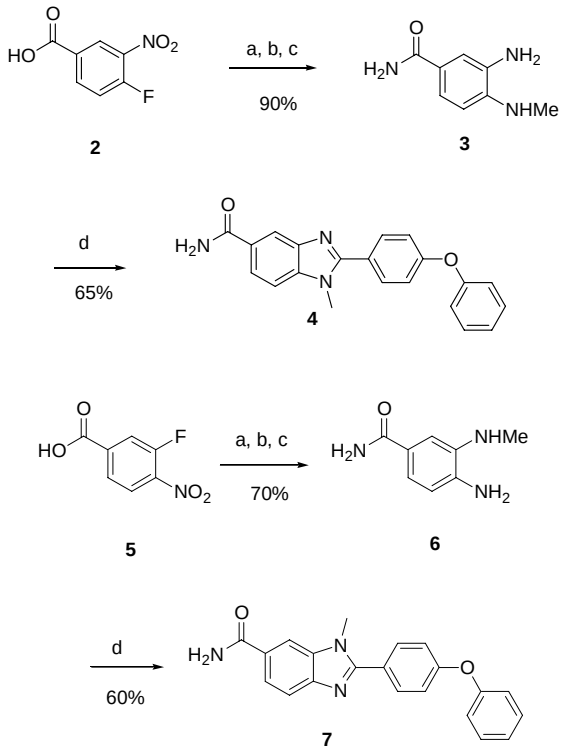
doi:10.1016/j.bmcl.2005.12.096

Table 1. Imidazole ring replacements



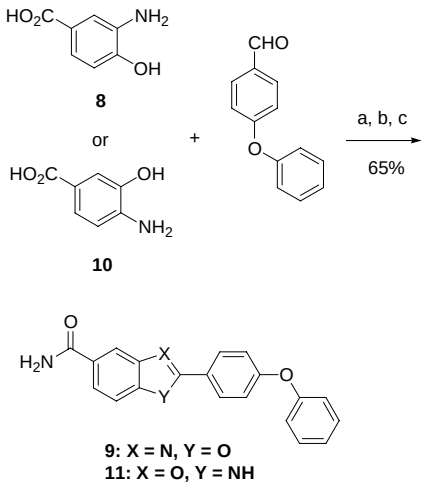
Compound	R	U	V	W	X	Y	Z	IC_{50} (nM)
1	H	NH_2	CH	CH	C	NH	N	55
1a	H	OH	CH	CH	C	NH	N	640
1b	4-Cl	OH	CH	CH	C	NH	N	133
1c	4-Cl	NH_2	CH	CH	C	NH	N	16
4	H	NH_2	CH	CH	C	NMe	N	>10,000
7	H	NH_2	CH	CH	C	N	NMe	1540
9	H	NH_2	CH	CH	C	O	N	>10,000
11	H	NH_2	CH	CH	C	N	O	>10,000
16	4-Cl	OH	CH	CH	C	CH	NH	5800
23	H	NH_2	CH	N	N	C	N	>10,000
27	4-Cl	NH_2	CH	N	C	NH	N	2000
32	4-Cl	NH_2	N	CH	C	NH	N	77



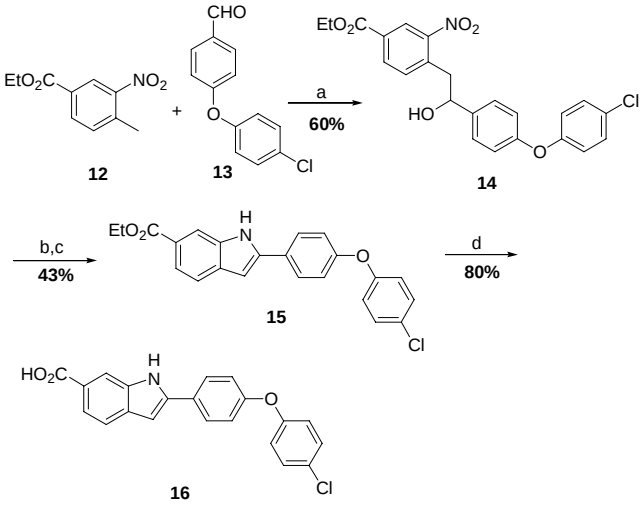
Scheme 1. Reagents and condition: (a) $MeNH_2$, DIPEA; (b) $SOCl_2$, NH_3; (c) H_2, Pd/C, EtOH; (d) $Na_2S_2O_5$, 4-phenoxybenzaldehyde, DMF, 100 °C.

Either of two aminohydroxybenzoic acids (**8** or **10**) were condensed with 4-phenoxybenzaldehyde giving the benzoxazolidine. This was then oxidized to the corresponding benzoxazole by treatment with lead tetraacetate.[6] The acid was converted to the acid chloride followed by displacement with ammonia yielding the primary amides **9** or **11**.

Indole **16** was prepared by the method outlined in Scheme 3. The anion of 4-methyl-3-nitroethyl benzoate was generated with sodium ethoxide and then



Scheme 2. Reagents and condition: (a) Neat, 150 °C; (b) $Pb(OAc)_4$, AcOH; (c) $SOCl_2$, NH_3.

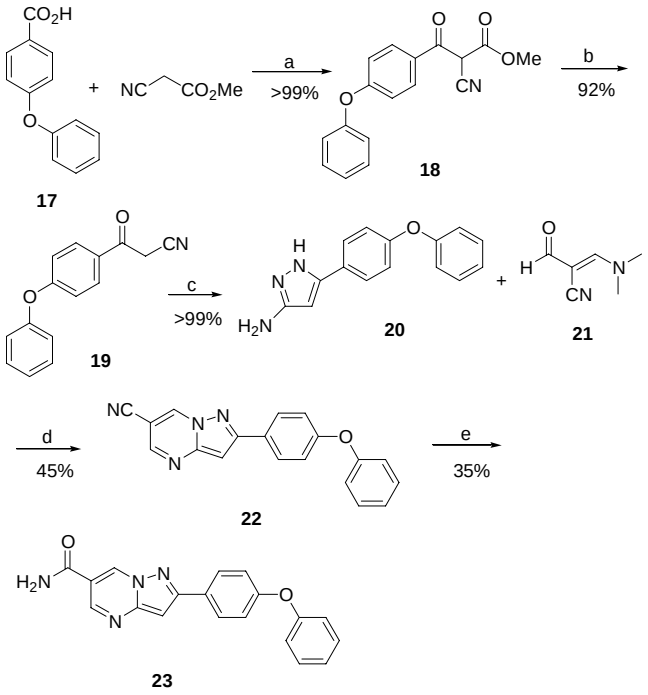


Scheme 3. Reagents and conditions: (a) NaOEt, EtOH, DMSO; (b) Fe, HCl/H_2O; (c) $RuCl_2(PPh_3)_4$, toluene, 100 °C; (d) 7 N KOH, THF, 80 °C.

condensed with the corresponding 4-chloro-phenoxy benzaldehyde giving compound **14** in good yield. Reduction of the nitro group with iron followed by ruthenium-catalyzed cyclization led to the indole intermediate **15**.[7] Finally, hydrolysis of the ester group gave the target compound **16**.

Heterocycle **23** was synthesized starting from commercially available 4-phenoxybenzoic acid (**17**) in a five-step sequence as outlined in Scheme 4.

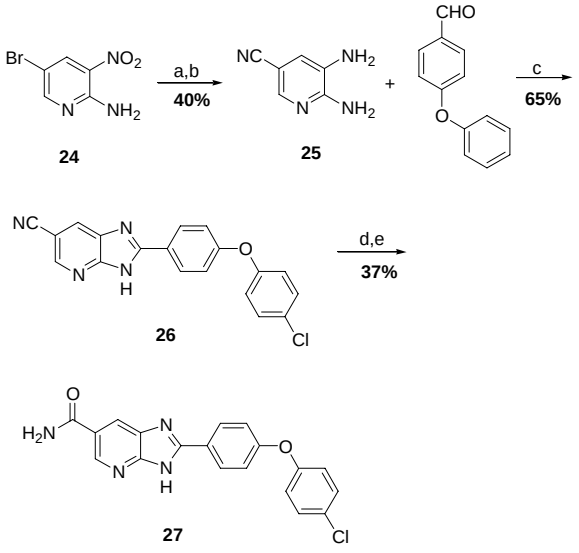
Benzoic acid **17** was first converted to β-ketoester **18** by treatment with methyl cyanoacetate. This intermediate was then decarboxylated giving the α-cyanoketone **19**. Formation of the amino pyrazole substrate **20** was achieved by addition of hydrazine. The key step in the synthesis involved the formation of the pyrazolo pyrimidine ring system, which was accomplished by treating the amino pyrazole with 3-dimethylamino-2-formyl-acrylonitrile[8] **21**. The nitrile was then converted to the



Scheme 4. Reagents and conditions: (a) DEPC, DIEA, DMF; (b) wet DMSO/NaCl; (c) N_2H_4, H_2O, EtOH, reflux; (d) EtOH, reflux; (e) BF_3·2AcOH, H_2O, 120 °C.

primary amide utilizing BF_3·2AcOH/H_2O yielding the target compound **23**.[9]

The imidazopyridine **27** in Table 1 was prepared as outlined in Scheme 5. The bromine on the commercially available pyridine **24** was first displaced with copper cyanide followed by reduction of the nitro group under standard hydrogenation conditions. Formation of the imidazopyridine ring **26** was accomplished using standard oxidative condensation conditions. Finally, the nitrile was converted to the primary amide by hydrolysis



Scheme 5. Reagents and conditions: (a) CuCN, NMP, 80 °C; (b) H_2, Pd/C, EtOH; (c) $Na_2S_2O_5$, DMF, 100 °C; (d) HCO_2H/HCl; (e) SO_2Cl, NH_3.

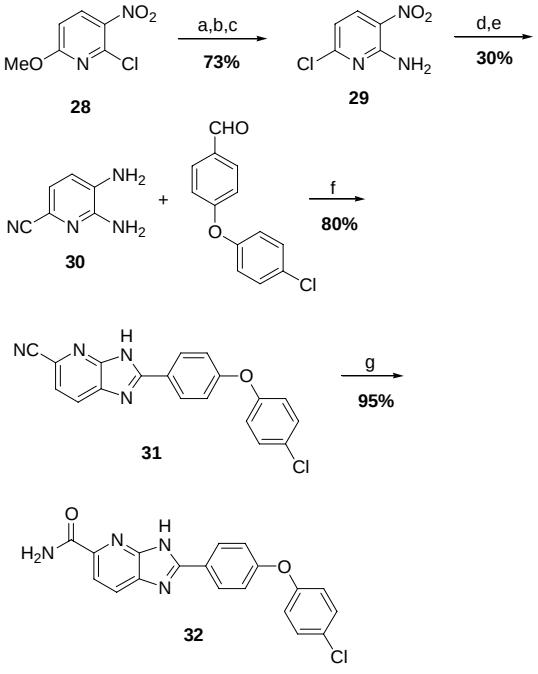
to the acid, followed by activation with thionyl chloride and displacement with ammonia giving the target compound **27**.

The regioisomeric imidazopyridine **32** was prepared as illustrated in Scheme 6.

Functional group interconversions of the commercially available pyridine **28** as described in preceding schemes led to the diaminopyridine intermediate **30** in good yield over five steps. The diaminopyridine **30** was then oxidatively condensed with the appropriate benzaldehyde. Finally, the nitrile was hydrolyzed to the primary amide by treatment with BF_3·2AcOH affording the compound **32**.

In a previous paper[1] on benzimidazole inhibitors of chk2, we described a binding model for this chemical series based on docking of these inhibitors into a homology model of the chk2 kinase as well as experimental evidence, suggesting that these compounds are ATP competitive inhibitors. The proposed binding model is pictured in Figure 1. It shows that the 5-amido group is involved in a key set of hydrogen bonding interactions with the backbone carbonyl and amide nitrogen of Met 90 in the ATP pocket and that the benzimidazole and the biaryl-ether groups lie in the hydrophobic region of the ATP pocket. SAR in this study focused on the biaryl-ether and 5-amido portions of the inhibitors.

In the present study, changes were made to the benzimidazole, which maintained the topological [5,6]-ring arrangement, but repositioned the heteroatoms to various positions about the 5,6-ring system. The activities of these analogs are shown in Table 1.



Scheme 6. Reagents and conditions: (a) NH_4OH, 3 days; (b) HBr, AcOH, 60 °C; (c) $POCl_3$, 100 °C to rt; (d) $Zn(CN)_2$, Pd_2dba_3, DPPF, DMF, H_2O, 100 °C; (e) H_2, Pd/C, EtOH; (f) $Na_2S_2O_5$, DMF, 100 °C; (g) BF_3·2AcOH, H_2O, 120 °C, 30 min.

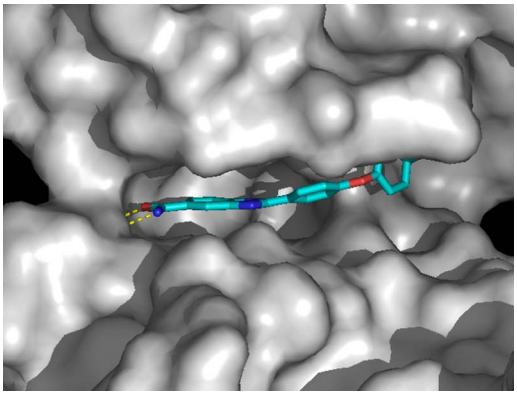


Figure 1. Binding model of compound 1 in ATP pocked of chk2 homology model.

It can be seen from analogue **4** that simple methylation of the Y-imidazole nitrogen altogether eliminates the binding affinity for the kinase while methylation of the Z-imidazole nitrogen reduces the binding affinity by 30-fold relative to compound **1**. In addition, replacement of the Y nitrogen with a CH group, as in compound **16**, leads to a compound that is much less active than compound **1** having an IC_{50} of 5.8 μM. These data strongly suggest that the unsubstituted imidazole nitrogen at the Y position is crucial for activity as well as the NH group at the Z position to a lesser extent. We can interpret these results in terms of our binding model if there is a hydrogen bond donor in proximity of the Y nitrogen and a hydrogen bond acceptor in proximity of the Z nitrogen. There are a number of examples of kinase inhibitors in which the X-ray crystal structure with the compound bound reveals that the conserved lysine at the bottom of the ATP pocket often forms a hydrogen bond with an acceptor group on the inhibitor.[10] Crystal structures also reveal that amino acid side chains on the edge of the ATP pocket can serve as hydrogen bond acceptors.[10] Both of these donor and acceptor sites occur in close proximity to the imidazole nitrogens in our models as depicted in Figure 2.

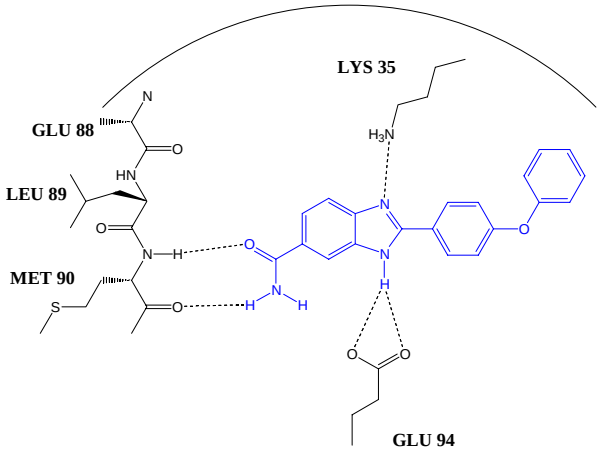


Figure 2. Proposed hydrogen binding model explaining the importance of benzimidazole for chk2 binding.

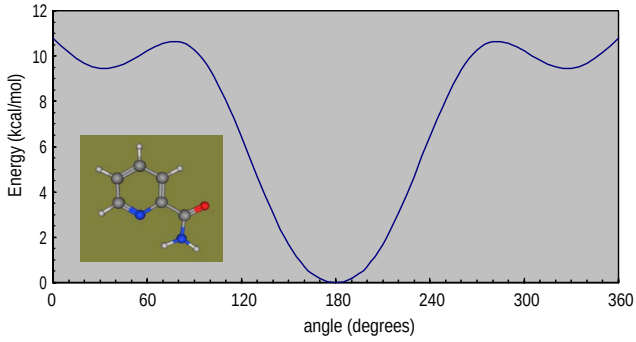


Figure 3. B3LYP/6-31G* calculated potential energy surface for picolinamide suggests an energy minimum favoring the amide in preferred orientation for chk2 binding.

The substitution of a nitrogen atom at the W position in **27** reduces activity considerably relative to **1**. This result is consistent with our proposed binding model since the polar nitrogen atom is being buried in the hydrophobic pocket, which is energetically unfavorable. On the contrary, substituting a nitrogen atom at the V position (**32**) leads to a compound that is comparable in activity to **1**. This is consistent with our model for two reasons. First, a nitrogen at the V position is more solvent exposed and second, a nitrogen at the V position can form an intra-molecular hydrogen bond with the additional hydrogen on the 5-amido group, which results in a conformationally restricted orientation of the 5-$CONH_2$ group. This latter notion that the 4-pyridyl-analog is conformationally constrained is corroborated by the X-ray crystal structure of picolinamide,[11] which has the amide group nitrogen oriented toward the pyridine nitrogen and appears to form an intra-molecular hydrogen bond. This idea is also supported by both ab initio DFT calculations that we performed as well.[12] In fact, one of the motivations for making this compound was to constrain the amido group Figure 3.

The aim of the work presented here was to determine the importance of the benzimidazole group in binding to the receptor and to gain a better understanding of the SAR around this series of molecules. A number of heterocyclic benzimidazole replacements were made to answer these questions. As can be seen from the decrease in binding affinity with almost all of the prepared analogues, the benzimidazole group seems to be preferred for binding to the receptor. An extension of the binding model to include key hydrogen bonding interactions between the benzimidazole nitrogens and Glu 94 and Lys 35 residues in the receptor would account for these findings. The comparable activity to the parent benzimidazole found in compound **32** can readily be explained by the solvent exposure to the pyridine nitrogen as well as the conformational restriction of the amide group via intramolecular hydrogen bonding to the pyridine nitrogen.

Acknowledgments

The authors thank Dr. Jiejun Wu for his help in the structure confirmation of the heterocyclic benzimidazole

replacements described in this paper. The authors also thank Dr. Michael Hack for potential energy calculations of picolinamide.

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