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# 3-(Indol-2-yl)indazoles as Chek1 kinase inhibitors: Optimization of potency and selectivity via substitution at C6

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**Abstract**—The development of 3-(indol-2-yl)indazoles as inhibitors of Chek1 kinase is described. Introduction of amides and heteroaryl groups at the C6 position of the indazole ring system provided sufficient Chek1 potency and selectivity over Cdk7 to permit escape from DNA damage-induced arrest in a cellular assay. Enzyme potency against Chek1 was optimized by the incorporation of a hydroxymethyl triazole moiety in compound **21** (Chek1 $IC_{50}$ = 0.30 nM) that was shown by X-ray crystallography to displace one of three highly conserved water molecules in the HI region of the ATP-binding cleft.


Although effective, DNA damaging agents used in chemotherapy show little selectivity for killing tumor cells over normal proliferating cells. Therefore, strategies targeting an increase in the therapeutic window of these agents are warranted. In normal cells, DNA damage causes cell cycle arrest through the tumor suppression protein p53 and activation of checkpoint kinase Chek1, allowing for repair.[1–3] In tumor cells that have impaired p53 function, a defect common to 50–70% of all cancers, survival from DNA damage relies primarily on activation of Chek1 which ultimately leads to inactivation of Cdc2 (Cdk1), a cyclin-dependent kinase whose activity is critical to cell cycle progression. In these tumors, Chek1 inhibition results in abrogation of arrest and premature cell cycle progression into mitosis where cell death occurs through mitotic catastrophe and apoptotic pathways. Accordingly, Chek1 inhibitors[4] have the potential to sensitize p53-impaired tumor cells to DNA damaging agents during chemotherapy and thereby increase the efficacy and alleviate the toxicity to normal cells associated with such treatments. In this paper, we report the optimization of Chek1 potency and selectivity of lead **1** (Table 1) through modification of the indazole substituent at C6.

Lead **1** was designed originally as a KDR kinase inhibitor and was subsequently identified as an ATP-competitive inhibitor of Chek1 from a directed screening campaign. Our initial efforts to improve the potency of **1** involved the introduction of a basic sidechain to C5′ of the indole ring system, a strategy successfully employed for this and closely related series of KDR kinase inhibitors.[5,6] Consistent with our previous findings, addition of a benzylic amine to C5′ provided a 10-fold improvement in Chek1 potency with **2**.[7] Not surprisingly, **1** and **2** were devoid of cellular activity in both a

*Keywords*: Chek1 kinase; Checkpoint escape; DNA damage; CDK7 kinase; Oncology; Indazoles; Indoles.
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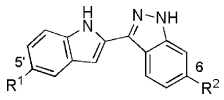
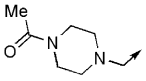
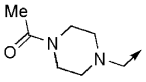
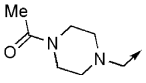
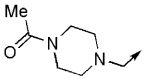
**Table 1.** Chek1 inhibitory and cellular activities of **1**–**5**



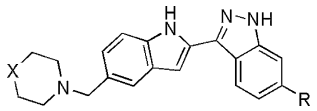
| Compound | $R^1$ | $R^2$ | Chek1 $IC_{50}$ (nM) | Phos $IC_{50}$ (nM) | CEA $IC_{50}$ (nM) |
|---|---|---|---|---|---|
| **1** | H |  | 27 | >10,000 | >10,000 |
| **2** |  |  | 2.3 | >10,000 | >10,000 |
| **3** |  | CN | 17 | 1000 | >10,000 |
| **4** |  | Cl | 120 | — | — |
| **5** |  | H | 640 | — | — |

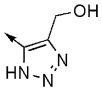
Chek1 autophosphorylation assay (Phos)[8] and in a functional checkpoint escape assay (CEA)[9] that measures release of H1299 tumor cells from DNA damage-induced cell cycle arrest and progression into mitosis following Chek1 inhibition. The lack of activity of **1** and **2** in these assays was attributed to a combination of low aqueous solubility and high polarity engendered by the acidic tetrazole group at C6. We postulated that cellular activity would be gained by replacement of the tetrazole with a neutral substituent, but potentially at the expense of enzyme potency, since an X-ray structure of **1** bound to Chek1 showed the tetrazole engaged in an ionic interaction with Lys38 located in hydrophobic region I (HI) of the ATP-binding cleft.[10] The Chek1 inhibitory activity of an initial set of compounds confirmed the latter prediction (Table 1), but encouragingly showed that the cyano group in **3** provided significant retention in potency when exchanged with the tetrazole moiety.

While **3** was active in the Phos assay, indicating that its intrinsic potency and cell membrane permeability[11] were sufficient to inhibit Chek1 activity in cells, it was found to be functionally inactive in the CEA. A potential explanation for the unexpected lack of cellular response in the CEA was revealed through the profiling of **3** against a broad panel of kinases where strong inhibitory activity was measured against Cdk7 ($IC_{50}$ = 39 nM) in particular.[12] Cdk7 is a member of the cyclin-dependent kinase family that plays a central role in the regulation of cell cycle transitions.[13,14] Specifically, Cdk7 activates other Cdks critical to cell cycling, including Cdc2, through phosphorylation of key threonine residues. Because Cdk7 activity is essential to cell cycle progression, we proposed that the inactivity of **3** in the CEA was due to counterproductive Cdk7 inhibition resulting in cellular arrest that precluded the expected cell cycle progression mediated by Chek1 inhibition. Further support for this hypothesis was gained in a follow-up study where we demonstrated that Cdk7 siRNA inhibited checkpoint escape of a functionally active Chek1 inhibitor in this assay (data not shown). Consequently, to achieve checkpoint escape, the desired cellular response to Chek1 inhibition, we set out to identify compounds in this series that showed selectivity for Chek1 inhibition over Cdk7.

Our design strategy to gain selectivity for Chek1 centered on modification of the C6 substituent whose bond vector is directed toward HI. The peptide sequences of Chek1 and Cdk7 differ in this back region. Most notably, the residue equivalent to Leu84 of Chek1,[15] the so-called 'gatekeeper' residue which forms part of HI, is a more sterically constraining Phe in Cdk7.[16] In an attempt to exploit this difference, we found that replacement of the C6 cyano group with a methyl ester (**7**) or small amides (**8**–**10**) retained Chek1 potency and afforded a significant enhancement in selectivity over Cdk7 relative to **6** (Table 2). Of greater gratification was that these compounds displayed cellular checkpoint escape with $EC_{50}$'s in the low micromolar range,[17] a finding consistent with our hypothesis concerning the importance of adequate Chek1 selectivity for functional activity in this assay. Heterocycles were also investigated as replacements for the cyano group, although with mixed results. Pyrazoles **11** and **13** and thiazole **14** were equipotent against Chek1 and Cdk7, and like **3** and **6** were inactive in the CEA.[18] Methylation of **11**, providing **12**, was attempted to improve Chek1 selectivity but was found detrimental to Chek1 potency. On the other hand, thiazole **15** maintained potent Chek1 inhibitory activity, displayed 80-fold selectivity for Chek1 over Cdk7, and accordingly demonstrated checkpoint escape in the functional assay. Incorporation of an N1-linked 1,2,3-triazole in **16** enhanced Chek1 potency and apparently provided sufficient selectivity to render this com-

**Table 2.** Inhibitory profiles of **6**–**21**



| Compound | X | R | Chek1 $IC_{50}$ (nM) | Cdk7 $IC_{50}$ (nM) (ratio) | CEA $EC_{50}$ (nM) |
|---|---|---|---|---|---|
| **6** | O | CN | 30 | 11 (0.37) | >50,000 |
| **7** | O | $CO_2Me$ | 62 | 6500 (100) | 1930 |
| **8** | O | $CONH_2$ | 12 | 1300 (110) | 980 |
| **9** | O | CONHMe | 13 | 4900 (380) | 650 |
| **10** | O | $CONMe_2$ | 80 | >100,000 (>1200) | 4000 |
| **11** | O |  | 10 | 25 (2.5) | >50,000 |
| **12** | O |  | 530 | — | — |
| **13** | O |  | 59 | 34 (0.58) | >50,000 |
| **14** | $CH_2$ |  | 12 | 18 (1.5) | >10,000 |
| **15** | $CH_2$ |  | 48 | 4000 (83) | 2000 |
| **16** | $CH_2$ |  | 2.6 | 57 (22) | 350 |
| **17** | $CH_2$ |  | 30 | 420 (14) | 1400 |
| **18** | $CH_2$ |  | 110 | 2300 (22) | >10,000 |
| **19** | $CH_2$ |  | 34 | 480 (14) | 4500 |
| **20** | $CH_2$ |  | 45 | 7000 (160) | 5000 |
| **21** | $CH_2$ |  | 0.30 | 56 (190) | 690 |

pound active in the CEA. Interestingly, methylation of the triazole to give **17** maintained an adequate selectivity ratio for functional activity, but reduced enzyme potency by an order of magnitude. N-Linked pyrazole **18** highlighted the importance of the 3-nitrogen atom of triazole **16** with regard to Chek1 and Cdk7 potency, while **19** showed that some of the loss could be recovered with an N2-linked 1,2,3-triazole. While the phenyl group of **20** provided adequate selectivity to induce checkpoint escape, potency was found to be only modest. Surprisingly, subnanomolar enzymatic potency for Chek1 was realized with the functionally active derivative **21**, a compound bearing a unique hydroxymethyl triazole group at C6.[19] To summarize, our data showed that while compounds equipotent against Chek1 and Cdk7 (**6**, **11**, **13**, and **14)** were inactive in the CEA, inhibitors with Cdk7/Chek1 $IC_{50}$ ratios as low as 14 (**17** and **19**) were functionally active.

Insight into the superior binding affinity of **21** was gained through analysis of an inhibitor-bound X-ray structure (Fig. 1).[20] The structure revealed that the hydroxyl group of the triazole had displaced one of three conserved water molecules that make up a key hydrogen bonding network in HI. While desolvation of the hydroxyl group may be an unfavorable thermodynamic event, the entropic gain of resolvation of the

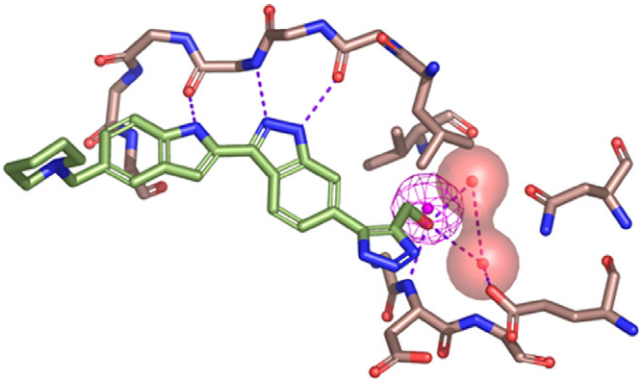
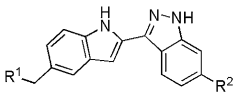
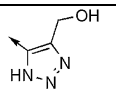
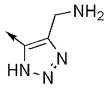
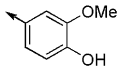


**Figure 1.** X-ray crystallographic structure of **21** (green) bound to Chek1 (light brown) with water molecules shown (red spheres and surface). Water molecule displaced by **21**, but present in X-ray structure of **1** shown as a mesh sphere (magenta).

**Table 3.** Inhibitory activities of **22–24**



| Compound | $R^1$ | $R^2$ | Chek1 $IC_{50}$ (nM) | Cdk7 $IC_{50}$ (nM) | CEA $EC_{50}$ (nM) |
|---|---|---|---|---|---|
| **22** |  |  | 0.30 | 180 | 340 |
| **23** |  |  | 0.11 | 300 | 610 |
| **24** |  |  | 0.25 | 310 | 92 |

ordered water molecule released from HI compensates for it.[21] Moreover, the X-ray structure of **21** showed that the hydroxyl group made an additional hydrogen bond to the protein backbone at Asp148. Taken together, we concluded that the gain in binding affinity with **21** was largely due to the 'anchoring' of the hydroxyl group within the depicted hydrogen bonding array.

In exploring the scope of this finding, we found that the aminomethyl triazole of **23** provided a small gain in Chek1 potency in the enzyme assay, but offered no improvement in cellular activity in the CEA relative to **22** (Table 3).[22] In **24**, we introduced the phenolic group that was recently reported as a potency-enhancing feature for a related series of Chek1 inhibitors.[23] For this series, the phenol conferred similar enzyme potency and greater cellular activity relative to the hydroxymethyl triazole. Interestingly, an X-ray structure of **24** showed displacement of all three water molecules from HI by the phenolic group, consistent with the reported finding.

The synthesis of **21** is shown in Figure 2.[24] The route began with the preparation of 6-bromoindazole by the method of Bartsch and Yang.[25] Iodination followed by a regioselective Suzuki cross-coupling reaction with the depicted indole boronic acid proceeded smoothly to form the 3-(indol-2-yl)indazole core structure. Palladium-catalyzed coupling of the product bromide with propargyl alcohol in pyrrolidine[26] and subsequent oxidation to the aldehyde set the stage for the construction of the triazole by way of a 3 + 2 cyclization with sodium azide.[27] Sodium borohydride reduction of the resulting aldehyde then provided **21**.

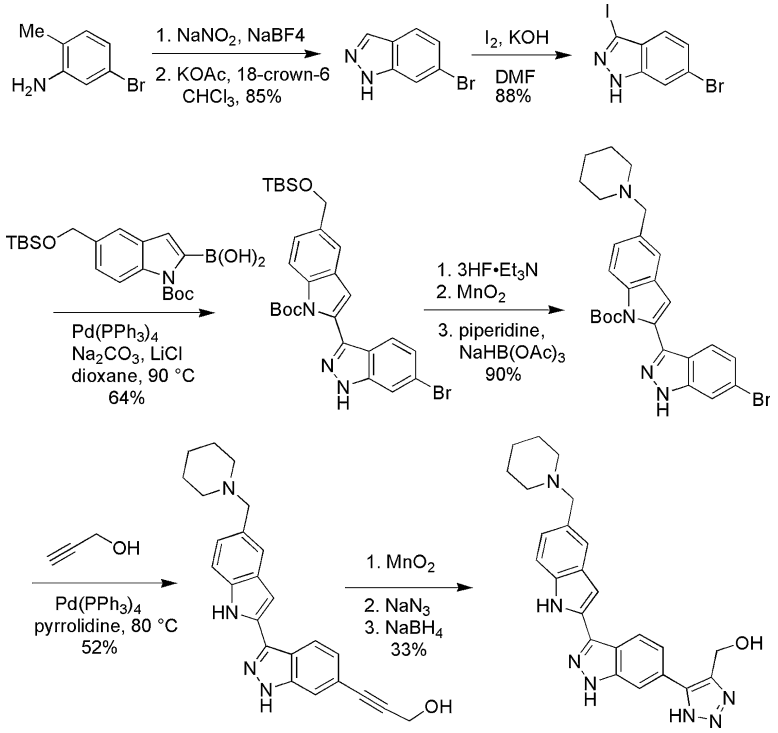



**Figure 2.** Synthesis of **21**.

In summary, we have described the effects of C6 substitution on Chek1 potency and selectivity for a series of 3-(indol-2-yl)indazoles. We showed that selectivity for Chek1 over Cdk7 was required for functional activity in a cell-based checkpoint escape assay for this series of compounds. The hydroxymethyl triazole group provided enhanced binding affinity, apparently through participation in a hydrogen bonding network in HI following displacement of a conserved water molecule.

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7. Chek1 inhibitory activity was measured using a homogeneous time-resolved fluorescence assay that detects phosphorylation of a biotinylated GSK-3 peptide as described by Barnett, S. F. et al. *Biochem. J.* **2005**, *385*, 399. The assay was run at 0.5 nM enzyme and 0.1 mM ATP. $IC_{50}$ values are reported as averages of at least two independent determinations; standard deviations are within ±25–50% of $IC_{50}$ values.
8. NCI-H1299 lung carcinoma cells were incubated with Chek1 inhibitors for 2 h and then treated with camptothecin for an additional 4 h. Inhibition of Chek1 autophosphorylation was measured by cyto-blot analysis using an antibody against phospho-S296 Chek1. For assay details, see WO2006086255. The values of $IC_{50}$ were measured with 10-point, 3-fold dilution series and are reported as an average of triplicate determinations.
9. NCI-H1299 lung carcinoma cells were arrested with 16 h treatment of camptothecin and then treated with Chek1 inhibitors for additional 8 h. Escape from arrest and progression into mitosis were measured by quantifying the mitosis-specific phosphorylation of nucleolin using an antibody-coated, bead-based assay. For assay details, see Fraley, M. E. et al. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1775, Ref. 16. The values of $EC_{50}$ were measured with 10-point, half-log dilution series and are reported as an average of tetraplicate determinations.
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12. Of note, compound **3** showed relatively weak activity against Cdc2 ($IC_{50} = 4200$ nM). Cdk7 and Cdc2 were assayed using IMAP-FP kits from Molecular Devices at 0.05 μM and 0.03 μM ATP, respectively. Enzymes were purchased from Upstate Biotechnology.
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17. Note that Chek1 inhibitory activity in the enzyme assay is measured at $K_m$ for ATP (0.1 mM). In the cellular assays, the ATP concentration is 2.0 mM resulting in an inherent 10-fold shift in potency between the enzyme and cellular assays.
18. Functionally inactive compounds **6**, **11**, **13**, and **14** were cell permeable as indicated by their activity in the Phos assay ($IC_{50}$s 200, 2800, 700, and 2300 nM, respectively).
19. The NH of the triazole is arbitrarily assigned.
20. PDB coordinates for **21**: 2HOG.
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22. The substantial shift in cellular activity relative to enzyme potency for **21**–**23** is believed to be due to suboptimal cell permeability as a result of high polar surface area (PSA > 120 Å$^2$, calculated by the method of Clark, D. E. *J. Pharm. Sci.* **1999**, *88*, 807). For discussions on the inverse relationship between PSA and cell permeability, see Papageorgiou, C.; Camenisch, G.; Borer, X. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1549, and references therein.
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