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Cyanopyridyl containing 1,4-dihydroindeno[1,2-*c*]pyrazoles as potent checkpoint kinase 1 inhibitors: Improving oral biovailability

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Abstract—A series of 1,4-dihydroindeno[1,2-*c*]pyrazole compounds with a cyanopyridine moiety at the 3-position of the tricyclic pyrazole core was explored as potent CHK-1 inhibitors. The impact of substitutions at the 6 and/or 7-position of the core on pharmacokinetic properties was studied in detail. Compounds carrying a side chain with an ether linker at the 7-position and a terminal morpholino group, such as **29** and **30**, exhibited much-improved oral biovailability in mice as compared to earlier generation inhibitors. These compounds also possessed desirable cellular activity in potentiating doxorubicin and will serve as valuable tool compounds for in vivo evaluation of CHK-1 inhibitors to sensitize DNA-damaging agents.

Cancer cells rely on various cell cycle checkpoints to restore their genomic integrity upon DNA-damaging treatments including ionized radiation, UV radiation, and chemotherapy. Studies have demonstrated that checkpoint kinase 1 (CHK-1) plays an important role in regulating G2/M and/or S phase checkpoints for commonly *p*53 deficient cancer cells in response to genotoxic stresses.[1] DNA damage triggers phosphorylation of CHK-1, a serine/threonine kinase, by ataxia-telangiectasia mutated (ATM) as well as ATM- and Rad3-related (ATR) kinases.[2] As a result, CHK-1 phosphorylates and degrades CDC25A leading to cell cycle arrest at G2/M or S phase.[3] Inhibition of CHK-1 can thus cause mitotic catastrophe to cancer cells since they are deprived of an opportunity to repair themselves at the G2/M or S checkpoints.

We have discovered molecules with a 1,4-dihydroindeno[1,2-*c*]pyrazole core as selective and potent CHK-1 inhibitors.[4,5] These compounds can engage in hydrogen bonding interactions with both the hinge and specificity regions of the catalytic pocket of the kinase. Although our earlier inhibitors possessed high inhibitory activity (IC_{50} value as low as 0.2 nM) and desirable potencies in both functional and mechanism-based cellular assays, they were deficient in oral biovailability ($F = 0$–9% in mice).[5] In this study, we disclose our efforts leading to a newer generation of potent CHK-1 inhibitors with much improved oral biovailability.

The primary series of our earlier CHK-1 inhibitors featuring a bi-aryl phenol unit at the 3-position of the tricyclic core (such as **1** in Table 1) have intrinsic pharmacokinetic (PK) liability due to their high lipophilicity (C log *P* at 5–6), potential metabolic instability, and poor aqueous solubility. Since variation of the substitutions at both the 6 and 7-positions and small modifications to the bi-aryl phenol moiety failed to provide compounds with acceptable oral biovailability,[5] we turned our attention to replacing the bi-aryl phenol in order to optimize the overall physicochemical properties of the inhibitors. To that end, we discovered that compounds with a cyanopyridine group at the 3-position (such as **2**) were equally potent as those with the bi-aryl phenol in the enzymatic biochemical assay.[6] The cyanopyridine group compensates for the intricate hydrogen

Keywords: 1,4-Dihydroindeno[1,2-*c*]pyrazole; Checkpoint kinase 1 inhibitors; CHK-1 inhibitors; Sensitizing DNA-damaging agents.
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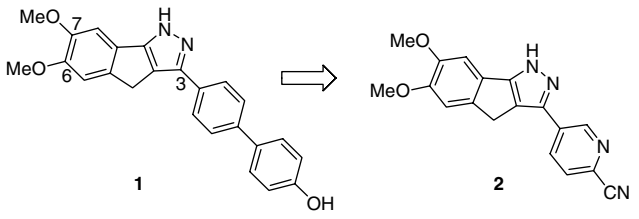
Table 1. From bi-aryl phenol to cyanopyridine

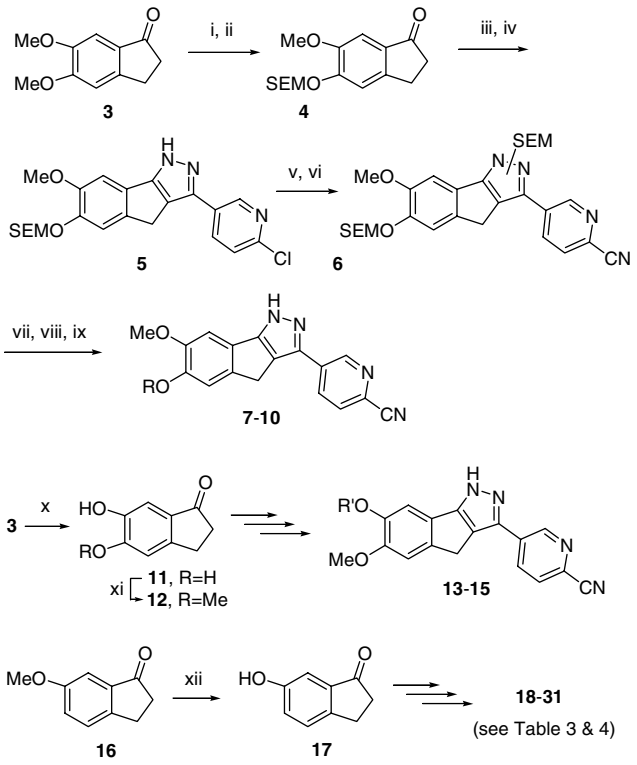


Compound	MW	CHK-1 IC_{50} (nM)	MTS EC_{50} (μM) S/C[a]	FACS EC_{50} (μM) S/C[a]	C log P[b]
1	384	1.6	>59/1.0	>10/0.15	5.4
2	313	0.81	>59/1.1	>10/0.27	2.6

[a] S/C, single/combo.
[b] Calculated by Biobyte methods.

bonding network lost by eliminating the interactions between the phenolic hydroxy group and Glu55 and Asn59 in the active site.[7] The comparison between the two compounds (**1** and **2**) shown in Table 1 reveals that with regard to better drug-like properties, the cyanopyridine group undoubtedly has superiority over the bi-aryl phenol. Compound **2** is not only about two log units lower in C log P than **1** (2.6 vs 5.4), and possesses much lower molecular weight, but also eliminates a potential site (phenol) for glucuronidation. In addition, as compared to **1**, compound **2** shows slightly better enzymatic activity, similar cellular potency in sensitizing the anti-proliferative potential of doxorubicin, a DNA-damaging agent, by at least 53-fold in an MTS assay,[8] and similar cellular profile in a mechanism-based FACS assay (i.e., not disturbing normal cell cycle by themselves with single $EC_{50} > 10$ μM but able to abrogate G2/M checkpoint in combination with doxorubicin with combo $EC_{50} < 0.3$ μM).[9] Although the cyanopyridyl compound **2** shows poor PK properties itself (AUC = 0.18 μg h/mL when dosed intraperitoneally in mice at 10 mg/kg), it does provide a better platform for lead optimization to discover potent CHK-1 inhibitors with desirable oral biavailability.

The chemistry leading to compounds with side chains at the 6 and/or 7-position of the tricyclic pyrazole core via an ether linker is shown in Scheme 1. 5,6-Dimethoxy-1-indanone **3** was selectively de-methylated at the 5-position in the presence of NaCN at 145 °C and the resulting hydroxy group was protected by a SEM group. Compound **4** was treated with NaH and phenyl 6-chloronicotinate followed by hydrazine and acetic acid in one pot to provide **5**. The pyrazole moiety of **5** was SEM-protected. The chloropyridine fragment was subsequently converted into a cyanopyridine under Pd-catalyzed conditions. The SEM on the hydroxy group of the intermediate **6** was removed selectively with HCl at room temperature. Next, the ether side chain at the 6-position was installed using a Mistunobu protocol. The acidic cleavage of SEM on the pyrazole led to compounds **7**–**10**. In a separate route, **3** was first de-methylated at both the 5 and 6-positions using BBr_3. Selective methylation

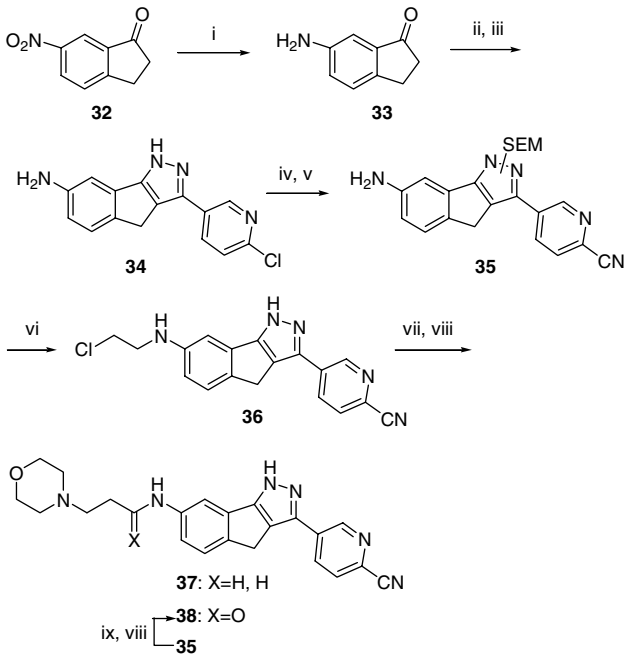


Scheme 1. Reagents and conditions: (i) NaCN, DMSO, 145 °C, 53%; (ii) SEMCl, DIEA, CH_2Cl_2, 72%; (iii) NaH, phenyl 6-chloronicotinate, THF; (iv) AcOH, hydrazine, EtOH, 90 °C, 81% (two steps); (v) NaH, SEMCl, DMF, 65%; (vi) Pd_2dba_3, dppf, Zn, $Zn(CN)_2$, DMA, 120 °C, 80%; (vii) HCl, MeOH, CH_2Cl_2, rt, 100%; (viii) DBAD, Ph_3P on polymer support, alcohols, THF, ~50–90%; (ix) HCl, EtOH, 75 °C, ~35–75%; (x) BBr_3, CH_2Cl_2, 98%; (xi) MeI, Li_2CO_3, DMF, 55 °C, 72%; (xii) $AlCl_3$, toluene, reflux, 85%.

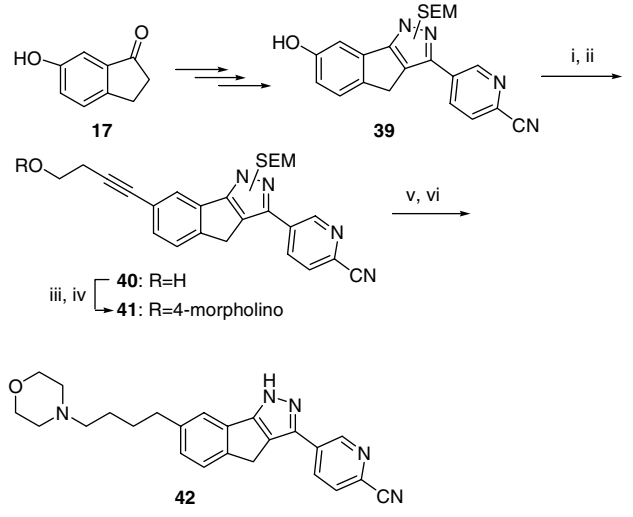
at the 5-position using MeI and Li_2CO_3 transformed **11** into **12**. Under similar conditions discussed above, compounds **13**–**15** were synthesized. Compounds **18**–**31**, with mono-ether side chain at the 7-position, were prepared from 6-methoxy-1-indanone **16**. De-methylation with $AlCl_3$ led to **17**. The final compounds were prepared following the well-established chemistry.

The synthesis of compounds **37** or **38** carrying a side chain with an amino or acetylamino linker at the 7-position and a terminal morpholine group is outlined in Scheme 2. Iron reduction converted 6-nitro-1-indanone **32** into the amino indanone **33**, which was in turn transformed into the intermediate **35** using similar procedures shown in Scheme 1. Compound **35** was treated with chloroacetaldehyde under reductive amination conditions to furnish **36**. The terminal chlorine was substituted by morpholine and subsequent SEM deprotection led to **37**. Compound **38** was derived from a HATU-mediated amide coupling reaction involving **35** and 3-morpholinopropanoic acid.

Intermediate **39** could be readily made from 6-hydroxy-1-indanone **17** in a similar fashion as described earlier (Scheme 3). The hydroxy group of **39** was converted into a triflate in the presence of NaH and $PhN(OTf)_2$. A Sonogashira reaction yielded compound **40**. The hydroxy



Scheme 2. Reagents and conditions: (i) Fe, NH_4Cl, EtOH, water, 90 °C, 68%; (ii) NaH, phenyl 6-chloronicotinate, THF; (iii) AcOH, hydrazine, EtOH, 90 °C, 66% (two steps); (iv) NaH, SEMCl, DMF, 73%; (vi) Pd_2dba_3, dppf, Zn, $Zn(CN)_2$, DMA, 180 °C, microwave, 20 min, 54%; (vi) chloroacetaldehyde in water, H_2SO_4, $NaBH_4$, THF, 0 °C, 28%; (vii) morpholine, Et_3N, CH_3CH_2CN, KI, 85 °C, 32%; (viii) HCl, CH_2Cl_2, EtOH, 75 °C, 68%; (ix) 3-morpholinopropanoic acid, HATU, Et_3N, THF, 71%.

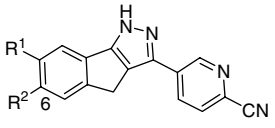


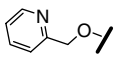
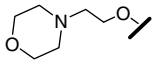
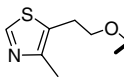
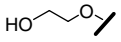
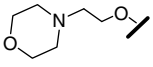
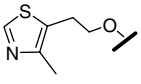
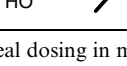
Scheme 3. Reagents and conditions: (i) NaH, $PhN(OTf)_2$, THF, 87%; (ii) 3-butyn-1-ol, CuI, $Pd(dppf)Cl_2$, Et_3N, DMF, 85 °C, 59%; (iii) MsCl, Et_3N, THF, 85%; (iv) morpholine, DMF, 90 °C, 52%; (v) Pd/C, H_2, THF, 81%; (vi) HCl, EtOH, 75 °C, 59%.

group on **40** was mesylated and displaced by morpholine. The acetylene moiety of **41** was reduced via catalytic hydrogenation followed by removal of the SEM to give **42**, a compound having a side chain with a carbon–carbon bond linker at the 7-position and a terminal morpholine group.

As we have demonstrated earlier, substitutions at both the 6 and 7-positions extend into the solvent exposed area in the kinase active site and can tolerate substantial variations.[5] In addition, the attachment of bis-alkoxy groups to an aromatic ring is known to exist in marketed orally active kinase inhibitors such as Iressa® and Tarceva®. Therefore, our initial strategy to improve the PK profile of **2** was to replace one of its methoxy groups with a different alkoxy moiety.[10] During our study, intraperitoneal (ip) dosing was the primary choice for the initial PK screening in mice. As shown in Table 2, although all the inhibitors (**7**–**15**) have potent inhibitory activity, the plasma exposure of representative compounds is low (AUC $\leqslant$ 0.93 μg h/mL). The location of the methoxy (6 or 7-position) and the nature of the terminal groups on the longer alkoxy side chain have minimal impact on the AUC values. These findings led us to investigate compounds with only one alkoxy substitution at the 7-position with elimination of the methoxy group, a potential metabolic liability. A series of compounds (**18**–**28**, Table 3) were prepared mostly with a solubilizing group capping the alkoxy side chain. Interestingly, the basicity of the side chain has a profound impact on the cellular anti-proliferative activity (data from the MTS assay) of the inhibitors. Non-basic side chain moieties such as thiophene and thiazole (**18** and **19**) led to compounds with no cellular potency (combo EC_{50} > 5.9 μM). On the other hand, basic functional

Table 2. Potency and PK evaluation of 6,7-substituted inhibitors



Compound	R_1	R_2	CHK-1 IC_{50} (nM)	AUC[a] (μg h/mL)
7	MeO		3.7	0.47
8	MeO		3.8	—
9	MeO		2.5	0[b]
10	MeO		3.1	0.30
13		MeO	3.0	0.93
14		MeO	1.1	—
15		MeO	2.6	—

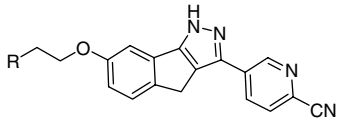
[a] Intraperitoneal dosing in mouse (10 mg/kg) except for **9**. Vehicle (for all mouse PK study unless indicated otherwise): 2.5% EtOH, 5% Tween 80, 25% PEG400, PBS.

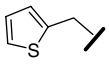
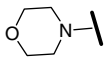
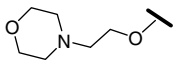
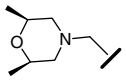
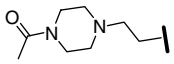
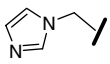
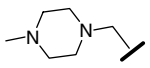
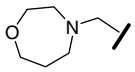
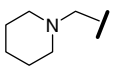
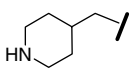
[b] Oral dosing in rat (5 mg/kg).

groups, such as piperidyl and homomorpholino, with pKa values over 9 resulted in compounds (**25**–**28**) with weak to fair potency in combination with doxorubicin. However, these compounds exhibited some cytotoxicity by themselves (single EC_{50} value between 2.7 and 6.2 μM). Only alkoxy side chains with pK_a around 6–7 gave inhibitors (**20**–**24**) with desirable results in the MTS assay, that is, weak or no single agent activity but high potentiation effect (at least 24-fold) on doxorubicin. More importantly, among this group of inhibitors, compound **20** possesses an AUC value (via ip dosing) in mice more than 6 times higher (5.7 vs 0.93 μg h/mL) than that of **13**, which has an extra methoxy at the 6-position. Therefore, compound **20** emerged as a new lead for further PK optimizations.

Table 4 summarizes the modification efforts around **20**. The carbon bridge between the morpholino group and the oxygen on the side chain of **20** was extended from two carbons up to five. Compounds **29** (with a three-carbon bridge) and **30** (with a four-carbon bridge) displayed plasma exposure comparable to **20** when dosed intraperitoneally in mice. Both compounds also possessed desirable cellular potency. Compound **29** was not cytotoxic as a single agent and was able to potentiate doxorubicin with an EC_{50} value at 1.6 μM. Mean-

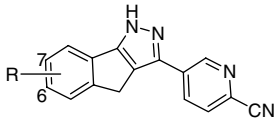
Table 3. Evaluation of inhibitors with an ether linker at the 7-position

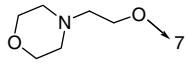
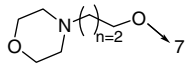
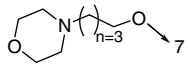
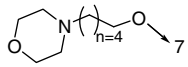
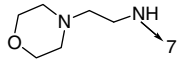
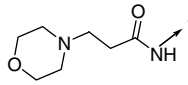
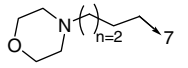
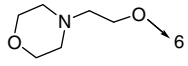
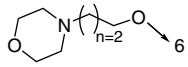


Compound	R	pK_a[a]	CHK-1 IC_{50} (nM)	MTS EC_{50} (μM) S/C[b]	AUC[c] (μg h/mL)
18		1.6	52	>59/ > 5.9	—
19		3.3	6.4	7.6/ > 5.9	—
20		5.7	5.9	>59/1.5	5.7
21		6.1	1.8	29/1.2	1.7
22		6.1	1.0	>52/1.6	3.0
23		6.9	1.5	33/0.51	2.2
24		7.2	1.1	>59/1.4	—
25		9.1	1.5	6.2/>5.9	—
26		9.1	0.92	5.4/1.1	0.031[d]
27		9.1	2.6	4.0/1.3	—
28		11	1.0	2.7/2.9	—

[a] Used PharmaAlgorithms protocol in calculation.
[b] S/C, Single/Combo.
[c] Intraperitoneal dosing in mouse (10 mg/kg) except for **26**.
[d] Oral dosing in mouse (10 mg/kg).

Table 4. Optimizing **20**



Compound	R	CHK-1 IC_{50} (nM)	MTS EC_{50} (μM) S/C[a]	AUC[b] (μg h/mL)
20		5.9	>59/1.5	5.7
29		3.2	>59/1.6	2.6
30		0.75	21/0.54	10.4[c]
31		1.4	8.1/1.9	—
37		7.0	>59/>5.9	—
38		16	33/2.3	—
42		1.2	9.7/1.6	—
43		6.5	>59/>5.9	—
44		6.4	>59/>5.9	—

[a] S/C, Single/Combo.
[b] Intraperitoneal dosing in mouse (10 mg/kg).
[c] Vehicle, 10% NMP, 20% Solutol, 70% PBS to achieve better solubility.

while, compound **30** had a slightly different profile with weak single agent activity (EC_{50} = 21 μM) but stronger anti-proliferative potency in the presence of doxorubicin (combo EC_{50} = 0.54 μM). Like its close analogs, **20** and **29**, compound **30** showed desired properties in the mechanism-based cellular assay (FACS analysis) with single-agent EC_{50} value more than 10 μM and combo EC_{50} value at 0.58 μM. When the carbon bridge was extended to five carbons, the inhibitor (**31**) started to show a deteriorated potentiation ratio (EC_{50} S/C) in the MTS assay. The linker between the side chain and the tricyclic core was also investigated. To this end, compounds with an amino (**37**), an acetylamino (**38**), and a carbon (**42**) linker were synthesized. Unfortunately, they showed no or only marginal potentiation of doxorubicin. The morpholino-capped alkoxy side chain was also moved from the 7 to the 6-position. The resulting compounds (**43** and **44**) showed no cellular activity at all, a dramatic contrast to their corresponding analogs at the 7-position (**20** vs **43**, and **29** vs **44**).

Compounds **29** and **30** were chosen to be further evaluated for their PK properties dosed via IV and PO in mice (Table 5). They both showed reasonable oral AUC values (3.0 μg h/mL for **29** and >3.3[11] for **30**) and oral biovailability (F = 44% and > 50% for **29** and **30**, respectively). These numbers represented a significant improvement from the earlier generation inhibitors with a bi-aryl phenol moiety at the 3-position[5] (oral AUC = 0–0.45 μg h/mL, F = 0–9%). The oral exposure of compound **30** was also studied in the Nude/Nude mice (Chart 1), a more appropriate model relevant to future in vivo efficacy studies. Dosed in two different vehicles (NMP and Phosal), the compound exhibited significant plasma exposures with C_{max} around 5 or 10 times the cellular EC_{50} values at 25 or 50 mkd dosage, respectively.

In summary, we explored a newer series of 1,4-dihydroindeno[1,2-*c*]pyrazole compounds with a cyanopyridine moiety at the 3-position as potent CHK-1 inhibitors. Replacing the bi-aryl phenol in the earlier series with the cyanopyridine not only retained the good enzymatic potency[12] for the inhibitors but also provided a better foundation for the PK improvement. The compounds with bis-alkoxy side chains failed to enhance PK properties. However, installing mono-

Table 5. Further pharmacokinetic studies on **29** and **30**

Compound	Mouse IV[a]				Mouse PO[b]		
	CL[c]	V_d[d]	$T_{1/2}$ (h)	AUC[e]	C_{max}[f]	AUC[e]	F (%)
29	3.8	7.6	1.4	2.0	0.66	3.0	44
30	3.4	6.9	1.4	2.1	0.77	>3.3	>50

[a] 3.0 mg/kg.
[b] 10 mg/kg.
[c] L/h kg.
[d] L/kg.
[e] μg h/mL.
[f] μM.

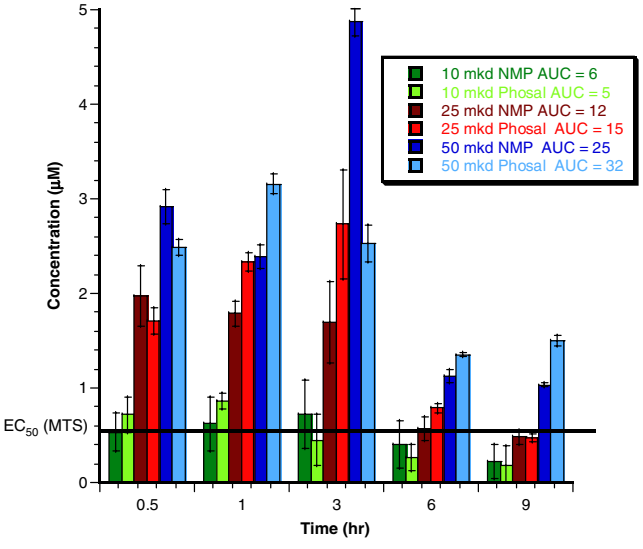


Chart 1. Plasma levels of **30** in Nu/Nu mice via oral dosing (vehicle: NMP or Phosal).

alkoxy with a morpholino terminal at the 7-position led to compounds, such as, **20**, **29**, and **30**, with much improved plasma exposures upon ip dosing in mice as well as desirable cellular anti-proliferative potentiation of doxorubicin. The window for achieving balanced properties appeared to be small since heterocyclic groups other than morpholine at the end of the side chains, extra alkoxy groups at the 6-position, linkers other than the ether off the tricyclic core, and placing the optimized side chains at the 6 instead of the 7-position all resulted in compounds with inferior overall profiles. With compounds **29** and **30** having oral biovailability at 44% or higher in mice, we have achieved tricyclic pyrazole compounds with much improved oral biovailability while maintaining good potencies. These two molecules will be used as tool compounds for in vivo evaluation of CHK-1 inhibitors to sensitize DNA-damaging agents.

References and notes

1. For reviews, see (a) Zhou, B. B.; Bartek, J. *Nat. Rev. Cancer* **2004**, *4*, 1; (b) Tao, Z-F.; Lin, N-H. *Anti-Cancer Agents Med. Chem.* **2006**, *6*, 377.
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6. The medicinal chemistry effort leading to discover of the cyanopyridine moiety will be published in a separate account.
7. See Ref. 4 for detailed structural biology information.
8. The MTS assay in HeLa cells was used as the functional assay. In this assay, the anti-proliferative effect was measured in the form of EC_{50} values for a CHK-1 inhibitor as a single agent and also for an inhibitor in the presence of doxorubicin (150 nM), a DNA-damaging agent. For the combination study, the baseline of the regression for EC_{50} calculation was adjusted to the inhibition level determined for 150 nM doxorubicin alone, a concentration known to cause G2/M arrest in HeLa cells. The ratio of the two corresponding EC_{50}s (combo/single) represents a relative scale of a CHK-1 inhibitor's function to potentiate doxorubicin. See Ref. 4 for assay conditions.
9. The cell cycle analysis (FACS assay) in H1299 cells examines the mechanism for the anti-proliferative function of the inhibitors. An EC_{50} was either the concentration of a CHK-1 inhibitor that reduces doxorubicin (500 nM)-induced G2/M cell population by half (combo), or it was measured in the absence of doxorubicin (single). See Ref. 4 for assay conditions and representative raw data.
10. For compounds with bis-alkoxy groups at the 6 and 7-positions and a bi-aryl phenol moiety at the 3-position of this class of CHK-1 inhibitors, see Tao, Z.-F.; Li, G.; Tong, Y.; Chen, Z.; Merta, P.; Kovar, P.; Zhang, H.; Rosenberg, S. H.; Sham, H. L.; Sowin, T. J.; Lin, N.-H. *Bioorg. Med. Chem. Lett.* **2007**. doi:10.1016/j.bmcl.2007.05.027.
11. The plasma concentration was sustained during the last several sampling time points.
12. CHK-1 Enzymatic Inhibition Assay: The assay was carried out using a recombinant CHK-1 kinase domain protein with amino acids from residue 1–289. A human biotinylated Cdc25c peptide was used as the substrate (Synpep Catalog# 02-1-22-1-ABB). The reaction mixture contained 25 mM of Hepes at pH 7.4, 10 mM $MgCl_2$, 0.08 mM Triton X-100, 0.5 mM DTT, 5 μM ATP, 4 nM ^{33}P ATP, 5 μM Cdc25c peptide substrate, and 5 nM of the recombinant CHK-1 protein. For potent compound with Ki below 1 nM, 0.5 nM of the recombinant CHK-1 protein and 8 nM of ^{33}P were used. The concentration of the vehicle, DMSO, in the final reaction is 2%. After 30 min at room temperature, the reaction was stopped by the addition of equal volume of 4 M NaCl and 0.1 M EDTA (pH 8.0). A 40 μL aliquot of the reaction was added to a well in a Flash Plate (NEN Life Science Products, Boston, MA) containing 160 μL of phosphate-buffered saline (PBS) without calcium chloride and magnesium chloride and incubated at room temperature for 10 min. The plate was then washed 3 times in PBS with 0.05% of Tween 20 and counted in a Packard TopCount counter (Packard BioScience Company, Meriden, CT).