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Discovery of 4′-(1,4-dihydro-indeno[1,2-*c*]pyrazol-3-yl)-benzonitriles and 4′-(1,4-dihydro-indeno[1,2-*c*]pyrazol-3-yl)-pyridine-2′-carbonitriles as potent checkpoint kinase 1 (Chk1) inhibitors

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Abstract—An extensive structure–activity relationship study of the 3-position of a series of tricyclic pyrazole-based Chk1 inhibitors is described. As a result, 4′-(1,4-dihydro-indeno[1,2-*c*]pyrazol-3-yl)-benzonitriles (**4**) and 4′-(1,4-dihydro-indeno[1,2-*c*]pyrazol-3-yl)-pyridine-2′-carbonitriles (**29**) emerged as new lead series. Compared with the original lead compound **2**, these new leads fully retain the biological activity in both enzymatic inhibition and cell-based assays. More importantly, the new leads **4** and **29** exhibit favorable physicochemical properties such as lower molecular weight, lower Clog *P*, and the absence of a hydroxyl group. Furthermore, structure–activity relationship studies were performed at the 6- and 7-positions of **4**, which led to the identification of ideal Chk1 inhibitors **49**, **50**, **51**, and **55**. These compounds not only potently inhibit Chk1 in an enzymatic assay but also significantly potentiate the cytotoxicity of DNA-damaging agents in cell-based assays while they show little single agent activity. A cell cycle analysis by FACS confirmed that these Chk1 inhibitors efficiently abrogate the G2/M and S checkpoints induced by DNA-damaging agent. The current work paved the way to the identification of several potent Chk1 inhibitors with good pharmacokinetics that are suitable for in vivo study with oral dosing.

Cancer is a top killer of human beings. There is great urgency to develop highly efficacious and minimally toxic treatments for cancer. Although tremendous progress has been achieved in the development of novel cancer treatments, most of the current cancer drugs usually exhibit high toxicity and are severely resisted by tumor cells in the clinic. This dilemma is particularly true for DNA-damaging agents, the mainstay of cancer treatment.[1] To improve the efficacy and lower the toxicity of DNA-damaging anticancer drugs, the development of adjuvant therapeutics has been aggressively pursued in recent years.[2,3] Such treatments may either sensitize tumor tissue or protect normal tissue from DNA damage.

Checkpoint kinase 1 (Chk1), a serine/threonine protein kinase which plays a key role in DNA damage-induced checkpoints,[4,5] has emerged as an attractive chemosensitization anticancer target.[6,7] Chk1 inhibitors have been demonstrated to abrogate the DNA damage-induced S and G2 checkpoints and disrupt the DNA repair process, resulting in premature chromosome condensation and leading to cell death; this preferentially sensitizes tumor cells, especially p53-null cells, to various DNA-damaging agents.[5–17] An optimal therapeutic window may be achieved for Chk1 inhibitors in the clinic because normal cells can be arrested in the G1 phase and are less affected by S and G2 checkpoint abrogation in response to DNA damage.[10]

Several classes of Chk1 inhibitors have been recently reported.[10–27] UCN-01 is the most extensively studied Chk1 inhibitor and is now in phase I/II clinical trials.[14,15] It abrogates both the S and G2 checkpoints and sensitizes tumor cells to a wide spectrum of

Keywords: Antitumor agent; Chk1 inhibitors; Checkpoint kinase 1; Pyrazole; Structure–activity relationship; DNA damage; Combination therapy.
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DNA-damaging agents.[16,17] Unfortunately, UCN-01 also potently inhibits a number of other kinases and exhibits strong single agent activity, and it has a high binding affinity for human plasma protein, which could limit its use in the clinic.[18] It remains a great challenge to identify ideal Chk1 inhibitors that show no single agent activity, but significantly potentiate DNA-damaging antitumor agents. We have discovered 4′-(1,4-dihydro-indeno[1,2-*c*]pyrazol-3-yl)-biphenyl-4-ols, exemplified by **1** and **2**, as a novel class of Chk1 inhibitors.[25–27] SAR studies on the 5-, 6-, and 7-positions disclosed that analogues with substituents on both the 6- and 7-positions stood out as the most potent subseries of tricyclic pyrazole-based Chk1 inhibitors.[26] For example, **2** is not only highly potent in the inhibition of Chk1 in an enzymatic assay, but also significantly potentiates the cytotoxicity of DNA-damaging agents to tumor cells without showing single agent activity.[26] However, these compounds show very poor pharmacokinetics (PK).[27] Preliminary ADME studies indicated that they exhibit low solubility due to the high lipophilicity of the biphenyl moiety.[27] In addition, the hydroxyl group is potentially labile to phase II biotransformations such as O-glucuronidation and O-sulfation. We have therefore performed extensive SAR studies on the 3-position in order to replace the biphenyl moiety of **2**. As a result, 4′-(1,4-dihydro-indeno[1,2-*c*]pyrazol-3-yl)-benzonitriles and 4′-(1,4-dihydro-indeno[1,2-*c*]pyrazol-3-yl)-pyridine-2′-carbonitriles were identified as new series of potent Chk1 inhibitors.

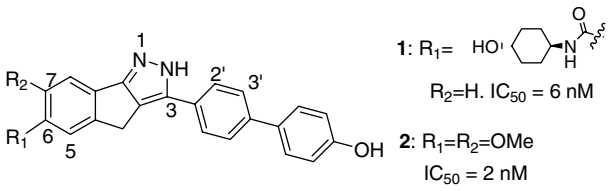
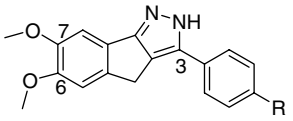
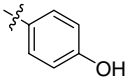
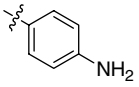
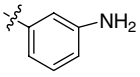
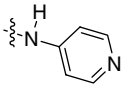
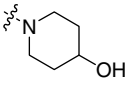
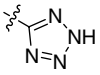
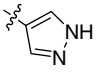
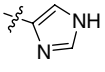
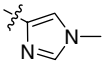
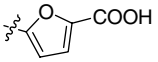


Table 1 shows the SAR results of analogues in which the terminal phenol group of **2** was replaced. Compounds **3**–**6**, which have polar substituents, showed potent Chk1 inhibition activity, suggesting possible hydrogen bonds in the polar region. However, the replacement of the hydroxyl group with an amino group (**7**, **8**) resulted in a substantial decrease in potency. Moving the amino group to the 3′-position of the phenyl ring produced a completely inactive compound (**9**). Other substituents such as bromide (**10**), pyridyl (**11**, **12**), morpholinyl (**13**), 4′-hydroxyl piperidinyl (**14**) caused considerable reduction of potency. Compounds with a variety of five-member ring heterocyclic substituents (**15**–**21**) were also evaluated. Only compound **15**, which contains the tetrazole, exhibits comparable potency as **2**. The analogues containing pyrazole (**16**) and pyrrole (**17**) are slightly less potent than **15**. Analogues containing imidazole (**18**–**20**) and furan (**21**) are much weaker Chk1 inhibitors.

Analogues with an alkyne spacer between the tricyclic core and the phenol are depicted in Table 2. These compounds (**22**–**27**) retain rigidity, are less lipophilic, and have lower molecular weight compared with the biphe-

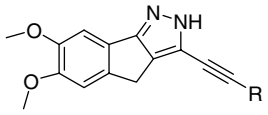
Table 1. Structure–activity relationship of phenyl analogues at the C-3 position

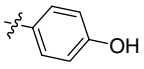
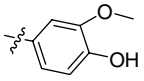
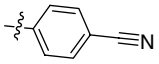
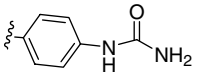
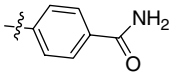


Compound	R=	Chk1 inhibition (IC_{50}, nM)[a]
2		2
3	OH	25
4	CN	7
5	CO_2H	2
6		25
7		779
8		355
9		>10,000
10	Br	73
11		>10,000
12		1288
13		6223
14		4579
15		11
16		47
17		77
18		1260
19		1374
20		3709
21		3003

[a] IC_{50} was measured by using 2 μM of substrate peptide in the presence of γ-[^{33}P]ATP (5 μM). For detailed enzymatic procedures, see Ref. 10.

Table 2. Structure–activity relationship at the C-3 position with an alkynyl spacer



Compound	R=	Chk1 inhibition (IC_{50}, nM)
22	H	5140
23		19
24		5
25		>10,000
26		1743
27		>10,000

nol **2**. The hydroxylphenyl analogues (**23**, **24**) exhibit comparable potency to **2** in an enzymatic assay, but had inferior cellular activity (vide infra). The replacement of the hydroxyl group with other polar groups such as cyano (**25**), urea (**26**), and amide (**27**) lost the Chk1 inhibition activity.

Examination of the X-ray co-crystal structure of **1**-Chk1 complex revealed that the hydroxyl group forms a hydrogen bond with Asn59 in the water pocket, an area of interest in gaining both potency and selectivity.[7,25] Similar interaction in the water pocket was reported for macrocyclic urea Chk1 inhibitors.[13] The 4′-N of the pyrazinyl ring of the macrocyclic inhibitors points to the water pocket and forms water-mediated hydrogen bonds with Asn59.[13] Figure 1a shows the overlay of co-crystal structures of **1** and a macrocyclic urea Chk1 inhibitor. The nitrogens at the 1- and 2-positions of **1** provide the hinge hydrogen-bonding replacement for the urea carbonyl and a NH of the macrocyclic inhibitor, respectively. The 4′-N of the urea pyrazinyl ring is situated in approximately the same position as C-3′ of **1**. We reasoned that a pyridyl analogue such as **28** would possess better activity by forming water-mediated hydrogen bonds in the water pocket (Table 3). Indeed, the potency of **2** was improved 4-fold by incorporation of a nitrogen atom at the 3′-position (**28**). The replacement of the terminal hydroxylphenyl group with a cyano group (**29**) fully retained the potency. Other substitutions on the pyridyl ring resulted in reduction of potency (**30**–**36**). Moving the N atom to the other position in the ring considerably decreased the activity (**29** vs **37**). The introduction of an additional N atom into the ring was also detrimental to potency (**38** vs **30**, **39** vs **30**). Molecular modeling indicates that three hydrogen bonds (between the CN and Lys38, between the pyridyl N and Ser147, and water-mediated hydrogen bonds between the pyridyl N and Asn59) may greatly contribute to the high potency of **29**.

Cell-based assays reveal that **4** and **29** significantly sensitize tumor cells to DNA-damaging agents while showing little cytotoxicity alone (vide infra). Thus, extensive SAR studies were performed on the 5-, 6-, and 7-positions to further optimize these two series. Table 4 shows the SAR results at the 6-position of compound **4**. Analogues with polar groups (**40**–**43**) at the 6-position exhibit low single digital nanomolar IC_{50} values against Chk1. Analogues with heteroaromatic groups (**44**–**49**) at the 6-position also potently inhibit Chk1 and their potencies were moderately affected by the type of pendant ring as well as the linker length between the tricyclic core and the pendant aromatic ring. Analogues with cyclic substituents (**50**–**52**) are the most potent compounds in this series. The potency difference between **53** and **54** suggests an important role of the carbonyl group of **53**. The above-discussed SAR results indicate that elaboration of the 6-position not only significantly improves the enzymatic activity (**52**, **50**, **51**, **49** vs **4**) but also the

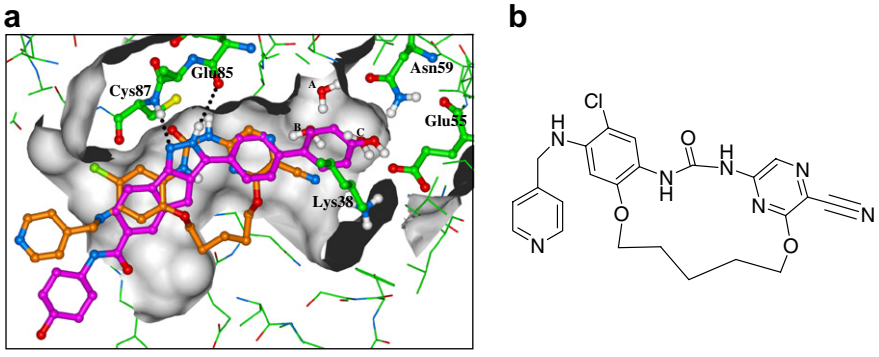
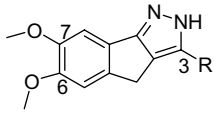
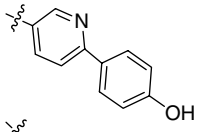
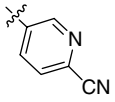
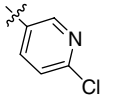
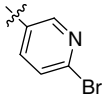
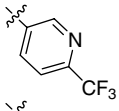
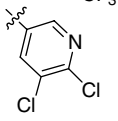
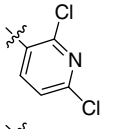
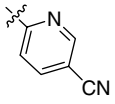


Figure 1. (a) Overlay of crystal structures of **1** (magenta carbons) and macrocyclic inhibitor (orange carbons) bound to Chk1 kinase. The connolly surface outlining the active site is shown in gray. Hinge hydrogen bonds for **1** are shown with dotted lines. Glu85 and Cys87 comprising the hinge and Lys38, Glu55, and Asn59 are shown with thick bonds. Ser147 is located directly behind the pyrazine of the macrocyclic inhibitor and is obscured by the active site surface. Three water molecules as observed in the crystal structure of the macrocyclic inhibitor, denoted A–C, are shown with one possible assignment of hydrogen positions. This network of intermolecularly hydrogen-bonded waters within the pocket is characteristic of Chk1 kinase subfamily within the kinase enzyme family. (b) The chemical structure of the macrocyclic urea compound.

Table 3. Structure–activity relationship at the C-3 position with a heteroaromatic ring spacer

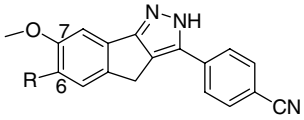


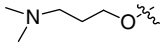
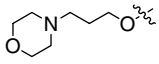
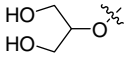
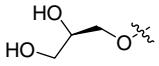
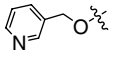
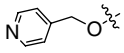
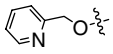
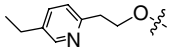
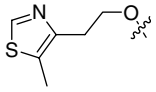
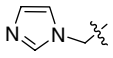
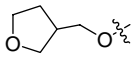
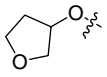
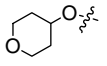
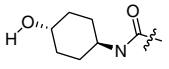
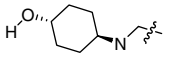
Compound	R=	Chk1 inhibition (IC_{50}, nM)
28		0.5
29		0.8
30		15
31		4
32		2
33		288
34		288
35		16
36		>10,000
37		44
38		89
39		44

potentiation ratio in the cellular assay (Table 5). SAR studies were also performed on the 7-position. In general, analogues with modifications at the 7-position are less potent than those with the same modifications at the 6-position (data not shown).

Potent Chk1 inhibitors were further evaluated in a MTS assay using p53-deficient HeLa cells in the absence or presence of doxorubicin (Dox).[28] Table 5 shows the

Table 4. Structure–activity relationship at the 6-position of compound **4**



Compound	R=	Chk1 inhibition (IC_{50}, nM)
40		4
41		3
42		3
43		6
44		14
45		20
46		10
47		77
48		9
49		2
50		3
51		2
52		1
53		3
54		17
55	OH	2

EC_{50} values and potentiation ratios of representative compounds. These compounds could be classified into three categories: (1) compounds that do not show cellular activity at all (**5**, **15**, **23**) or only show little or no potentiation of the cytotoxicity of Dox (**24**, **54**, **32**); (2) compounds that show significant potentiation but also have single agent activity (**28**, **43**); (3) compounds that

Table 5. Cellular activities in the presence and absence of Dox

Compound	MTS EC_{50} (μM) Cmpd + Dox/(−Dox)	Potentiation ratio	FACS EC_{50} (μM) Cmpd + Dox/(−Dox)
4	1.3/33.6	26	1.3/>50
5	>5.9/>59.3	N/A	>10/>10
15	>5.9/>59.3	N/A	>10/>10
23	>5.9/>59.3	N/A	4.8/>10
24	4.4/2.5	0.5	6.1/>10
28	0.8/10.6	13	0.3/3.5
29	1.1/>59.3	>54	0.3/>10
30	4.8/>59.3	>12	4.2/>10
31	3.9/>59.3	>15	1.4/>10
32	2.1/14.2	7	0.68/8.2
40	1.8/14.2	8	1.3/>10
41	1.5/13.9	9	2.7/>10
42	1.4/10.8	8	2.7/>10
43	1.0/18.3	18	2.1/>10
49	1.0/42.0	42	0.7/>10
50	1.9/>59.3	>31	2.6/>10
51	0.8/46.5	57	1.9/>10
52	1.6/>59.3	>37	1.3/>10
54	4.0/16.3	4	1.3/>10

show little or no antiproliferative activity alone but significantly potentiate Dox (**4**, **29**, **49**, **50**, **51**, and **52**), which have been defined as ideal Chk1 inhibitors. The lack of a good correlation between Chk1 enzymatic inhibition potency and cellular antiproliferative activity may be due to variation in physicochemical properties such as cellular permeability and potential off-target activity.

Fluorescence-activated cell sorting (FACS) analysis of cell cycle profiles was used to study the mechanism of action of Chk1 inhibitors.[29] Table 5 shows the EC_{50} values for abrogation of G2/M caused by Dox in the presence of various concentrations of Chk1 inhibitors. In accord with the antiproliferative activity cited above, ideal Chk1 inhibitors such as **4**, **29**, **49**, **50**, **51**, and **52** do not affect the regular cell cycle profile at high concentration (up to 50 μM), but efficiently abrogated the Dox-induced-G2/M checkpoint with nanomolar to low single digit micromolar EC_{50} values. Furthermore, we confirmed that representative Chk1 inhibitors efficiently abrogated camptothecin (CPT)-induced S arrest (data not shown).

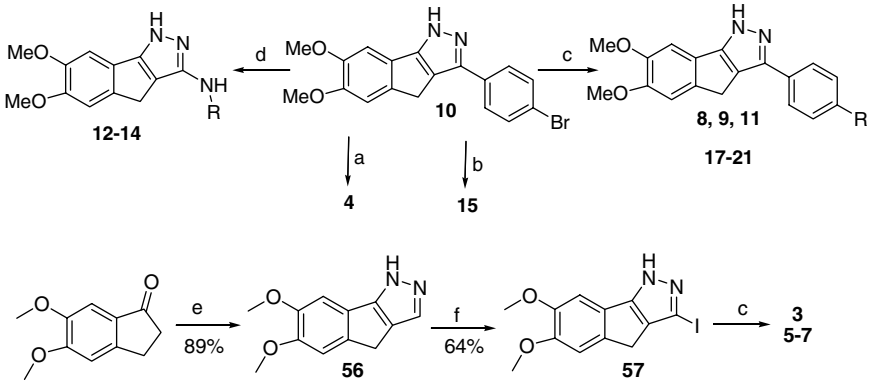
The syntheses of **3**–**15** and **17**–**21** are shown in Scheme 1. Compound **10**, prepared by a literature procedure,[26] was smoothly coupled with various boronic acids under Suzuki conditions in a microwave synthesizer to provide **8**, **9**, **11**, and **17**–**21**. A similar Suzuki coupling of **57**, prepared from 5,6-dimethoxy-indan-1-one in three steps, gave **3** and **5**–**7**.

Compounds **12**–**14** were obtained by amination of **10** under Buckwald conditions in a microwave synthesizer. The displacement of bromide from **10** by cyanide was effected in $Pd(PPh_3)_4$–$Zn(CN)_2$–DMF to produce **4**. Compound **15** was obtained through cyanylation of **10** followed by in situ cyclization with sodium azide in one pot under microwave conditions.

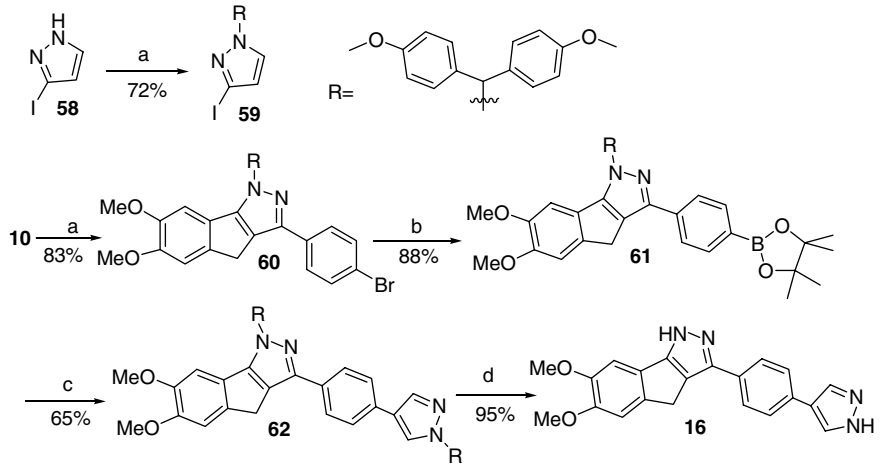
Scheme 2 depicts the synthesis of **16**. Protected tricyclic pyrazole bromide **60** (the protecting group could be on either of the N atoms, only one isomer is shown), prepared from **10**, was transformed into boronic ester **61** in excellent yield. Compound **61** was coupled with iodide **59** to provide **62**. Deprotection of the two pyrazoles of **62** produced the final product **16** in quantitative yield.

The syntheses of tricyclic pyrazoles bearing an alkyne spacer are shown in Scheme 3. The syntheses featured two Sonogashira reactions. Coupling of compound **57** with TMS-protected acetylene followed by deprotection provided **22**. A second Sonogashira reaction between **22** and various arylbromides in a microwave synthesizer smoothly produced **23**–**27**.

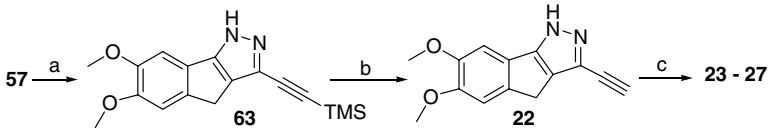
The synthesis of heteroaromatic analogues is shown in Scheme 4. 6-Chloronicotinic acid **64** was activated by DCI and then coupled with 5,6-dimethoxy-indanone in the presence of NaH to provide the diketone **66**. This diketone was treated with hydrazine in ethanol to produce **31**. Compound **28** was obtained by the Suzuki coupling of **31** with 4-hydroxyphenyl boronic acid. The cyanylation of **31** provided **29**. Compounds **32**–**38** were prepared by using synthetic procedures analogous to



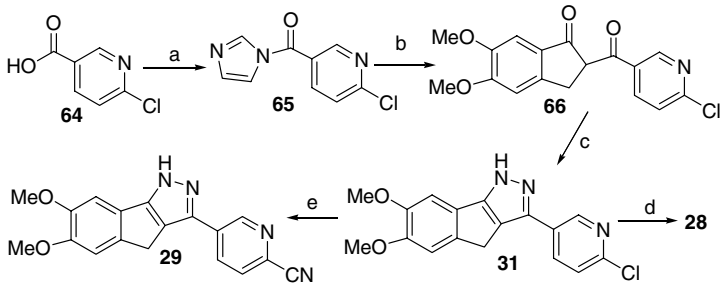
Scheme 1. Reagents: (a) $Pd(PPh_3)_4$, $Zn(CN)_2$, DMF; (b) $Pd(PPh_3)_4$, $Zn(CN)_2$, DMF; then, NaN_3, NH_4Cl, microwave; (c) boronic acids, Na_2CO_3, $Pd(PPh_3)_2Cl_2$, DME–EtOH–H_2O; (d) corresponding amines, biphenyl-2-yldi-*tert*-butylphosphine, NaO*t*-Bu, $Pd(OAc)_2$, toluene; (e) formic acid ethyl ester, NaH, benzene; then, NH_2NH_2, HOAc, EtOH; (f) NIS, DMF.



Scheme 2. Reagents and condition: (a) 4,4′-(chloromethylene)bis(methoxybenzene), TEA, THF; (b) bis(pinacolato)dibrone, $PdCl_2(dppf)CH_2Cl_2$; (c) **59**, Na_2CO_3, $Pd(PPh_3)_2Cl_2$, DME–EtOH–H_2O, microwave, 160 °C; (d) HCl, dioxane.



Scheme 3. Reagents and condition: (a) (trimethylsilyl)acetylene, $PdCl_2(PPh_3)_2$, CuI, PPh_3, TEA, DMF, microwave, 120 °C; (b) TBAF, THF; (c) arylbromides, $PdCl_2(PPh_3)_2$, CuI, PPh_3, TEA, DMF, microwave, 120 °C.

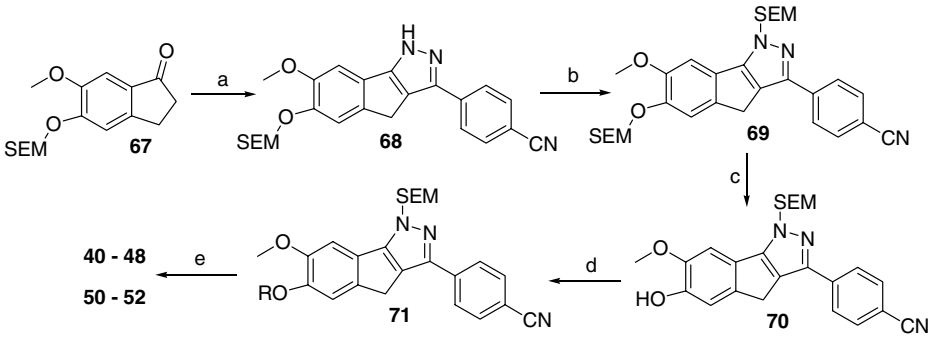


Scheme 4. Reagents and condition: (a) DCI, DMF; (b) 5,6-dimethoxy-indanone, NaH, THF; (c) NH_2NH_2, HOAc, ethanol; (d) 4-hydroxyphenyl boronic acid, Na_2CO_3, $Pd(PPh_3)_2Cl_2$, DME–EtOH–H_2O, microwave, 160 °C; (e) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF.

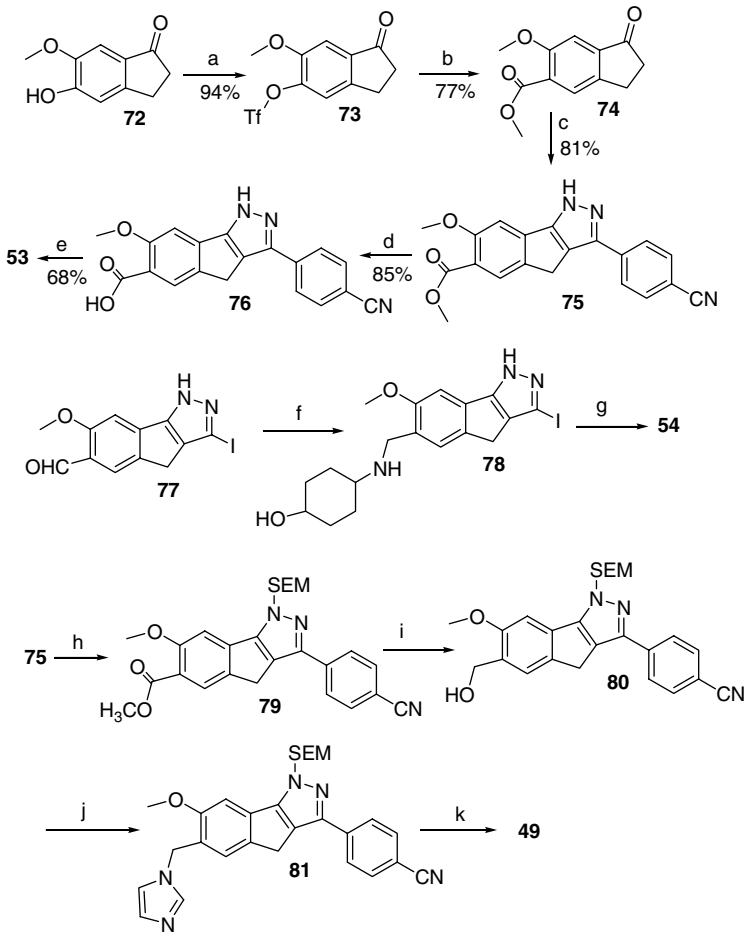
those described for **31**. Compounds **30** and **39** were synthesized by Suzuki coupling of **57** with the corresponding boronic acids.

Scheme 5 outlines the synthesis of compounds **40**–**48**, **50**–**52**, and **55**. Starting from the O-protected indanone **67**,[26] tricyclic pyrazole **68** was synthesized in two steps by a synthetic procedure analogous to that described for **31**. The treatment of **68** with SEMCl produced the protected pyrazole **69** as a mixture of two regioisomers with the SEM group attached to either of the nitrogen atoms (for clarity, only one isomer is shown). Selective removal of the SEM group at the 6-position of **69** in a diluted HCl–ethanol system produced the key intermediate **70**. Compound **71**, prepared by coupling of **70** with an alcohol under Mitsunobu conditions, was deprotected to provide Chk1 inhibitors **48**, **50**–**52**. Removal of both SEM groups from **69** gave **55**.

Scheme 6 depicts the synthesis of **49**, **53**, and **54**. 5-Hydroxy-6-methoxy-indanone (**72**)[26] was converted into trifilate **73** in excellent yield. Carbonylation of **73** was smoothly achieved under palladium-catalyzed conditions to provide **74**. Intermediate **75** was obtained in two steps by following a sequence similar to that described for **31**. The saponification of **75** gave acid **76**. The coupling of **76** and 4-aminocyclohexanol in the presence of Bop reagent provided the final product **53**. Compound **78**, prepared by the reductive amination of **77**, was coupled with the cyanophenyl boronic acid under Suzuki conditions to provide **54**. Compound **79**, obtained by the SEM protection of **75** (the SEM group could be on either of the nitrogen atoms; for clarity, only one isomer is shown), was reduced to alcohol **80**. The coupling of **80** and DCI in acetonitrile gave **81**. The removal of the SEM group from **80** under acidic conditions provided the final product **49**.



Scheme 5. Reagents: (a) 5-(imidazole-1-carbonyl)-pyridine-2-carbonitrile, NaH, THF; then, NH_2NH_2, HOAc, ethanol; (b) SEMCl, NaH, DMF; (c) HCl (diluted), dioxane; (d) ROH, DBAD, polymer-supported PPh_3, THF; (e) HCl, EtOH.



Scheme 6. Reagents and conditions: (a) triflic anhydride, 2,6-lutidine, 4-DMAP; (b) CO, $PdCl_2(dppf)$-CH_2Cl_2, TEA, CH_3OH; (c) 5-(imidazole-1-carbonyl)-pyridine-2-carbonitrile, NaH, THF; then, NH_2NH_2, HOAc, ethanol; (d) NaOH, H_2O, EtOH; (e) 4-aminocyclohexanol, TEA, Bop reagent, DMF; (f) *trans*-4-aminocyclohexanol, K_2CO_3, EtOH; then $NaBH_4$; (g) 4-cyanophenyl boronic acid, Na_2CO_3, $Pd(PPh_3)_2Cl_2$, DME–EtOH–H_2O, microwave, 160 °C; (h) SEMCl, NaH, DMF; (i) $NaBH_4$, THF–MeOH; (j) DCI, CH_3CN, 80 °C, 24 h; (k) HCl, EtOH.

In summary, an extensive structure–activity relationship at the 3-position of tricyclic pyrazole-based Chk1 inhibitors was described. In this endeavor, 4′-(1,4-dihydro-indeno[1,2-*c*]pyrazol-3-yl)-benzonitriles (**4**) and 4′-(1,4-dihydro-indeno[1,2-*c*]pyrazol-3-yl)-pyridine-2′-carbonitriles (**29**) emerged as new lead series. Compared with the original lead compound **2**, these new leads fully retain the biological activity in both enzymatic inhibition and cell-based assays. More importantly, the new leads **4** and **29** exhibit favorable physicochemical properties such as lower molecular weight, lower Clog *P*, and the absence of the hydroxyl group. SAR on the 6-position of **5** suggested that both the enzymatic activity (**49**, **50**, **51**, **55** vs **4**) and the potentiation ratio in the cellular assay could be improved significantly by modifying the 6-position. The discovery of lead compound **29** has paved

the way to the identification of several potent Chk1 inhibitors with good PK that are suitable for in vivo study with oral dosing.

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28. The EC_{50} values for the compounds were determined either alone or in the presence of 150 nM of doxorubicin (Dox), a clinical topoisomerase II inhibitor known to arrest the G2/M checkpoint at this concentration in HeLa cells. The EC_{50} values for Chk1 inhibitors in combination with Dox were calculated from the percentage of inhibition by Chk1 compounds at various concentrations above the background inhibition by 150 nM Dox. The ability of Chk1 inhibitors to potentiate Dox is represented by the ratio of the EC_{50} values of the inhibitor alone and the inhibitor with Dox. For more details about the assay, see Ref. 10.
29. H1299 cells were treated with Chk1 inhibitors in the presence and absence of Dox (500 nM) and their detailed cell cycle kinetics were analyzed by using FACS. For more details about the assay, see Ref. 10.