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Macrocyclic ureas as potent and selective Chk1 inhibitors: An improved synthesis, kinome profiling, structure–activity relationships, and preliminary pharmacokinetics

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Abstract—A new series of potent macrocyclic urea-based Chk1 inhibitors are described. A detailed SAR study on the 4-position of the phenyl ring of the 14-member macrocyclic ureas **1a** and **d** led to the identification of the potent Chk1 inhibitors **2**, **5**–**7**, **10**, **13**, **14**, **19**–**21**, **25**, **27**, and **31**–**34**. These compounds significantly sensitize tumor cells to the DNA-damaging antitumor agent doxorubicin in a cell-based assay and efficiently abrogate the doxorubicin-induced G2/M and camptothecin-induced S checkpoints, indicating that the potent biological activities of these compounds are mechanism-based through Chk1 inhibition. Kinome profiling analysis of a representative macrocyclic urea **25** against a panel of 120 kinases indicates that these novel macrocyclic ureas are highly selective Chk1 inhibitors. Preliminary PK studies of **1a** and **b** suggest that the 14-member macrocyclic inhibitors may possess better PK properties than their 15-member counterparts. An improved synthesis of **2** and **20** by using 2-(trimethylsilyl)ethoxycarbonyl (Teoc) to protect the amino group not only readily provided the desired compounds in pure form but also facilitated the scale up of potent compounds for various biological studies.

Since nitrogen mustards were developed for cancer chemotherapy more than six decades ago,[1] DNA-damaging agents have been a mainstay of cancer treatment. However, their clinical use is increasingly limited by their severe toxicity to normal cells and resistance by tumor cells.[2] To overcome this limitation, the development of adjuvant therapeutics that improve the efficacy and selectivity of DNA-damaging agents in the clinic has recently attracted much attention.[3,4] Such treatments may either sensitize tumor tissue or protect normal tissue from DNA damage.

Checkpoint kinase 1 (Chk1) is a serine/threonine protein kinase which plays a critical role in DNA damage-induced checkpoints, and Chk1 has emerged as an attractive chemosensitization target.[5,6] In response to DNA damage, ATM and ATR kinases activate Chk1 through phosphorylation in the SQ/TQ domain to arrest cells at various DNA-damaging checkpoints (G1, S, G2) to initiate the DNA repair process.[7,8] Since p53-deficent tumor cells lack the G1 checkpoint, they are selectively arrested at the S or G2 checkpoint after DNA damage. The inhibition of Chk1 abrogates the S and G2 checkpoints and disrupts the DNA repair process, resulting in premature chromosome condensation, leading to cell death. Tumor cells, especially p53-null cells, are thereby preferentially sensitized to various DNA-damaging agents by inhibition of Chk 1.[9,10] In contrast, normal cells can still arrest in the G1 phase and are less affected by S and G2 checkpoint abrogation, suggesting that a favorable therapeutic window may be achieved for G2 and/or S abrogators.[11]

Several classes of Chk1 inhibitors have been reported.[6] However, it still remains a great challenge to identify ideal Chk1 inhibitors that significantly potentiate DNA-damaging antitumor agents without showing single agent activity. Based on the crystallographic analysis of a urea–Chk1 complex and molecular modeling, we have recently discovered a class of macrocyclic Chk1 inhibitors (**1**).[12–14] SAR studies based on **1b**, a 15-member macrocycle, have led to the identification of several compounds that not only potently inhibit Chk1 in an enzymatic assay, but also significantly potentiate the

Keywords: Checkpoint kinase 1; Chk1; Ureas; Kinase inhibitors; Macrocycles; Ring-closure metathesis; DNA damage.
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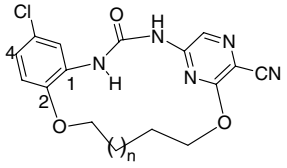
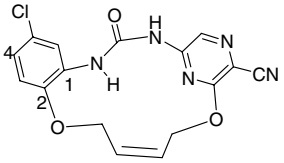
cytotoxicity of DNA-damaging agents to tumor cells.[12] Preliminary SAR on the ring size revealed that the enzymatic potency of **1a–c** decreased in the order of **1a** (IC_{50} = 6 nM, 14-member) $\geqslant$**1b** (IC_{50} = 7 nM, 15-member) > **1c** (IC_{50} = 28 nM, 16-member). This indicates that the additional conformational flexibility resulting from the presence of more rotatable bonds in the carbon chain linker is detrimental. This is consistent with other literature examples that show that the restriction of conformation produces more potent inhibitors.[15] We therefore further elaborated the more constrained macrocycle **1a**. Described herein are: (1) SAR based on the 14-member macrocycle **1a** and its olefin counterpart **1d**; (2) the kinome profiling of a representative compound; (3) preliminary pharmacokinetics (PK); (4) an improved synthesis of macrocyclic ureas.



1a: n=1, 14-member ring, IC_{50}=6 nM
1b: n=2, 15-member ring, IC_{50}=7 nM
1c: n=3, 16-member ring, IC_{50}=28 nM



1d

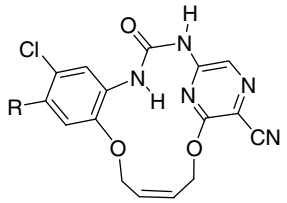
Examination of an X-ray co-crystal structure of a macrocyclic urea–Chk1 complex[12] reveals that the 4-position of the phenyl ring is very open to a solvent accessible region, which should be able to accommodate a variety of groups for further optimization. Thus, an extensive SAR on the 4-position of the phenyl ring was performed. The substituents at the 4-position were selected based on our previous SAR results of the macrocyclic urea-based Chk1 inhibitors.[12] These groups may improve the cellular activity or physical properties. Table 1 shows the analogues (**2–18**) derived from **1d**. As predicted, all the analogues exhibited potent activity for Chk1 inhibition.[16] Compounds **2–12** are macrocyclic ureas in which a substituent is linked to the phenyl ring by a nitrogen linker. The amino analogue **2** exhibits a subnanomolar IC_{50} against Chk1 (Table 1). The dimethylamino (**3**) and mono-methylamino analogues (**4**) are equally potent to one another. Analogues **5** and **6**, with extended alkyl tails, not only fully retain low single digit nanomolar potency but also potentially have better solubility due to the presence of the terminal polar hydroxyl groups. The introduction of an aromatic group such as a thiazolyl group (**7**) to the terminal position of the substituent gave Chk1 inhibition activity comparable to **4**. Amides (**8–10**) and carbamates (**11** and **12**) are also very potent but are slightly weaker than the alkyl amino analogues. Compounds **13–18** are macrocyclic ureas in which a substituent is linked to the phenyl ring by an oxygen atom. All these compounds have polar moieties on the terminus of the substituent that are intended to point into the solvent exposed area. The polar moieties may be solvated and therefore may boost the potency as previously observed.[12] Indeed, compounds **13–18** are very potent Chk1 inhibitors with IC_{50} values ranging from subnanomolar to low single digital nanomolar.

Table 2 summarizes the SAR results at the 4-position of the phenyl ring based on macrocyclic urea **1a**. These compounds (**19–36**) were also found to be potent Chk1 inhibitors, and the SAR based on **1a** is very similar to that based on **1d** (Table 1); for the same substituent at the 4-position of the phenyl ring, analogues derived from the saturated core **1a** exhibit the same or very comparable potency as those derived from the olefin core **1d** (**3** vs **21**, **4** vs **22**, **7** vs **25**, **18** vs **36**, etc.). Notably, the diethylamino analogue **23** is 11-fold less potent than the dimethylamino analogue **21**, indicating that the larger ethyl groups may clash with the Chk1 protein. Compound **31** is a phosphate derivative of **30**. Although the enzymatic activity of **31** is weaker than that of its parent compound **30**, the cellular potency of **31** shows dramatic improvement. Furthermore, the phosphate group of **31** should improve the aqueous solubility.

All the potent Chk1 inhibitors identified in the enzymatic assay were further evaluated in an MTS assay using HeLa cells, a p53-null human cervical cancer cell line. 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 inhibitors 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} value of the inhibitor with Dox to that of the inhibitor alone. As shown in Tables 1 and 2, these compounds fit into three categories: the first class of compounds show no cellular activity at all (**4**, **8**, **11**, **12**, **15**, **22–24**, and **28–30**) or show little or no potentiation of the cytotoxicity of Dox (**9**, **16–18**, **26**, **35**, and **36**); the second class of compounds show significant potentiation but also have single agent activity (**20**); the third class of compounds show little or no antiproliferative activity alone but significantly potentiate Dox (**2**, **5–7**, **10**, **13**, **14**, **19**, **21**, **25**, **27**, and **31–34**). This latter class of compounds fit the definition of 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.

To confirm the cellular activity obtained in the above-described MTS assay, and to make sure the biological activity of the Chk1 inhibitors is truly expressed through the abrogation of DNA damage-induced checkpoints, fluorescence-activated cell sorting (FACS) analysis of cell cycle profiles was performed. H1299 cells were treated with Chk1 inhibitors in the presence and absence of Dox and their detailed cell cycle kinetics were assessed by FACS analysis. As predicted, Dox itself induced a remarkable G2/M-phase arrest at a concentration of 500 nM. The EC_{50} values for abrogation of the G2/M checkpoint caused by Dox in the presence of various concentrations of Chk1 inhibitors are shown in Tables 1 and 2. FACS analysis charts of H1299 cells treated with Dox in the presence or absence of Chk1 inhibitor

Table 1. SAR and cellular activities of unsaturated macrocyclic Chk1 inhibitors



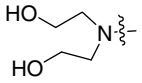
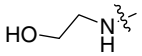
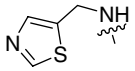
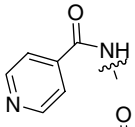
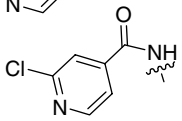
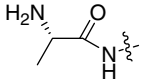
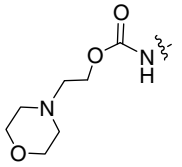
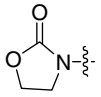
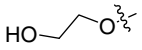
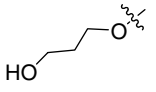
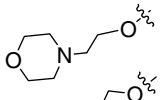
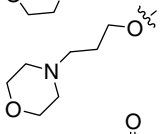
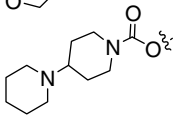
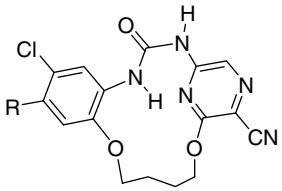
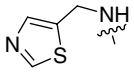
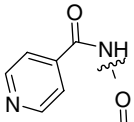
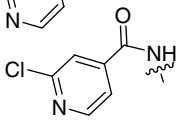
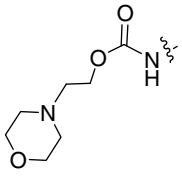
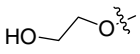
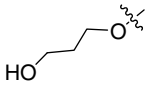
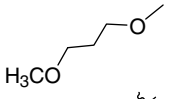
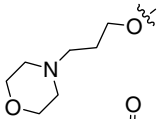
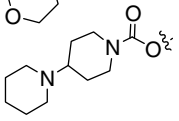
Compound	R=	Chk1 inhibition (IC_{50}, nM)	MTS EC_{50} (μM) compound + Dox/ (compound alone)	Potentiation ratio	FACS EC_{50} (μM) compound + Dox/ (compound alone)
2	NH_2	0.3	1.2/>59.3	>49	<0.03/>10
3		2	1.9/18.7	10	1.7/>10
4		2	>5.9/>59.3	NA	>10/>10
5		1	1.5/34.1	22	NA
6		2	2.3/>59.3	>26	1.3/>10
7		4	0.59/13.6	25	0.2/>10
8		6	>5.9/>59.3	NA	>10/>10
9		6	2.9/5.4	2	>10/>10
10		3	1.9/>59.3	>31	0.09/>10
11		3	>5.9/>59.3	NA	>10/>10
12		10	>5.9/>59.3	NA	NA
13	OH	0.3	0.93/45.7	49	0.7/>10
14		1	2.3/>59.3	>26	1.9/>10
15		2	>5.9/>59.3	NA	NA
16		1	4.2/14.1	3	>10/>10
17		0.3	1.1/1.9	2	>10/>10
18		1	0.7/1.2	2	>10/>10

Table 2. SAR and cellular activities of saturated macrocyclic Chk1 inhibitors



Compound	R=	Chk1 inhibition (IC_{50}, nM)	MTS EC_{50} (μM) compound + Dox/ (compound alone)	Potentiation ratio	FACS EC_{50} (μM) compound + Dox/ (compound alone)
19	H	6	3.08/57.6	19	1.3/>10
20	NH_2	1	0.2/3.4	28	<0.03/2.6
21		2	1.8/54.5	30	0.54/>10
22		2	>5.9/>59.3	NA	>10/>10
23		23	>5.9/>59.3	NA	>10/>10
24		3	>5.9/>59.3	NA	NA
25		4	1.0/59.3	>59	NA
26		4	5.1/24.3	5	NA
27		4	2.6/>59.3	23	NA
28		3	>5.9/>59.3	NA	>10/>10
29		6	>5.9/>59.3	NA	NA
30	OH	0.3	>5.9/>59.3	NA	NA
31		8	0.23/17.4	78	NA
32		8	2.0/>59.3	>30	3.2/>10
33		1	1.5/>59.3	>40	2.5/>10
34		3	0.9/13.9	18	NA
35		1	2.1/5.0	2	>10/>10
36		1	1.2/2.8	2	>10/>10

2 are shown in Figure 1a. Ideal Chk1 inhibitors (**2**, **6**, **7**, **10**, **13**, **14**, **19**, **21**, **32**, and **33**) did not affect the regular cell cycle profile even at high concentrations (up to 10 μM), but efficiently abrogated the Dox-induced-G2/M checkpoint with subnanomolar to low single digital micromolar EC_{50} values. Notably, compounds that do not show cellular activity at all (**4**, **8**, **11**, **22**, **23**, and **28**) or only show little or no potentiation of the cytotoxicity of Dox (**9**, **16**–**18**, **35**, and **36**) do not abrogate the Dox-induced-G2/M checkpoint even at 10 μM concentration.

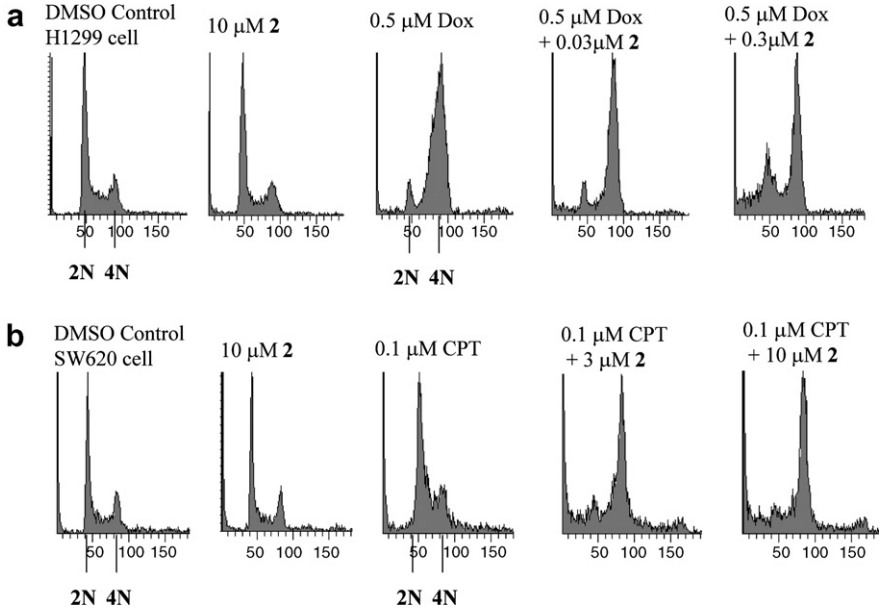


Figure 1. FACS profiles of cancer cells treated with DNA-damaging agents in the presence or absence of Chk1 inhibitor **2**. The cells were stained with propidium iodide for DNA contents. G0/G1 cells contain 2N DNA while cells in G2/M phase have 4N DNA. S phase cells have DNA content between 2N and 4N. Apoptotic cells contain less than 2N DNA. (a) H1299 cells treated with Dox. (b) SW620 cells treated with CPT.

Table 3. Kinase selectivity of macrocyclic Chk1 inhibitor **25**

Kinase	K_i[a] (μM)	Kinase	K_i[a] (μM)	Kinase	K_i[a] (μM)	Kinase	K_i[a] (μM)
CHK1	0.004	CDK7	>1.525	IKKα	>3.75	PKCγ	>8.750
GSK3α	0.911	CDK9	>1.644	IKKβ	>2.338	PKCζ	>8.333
PLK3	1.085	CHK2	>5.578	INSR	>5.238	PKG1A	>1.777
SRPK1	2.307	CLK2	>8.753	JAK2	>1.453	PKG1B	>1.683
AUR1	2.025	CLK4	>8.951	JAK3	>1.722	PKN2	>8.195
CDK2	3.088	CTAK-1	>6.667	JNK1a1	>3.056	PRAK	>1.525
IRAK1	3.011	CAMK1	>1.677	JNK2a2	>4.118	PRKX	>1.488
AKT1	3.698	CAMK4	>8.571	KDR	>8.876	PIM2	>1.424
MK2	3.886	CK2	>5.833	LCK	>2.701	PKD2	>8.980
DYRK1A	3.401	CK1α 1	>6.916	LIMK1	>9.546	PRKCN	>9.217
MSK2	0.949	CK1δ	>7.826	LYN	>1.667	ROCK1	>9.105
EMK	4.266	CK1γ2	>7.166	MINK1	>8.493	ROCK2	>7.500
AKT2	4.800	DCAMKL2	>9.570	MK3	>9.234	RSK2	>7.368
AMPK	1.539	DYRK3	>5.804	MLK1	>1.667	STK31	>1.442
PBK	5.195	EGFR	>1.803	MNK2	>9.350	STK33	>1.638
AUR2	1.911	EPHA2	>8.000	MSK1	>1.892	SRC	>1.936
PLK1	6.560	ERBB2	>1.438	MSSK1	>1.736	TAK1-TAB1	>1.606
AKT3	7.157	ERBB4	>3.750	MUSK	>1.650	TAOK2	>9.334
SGK	8.176	ERK2	>8.750	MAP4K2	>6.516	TBK1	>1.525
PDK1	3.482	FGFR3	>1.863	NLK	>1.611	TSSK1	>1.596
CKIT	1.119	FGFR	>4.737	NEK11	>1.549	TSSK2	>1.526
FLT4	6.846	FLT1	>0.440	NEK2	>9.242	TYK2	>1.936
P38δ	6.812	FLT3	>2.481	NEK3	>1.667	TRKA	>2.481
PIM1	9.182	FYN	>0.809	NEK4	>6.711	TRKB	>4.536
ARK5	>1.503	GSK3β	>7.059	P70S6K	>6.668	WNK2	>1.518
ABL	>0.706	HIPK2	>3.338	PAK1	>9.727	ZAK	>8.573
BLK	>1.453	HIPK4	>7.664	PAK4	>5.454	ZIPk	>1.391
CDC42BPA	>9.524	IGF1R	>0.991	PHKλ2	>4.902	CMET	>2.857
CDK5	>9.275	IRAK4	>9.921	PKA	>7.500	CDC2	>8.913
CDK6	>1.549	ITK	>1.525	PKCδ	>8.889	p38γ	>7.500

[a] The inhibition constant (K_i) values are calculated from the Cheng-Prusoff equation, $K_i = IC_{50}/1 + ([ATP]/K_m)$.

The results obtained from the MTS assays are very consistent with those derived from FACS analysis. In addition, we found that the Chk1 inhibitors efficiently abrogate camptothecin (CPT)-induced S arrest. CPT damages DNA through the inhibition of topoisomerase I and is known to cause S phase arrest. As shown in Figure 1b, compound **2** alone did not alter the regular cell cycle profile, but it efficiently abrogated CPT-induced S arrest and forced the cells further into the G2/M phase. The subG0/G1 population indicates the resulting apoptosis.

The macrocyclic urea Chk1 inhibitor **25** was tested against a panel of 120 kinases to obtain a selectivity profile, and excellent selectivity was observed. As shown in Table 3, the compound exhibited no inhibitory activity toward a majority of kinases in this panel even at the highest concentration tested. Moreover, compound **25** does not inhibit Chk2, an important DNA damage-involving checkpoint kinase, or Cdk7, a cyclin-dependent kinase that is critical in the regulation of cell cycle transition. Recently it has been reported that selectivity for Chk1 over Cdk7 is essential for the abrogation of DNA damage-induced checkpoint arrests.[17]

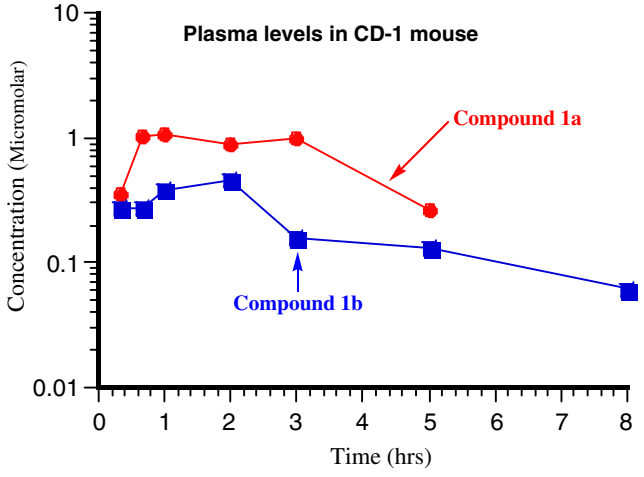
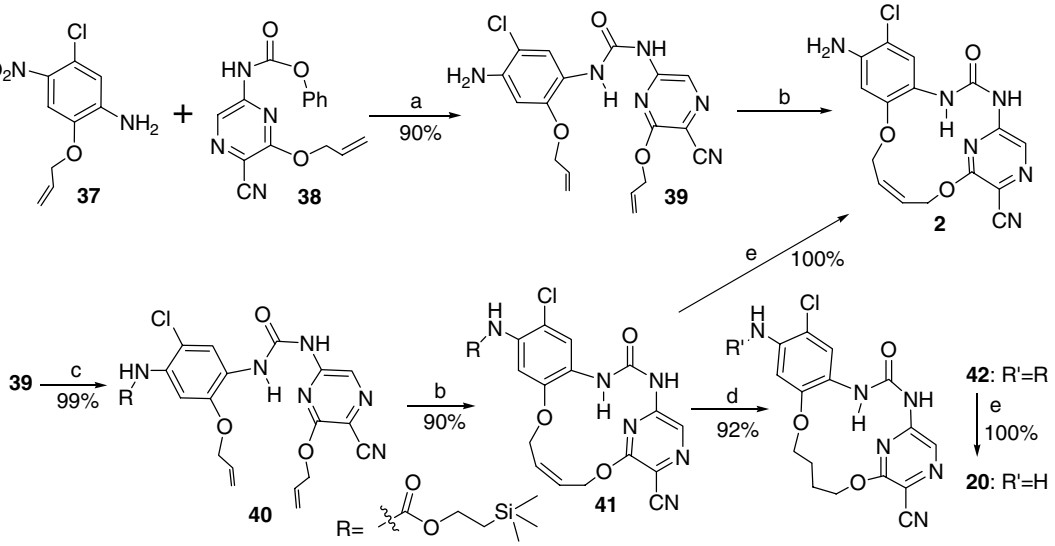


Figure 2. Plasma levels of compounds **1a** and **b** with intraperitoneal (IP) dosing in CD-1 mice.

To investigate how the ring size affects PK, compounds **1a** and **b** were intraperitoneally administered to mice with a dosage of 10 mg/kg. As shown in Figure 2, both compounds show moderate plasma exposure with AUC values of 3.8 μmol h/L for **1a** and 1.5 μmol h/L for **1b**. It is very interesting to note that the 14-member macrocycle **1a** exhibits over 2-fold more exposure than the 15-member **1b**.

Scheme 1 outlines the syntheses of **2** and **20**, which are the key intermediates toward macrocyclic ureas in which a nitrogen atom links the substituent to the phenyl ring. Compound **39** was prepared in high yield by the coupling of **37** and **38**, both of which were prepared as previously described,[18] followed by reduction of the nitro group. The macrocyclization of **39** was first attempted with Grubbs catalysts (1st and 2nd generation) in DCM under reflux. LCMS indicated a 40–60% conversion of **39** to **2**. Hoveyda-Grubbs catalyst (2nd) was then tried and gave better conversion (70–90%). However, it was very difficult to obtain **2** in pure form due to contaminants of dark green color originating from the catalysts. Attempts to remove the color by using reported methods[19–21] were not successful. In addition, the conversion of **2** to **20** by hydrogenation also proved to be problematic. We therefore decided to protect the amino group of **39**. After screening several protecting groups, 2-(trimethylsilyl)ethoxycarbonyl (Teoc)[22] stood out as the best. Thus, **39** was coupled with Teoc-Cl in the presence of pyridine to give **40** in quantitative yield. Cyclization of **40** in the presence of Hoveyda-Grubbs catalyst (2nd) provides **41** in 90% isolated yield. Deprotection of **41** in TFA at 0 °C quantitatively produced **2**. Saturation of olefin **41** in the presence of Wilkinson's catalyst smoothly gave **42**. Compound **20** was obtained by the deprotection of **42** in quantitative yield. This improved

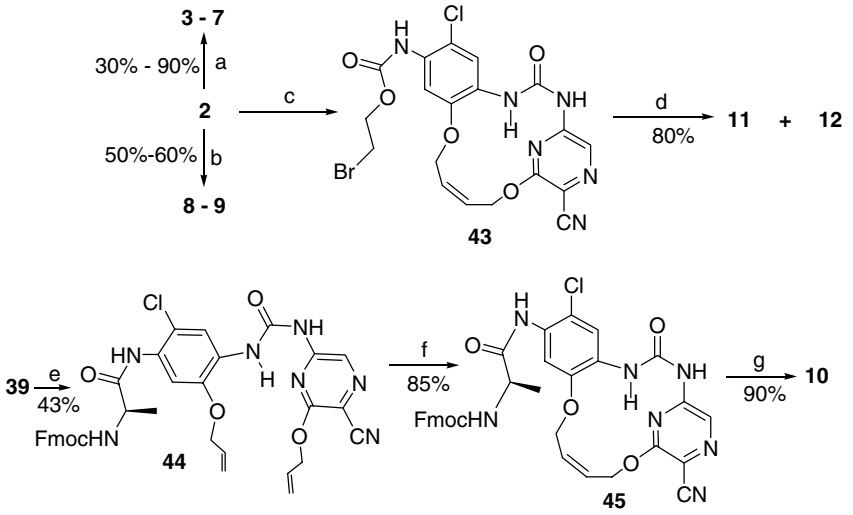


Scheme 1. Reagents and conditions: (a) i—DMF, 50 °C; ii—iron powder, NH_4Cl, EtOH, H_2O, 80 °C; (b) Hoveyda-Grubbs catalyst, CH_2Cl_2, reflux; (c) RCl, THF, pyridine; (d) H_2, Wilkinson's catalyst, THF, rt; (e) TFA, 0 °C.

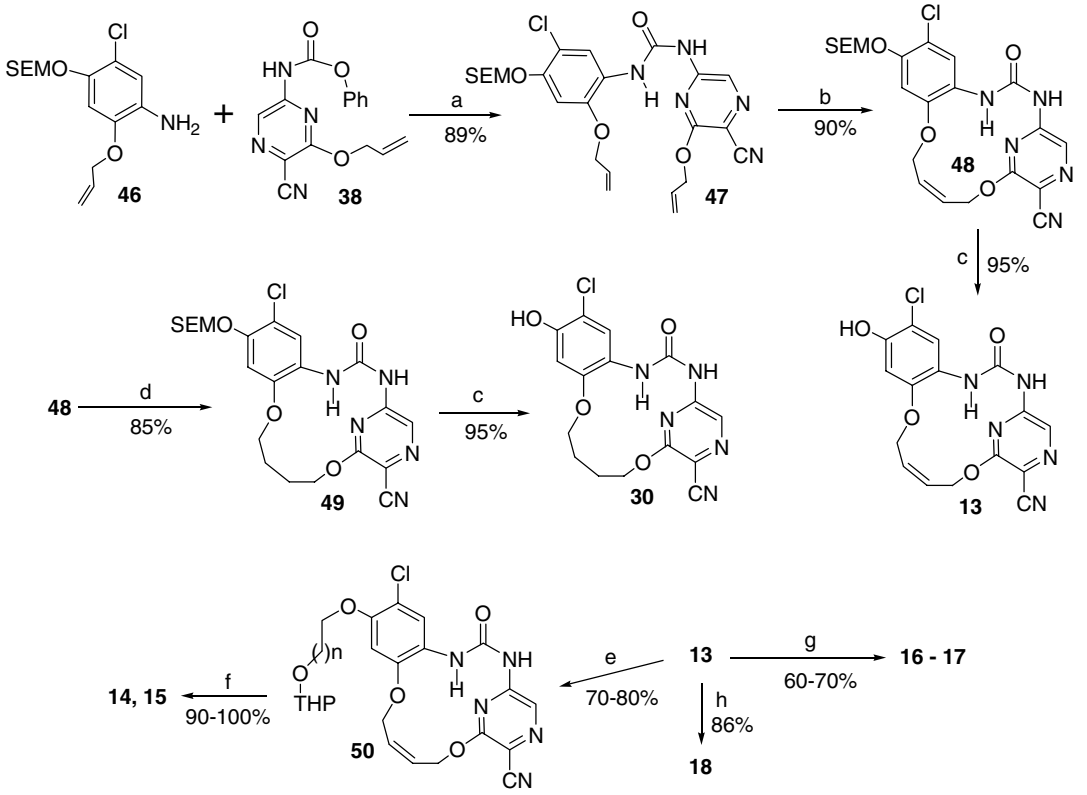
synthetic sequence not only readily provided **2** and **20** in pure form, but also facilitated the scale up of potent compounds for various biological studies.

Scheme 2 depicts the synthesis of compounds **3**–**12**. Compounds **3**–**7** were prepared by the reductive amination of **2** in moderate to good yield. The coupling of **2** with the corresponding acyl chlorides gave amides **8** and **9**. Compound **43**, obtained through the coupling of **2** with bromoethyl chloroformate, was treated with morpholine to provide **11** and **12** concurrently. Amidation of **39** gave **44**, which was readily cyclized to provide **45**. Removal of the Fmoc group in piperidine produced **10**. Compounds **21**–**29** were prepared using routes similar to those in Scheme 2.

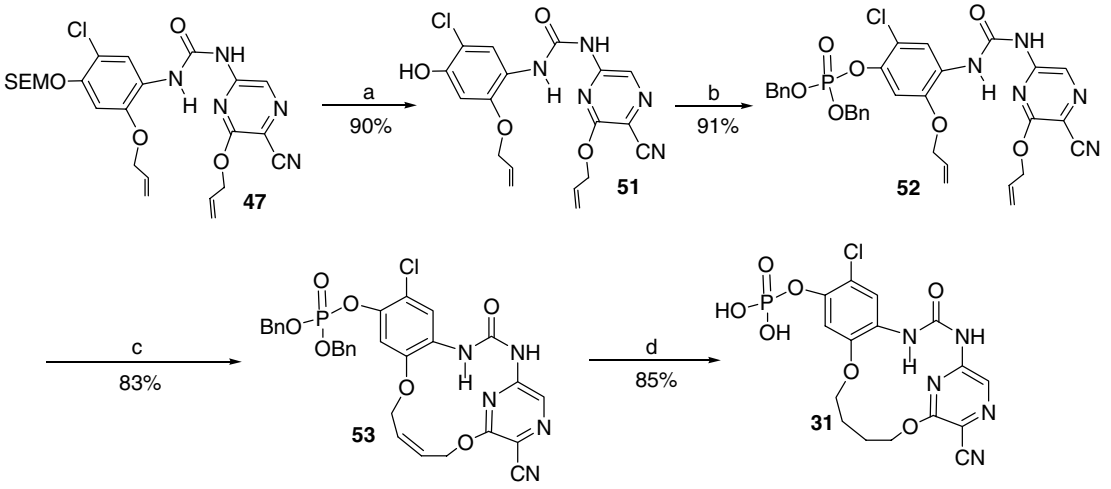
Scheme 3 shows the synthetic routes to macrocyclic ureas in which an oxygen atom links the substituent to the



Scheme 2. Reagents and conditions: (a) RCHO, H_2SO_4, THF, then $NaBH_4$, 0 °C; (b) RCOCl, pyridine, CH_2Cl_2, rt; (c) bromoethyl chloroformate, pyridine, DMF–THF; (d) morpholine, DMF–THF; (e) Fmoc-L-Ala-Cl, aqueous Na_2CO_3 (1 M), DCM; (f) Hoveyda-Grubbs catalyst (2nd), DCM, rt; (g) piperidine, DMF.



Scheme 3. Reagents and conditions: (a) DMF, 50 °C; (b) Hoveyda-Grubbs catalyst, CH_2Cl_2, reflux; (c) HCl, dioxane–CH_2Cl_2–EtOH; (d) H_2, Wilkinson's catalyst, THF, rt; (e) RBr, Cs_2CO_3, DMF; (f) HOAc, THF–H_2O; (g) ROH, polymer-supported PPh_3, DBAD, THF; (h) 1,4′-bipiperidine-1′-carbonyl chloride, pyridine, DMF–THF.



Scheme 4. Reagents and conditions: (a) HCl, dioxane–CH_2Cl_2–EtOH; (b) $P(H)(O)(OBn)_2$, CCl_4, DIEA, DMAP, CH_3CN; (c) Hoveyda-Grubbs catalyst, CH_2Cl_2, reflux; (d) H_2, Wilkinson's catalyst, THF, rt.

4-position of the phenyl ring. Compound **46** was prepared according to a literature procedure.[18] Macrocyclic precursor **48** was synthesized by the olefin metathesis of **47** in the presence of Hoveyda-Grubbs catalyst (2nd). Deprotection of **48** under acidic conditions gave **13** in excellent yield. Hydrogenation of **48** smoothly produced **49**, which was deprotected to provide **30**. Alkylation of **13** with organic bromides gave ethers **50**, which were treated with acetic acid to provide alcohols **14** and **15**. Alternatively, alkylation of **13** by alcohols under Mitsunobu's conditions gave **16** and **17** in good yield. Compound **18** was obtained by the pyridine-catalyzed coupling of **13** with 1,4′-bipiperidine-1′-carbonyl chloride. Compounds **32**–**36** were prepared using routes similar to those in Scheme 3.

The synthesis of **31** is shown in Scheme 4. Compound **51**, obtained by the deprotection of **47**, was phosphorylated with dibenzyl phosphate using a recently reported procedure[23] to give **52** in excellent yield. The ring-closure olefin metathesis of **52** was effected by Hoveyda-Grubbs catalyst to provide **53**. The debenzylation and olefin saturation of **53** were effected simultaneously in the presence of Wilkinson's catalyst under a hydrogen atmosphere to give the final product **31**.

In summary, a new series of potent macrocyclic urea-based Chk1 inhibitors are described. A detailed SAR study on the 4-position of the phenyl ring of **1a** and **d** led to the identification of the potent Chk1 inhibitors **2**, **5**–**7**, **10**, **13**, **14**, **19**, **21**, **25**, **27**, and **31**–**34**. These compounds significantly sensitize tumor cells to the DNA-damaging antitumor agent Dox in a cell-based assay and efficiently abrogate the Dox-induced G2/M and CPT-induced S checkpoints, indicating that their potent biological activities are mechanism-based through Chk1 inhibition. Kinome profiling analysis of a representative macrocyclic urea **25** against a panel of 120 kinases indicates that these novel macrocyclic ureas are highly selective Chk1 inhibitors. The preliminary PK (IP) studies of **1a** and **b** suggest that the 14-member macrocyclic inhibitors may possess better PK properties than their 15-member counterparts. The improved synthesis of **2** and **20** by using Teoc as an amine protecting group not only readily provided the desired compounds in pure form but also facilitated the scale up of potent compounds for various biological studies.

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References and notes

1. Goodman, L. S.; Wintrobe, M. M.; Dameshek, W.; Goodman, J. J.; Gilman, A. *JAMA* **1946**, *132*, 126.
2. Hurley, L. H. *Nat. Rev. Cancer* **2002**, *2*, 188.
3. Goldhirsch, A.; Coates, A. S.; Colleoni, M.; Castiglione-Gertsch, M.; Gelber, R. D. *J. Clin. Oncol.* **1998**, *16*, 1358.
4. Ding, J.; Miao, Z.-H.; Meng, L.-H.; Geng, M.-Y. *Trends Pharmacol. Sci.* **2006**, *27*, 338.
5. Zhou, B.-B.; Bartek, J. *Nat. Rev. Cancer* **2004**, *4*, 216.
6. Tao, Z.-F.; Lin, N.-H. *Anticancer Agents Med. Chem.* **2006**, *6*, 377.
7. Sancar, A.; Lindsey-Boltz, L. A.; Unsal-Kacmaz, K.; Linn, S. *Annu. Rev. Biochem.* **2004**, *73*, 39.
8. Kastan, M. B.; Bartek, J. *Nature* **2004**, *432*, 316.
9. Chen, Z.; Xiao, Z.; Chen, J.; Ng, S. C.; Sowin, T. J.; Sham, H.; Rosenberg, S.; Fesik, S.; Zhang, H. *Mol. Cancer Ther.* **2003**, *2*, 543.
10. Xiao, Z.; Chen, Z.; Gunasekera, A. H.; Sowin, T. J.; Rosenberg, S. H.; Fesik, S.; Zhang, H. *J. Biol. Chem.* **2003**, *278*, 21767.
11. Chen, Z.; Xiao, Z.; Gu, W.-Z.; Xue, J.; Bui, M.; Kovar, P.; Li, G.; Wang, G.; Tao, Z.-F.; Tong, Y.; Lin, N.-H.; Sham,

H. L.; Wang, J. Y.; Sowin, T. J.; Rosenberg, S. H.; Zhang, H. Y. *Int. J. Cancer* **2006**, 2784.
12. Tao, Z.-F.; Wang, L.; Stewart, K. D.; Chen, Z.; Gu, W.; Bui, M.; Merta, P.; Zhang, H.; Kovar, P.; Johnson, E.; Park, C.; Judge, R.; Rosenberg, S.; Sowin, T.; Lin, N.-H. *J. Med. Chem.* **2007**, *50*, 1514.
13. Tao, Z.-F.; Lin, N.-H.; Wang, L.; Sowin, T. J. Macrocyclic kinase inhibitors. US20050215556, 2005.
14. Tao, Z.-F.; Lin, N.-H.; Wang, L.; Sowin, T. J.; Sullivan, G. M.; Tong, Y.; Przytulinska, M.; Li, G. Macrocyclic kinase inhibitors. WO 2005047294, 2005.
15. Chen, X.; Wang, W. *Annu. Rep. Med. Chem.* **2003**, *38*, 333.
16. The Chk1 enzymatic assay was carried out using recombinant Chk1 kinase domain protein covering amino acids from residue 1 to 289 and a polyhistidine tag at the C-terminal end. Human Cdc25c peptide substrate contained a sequence from amino acid residue 204–225. 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 6.3 nM of the recombinant Chk1 protein. For more details, see Refs. 11 and 12.
17. Fraley, M. E.; Steen, J. T.; Brnardic, E. J.; Arrington, K. L.; Spencer, K. L.; Hanney, B. A.; Kim, Y.; Hartman, G. D.; Stirdivant, S. M.; Drakas, B. A., et al. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6049.
18. Tao, Z.-F.; Sowin, T. J.; Lin, N.-H. *Synlett*. **2007**, in press.
19. Ahn, Y. M.; Yang, K.; Georg, G. I. *Org. Lett.* **2001**, *3*, 1411.
20. Maynard, H. D.; Grubbs, R. H. *Tetrahedron Lett.* **1999**, *40*, 4137.
21. Paquette, L. A.; Schloss, J. D.; Efremov, I.; Fabris, F.; Gallou, F.; Mendez-Andino, J.; Yang, J. *Org. Lett.* **2000**, *2*, 1259.
22. Shute, R. E.; Rich, D. H. *Synthesis* **1987**, *4*, 346.
23. Silverberg, L. J.; Dillon, J. L.; Vemishetti, P. *Tetrahedron Lett.* **1996**, *37*, 771.