Bioorganic & Medicinal Chemistry 20 (2012) 1475–1481

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

# Synthesis and evaluation of debromohymenialdisine-derived Chk2 inhibitors

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ARTICLE INFO

*Article history:*
Received 11 November 2011
Revised 20 December 2011
Accepted 23 December 2011
Available online 11 January 2012

*Keywords:*
Checkpoint kinases
Chk2
Natural products
Debromohymenialdisine
Hymenialdisine
Radioprotection

ABSTRACT

Natural products have been the subject of interest for drug discovery and as tools for understanding the underlying cellular pathways in various diseases. We present herein the synthesis and evaluation of new analogs of the marine sponge metabolite, debromohymenialdisine, as checkpoint kinase 2 (Chk2) inhibitors. We illustrate herein that slight modifications to the natural product scaffold can induce strong selectivity for Chk2 over Chk1. These Chk2 inhibitors can serve as drug templates or molecular tools to gain insight in Chk2 mediated radioprotection.



## 1. Introduction

The induction of DNA damage by chemotherapeutic agents or ionizing radiation (IR) is one of the more effective therapy to reduce tumor growth. The effectiveness of the therapy lies in its ability to damage DNA of cancer cells, resulting in an apoptotic cell signal. Unfortunately, the DNA of normal cells is also damaged resulting in part in the harmful side effects of these treatments. Thus, strategies that induce DNA repair in normal cells may have the potential to improve the overall therapeutic treatment.

DNA damage induced by ionizing radiation or chemotherapeutics leads to the activation of DNA sensors including the ataxia telangiectasia mutated (ATM) or/and ataxia telangiectasia mutated and rad-related (ATR) kinases.[1–3] These sensors activate pathways that induce DNA repair or apoptosis. Although many cross-over pathways are possible, activation of the ATR pathway is typically seen following single strand DNA breaks,[4] whereas ATM is activated upon double strand breaks (DSBs),[5] as seen in ionizing radiation (IR) therapy. Following DNA DSBs, ATM is recruited to damage site, undergoes autophosphorylation, and subsequently activates and phosphorylates downstream effector kinases, including checkpoint kinase 2 (Chk2).[6] Chk2 plays a pivotal role in the cell cycle regulation, and thus has become an attractive target in cancer therapy.[1,6–9] Inhibition of Chk2 has been postulated to significantly augment current cancer therapies in two ways.[10] Firstly, some cancerous cells overexpress Chk2 and require high Chk2 levels for survival.[11] Inhibition of Chk2 in those cancer types results in a robust apoptotic response in those cancer types.[12] Secondly, in normal cells, inhibition of Chk2 has indicated radioprotective effects by inhibiting the IR-induced p53 mediated apoptosis,[13] thus indirectly inducing DNA repair. In vivo studies have validated this radioprotective approach and illustrated that the Chk2 −/− mice appear normal, fertile but resistant to IR induced apoptosis and showed increased survival over the wild type mice.[14]

In the recent years inhibition of Chk2 has attracted great attention as a therapeutic target, however only a few Chk2 inhibitors have been reported in the literature, which include the indolocarbazole UCN-01,[15] Gö6976,[16] EXEL-9844,[17] NSC109555,[18,19] PV1019,[12] VRX0466617,[20] 2-(quinazolin-2-yl)phenol (2QP) based inhibitors like CCT241533,[21,22] 2-aminopyridine based inhibitors (2AP),[23] the aryl benzimidazole based inhibitors[24–26] and the natural products hymenialdisine (HMD) and debromohymenialdisine (DBH).[13,27] The use of these Chk2 inhibitors for potentiating DNA-damage agents in cancerous cells and radioprotection of normal cells has become an increasingly promising strategy to improve current chemotherapeutic regimens.

However, several studies report high variability in effectiveness as not all inhibitors of Chk2 demonstrate similar physiological effects. For example, Chk2 inhibitor PV1019 showed potentiation of the DNA-damaging agents, topotecan and camptothecin, in OVCAR-4 and OVCAR-5 human tumor cells.[12] However, the Chk2 inhibitor CCT241533 does not potentiate the cytotoxicity of a selection of genotoxic agents, but potentiates the selectivity of PARP inhibitors in p53 defective cancer cell lines.[21]

Similarly VRX046617 does not potentiate the cytotoxicity of anticancer drugs like doxorubicin, taxol and cisplatin, but selectively inhibits IR-induced Chk2-dependent degradation of Hmdx.[20]

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doi:10.1016/j.bmc.2011.12.054

These studies indicate that different structural classes of Chk2 inhibitors may invoke very different physiological effects when the cells are treated by mechanistically different classes of chemotherapeutics. Thus, the development of different and selective Chk2 inhibitors can be used as tools to aid our understanding of Chk2 mediated phenotypical responses and as potential lead agents for pharmaceutical development.

Previously, debromohymenialdisine (DBH, **1**) and our DBH-derived indoloazepine (**2**) Chk2 inhibitor (Fig. 1) have been found to inhibit both Chk1 and Chk2.[27] We and others demonstrated that, while DBH is a good inhibitor of Chk2, it lacked in kinase selectivity.[13,27–30] However, the DBH-derived indoloazepine was found to be more selective towards the checkpoint kinases and was found to be a nanomolar inhibitor of Chk2 ($IC_{50}$ 8 nM) and Chk1 ($IC_{50}$ 234 nM).[27] More excitingly, the DBH-derived indoloazepine induced strong radioprotection of normal cells from ionizing radiation, without affecting the survival of cancerous cells.[13] In an effort to elucidate the role of the Chk2 pathway involved chemosensitization of cancer cells and radioprotection of normal cells, we set out to improve the selectivity of Chk2 inhibition over Chk1 by this potent class of radioprotecting agents. Herein, we report the synthesis and evaluation of new derivatives of DBH (**3–11**), with significant improvement of Chk2 over Chk1 selectivity.

## 2. Results and discussion

The design of our modifications to the DBH-scaffold was based on the previously reported X-ray crystal structure of DBH-bound in the Chk2 binding pocket.[31] The first set of modifications involved the substitution of hydrogen-atom at the C2-pyrrole position of DBH with an aryl moiety. This change will allow flexibility to the phenyl ring to orient better inside the binding pocket (Fig. 2a), as compared to the DBH-derived indoloazepine (Fig. 1), where phenyl ring was fused with the pyrrole ring making the rigid and planar indolic structure. The second set of modifications involved the alkyl substitution on the exocyclic amine of the glycocyamidine ring. This amine is projected towards the adjacent cavity as shown in Figure 2b. We envisioned that placing substitution at this position will allow for additional interactions in the adjoining cavity.

### 2.1. Chemistry

Compound **12** was prepared by previously published procedure.[32] In order to prepare compounds **13–15** from compound **12**, we utilized the chemistry that we have reported recently (Scheme 1). Treatment of compound **12** with appropriate aryl boronic acid yielded the respective 2-aryl aldisine **13–15**, smoothly in good yields. The aryl aldisines **13–15** were subsequently condensed with 2-(methylthio)-1*H*-imidazol-4(5*H*)-one to give aldol adducts **16–18**. Subsequent treatment of the methylthio-imidazolones **16–18** with ammonium hydroxide led to the formation of respective DBH-analogs **3–5** in 69%, 65% and 52% yields, respectively.

The exocyclic amine on glycocyamidine ring was subsequently modified, in order to explore the effects on Chk2 inhibition. Previous studies had already indicated that secondary amines were depleted of all biological activity,[13] thus only a range primary amines were prepared in these studies. The primary exocyclic amines were prepared by heating thio-imidazolone **16** with appropriate amine in sealed tube, which provided the analogs **6–11** in good yields (Scheme 2).

### 2.2. Biological evaluation and structure–activity relationships

Analogs **3–11** were examined for their ability to inhibit both Chk1 and Chk2 using a HTRF serine/threonine KinEASE assay. All compounds were compared to their parent agent DBH and its indoloazepine analog **2**, with the goal to improve Chk2 selectivity over Chk1, while maintaining low nanomolar Chk2 activity. The results

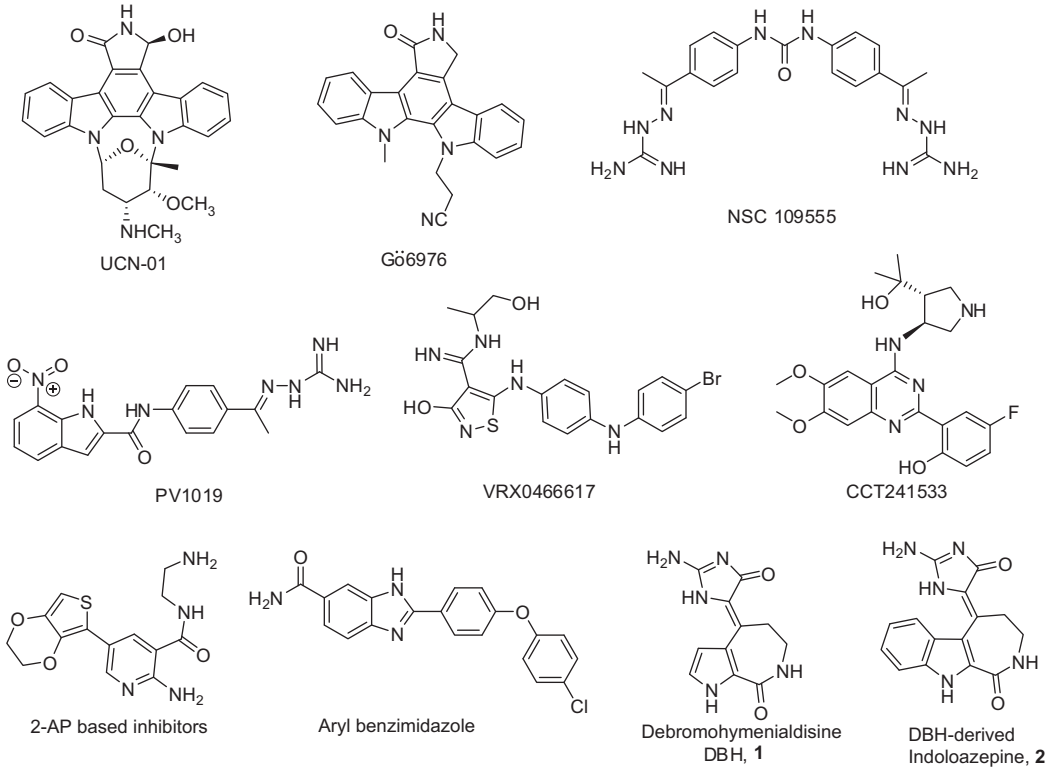



**Figure 1.** Structures of Chk2 inhibitors.

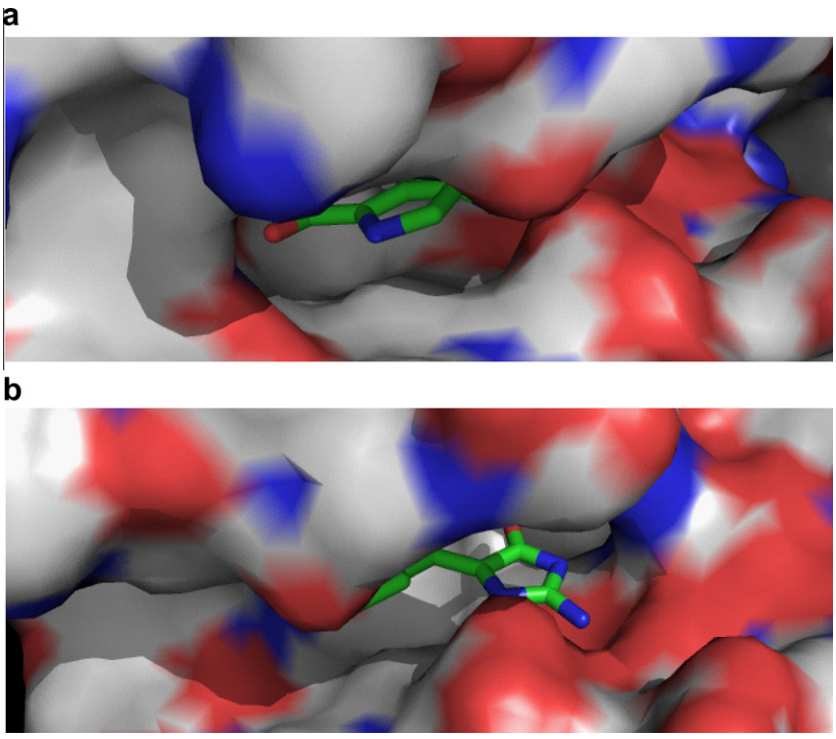
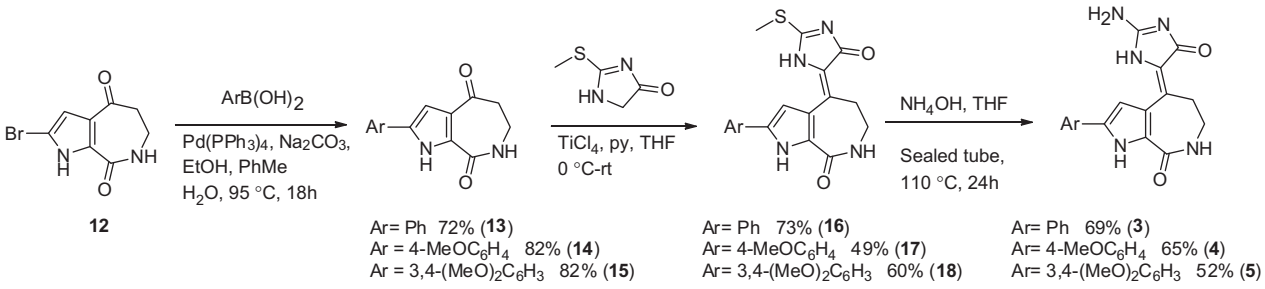
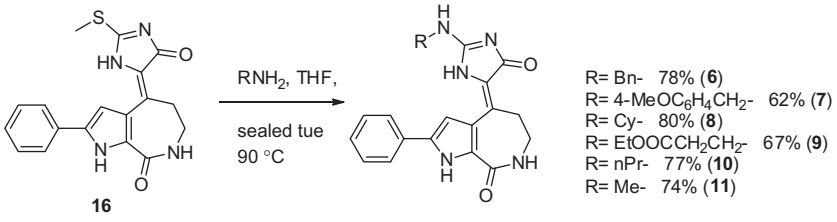



**Figure 2.** Crystal structure of Chk2 with DBH in the binding pocket (pdb code: 2CN8) (a) showing the pyrrolic N-atom and C2-carbon atom (b) showing the amine of glycocyamidine projecting into the adjacent cavity.




**Scheme 1.** Synthesis of compounds **3–5**.




**Scheme 2.** Synthesis of compounds **6–11**.

are shown in Table 1, where the $IC_{50}$ values shown are averages of a minimum of three independent experiments, each performed in duplicate (see Supplementary data Table SI-1 for error margins, means and individual $IC_{50}$ values). As indicated in Table 1, placement of the 2-aryl-pyrole moiety led to significant gains in the selectivity favoring Chk2, while gaining, excellent low nanomolar potency towards Chk2. Incorporation of methoxy substituents did not gain any significant potency over the phenyl analogue **3**. The 3,4-dimethoxy substituted Chk2 inhibitor **5**, achieved excellent selectivity for Chk2 (>100-fold Chk2 over Chk1) while maintaining excellent potency ($IC_{50}$ 14 nM).

Next, we evaluated the activities of various substitutions on the exocyclic amine of the aminoimidazolone moiety. These analogs (compounds **6–11**) were subjected to the same HTRF serine/threonine KinEASE assay and the results are shown in Table 2. Interestingly, the alkylation on the exocyclic nitrogen (compounds **6–11**) resulted in a strong decrease of Chk1 activity compared to the free amine (compound **3**). However, the activity for Chk2 was also

**Table 1**
Kinase profiling of compounds **3–5**

| Compound No. | Ar | $IC_{50}$ in nM (std. dev.) | | Fold |
|---|---|---|---|---|
| | | Chk1 | Chk2 | Chk1/Chk2 |
| DBH (**1**) | H– | 725 | 183 | 4 |
| Indoloazepine-analog (**2**) | Indole | 234 | 8 | 29 |
| **3** | Ph– | 1310 (475) | 20 (5) | 66 |
| **4** | 4-$MeOC_6H_4$– | 867 (239) | 14 (5) | 62 |
| **5** | 3,4-$(MeO)_2C_6H_3$– | 1554 (217) | 14 (13) | 111 |

Standard deviations of all new compounds are shown in parentheses.

**Table 2**
Kinase profiling of compounds **6–11**

| Compound No. | R | $IC_{50}$ in nM (std. dev.) | | Fold |
|---|---|---|---|---|
| | | Chk1 | Chk2 | Chk1/Chk2 |
| **3** | H– | 1310 | 20 | 66 |
| **6** | Bn– | 905 (38) | 28 (13) | 32 |
| **7** | 4-$MeOC_6H_4CH_2$– | >10,000 | 588 (72) | >17 |
| **8** | Cy– | >10,000 | 1526 (90) | >7 |
| **9** | $EtOOCCH_2CH_2$– | >10,000 | 277 (140) | >36 |
| **10** | $^n$Pr– | >10,000 | 156 (46) | >64 |
| **11** | Me– | 6558 (1913) | 171 (37) | 38 |

Standard deviations of all new compounds are shown in parentheses.

decreased in most cases. Simple aliphatic chains, such as seen in compound **10**, indicated good selectivity towards Chk2 over Chk1, comparable to compound **3**, yet at a lower overall potency for Chk2.

Overall, the results indicate that unlike secondary amines, the primary amines show a size based inhibitory ability for Chk2 and significant depletion of Chk1 inhibitory activity. The results also indicate that smaller substituents at this glycocyamidine amine exhibited better $IC_{50}$ values for Chk2 and as the size of the substituent increased, the $IC_{50}$ values deteriorated. The compound **6** showed some deviation to the observation. Nonetheless, the fact that these compounds show the depletion of Chk1 inhibitory activity make them attractive candidates to study the Chk2 mediated phenotypical responses.

## 3. Conclusion

In conclusion, we have presented the synthesis and evaluation of a series of Chk2 inhibitors. Changing some of the substituents on the pyrrolic region has resulted in remarkable increases in the selectivity as compared to the natural product, debromohymenialdisine (DBH), while maintaining excellent low nanomolar potency. Alkylation of the exocyclic amine moiety did present good selectivity for Chk2, but at a cost of potency. We also demonstrated that while the secondary amines at this position lead to the loss of the activity, the analogs with primary exocyclic amines are potent inhibitors of Chk2. These inhibitors could also provide potential lead to the new drug candidates, but in the mean time serve as excellent tools to elucidate some of the complexity of the Chk1–Chk2-mediated cell cycle regulation.

## 4. Experimental section

### 4.1. General methods

All commercial reagents were used without further purification. All solvents were reagent grade. THF was freshly distilled from sodium/benzophenone under nitrogen. $CH_2Cl_2$ was freshly distilled from $CaH_2$ under nitrogen. Column chromatography was carried out on silica gel 60 (230–400 mesh) supplied by EM Science. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. Infrared spectra were recorded on a Nicolet IR/42 spectrometer. Proton and carbon NMR spectra were recorded on a Varian Inova-300 spectrometer or a Varian Inova-500 spectrometer or Varian Inova-600 MHz. High resolution mass spectra were obtained at the Mass Spectrometry Laboratory of the Michigan State University with a QTof-Ultima mass spectrometer.

### 4.2. General procedure for preparing compounds 13–15

Compound **12** (1 mmol) was added in a mixture of toluene and ethanol (3:1, 40 mL) and aryl boronic acid (1.2 mmol) and tetrakis (triphenylphosphine) palladium (5 mol %) were added to the reaction mixture. Then a solution of sodium carbonate (3 mmol) in water (3 mL) was added to the reaction mixture and the mixture was heated to 95 °C for 18 h. Then the reaction mixture was evaporated and the contents were dissolved in ethyl acetate (50 mL) and transferred to a separatory funnel. Then the contents of the flask were washed with 10% aqueous sodium bicarbonate solution (50 mL) and brine (50 mL). The organic layer was dried over anhydrous sodium sulfate (500 mg). The solvent was removed and the crude material was purified by column chromatography (silica, ethyl acetate) to afford product.

#### 4.2.1. 2-Phenyl-6,7-dihydropyrrolo[2,3-*c*]azepine-4,8(1*H*,5*H*)-dione (13)

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.90 (2H, d, $J$ = 7.5 Hz), 7.38 (2H, t, $J$ = 7.5 Hz), 7.28 (1H, t, $J$ = 7.5 Hz), 6.98 (1H, s), 3.48–3.50 (2H, m), 2.78–2.80 (2H, m); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 194.5, 162.1, 135.3, 130.6, 128.9, 128.7, 127.6, 125.4, 124.6, 107.0, 43.7, 36.5; IR (film): 3204, 1644, 1510, 1513, 1467, 1437, 1399, 1363; MS (ES+) $m/z$: 241.1 $[M+H]^+$; mp 222 °C; HRMS (ES+): $m/z$ calcd for $C_{14}H_{13}N_2O_2$ $[M+H]^+$ 241.0977, found, 241.0982.

#### 4.2.2. 2-(4-Methoxyphenyl)-6,7-dihydropyrrolo[2,3-*c*]azepine-4,8(1*H*,5*H*)-dione (14)

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.83 (2H, d, $J$ = 8.6 Hz), 6.94 (2H, d, $J$ = 8.8 Hz), 6.86 (1H, d, $J$ = 2.4 Hz), 3.77 (3H, s), 3.35–3.38 (2H, m), 2.70–2.72 (2H, m); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 194.5, 162.1, 158.9, 135.4, 128.3, 126.8 (s), 124.6, 123.3, 114.1 (s), 105.9 (s), 55.2 (t), 43.7 (d), 36.5 (d); IR (film): 3212, 3135, 2897, 1653, 1636, 1456, 1256 cm$^{-1}$; MS (ES+) $m/z$: 271.1 $[M+H]^+$, mp decomposes above 255–256 °C; HRMS (ES+) calcd for $C_{15}H_{15}N_2O_3$ $[M+H]^+$ 271.1083, found 271.1085.

#### 4.2.3. 2-(3,4-Dimethoxyphenyl)-6,7-dihydropyrrolo[2,3-*c*]azepine-4,8(1*H*,5*H*)-dione (15)

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.57 (1H, d, $J$ = 2.0 Hz), 7.41 (1H, dd, $J$ = 8.4, 2.1 Hz), 6.94 (2H, dd, $J$ = 8.5 Hz), 6.8 (1H, d, $J$ = 2.4 Hz), 3.84 (3H, s), 3.76 (3H, s), 3.38–3.40 (2H, m), 2.72–2.74 (2H, m); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 194.36, 162.3, 148.8, 148.4,

135.7, 128.3, 124.7, 123.5, 117.9 (s), 112.0 (s), 109.2 (s), 106.2 (s), 55.7 (t), 55.5 (t), 43.7 (d), 36.6 (d); IR (film): 2923, 2849, 1653, 1644, 1636, 1491, 1468, 1256 $cm^{-1}$; MS (ES+) *m/z*: 301.1 $[M+H]^+$; mp decomposes above 180 °C; HRMS (ES+) calcd for $C_{16}H_{17}N_2O_4$ $[M+H]^+$ 301.1188, found 301.1192.

### 4.3. General procedure for preparing compounds 16–18

Respective compound **13–15** (1 mmol) was dissolved in THF (25 mL) and 2-(methylthio)-1*H*-imidazol-4(*5H*)-one (2 mmol) was added to the reaction flask. The reaction mixture was cooled to 0 °C and 1 M solution of $TiCl_4$ in DCM (4 mmol) was added to the reaction mixture in drop-wise manner. The reaction mixture was stirred for 30 min and pyridine (8 mmol) was added to the reaction mixture dropwise over 15 min. The reaction mixture was stirred for an additional 14 h allowing it to gradually warm to room temperature. At this point saturated $NH_4Cl$ solution (40 mL) was added to the reaction mixture and contents of the flask were transferred to the separatory funnel. Then the crude product was extracted with ethyl acetate (50 mL × 3). The ethyl acetate fractions were combined and dried over anhydrous $Na_2SO_4$ (500 mg). The solvent was removed and the crude material was purified by column chromatography (silica, EtOAc) to afford product.

#### 4.3.1. (*Z*)-4-(2-(Methylthio)-4-oxo-1*H*-imidazol-5(4*H*)-ylidene)-2-phenyl-4,5,6,7-tetrahydropyrrolo[2,3-*c*]azepin-8(1*H*)-one (16)

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.01 (1H, s), 7.82 (2H, d, *J* = 7.5 Hz), 7.26 (1H, t, *J* = 7.5 Hz), 3.44–3.46 (2H, m), 3.27–3.28 (2H, m), 2.65 (3H, s); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 170.6, 162.8, 158.3, 135.4, 134.3, 133.6, 131.3, 128.7, 128.4, 127.1 (s), 125.0 (s), 124.0, 111.8 (s), 39.1 (d), 30.3 (d), 12.1 (t); IR (film): 3184, 3046, 2929, 1691, 1658, 1623, 1605, 1508, 1470, 1436, 1411 $cm^{-1}$; MS (ES+) *m/z*: 353.1 $[M+H]^+$; mp decomposed over 250 °C; HRMS (ES+) *m/z* calcd for $C_{18}H_{17}N_4O_2S$ $[M+H]^+$ 353.1072, found, 353.1082.

#### 4.3.2. (Z)-2-(4-Methoxyphenyl)-4-(2-(methylthio)-4-oxo-1*H*-imidazol-5(4*H*)-ylidene)-4,5,6,7-tetrahydropyrrolo[2,3-*c*]azepin-8(1*H*)-one (17)

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.90 (1H, s), 7.76 (2H, d, *J* = 8.3 Hz,), 6.95 (2H, d, *J* = 8.3 Hz), 3.77 (3H, s), 3.42–3.45 (2H, m), 3.25–3.27 (2H, m), 2.4 (3H, s); $^{13}$C NMR (125 MHz; DMSO-$d_6$) $\delta$ 170.6, 162.8, 158.6, 135.5, 134.4, 133.5, 131.5, 127.8, 126.4 (s), 124.0, 123.9, 114.2 (s), 110.7 (s), 55.1 (t), 39.2 (d), 30.3 (d), 12.2 (t); IR (film): 2980, 2910, 1676, 1632, 1588, 1478, 1456, 1435, 1252, 1179 $cm^{-1}$; MS (ES+) *m/z*: 383.1 $[M+H]^+$; mp decomposes above 240 °C; HRMS (ES+) calcd for $C_{19}H_{19}N_4O_3S$ $[M+H]^+$ 383.1178; found 383.1185

#### 4.3.3. (*Z*)-2-(3,4-Dimethoxyphenyl)-4-(2-(methylthio)-4-oxo-1*H*-imidazol-5(4*H*)-ylidene)-4,5,6,7-tetrahydropyrrolo[2,3-*c*] azepin-8(1*H*)-one (18)

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.99 (1H, d, *J* = 2.9 Hz), 7.51 (1H, d, *J* = 2.0 Hz), 7.33 (1H, dd, *J* = 8.3, 2.0 Hz), 6.98 (1H, d, *J* = 8.6 Hz), 3.77 (3H, s), 3.83 (3H, s), 3.43–3.45 (2H, m), 3.26–3.28 (2H, m), 2.66 (3H, s); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 170.6, 162.9, 158.1, 148.8, 148.1, 135.5, 134.6, 133.4, 127.8, 124.1, 124.0, 117.2 (s), 112.0 (s), 111.0 (s), 108.8 (s), 55.5 (t), 55.4 (t), 39.2 (d), 30.2 (d), 12.3 (t); IR (film): 2921, 2860, 1686, 1678, 1653, 1636, 1507, 1487, 1456, 1437, 1385, 1248, 1184, 1136 $cm^{-1}$; MS (ES+) *m/z*: 413.2 $[M+H]^+$; mp decomposes above 250 °C; HRMS (ES+) calcd for $C_{20}H_{21}N_4O_4S$ $[M+H]^+$ 413.1284, found 413.1293.

### 4.4. General procedure for preparing compounds 3–5

The methylthio-imidazolone precursor **16–18** (0.32 mmol) was added to THF (5 mL) in a sealable tube and ammonia solution (30%, 5 mL) was added to the solution. The tube was sealed and the reaction mixture was heated at 90 °C for 24 h and then allowed to cool to room temperature. Then the reaction mixture was concentrated and the crude material was purified by column chromatography (silica, MeOH/DCM 1:4) to afford the product.

#### 4.4.1. (*Z*)-4-(2-Amino-4-oxo-1*H*-imidazol-5(4*H*)-ylidene)-2-phenyl-4,5,6,7-tetrahydropyrrolo[2,3-*c*]azepin-8(1*H*)-one (3)

$^1$H NMR (500 MHz, DMSO-$d_6$ + $CF_3COOH$) $\delta$ 7.89 (2H, d, *J* = 7.4 Hz), 7.38 (2H, t, *J* = 7.4 Hz), 7.27 (1H, t, *J* = 7.4 Hz), 6.86 (1H, s), 3.29 (4H, br r); $^{13}$C NMR (125 MHz, DMSO-$d_6$ + $CF_3COOH$) $\delta$ 163.7, 163.5, 154.6, 135.9, 131.1, 130.2, 128.9 (s), 128.2 (s), 127.8, 125.8 (s), 121.6, 121.0, 107.6 (s), 39.2 (d), 33.2 (d); IR (film): 2915, 2857, 2444, 1697, 1683, 1650, 1634, 1621, 1607, 1578, 1560, 1542, 1509, 1470, 1456 $cm^{-1}$; MS (ES+) *m/z*: 322.1 $[M+H]^+$; mp decomposed above 250 °C; HRMS (ES+) *m/z* calcd for $C_{17}H_{16}N_5O_2$ $[M+H]^+$ 322.1310, found 322.1304.

#### 4.4.2. (*Z*)-4-(2-Amino-4-oxo-1*H*-imidazol-5(4*H*)-ylidene)-2-(4-methoxyphenyl)-4,5,6,7-tetrahydropyrrolo[2,3-*c*]azepin-8(1*H*)-one (4)

$^1$H NMR (500 MHz, DMSO-$d_6$ + drop of $CF_3COOH$) $\delta$ 7.84 (2H, d, *J* = 8.5 Hz), 6.96 (2H, d, *J* = 8.5 Hz), 6.76 (1H, s), 3.77 (3H, s), 3.29 (4H, br r); $^{13}$C NMR (125 MHz, DMSO-$d_6$ + drop of $CF_3COOH$) $\delta$ 163.7, 163.6s, 154.7, 136.1, 130.6, 127.8, 127.4 (s), 126.9, 123.9, 121.7, 120.9, 114.5 (s), 106.6 (s), 55.6 (t), 39.6 (d), 33.2 (d); IR (film): 2995, 2935, 1696, 1684, 1653, 1636, 1617, 1559, 1539, 1491, 1456, 1437, 1385, 1260, 1206, 1138 $cm^{-1}$; MS (ES+) *m/z*: 352.1 $[M+H]^+$; mp decomposes above 250 °C; HRMS (ES+) calcd for $C_{18}H_{18}N_5O_3$ $[M+H]^+$ 352.1410, found 352.1412.

#### 4.4.3. (*Z*)-4-(2-Amino-4-oxo-1*H*-imidazol-5(*4H*)-ylidene)-2-(3,4-dimethoxyphenyl)-4,5,6,7-tetrahydropyrrolo[2,3-*c*]azepin-81*H*) -one (5)

$^1$H NMR (600 MHz, DMSO-$d_6$ + drop of $CF_3COOH$) $\delta$ 7.57 (1H, d, *J* = 2.2 Hz), 7.41 (1H, dd, *J* = 8.3, 2.0 Hz), 6.96 (1H, d, *J* = 8.5 Hz), 6.77 (1H, d, *J* = 2.4 Hz), 3.84 (3H, s), 3.77 (3H, s), 3.31 (4H, br r); $^{13}$C NMR (150 MHz, DMSO-$d_6$ + drop of $CF_3COOH$) $\delta$ 163.5, 163.2, 154.4, 149.1, 148.7, 136.0, 130.4, 127.4, 123.9, 121.4, 120.6, 118.2 (s), 112.4 (s), 109.7 (s), 106.4 (s), 55.8 (t), 55.7 (t), 39.2 (d), 32.9 (d); IR (film): 2921, 2851, 1701, 1684, 1653, 1558, 1489, 1473, 1456, 1258, 1204, 1181 $cm^{-1}$; MS (ES+) *m/z*: 382.2 $[M+H]^+$; mp decomposes above 250 °C; HRMS (ES+) calcd for $C_{19}H_{20}N_5O_4$ $[M+H]^+$ 382.1515, found 382.1526.

### 4.5. General procedure for preparing compounds 6–11

Compound **16** (1 mmol) was added to THF (5 mL) in a sealable tube and appropriate alkylamine (4 mmol) was added to the solution. The tube was sealed and the reaction mixture was heated at 90 °C for 24 h and then allowed to cool to room temperature. Then the reaction mixture was concentrated and the crude material was purified by column chromatography (silica, MeOH/DCM 1:9) to afford the product.

#### 4.5.1. (*Z*)-4-(2-(Benzylamino)-4-oxo-1*H*-imidazol-5(4*H*)-ylidene)-2-phenyl-4,5,6,7-tetrahydropyrrolo[2,3-*c*]azepin-8(1*H*)-one (6)

$^1$H NMR (600 MHz, DMSO-$d_6$ + drop of $CF_3COOH$) $\delta$ 7.86 (2H, d, *J* = 7.8 Hz), 7.35–7.39 (5H, m), 7.25–7.30 (2H, m), 6.87 (1H, s), 4.59 (2H, br r), 3.31 (4H, br r); $^{13}$C NMR (150 MHz, DMSO-$d_6$ + drop of $CF_3COOH$) $\delta$ 163.8, 163.7, 154.0, 136.5, 136.0, 131.1, 130.6, 128.9 (s), 128.4, 128.1 (s), 127.9 (s), 127.7 (s), 127.6, 125.8 (s), 121.7, 121.0, 107.7 (s), 46.3 (d), 39.2 (d), 33.2 (d); IR (film): 3077, 2924, 2800, 1690, 1680, 1640, 1489, 1427, 1200, 1136 $cm^{-1}$; MS (ES+) *m/z*: 412.2 $[M+H]^+$; mp decomposes above 220–222 °C HRMS (ES+) calcd for $C_{24}H_{22}N_5O_2$ $[M+H]^+$ 412.1774, found 412.1778.

#### 4.5.2. (*Z*)-4-(2-((4-Methoxybenzyl)amino)-4-oxo-1*H*-imidazol-5(4*H*)-ylidene)-2-phenyl-4,5,6,7-tetrahydropyrrolo[2,3-*c*]azepin-8(1*H*)-one (7)

$^1$H NMR (600 MHz, DMSO-$d_6$ + drop of $CF_3COOH$) $\delta$ 7.88 (2H, d, $J$ = 7.6 Hz), 7.36–7.40 (2H, m), 7.26–7.32 (3H, m), 6.92–6.96 (2H, m), 6.87 (1H, s), 4.52 (2H, d, $J$ = 5.4 Hz), 3.87 (1H, s), 3.73 (3H, s), 3.31 (4H, br r); $^{13}$C NMR (150 MHz, DMSO-$d_6$ + drop of $CF_3COOH$) $\delta$ 163.7, 163.4, 159.2, 153.6, 135.7, 130.9, 130.2, 129.1 (s), 129.0, 128.6 (s), 128.1, 127.7 (s), 125.6 (s), 121.4, 120.7, 114.4 (s), 107.5 (s), 55.2 (t), 45.6 (d), 39.3 (d), 32.9 (d); IR (film): 2922, 2849, 1696, 1680, 1634, 1516, 1476, 1433, 1203, 1180, 1132 cm$^{-1}$; MS (ES+) $m/z$: 442.2 $[M+H]^+$; mp decomposes above 250 °C; HRMS (ES+) calcd for $C_{25}H_{24}N_5O_3$ $[M+H]^+$ 442.1879, found 442.1880.

#### 4.5.3. (*Z*)-4-(2-(Cyclohexylamino)-4-oxo-1*H*-imidazol-5(4*H*)-ylidene)-2-phenyl-4,5,6,7-tetrahydropyrrolo[2,3-*c*]azepin-8(1*H*)-one (8)

$^1$H NMR (500 MHz, $CD_3OD$ + drop of $CF_3COOH$, 50 °C) $\delta$ 7.75 (2H, d, $J$ = 7.6 Hz), 7.42 (2H, t, $J$ = 7.6 Hz), 7.33 (1H, t, $J$ = 7.6 Hz), 6.89 (1H, s), 3.46 (4H, br r), 1.98 (2H, br r), 1.80 (2H, br r r), 1.66 (1H, d, $J$ = 12.9 Hz), 1.41–1.47 (4H, m), 1.28 (1H, br r); $^{13}$C NMR (125 MHz, $CD_3OD$ + drop of $CF_3COOH$, 50 °C) $\delta$ 166.0, 164.1, 160.3, 154.0, 138.5, 132.4, 132.1, 130.0, 129.2, 128.3 (s), 126.5 (s), 124.0 (s), 105.7 (s) 54.4, 40.9 (d), 33.5 (d), 32.0 (d), 26.0 (d), 25.3 (d); IR (film): 2930, 2855, 2800, 1727, 1700, 1676, 1615, 1663, 1516, 1466, 1450, 1205, 1181, 1136 cm$^{-1}$; MS (ES+) $m/z$: 404.2 $[M+H]^+$; mp decomposes above 250 °C; HRMS (ES+) calcd for $C_{23}H_{26}N_5O_2$ $[M+H]^+$ 404.20887, found 404.2088.

#### 4.5.4. (*Z*)-Ethyl 3-((4-oxo-5-(8-oxo-2-phenyl-5,6,7,8-tetra hydropyrrolo[2,3-*c*]azepin-4(1*H*)-ylidene)-4,5-dihydro-1*H*-imidazol-2-yl)amino)propanoate (9)

$^1$H NMR (500 MHz, DMSO-$d_6$ + drop of $CF_3COOH$) $\delta$ 7.88 (2H, d, $J$ = 7.6 Hz), 7.39 (2H, t, $J$ = 7.6 Hz), 7.29 (1H, t, $J$ = 7.6 Hz), 6.84 (1H, br r), 4.08 (2H, q, $J$ = 7.1 Hz), 3.59 (2H, br r), 3.31 (4H, br r), 2.71–2.65 (2H, m), 1.18 (3H, t, $J$ = 7.1 Hz); $^{13}$C NMR (125 MHz, DMSO-$d_6$ + drop of $CF_3COOH$) $\delta$ 171.0, 163.6, 163.5, 153.9, 135.8, 131.0, 130.2, 128.8 (s), 128.2, 127.8 (s), 125.7 (s), 121.4, 120.7, 107.3 (s), 60.5 (d), 39.9 (d), 39.8 (d), 33.3 (d), 33.1 (d), 14.1 (t); IR (film): 3222, 2921, 2993, 1728, 1717, 1696, 1686, 1653, 1636, 1617, 1203, 1138 cm$^{-1}$; MS (ES+) $m/z$: 422.2 $[M+H]^+$; mp 227–229 °C; HRMS (ES+) calcd for $C_{22}H_{24}N_5O_4$ $[M+H]^+$ 422.1828, found 422.1831.

#### 4.5.5. (*Z*)-4-(4-Oxo-2-(propylamino)-1H-imidazol-5(4H)-ylidene)-2-phenyl-4,5,6,7-tetrahydropyrrolo[2,3-*c*]azepin-8(1*H*)-one (10)

$^1$H NMR (600 MHz, DMSO-$d_6$ + drop of $CF_3COOH$) $\delta$ 7.88 (2H, d, $J$ = 7.6 Hz), 7.39 (2H, t, $J$ = 7.7 Hz), 7.28 (1H, t, $J$ = 7.7 Hz), 6.86 (1H, d, $J$ = 2.2 Hz), 3.22–3.36 (6H, m), 1.49–1.58 (2H, m), 0.88 (3H, t, $J$ = 7.3 Hz); $^{13}$C NMR (150 MHz, DMSO-$d_6$ + drop of $CF_3COOH$) $\delta$ 163.7, 163.4, 153.6, 135.7, 130.9, 129.7, 128.7 (s), 128.0, 127.6 (s), 125.6 (s), 121.4, 120.7, 107.4 (s), 44.5 (d), 39.2 (d), 32.9 (d), 22.3 (d), 10.7 (t); IR (film): 2920, 1700, 1684, 1635, 1472, 1435, 1385, 1264, 1206, 1132 cm$^{-1}$; MS (ES+) $m/z$: 364.2 $[M+H]^+$; mp decomposes above 270 °C; HRMS (ES+) calcd for $C_{20}H_{22}N_5O_2$ $[M+H]^+$ 364.1774, found 364.1777.

#### 4.5.6. (*Z*)-4-(2-(Methylamino)-4-oxo-1*H*-imidazol-5(4*H*)-ylidene)-2-phenyl-4,5,6,7-tetrahydropyrrolo[2,3-*c*]azepin-8(1*H*)-one (11)

$^1$H NMR (500 MHz, DMSO-$d_6$ + drop of $CF_3COOH$) $\delta$ 7.89 (2H, d, $J$ = 7.8 Hz), 7.37–7.41 (2H, t, $J$ = 7.8 Hz), 7.22 (1H, t, $J$ = 7.8), 6.85 (1H, s), 3.31 (4H, br r), 2.97 (3H, d, $J$ = 4.6 Hz); $^{13}$C NMR (125 MHz, DMSO-$d_6$ + drop of $CF_3COOH$) $\delta$ 163.6, 163.3, 154.3, 135.6, 130.9, 129.7, 128.7 (s), 128.1, 127.6 (s), 125.6 (s), 121.4, 120.8, 107.5 (s), 39.1 (d), 32.8 (d), 29.6 (t); IR (film): 2975, 2920, 1701, 1684, 1635, 1617, 1676, 1559, 1437, 1385, 1265, 1198, 1191, 1128 cm$^{-1}$; MS (ES+) $m/z$: 336.1 $[M+H]^+$; mp 240-242 °C; HRMS (ES+) calcd for $C_{18}H_{18}N_5O_2$ $[M+H]^+$ 336.1461, found 336.1464.

### 4.6. Biological methods

The HTRF KinEASE STK1 kit (from Cisbio/Millipore) was used to evaluate the serine/threonine kinase activity of Chk1 and Chk2 according to the manufacturer's instructions. In short, Chk1 and Chk2 activity was analyzed in a white 96-well half volume plate in a final reaction volume of 50 μL, human Chk1 or Chk2 (5–10 m U) was incubated with various concentrations of test agent or vehicle (a final DMSO vehicle concentration of 0.2%), STK1 substrate (50 nM for Chk1 and 1 μM for Chk2) in 50 mM HEPES pH 7.0 supplemented with 5 mM $MgCl_2$ and 1 mM DTT. The kinase reaction was initiated with the addition of 100 μM ATP. After incubation for 10 min at 30 °C, the reaction was stopped by the addition of 25 μL Sa-XL665 and 25 μL STK Antibody-Eu(K) in EDTA. The plate was sealed and incubated for 1 h at room temperature. The resulting TR-FRET signal was measured on a SpectaMaxM5e plate reader. The fluorescence emission was measured at 620 nm (cryptate) and 665 nm (XL665). A ratio was calculated (665/620) for each well and the results were expressed as follows: specific signal = ratio (sample)−ratio (negative control), where ratio = (665 nm/620 nm) × $10^4$.

### Acknowledgments

The authors gratefully acknowledge financial support in part from Michigan State University and the Fulbright Scholarship Program for funding for this work.

### Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.12.054.

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