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# Benzothiophene inhibitors of MK2. Part 2: Improvements in kinase selectivity and cell potency

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ABSTRACT

Optimization of kinase selectivity for a set of benzothiophene MK2 inhibitors provided analogs with potencies of less than 500 nM in a cell based assay. The selectivity of the inhibitors can be rationalized by examination of X-ray crystal structures of inhibitors bound to MK2.



In Part 1 of this Letter we described a new class of benzothiophene inhibitors of mitogen activated protein kinase-activated protein kinase 2 (MK2). While these compounds were potent inhibitors of MK2 with sub-micromolar cellular potencies, selectivity against other kinases, including CDK2, was not achieved. In this Letter, strategies used to improve the selectivity of this class of inhibitors are described.

Previously we had disclosed that placing a rigid group near the hinge binding element resulted in remarkable selectivity enhancement for a different chemical class of MK2 inhibitors.[1] For example, compound **1** was found to possess good selectivity against a number of kinases, and this selectivity was attributed to the rigid aryl group attached to the 2-position of the pyridine. Based on this precedent, we sought to modify the hinge binding element in the benzothiophene class (e.g., **2**) to provide an attachment point for this selectivity element as shown in **3**.

To assess this hypothesis, compounds were prepared with an additional ring fused to the benzothiophene ring, which would provide a rigid attachment point for a selectivity element. To verify that the hetero atom, as part of an additional fused ring, could be an effective hinge-binding element, compounds without the selectivity element were first prepared (Fig. 1).

The synthesis of furan and dihydro–furan analogs is shown in Scheme 1. Chlorobenzothiophene **4**[2] was demethylated with $BBr_3$ and alkylated with allyl bromide to provide **5**. Heating **5** to 200 °C in diethylaniline regioselectively provided **6** in quantitative yield. Treatment of **6** with $OsO_4/NaIO_4$ gave a tautomeric mixture of **7** and **8**. This mixture was converted to furan **9** by dehydration in phosphoric acid. Dihydrofuran **10** was prepared by sodium borohydride reduction, mesylate formation of the resulting primary alcohol and cyclization with sodium bicarbonate. Chlorobenzothiophenes **9** and **10** were then elaborated as described in Part 1 of this series by Buchwald coupling with **11**, followed by deprotection and cyclization to afford diazapenes **12** and **13**.

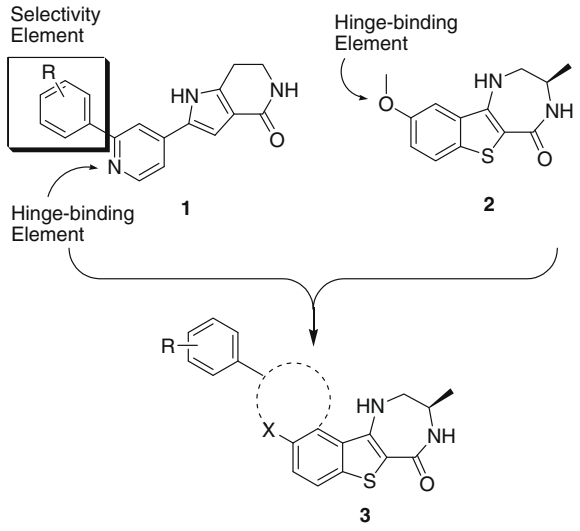


**Figure 1.** Rationale for improving selectivity of benzothiophene inhibitors of MK2.

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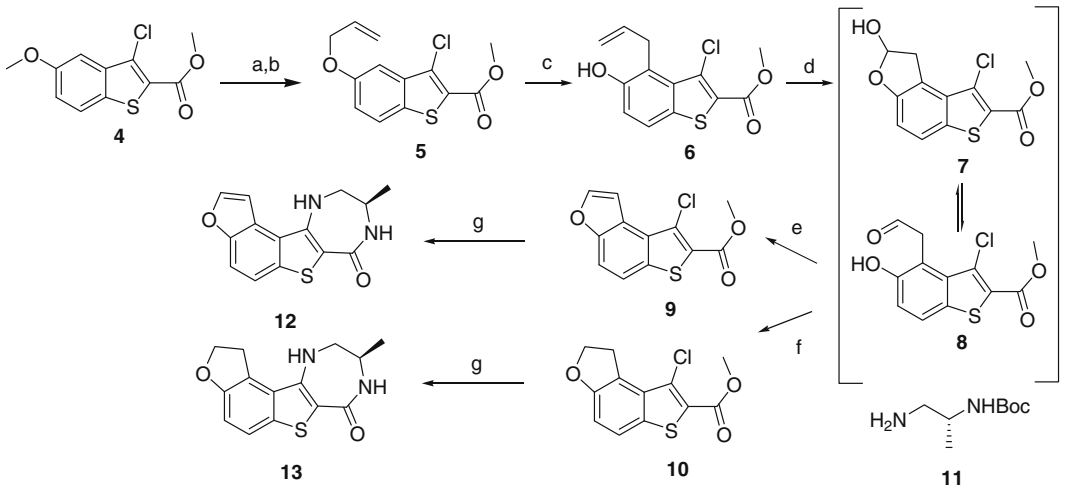
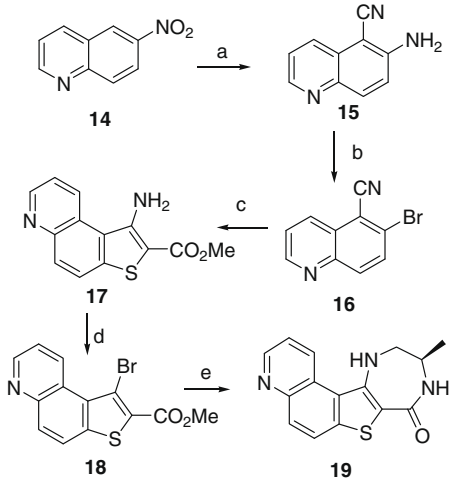


**Scheme 1.** Reagents and conditions: (a) $BBr_3$; (b) allyl bromide, $K_2CO_3$, DMF; (c) $PhNEt_2$, 200 °C; (d) $OsO_4/NaIO_4$; (e) $H_3PO_4$, 120 °C; (f) (i) $NaBH_4$; (ii) MsCl; (iii) $NaHCO_3$; (g) (i) (*R*)-propane-1,2-diamine, $Pd_2(dba)_3$ (5 mol %), (±)BINAP (10 mol %), $Cs_2CO_3$ (2 equiv) toluene, 110 °C, 24 h; (ii) TFA/$CH_2Cl_2$; (iii) NaOMe/MeOH.

The synthesis of an analog with a pyridine-containing binding element is shown in Scheme 2. Nitroquinoline **14** was treated with ethyl cyanoacetate and KOH in DMF to provide aminocyanoquinoline **15**.[3] The amino group was converted to bromide via diazotization to provide **16**. Nucleophilic aromatic substitution on the resulting bromide with methyl thioglycolate and subsequent thiophene formation produced **17**. Conversion of the amino group in **17** to bromide **18** was accomplished by diazotization and substitution. The resulting bromide was then elaborated as described for **9** and **10** to produce diazapene **19**.

The potencies of the analogs prepared in Schemes 1 and 2 for MK2, CDK2 and TNFα production in LPS-stimulated U937 cells are reported in Table 1. All three hinge binding replacements are potent MK2 and CDK2 inhibitors. Furan analog **12** and pyridine analog **19** in particular were found to be exceptionally potent inhibitors of MK2 and had $IC_{50}$ values of less than 100 nM in the cell assay. Broad panel kinase selectivity screening of **12** was conducted. Potencies for a total of 109 kinases were evaluated at 1 μM concentration and 26 kinases (24%) showed >70% inhibition.

In an attempt to improve the selectivity of these inhibitors for MK2, compounds with aromatic substituents in the 2-position



**Scheme 2.** Reagents and conditions: (a) (i) ethyl cyanoacetate, KOH, DMF; (ii) HCl; (b) (i) $NaNO_2$; (ii) HBr; (c) Methyl mercaptoacetate, NaOMe, MeOH; (d) t-BuONO, $CuBr_2$; (e) (i) (*R*)-propane-1,2-diamine, $Pd_2(dba)_3$ (5 mol %), (±)BINAP (10 mol %), $Cs_2CO_3$ (2 equiv) toluene, 110 °C, 24 h; (ii) TFA/$CH_2Cl_2$; (iii) NaOMe/MeOH.

**Table 1**
MK2 and CDK2 potencies of analogs **2**, **12–13**, **19**

| Compound number | MK2 inhibition $IC_{50}$[a] (μM) | CDK2 inhibition $IC_{50}$[a] (μM) | U937 TNFα release $IC_{50}$[a] (μM) |
|---|---|---|---|
| **2** | 0.04 | 0.012 | 0.7 |
| **12** | 0.016 | 0.001 | 0.09 |
| **13** | 0.028 | 0.005 | 0.26 |
| **19** | 0.001 | 0.0008 | 0.05 |

[a] Values are means of at least three experiments and standard deviations were within 50% of the reported value Assays conditions are the same as described in Ref. 1.

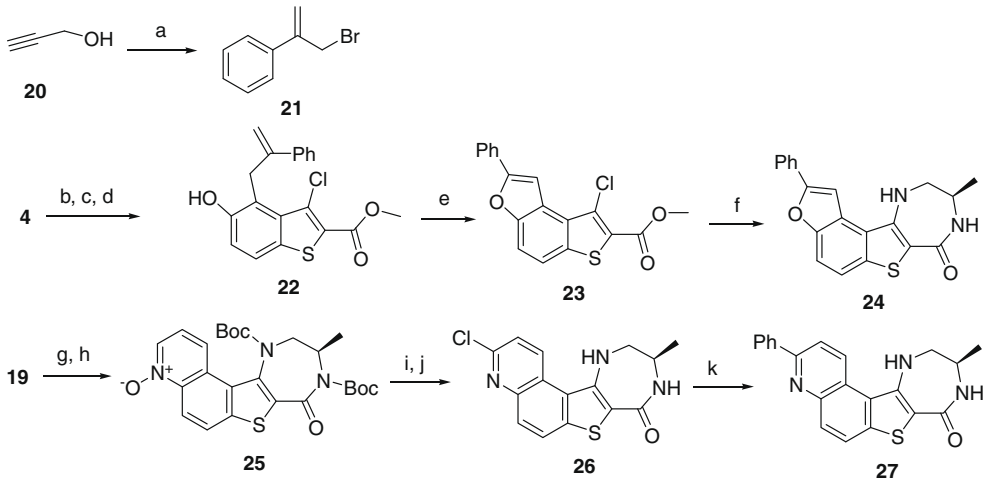
were prepared. The synthesis of these analogs is shown in Scheme 3. Propargyl alcohol (**20**) was converted to allyl bromide **21** and was used in the alkylation as described previously in the conversion of **4** to **5**. The remaining sequence of rearrangement, oxidative cleavage of the alkene and dehydration produced **24**. Analog **19** was doubly protected with boc groups and the pyridine oxidized to form **25**. Reaction with oxalyl chloride and removal of the boc protecting groups provided intermediate **26**. Suzuki coupling with phenyl boronic acid yielded compound **27**.

The potencies of these analogs for MK2, CDK2 and in cell based TNFα assay are shown in Table 2. Substitution of the furan ring did not improve selectivity for MK2 over CDK2 and a loss of overall potency was observed for both kinases. Similarly, a loss of cellular potency was also observed. Broad panel kinase selectivity screening of **24** was conducted. A total of 126 kinases were profiled at 1 μM concentration and 14 kinases (11%) showed >70% inhibition.

Substitution at the pyridine ring was less detrimental to potency. Less than a 10-fold drop in MK2 potency was observed comparing **19** to **27**; however the ratio of MK2 to CDK2 potency was improved approximately 20-fold. This improvement in selectivity may be rationalized by the different trajectories of the aromatic ring emanating from a 5-versus a 6-membered ring. Furthermore, the cell potency was well below 1 μM. Compound **26** had MK2 potency and CDK2 selectivity intermediate between **19** and **27** suggesting that the size of the 2-substituent may play a role in both potency and selectivity.

Intermediate **26** provided a convenient late stage intermediate for facile analog synthesis via Suzuki coupling. Results are summarized in Table 3.

The analogs described in Table 3 are within approximately 10-fold in potency for MK2 indicating that most substitutions are well tolerated. Many analogs have improved selectivity ratios compared




**Scheme 3.** Reagents and conditions: (a) (i) PhMgBr, CuI; (ii) $PPh_3$, $CBr_4$; (b) BBr; (c) $K_2CO_3$; (d) $PhNEt_2$, 200 ° C; (e) (i) $OsO_4$, $NaOI_4$; (ii) $H_3PO_4$, 120 °C; (f) (i) (*R*)-propane-1,2-diamine, $Pd_2(dba)_3$ (5 mol %), (±)BINAP (10 mol %), $Cs_2CO_3$ (2 equiv), toluene, 110 °C, 24 h; (ii) TFA/$CH_2Cl_2$; (iii) NaOMe/MeOH; (g) $Boc_2O$, DMAP, TEA; (h) *m*-CPBA (i) $(COCl)_2$, DMF; (j) HCl; (k) $PhB(OH)_2$, $Pd(PPh_3)_4$, $Na_2CO_3$, 80 °C.

**Table 2**
MK2 and CDK2 potencies of analogs **24**, **26–27**

| Compound number | MK2 inhibition $IC_{50}$[a] (μM) | CDK2 inhibition $IC_{50}$[a] (μM) | U937 TNFα release $IC_{50}$[a] (μM) |
|---|---|---|---|
| **24** | 0.15 | 0.25 | 6.8 |
| **26** | 0.002 | 0.025 | 0.13 |
| **27** | 0.009 | 0.2 | 0.18 |

[a] Values are means of at least three experiments and standard deviations were within 50% of the reported value. Assays conditions are the same as described in Ref. 1.

to **27**, however, indicating that substitution in this region has an impact on selectivity. Compounds **29** and **35** have cell potency values below 500 nM and have >1000-fold selectivity for MK2 versus CDK2.

To better understand the potency and selectivity of these compounds, a crystal structure of **35** in MK2 was obtained at 3.7 Å and is shown in Figure 2. While this is a low resolution crystal structure, some analysis may be made. The lactam carbonyl interacts with the conserved Lys93 and the Asp207 of the activation loop. The 3-pyridyl nitrogen appears to make no meaningful interaction with MK2, but the group occupies the same space as the previously described selective series of MK2 inhibitors.

**Table 3**
MK2 and CDK2 potencies of analogs **28–35**

| Compound number | R | MK2 inhibition $IC_{50}$[a] (μM) | CDK2 inhibition $IC_{50}$[a] (μM) | U937 TNFα release $IC_{50}$[a] (μM) |
|---|---|---|---|---|
| **28** | 4-Pyridyl | 0.014 | 0.39 | 0.74 |
| **29** | 3-Pyridyl | 0.005 | 6.33 | 0.22 |
| **30** | 5-Pyrimidinyl | 0.023 | 10.8 | 0.64 |
| **31** | 2-Methoxyphenyl | 0.020 | 2.73 | 1.04 |
| **32** | 3-Methoxyphenyl | 0.041 | 2.04 | 0.69 |
| **33** | 2-Fluorophenyl | 0.014 | 10.9 | 0.93 |
| **34** | 2-Methylphenyl | 0.03 | 2.21 | 3.36 |
| **35** | 4-Methyl-3-pyridyl | 0.005 | 7.92 | 0.15 |

[a] Values are means of at least three experiments and standard deviations were within 50% of the reported values. Assays conditions are the same as described in Ref. 1.

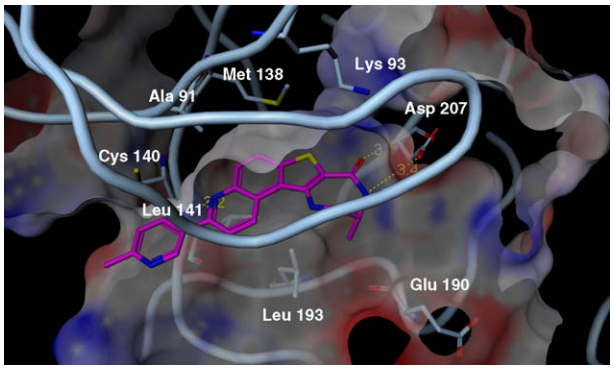



**Figure 2.** X-ray crystal structure of **35** in MK2 (3.7 Å resolution 3FYJ).

The selectivity of **29** and **35** was investigated by screening against a subset panel of kinases comprised of targets that previous series of compounds had inhibitory activity against. Of the 50 kinases targeted for screening, only 4 had >70% inhibition at 1 μM (AMPK, PIM1, MEKK5 and BRSK1).

The mechanism of action within the cell based assay was assessed by comparing inhibition of phosphorylation of Ser78 on HSP27 within the cell to TNFα suppression as described previously.[1] The $IC_{50}$ values for phosphorylated HSP27 were within 2 fold of TNFα $IC_{50}$ values for both of these inhibitors with no effect on levels of phospho-p38 or phospho-JNK2. The correlation between a target biomarker and suppression of cytokine release indicates that both **29** and **35** are inhibiting TNFα expression by selectively inhibiting MK2.

In summary, we have discovered a new, potent and selective class of MK2 inhibitors that have less than 500 nM potency in a cell-based assay. Selectivity was improved by modification of the hinge binding element into a ring to provide a scaffold for the required selectivity element. The in vivo pharmacology of these inhibitors will be described in a future communication.

## References and notes

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