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Benzothiophene inhibitors of MK2. Part 1: Structure–activity relationships, assessments of selectivity and cellular potency

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ABSTRACT

Identification of potent benzothiophene inhibitors of mitogen activated protein kinase-activated protein kinase 2 (MK2), structure–activity relationship (SAR) studies, selectivity assessments against CDK2, cellular potency and mechanism of action are presented. Crystallographic data provide a rationale for the observed MK2 potency as well as selectivity over CDK2 for this class of inhibitors.

Mitogen activated protein kinase-activated protein kinase 2 (MK2) is a Ser/Thr protein kinase in the calcium/calmodulin-dependent protein kinase family. MK2 is a direct substrate of p38 kinase and is important for cytokine production.[1–3] MK2 knockout mice show reduced expression of TNFα when stimulated with lipopolysaccharide (LPS) and are resistant to developing disease in arthritis models.[2,3] MK2 knockout mice have a normal phenotype suggesting that MK2 may be a safe target for therapeutic intervention.

A number of chemotypes have been reported as MK2 inhibitors by us as well as others.[4–9] In the current study we report a different chemical class, benzothiophenes, with a general structure shown in Figure 1.

This chemotype is similar to previously reported structures in that it has a five-membered ring fused to a lactam.[5–9] We anticipated a similar binding mode with the 7-methoxy group forming a hydrogen bond in the hinge region (Leu141 backbone NH) and the lactam portion binding to the conserved catalytic Lys93 and Asp207 in the active site of MK2 (Fig. 2a).

The synthesis of this class is shown in Scheme 1. Cinnamic acid **2** was converted to intermediate **3** with thionyl chloride in chlorobenzene.[10] Direct displacement of the chlorides with amines was not successful, but palladium catalyzed coupling of **3** and Boc-protected diamines **4–8** using (±)-BINAP as the catalyst gave the desired adduct in good yields.[11] Sequential deprotection with TFA and cyclization with NaOMe/MeOH gave the target compounds.

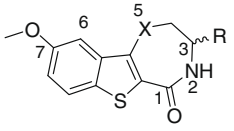

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Fig. 1. General structure of benzothiophene MK2 inhibitors.

Protected diamines **5–7** were prepared via borane reduction of the primary amides of each enantiomer of alanine.[12]

Scheme 2 shows the synthesis of compounds designed to test the role of the nitrogen atom at the 5-position and further elaboration of compound **13**. In order to replace the nitrogen with sulfur, the chlorine on intermediate **3** was displaced with a protected amino thiol **14** followed by deprotection and cyclization to yield analog **15**. For comparison, the carbon analog was prepared by treating protected allyl amine **16** with 9-BBN followed by Suzuki coupling to **3**. Deprotection and cyclization of **3** produced analog **17**. Alcohol **13** was activated as the mesylate and displaced with sodium azide. Reduction with triphenyl phosphine gave the primary amine **18**.

The potencies of the analogs described in Schemes 1 and 2 for MK2, CDK2 and TNFα production an LPS-stimulated U937 TNFα cell assay are reported in Table 1.

Nitrogen containing analogs **9–11** were more potent than the carbon and sulfur analogs (compare **9, 15**, and **17**). The configuration of the stereogenic center in **10** and **11** appears to be important with analog **10** being about 10-fold more potent against both MK2 and CDK2 compared to its enantiomer. Interestingly, this improvement in enzyme potency is reflected in improved cell potency as

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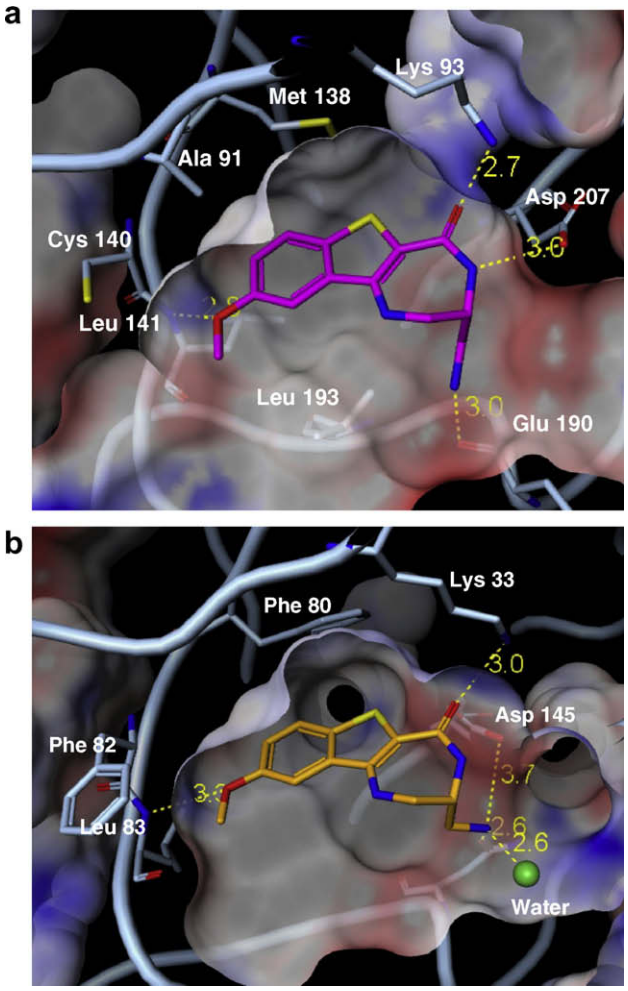
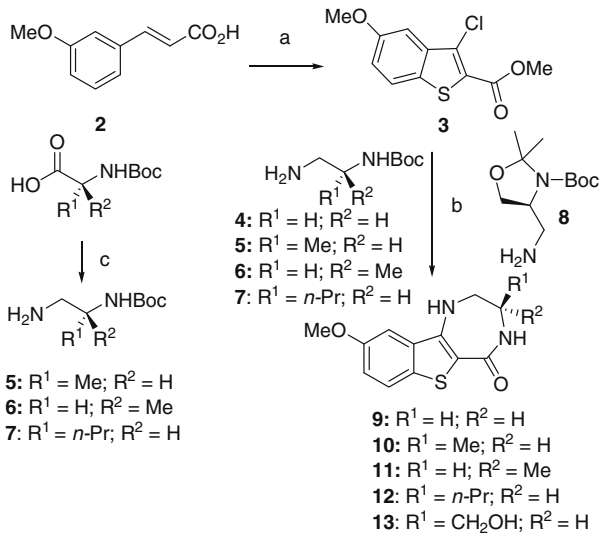


Fig. 2. X-ray crystal structures of **18** in the complex with a) MK2 (3.5 Å resolution, 3FYK) and b) CDK2 (1.9 Å resolution, 3FZ1). The structures revealed different engagement of the aminomethyl in the two protein kinases that provides rational for selectivity for MK2 over CDK2.

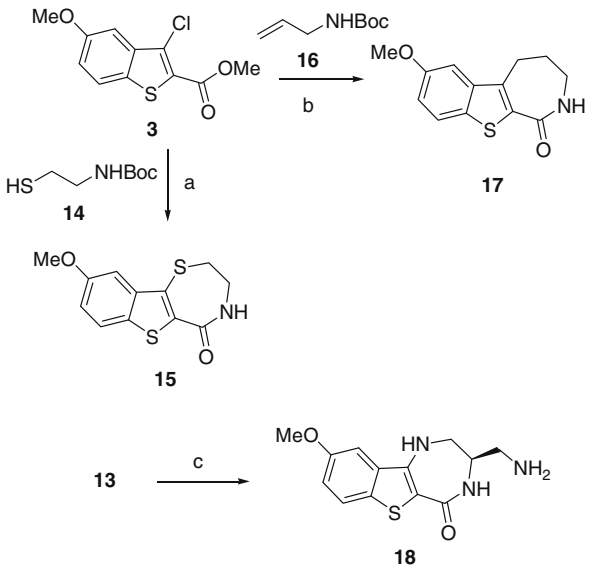
well. Compounds **13** and **18** introduce a heteroatom onto the diazapenone substituent resulting in a gain in potency for MK2 and in the case of **18** an improvement in CDK2 selectivity. However, only for alcohol **13** is this improvement in enzyme potency manifested in an improvement in cell potency. Presumably this is due to reduced permeability of the analog with the primary amine being predominantly ionized in solution. Extension of the chain without a heteroatom did result in a loss of potency (compound **12**) suggesting that the heteroatom (oxygen or nitrogen) could be engaged in a direct or water-mediated hydrogen bonding interaction with the protein.

Most of these compounds (with the exception of **18**) are not selective for MK2 over CDK2: they are nearly equipotent to both MK2 and CDK2. Broad kinase panel profiling of **10** revealed that it is a potent inhibitor of numerous kinases with less than 10-fold selectivity against 18 of the 90 kinases profiled. To better understand contributions to potency and selectivity, X-ray crystal structures of compound **18** were obtained with MK2 (at 3.5 Å resolution) and CDK2 (at 1.9 Å resolution) co-crystals (Fig. 2). While the resolution of the MK2 structure is not very high, some analysis can be done to account for the discrepancy of the observed potencies.

Although MK2 and CDK2 are quite similar in the phosphate region (with the exception of Thr206 in MK2 vs Ala144 in CDK2) the aminomethyl does not bind in a similar fashion to both kinases. In the MK2 structure, it forms a hydrogen bond to the backbone carbonyl of Glu190 in the solvent exposed region while in the CDK2 structure it forms a hydrogen bond with both side chains of the catalytic Asp145 and conserved Asn132. In the MK2 structure the lactam nitrogen is hydrogen bonded to the catalytic Asp207 while the lactam carbonyl is engaging catalytic Lys93. It is worth emphasizing that the lactam nitrogen is not engaged in a hydrogen bond interaction with the catalytic Asp145 in the CDK2 structure since the Asp145 side chain has undergone a conformational change and is interacting with the aminomethyl of compound **18** instead. However, in both structures the lactam carbonyl interacts with the catalytic Lys while the 7-methoxy group forms a hydrogen bond to the hinge region. The hydrogen bond to the hinge, which is also considered as a key interaction in a large number of kinases, is a bit longer (3.3 Å) in the CDK2 structure compared to that of MK2

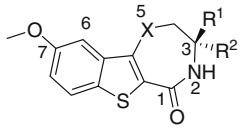


Scheme 1. Reagents and conditions: (a) (i) $SOCl_2$, chlorobenzene, 120 °C; (ii) MeOH; (b) (i) $Pd_2(dba)_3$ (5 mol %), (±)BINAP (10 mol %), Cs_2CO_3 (2 equiv) toluene, 110 °C, 24 h; (ii) TFA/CH_2Cl_2; (iii) NaOMe/MeOH; (c) (i) CDI; (ii) NH_4OH; (iii) BH_3·THF.



Scheme 2. Reagents and conditions: (a) (i) DBU/DMF; (ii) TFA/CH_2Cl_2; (iii) NaOMe/MeOH; (b) (i) 9-BBN, **12**, THF 0 °C; (ii) $Pd(PPh_3)_4$, Na_2CO_3; (iii) TFA/CH_2Cl_2; (iv) NaOMe/MeOH; (c) (i) MsCl, NEt_3, CH_2Cl_2; (ii) NaN_3, DMF; (iii) PPh_3, THF, H_2O.

Table 1
MK2 and CDK2 potencies of analogs **9–13**, **15**, **17**, **18**



Compound number	X	R^1, R^2	MK2 inhibition IC_{50}[a](μM)	CDK2 inhibition IC_{50}[a] (μM)	U937 TNFα release IC_{50}[a] (μM)
9	N	H,H	0.18	0.16	1.4
10	N	Me, H	0.04	0.012	0.7
11	N	H, Me	0.30	0.26	3.8
12	N	*n*-Pr, H	1.52	—	5.45
13	N	CH_2OH, H	0.014	0.025	0.4
15	S	H,H	1.09	0.15	5.5
17	C	H,H	0.50	0.36	4.7
18	N	CH_2NH_2,H	0.005	0.146	2.6

[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. 5.

(2.8 Å) which may account to a certain extent for the discrepancy in potency. While the nitrogen at the 5-position seems to be important for potency against both MK2 and CDK2, it does not appear to be making a direct hydrogen bond to either kinase. However, it might form a water-mediated hydrogen bond to the backbone carbonyl of Ile10 of MK2 and of Leu70 of CDK2. Another contributing factor could be the 5-position nitrogen donates some electron density to the lactam carbonyl oxygen via the thiophene ring. Alternatively, it could be also influencing the conformation of the seven-membered lactam ring (sp2 nitrogen vs sp3 carbon) to facilitate hydrogen bonding interaction of the lactam carbonyl and NH to the MK2 catalytic Asp/Lys pair.

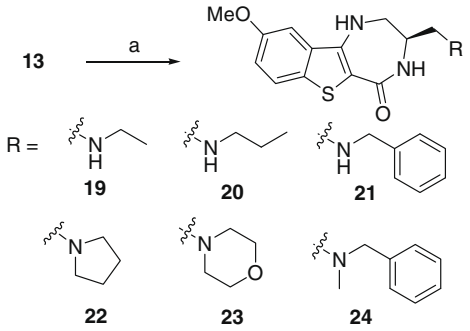
In an attempt to improve the cell potency of **18**, additional amino substituents were prepared and evaluated as described in Scheme 3. Alcohol **13** was activated as the mesylate and displaced with amines. The results are summarized in Table 2.

Simple alkyl substitution resulted in about 100-fold loss in potency for MK2 and a loss of cell potency. Cyclic tertiary amines **22** and **23** had no measurable potency towards MK2. None of the tested analogs had superior potency compared to the unsubstituted diazapenone **9**.

In our previous report[5] we described a strategy for gaining selectivity against CDK2 by exploiting differences in the amino acid sequence in the hinge region between MK2 and CDK2. Alteration of the hinge binding element was also examined in an effort to improve MK2 potency, selectivity and cellular potency. The 7-methoxy group of **10** was de-methylated to yield phenol **24.** Further elaboration gave analogs **26–31**. (Scheme 4, potency data is in Table 3).

None of these modifications afforded a compound with improved selectivity, enzyme or cellular potency as compared to compound **10**. Only compound **29** was equally potent against MK2, but with no improvements in selectivity against CDK2. The lack of selectivity of these analogs may be due to the conformational flexibility of the ether groups. A detailed study of more rigid systems is the subject of Part 2 of this Letter.

To verify that suppression of TNFα production in the U937 cell assay correlated with MK2 inhibition, we determined the amount of phospho-Ser78 present on HSP27 in LPS-stimulated U937 cells pre-incubated with **13**. HSP27 is a substrate of MK2 and may be used as an intracellular target biomarker to correlate with TNFα production as previously shown.[5] Amounts of phospho-p38 and phospho-JNK2 were also determined. Compound **13** showed no effect on levels of phospho-p38 and phospho-JNK2, but did inhibit

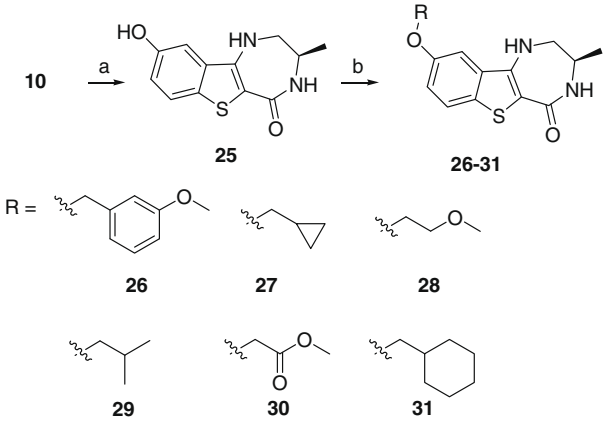


Scheme 3. Reagents and conditions: (a) (i) MsCl, NEt_3, CH_2Cl_2; (ii) amines, K_2CO_3, KI, 100 °C, microwave.

Table 2
MK2 and CDK2 potencies of analogs **19–24**

Compound number	MK2 inhibition IC_{50}[a] (μM)	CDK2 inhibition IC_{50}[a] (μM)	U937 TNFα release IC_{50}[a] (μM)
19	0.57	3.1	21.6
20	0.45	2.6	14.9
21	0.57	2.7	21
22	>20	—	—
23	>20	—	—
24	1.91	3.17	24

[a] IC_{50} 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. 5.



Scheme 4. Reagents and conditions: (a) (i) $MsCl/NEt_3$; (ii) amines

Table 3
MK2 and CDK2 enzyme potencies of analogs **25–31**

Compound number	MK2 inhibition IC_{50}[a] (μM)	CDK2 inhibition IC_{50}[a] (μM)	U937 TNFα release IC_{50}[a] (μM)
25	0.19	0.026	3.5
26	0.11	0.064	4.6
27	1.32	0.052	27
28	0.27	0.081	3.1
29	0.03	0.011	1.8
30	3.44	3.09	78

[a] IC_{50} 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. 5.

the phosphorylation of HSP27 in a dose- dependant manner with an IC_{50} value within twofold of the TNFα IC_{50} value. Since **13** inhibited the downstream marker (HSP27) but not the upstream kinase

pathway (p38) it is reasonable to conclude that the observed cell potency was primarily due to inhibition of MK2, thus, confirming that the inhibition of TNFα production with our MK2 inhibitors is within the mechanism of action.

In summary, we have reported an additional chemical series of potent MK2 inhibitors with improved cellular potency and that the observed cellular potency is within the mechanism of action. Improvements in MK2 potency may be achieved by modification of the diazapenone substituents. However, these changes did not result in improved cellular potency. Examination of crystal structures revealed additional opportunities for improvement of selectivity for MK2 over CDK2. It was observed that the engagement of the protonated aminomethyl is slightly different in MK2 compared to CDK2 resulting to a weaker hydrogen bond between the 7-methoxy group to the hinge for CDK2 compared to MK2 (0.5 Å difference in the length of the hydrogen bond). Discovery of MK2 selective inhibitors within this series will be the focus of Part 2.

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