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Novel 1-(2-aminopyrazin-3-yl)methyl-2-thioureas as potent inhibitors of mitogen-activated protein kinase-activated protein kinase 2 (MK-2)

Songnian Lin *, Matthew Lombardo, Sunita Malkani, Jeffrey J. Hale, Sander G. Mills, Kevin Chapman, James E. Thompson, Wen Xiao Zhang, Ruixiu Wang, Rose M. Cubbon, Edward A. O'Neill, Silvi Luell, Ester Carballo-Jane, Lihu Yang

Merck Research Laboratories, Rahway, NJ 07065, USA

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ABSTRACT

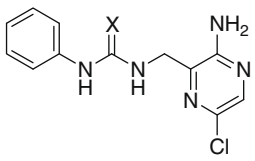
Novel 1-(2-aminopyrazin-3-yl)methyl-2-thioureas are described as inhibitors of mitogen-activated protein kinase-activated protein kinase 2 (MK-2). These compounds demonstrate potent in vitro activity against the enzyme with IC_{50} values as low as 15 nM, and suppress expression of TNFα in THP-1 cells and in vivo in an acute inflammation model in mice. The synthesis, structure–activity relationship (SAR), and biological evaluation of these compounds are discussed.

Tumor necrosis factor-α (TNFα) is a cytokine that is over-produced in several inflammatory disease states such as rheumatoid arthritis (RA).[1] Anti-TNFα biologics have been very successful in the treatment of several autoimmune diseases like RA, Crohn's disease and psoriasis.[2] Considerable effort has been devoted to the search for biological targets which are amenable for modulation with small molecules and a number of targets have been identified. Among them, p38 mitogen-activated protein kinase (MAPK) is most notable,[3] with inhibitors of p38 MAPK demonstrating efficacy in preclinical in vivo models as well as in RA patients in clinical trials.[4]

Mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK-2 or MK-2) is a direct substrate of p38 MAPK and plays a critical role in the signal transduction pathway regulating the production of TNFα.[5] MK-2 knockout mice produce significantly less TNFα when challenged with lipopolysaccharide (LPS),[5a] in addition to being fertile, healthy, and resistant to developing diseases in arthritis models.[5,6] MK-2 inhibition therefore may provide an effective treatment for TNFα-mediated diseases. Recently, several structural classes of compounds have been reported to be inhibitors of MK-2, among them aminocyanopyridines,[7] pyrrolopyridones,[8] tertrahydro-β-carbolines,[9] tricyclic indole derivatives,[10] pyrrolo-pyrimidones,[11] and benzothiophenes.[12]

Our effort to develop small molecule MK-2 inhibitors began with high throughput screening that identified several 1-(2-aminopyrazin-3-yl)methyl-2-thioureas as moderate MK-2 inhibitors as represented by phenyl analog **1a** (Fig. 1). Compound **1a** exhibits a 2.0 μM IC_{50} value in an in vitro MK-2 enzyme assay[13] and 7.9 μM IC_{50} value in a cell-based assay that measures LPS-stimulated TNFα production from THP-1 cells.[14] This prompted us to start a chemistry program to further explore this class of compounds. In this Letter, we would like to disclose a series of potent MK-2 inhibitors discovered through this effort.

Compounds **1–12** in Figures 1 and 2 and Tables 1–7 were synthesized according to Schemes 1 and 2. Substituted 2-aminopyrazin-3-carbamides **13**[15] were converted to corresponding cyanides



1a: X =S
1b: X = O

Figure 1. HTS hit **1a** and its analog **1b**.

* Corresponding author. Tel.: +1 732 594 0585; fax: +1 732 594 2840.
E-mail address: Songnian_lin@merck.com (S. Lin).

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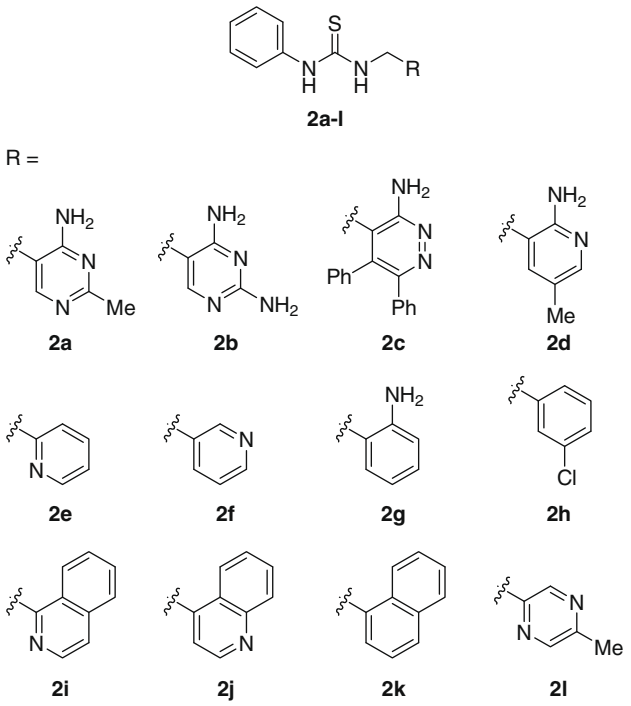
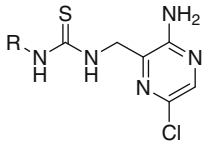


Figure 2. Preliminary SAR on aminopyrazine.

14 upon treatment with $POCl_3$ in DMF followed by aqueous HCl. Reduction of cyanides **14** with $LiAlH_4$ afforded desired amines **15** in good yields. Treatment of amines **15** with various of amines in the presence of 1,1′-thiocarbonyldiimidazole (TCI), or directly with isothiocyanides, provided corresponding 1-(2-aminopyrazin-3-yl)-methyl-2-thioureas **1a** and **3–12** in moderate to high yields (Scheme 1). Urea **1b** was prepared readily from amine **15a** with phenyl isocyanate, and compounds **2a–l** were prepared straightforwardly from phenyl isothiocyanate **16** with corresponding amines (Scheme 2).

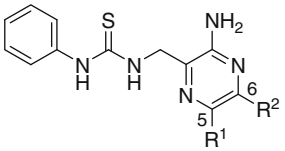
We began the SAR study on compound **1a** with a preliminary exploration of the minimal structural features that are required for MK-2 activity. A simple replacement of the central thiourea linker with an urea linker resulted in compound **1b** (Fig. 1), which is inactive at 40 μM concentration in the MK-2 enzyme assay. We

Table 1
Preliminary SAR on phenyl moiety



Compound	R	MK-2 IC_{50} (μM)
1a	Ph	2.0
3a	Et	20
3b	*i*-Pr	13
3c	*t*-Bu	2.5
3d	*c*-Pr	12
3e	*c*-Pentyl	53
3f	*c*-Heptanyl	35
3g	Bn	36
3h	$PhCH_2CH_2$	>50
3i	$MeOCH_2CH_2$	22
3j	$Et_2NCH_2CH_2CH_2$	>50
3k	Benzoyl	91
3l	1-Naphthyl	3.2

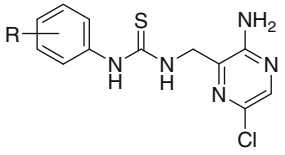
Table 2
SAR on the aminopyrazine moiety



Compound	R^1	R^2	MK-2 IC_{50} (μM)
1a	Cl	H	2.0
4a	H	H	32
4b	Me	H	4.0
4c	Et	H	1.5
4d	*n*-Pr	H	2.3
4e	CF_3	H	>50
4f	*c*-Pr	H	0.47
4g	Ph	H	~50
4h	Cl	NMe_2	>50
4i	Cl	OEt	>50
4j	Cl	SMe	>50

further explored the right-hand side aminopyrazine subunit. As shown in Figure 2, aminopyrazine was replaced with aminopyrimidines (**2a** and **2b**), aminopyridazine (**2c**), aminopyridine (**2d**), pyridines (**2e** and **2f**), quinolines (**2j**), isoquinolines (**2i**), naphthylenes (**2k**), and benzenes (**2g** and **2h**), and all these replacements resulted in the loss of activity (IC_{50} > 40 μM). The deletion of the 2-amino group and the replacement of a 5-Cl with a 6-methyl

Table 3
SAR on the phenyl ring



Compound	R	MK-2 IC_{50} (μM)
5a	4-Me	2.7
5b	4-Cl	3.7
5c	4-MeO	1.9
5d	4-*i*-Pr	2.6
5e	4-*t*-Bu	13.7
5f	4-Br	3.1
5g	$4\text{-}NO_2$	9.9
5h	4-CN	6.9
5i	4-Tetrazole	>40
5j	4-BnO	2.7
5k	4-Ac	6.9
5l	4-EtOC(=O)–	10.7
5m	$4\text{-}NMe_2$	7.0
5n	4-(Morphorin-1-yl)	17.3
5o	4-AcNH–	5.2
5p	$4\text{-}NH_2$	2.0
5q	4-BnOC(=O)NH–	0.46
6a	2-Me	3.6
6b	2-Cl	1.1
6c	2-MeO	2.6
6d	2-F	2.0
6e	2-MeS	2.5
6f	2-Ph	>20
7a	3-Me	9.3
7b	3-Cl	11.3
7c	3-MeO	14.5
8a	2,4-Di-MeO	2.5
8b	2,4-Di-Cl	4.3
8c	3,4-Di-MeO	~40
8d	3,4-Di-Cl	~40
8e	3,5-Di-Cl	27