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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

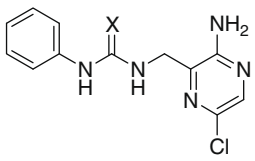
Novel 1-(2-aminopyrazin-3-yl)methyl-2-thioureas are described as inhibitors of mitogen-activated protein kinase-activated protein kinase 2 (MK-2). These compounds demonstrate potent in vitro activity against the enzyme with IC_{50} values as low as 15 nM, and suppress expression of TNFα in THP-1 cells 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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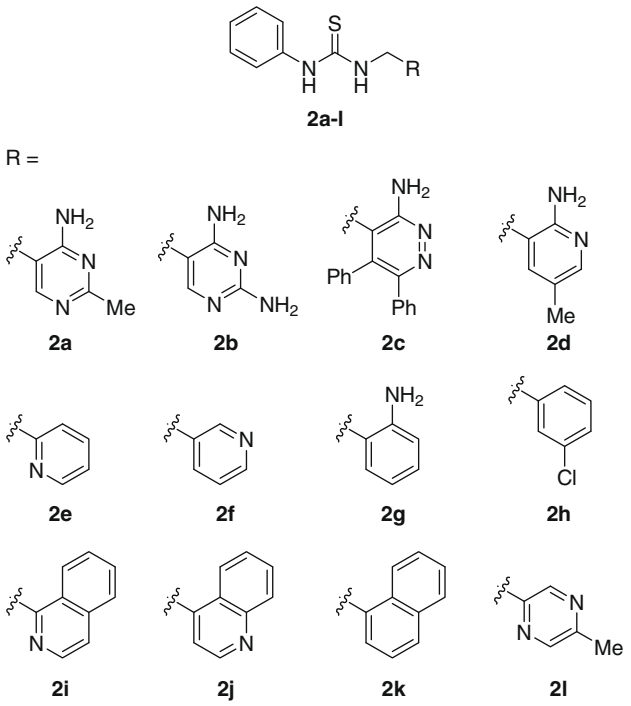
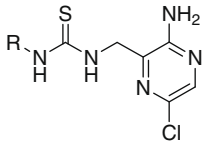


Figure 2. Preliminary SAR on aminopyrazine.

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).

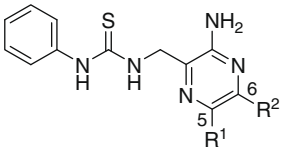
We began the SAR study on compound **1a** with a preliminary exploration of the minimal structural features that are required for MK-2 activity. A simple replacement of the central thiourea linker with an urea linker resulted in compound **1b** (Fig. 1), which is inactive at 40 μM concentration in the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

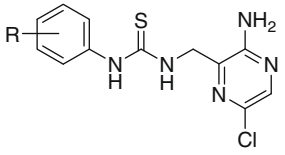
Table 2
SAR on the aminopyrazine moiety



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

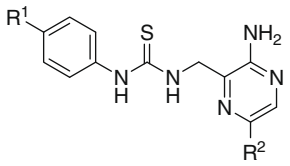
further explored the right-hand side aminopyrazine subunit. As shown in Figure 2, aminopyrazine was replaced with aminopyrimidines (**2a** and **2b**), aminopyridazine (**2c**), aminopyridine (**2d**), pyridines (**2e** and **2f**), quinolines (**2j**), isoquinolines (**2i**), naphthylenes (**2k**), 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

Table 4
SAR on C4 position of phenyl ring

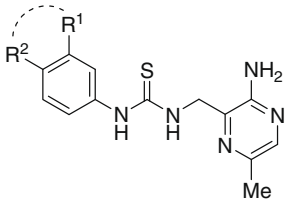


Compound	R^1	R^2	MK-2 IC_{50} (μM)
5q	BnOC(=O)NH–	Cl	0.46
9a	MeOC(=O)NH–	Cl	0.94
9b	EtOC(=O)NH–	Cl	0.31
9c	*n*-BuOC(=O)NH–	Cl	0.23
9d	*i*-BuOC(=O)NH–	Cl	0.15
9e	*t*-BuOC(=O)NH–	Cl	0.19
9f	*i*-BuOC(=O)N(Ph)–	Cl	1.5
9g	MeNHC(=O)NH–	Cl	1.7
9h	*t*-BuNHC(=O)NH–	Cl	0.70
10a	*t*-BuOC(=O)NH–	Me	0.21
10b	PhOC(=O)NH–	Me	0.73
10c	EtOC(=O)N(Me)–	Me	10.0
10d	*t*-BuOC(=O)O–	Me	2.9
10e	EtC(=O)NH–	Me	13.3
10f	*n*-PrC(=O)NH–	Me	2.3
10g	*n*-BuC(=O)NH–	Me	1.5
10h	BnC(=O)NH–	Me	3.8
10i	PhC(=O)NH–	Me	0.58

group on aminopyrazine (**2l**) led to inactive compounds as well (IC_{50} > 40 μM). In contrast, the left-hand phenyl group is more amenable to the modifications as can be seen in Table 1. Replacements with sizable alkyl groups (**3a–c**), cycloalkyl groups (**3d–f**), methoxyethanyl group (**3i**), benzyl group (**3g**), or a naphthyl group (**3l**) retain a certain level of activity, albeit most of them are weaker than that of the original phenyl group. The key structural features of **1a**, that is, the aminopyrazine subunit, the phenyl group, and the thiourea linker, were therefore kept in our further SAR explorations.

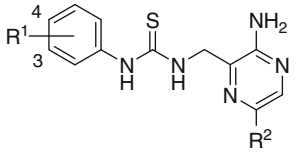
We then focused the SAR effort on the right hand side of compound **1a**, with emphasis on the C5 and C6 positions of the aminopyrazine subunit. The results are summarized in Table 2. C6 position was found not tolerant to bulky groups, as substitutions with alkoxyl, amino, or mercaptan groups all led to inactive compounds (**4h–j**). Deletion of chlorine substitution at C5 position (**4a**), or replacing it with a phenyl group (**4g**), resulted in ca. 15–25-fold weaker activity. While replacement of the C5 chlorine with a small alkyl group such as a methyl, an ethyl or a *n*-propyl group retains a similar level of potency (**4b–d**), replacement with a trifluoromethyl group completely loses the activity (**4e**). Introduction of a cyclopropyl group at C5 position, however, significantly improves the potency, which led to the identification of the first compound in this series that possesses sub-micromolar activity (**4f**, IC_{50} = 0.46 μM).

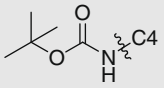
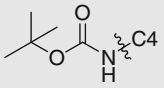
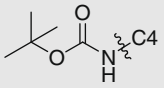
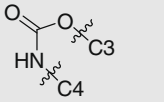
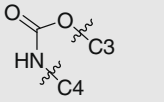
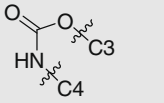
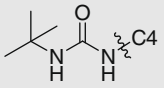
Table 5
SAR on the bi-cyclic left-hand moiety



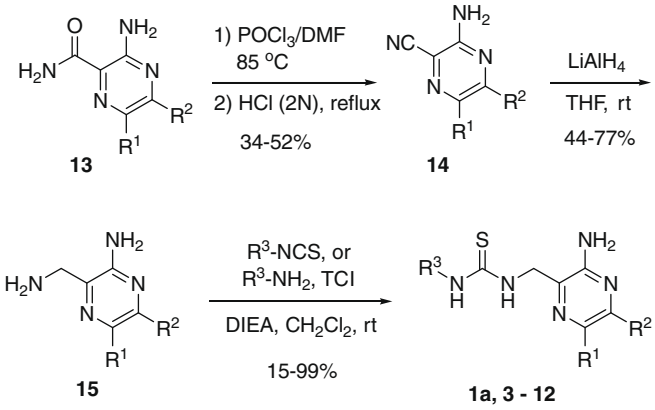
Compound	R^1–R^2	MK-2 IC_{50} (μM)
11a	3-OC(=O)NH-4	0.21
11b	3-NHC(=O)NH-4	15
11c	3-CH_2C(=O)NH-4	27
11d	3-SC(=O)NH-4	15
11e	3-NHC(=O)O-4	14
11f	3-NHC(=O)N(Me)-4	>20

Table 6
Selected SAR on both phenyl group and aminopyrazine

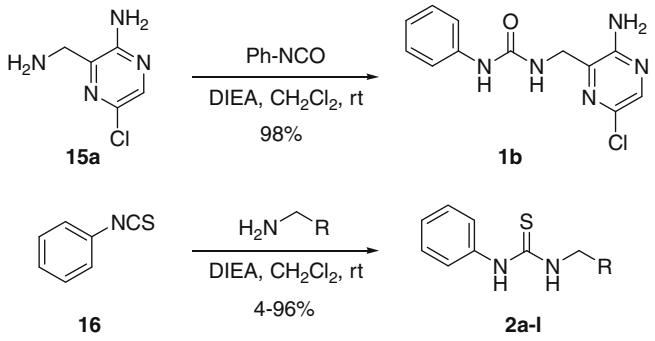


Compound	R^1	R^2	MK-2 IC_{50} (μM)	THP-1 IC_{50} (μM)
12a		Et	0.14	4.0
12b		*n*-Pr	0.045	3.0
12c		*c*-Pr	0.103	3.2
12d		*n*-Pr	0.020	0.75
12e		*n*-Bu	0.030	3.8
12f		*c*-Pr	0.015	1.7
12g		*c*-Pr	0.083	1.4

The SAR exploration on the left side phenyl subunit of compound **1a** utilizing the initial 5-Cl right-hand side was carried out simultaneously. A number of compounds with mono- or di-substitution on the phenyl ring were synthesized, and wide-ranged substituents such as halides, alkyl, aryl, alkoxy, amino, cyano, nitro, acyl, carboxylic, tetrazole, and amido groups were investigated. As shown in Table 3, mono-substitution on the 4-position (**5a**, **5b**, and **5c**, 4-Me, Cl, and MeO, respectively) or the 2-position (**6a**, **6b**, and **6c**, 2-Me, Cl, and MeO, respectively) of the phenyl group is clearly preferred over 3-position (**7a**, **7b**, and **7c**, 3-Me, Cl, and MeO, respectively), with 3–10-fold differences in potency. Similarly, 2,4-disubstitution (**8a** and **8b**, 2,4-di-MeO and Cl, respectively) exhibits 6–15-fold better activity compared with 3,4-disubstitution (**8c** and **8d**, 3,4-di-MeO and Cl, respectively) or 3,5-disubstitution (**8e**, 3,5-di-Cl). Overall, however, most of these modifications resulted in similar or weaker activities compared with that of **1a**, which has an unsubstituted phenyl group. The highlight of this SAR study, however, was discovered when a benzyl carbamate group was incorporated at the C4 position, which resulted in over 4-fold improvement in potency (**5q**, IC_{50} = 0.47 μM).



Scheme 1. Synthesis of compounds **1a**, **3–12**. See Table 2 for R^1 and R^2.



Scheme 2. Synthesis of compound **1b** and **2**. See Figure 2 for R.

To follow up with the 4-carbamate SAR of compound **5q**, a series of 4-amino derivatives was synthesized and evaluated for their inhibitory activity against MK-2, utilizing both 5-Cl and 5-Me right-hand side (Table 4). Alkyl and aryl carbamates were first explored, and all compounds prepared in this series exhibit improved potency over compound **1a** (**9a–e** and **10a–b**, IC_{50} = 0.15–0.94 μM). *tert*-Butyl (**9e** and **10a**, IC_{50} = 0.19 and 0.21 μM, respectively) and *iso*-butyl carbamates (**9d**, IC_{50} = 0.15 μM) were identified to be the most potent moieties. Replacement of the carbamate moiety with a urea moiety was found well-tolerated with only slight compromise in activity (**9g** and **9h,** IC_{50} = 1.7 and 0.70 μM, respectively). However, the free NH is found to be necessary to keep the high potency for the carbamate series, as substituting it with a phenyl group (**9f**) or a methyl group (**10c**), or replacing it with an oxygen (**10d**) resulted in significant loss of potency. Replacing the carbamate with an amide moiety was also investigated. As observed earlier with compound **5o** (Table 3), the aliphatic amides do not have much influence on activity (**10e–h**, Table 5). Aromatic amides, that is, benzamide (**10i**, IC_{50} = 0.58 μM) and 2-furanyl amide (**10j**, IC_{50} = 0.61 μM), however, do improve the activity markedly compared to compounds **4b** and **1a**.

As a further extension of the SAR on the 4-amino series, we also synthesized several compounds where 3- and 4-substituents are joined to form a ring (Table 5). Very intriguingly, none of these compounds show good potency against MK-2 in the in vitro enzyme assay, except cyclic carbamate compound **11a**. Compound **11a** exhibits an IC_{50} of 0.21 μM, which is over 70-fold better than that of other closely related compounds.

Having identified potent moieties on both the left-hand side and the right-hand side of compound **1a**, we then incorporated these moieties onto the same molecules. Selected compounds were synthesized as showed in Table 6. As expected, many of these compounds show further improved activity, with value of IC_{50} in the MK-2 enzyme assay as low as 15 nM (**12f**). In the THP-1 cellular assay, these compounds were all active and exhibited up to 10-fold improved potency (**12d**, IC_{50} = 0.78 μM) compared to compound **1a** (IC_{50} = 7.9 μM) in reducing LPS-stimulated TNFα production from THP-1 cells. As is clearly evident from literature,[7–12] there are few known potent MK-2 inhibitors shown to possess submicromolar cellular potency. These thioureas, therefore, represent a novel class of highly potent MK-2 inhibitors.

Compounds **12b**, **12d**, and **12f** were selected for further study in vivo. In an acute mouse inflammation model (mouse LPS model),[16] all three compounds were found to be highly active (10 mg/kg, iv), with 62%, 70%, and 75% inhibition of TNFα production, respectively for **12b**, **12d**, and **12f**, one and half hours after LPS challenge.[17]

In summary, we have discovered a novel series of MK-2 inhibitors based on 1-(2-aminopyrazin-3-yl)methyl-2-thiourea. Extensive SAR studies were carried out on both the left-hand phenyl moiety and the right hand aminopyrazine moiety of initial HTS lead compound **1a**, which resulted in the identification of potent inhibitors with IC_{50} values as low as 15 nM. These compounds suppress the expression of TNFα in THP-1 cells and in vivo in an acute inflammation model in mice.[18]

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13. Protocol of the MK-2 enzyme assay: The assay is started by mixing 12.5 μL of 0.5 nM MK-2 with 12.5 μL of 4 μM biotinylated HSP27 72-90 (biotin-LC-AYSRALSRQLSSGVSEIRH) and 1.0 μM ATP/30 μCi/mL ^{33}P-ATP with or without 1 μL of compound titration in kinase assay buffer (KB), which includes 50 mM Hepes (pH 7.5), 10 mM MgCl, 1 mM DTT, and 1 mg/mL BSA. The reaction was incubated at room temperature for 30 min. The reaction was quenched by addition of 125 μL of quench solution (PBS, pH 7.5, 50 mM EDTA, and 0.1% Triton-100) containing 800 pmol/mL SPA bead to the above reaction mixture. The plate was spun at 2000 rpm for 5 min, and counted at top counter (Packard).

14. For protocol of the THP-1 assay, see: Natarajan, R. S.; Wisnoski, D. D.; Singh, S. B.; Stelmach, J. E.; O'Neill, E. A.; Schwartz, C. D.; Thompson, C. M.; Fitzgerald, C. E.; O'Keefe, S. J.; Kumar, S.; Hop, C. E. C. A.; Zaller, D. M.; Schmatz, D. M.; Doherty, J. B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 273.
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16. Mouse LPS challenge assay: 12-week-old female Balb/c mice were dosed with a MK-2 inhibitor (iv, 10 mg/kg in DMSO/Cremophor/saline) or vehicle, just prior to injection of 10 μg/mouse LPS (*Escherichia coli* Sero-type 0111:b4, sigma) and 800 mg/kg D-galactosamine (sigma) in saline. Animals were euthanized 90 min later, and plasma TNFα was measured by ELISA. See also: O'Keefe, S. J.; Mudgett, J. S.; Cupo, S.; Parsons, J. N.; Chartrain, N. A.; Fitzgerald, C.; Chen, S.-L.; Lowitz, K.; Rasa, C.; Visco, D.; Luell, S.; Carballo-Jane, E.; Owens, K.; Zaller, D. M. *J. Biol. Chem.* **2007**, *282*, 34663.
17. To further support that the activity of these compounds was primarily due to MK-2 inhibition, the kinase selectivity of compounds **12b** and **12d** was investigated by screening against a panel of 33 kinases, including related MAPKAP kinases, p38 MAP kinases, CDK2, and other kinases associated with TNFα production. Of these kinases targeted for screening, only two had >70% inhibition at 1 μM for compound **12d** (MK-5 and AMPK) and only four had >70% inhibition at 10 μM for compound **12b** (RPS6KA1, RPS6KA5, AMPK, and LCK). The pharmacokinetic properties of these two compounds were evaluated in rat and the results are shown below in Table 7.

Table 7
Pharmacokinetic properties of **12b** and **12d** in rat

Compound	Cl (mL/min/kg)	$T_{1/2}$ (h)	AUCN (μM h kg/mg)	F (%)
12b	2.5	0.8	3.3	19
12d	12.9	0.3	0.3	8

18. Modeling effort is being undertaken to further understand how these novel inhibitors interact with MK-2 in light of the recently available information on the binding site of MK-2 (see Refs. 7, 9a, 10a, and 12). The results of this study, together with the results of our continuous SAR effort to develop novel aminopyrazine-based non-thiourea MK-2 inhibitors, will be communicated separately in the near future.